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**Commentary**

In this issue of the *JCI*, Semple and colleagues report phenotypic evaluation of patients with a germline mutation in the gene encoding serine/threonine kinase AKT2 (see the related article beginning on page 315). Their findings support the idea that the postreceptor actions of insulin in the liver — suppression of gluconeogenesis and stimulation of lipogenesis — are mediated through divergent pathways that can be uncoupled. The results appear to refine the arrangement of crucial steps along these pathways and show how comprehensive study of the phenotype, "deep phenotyping," of patients who carry rare mutations might complement other types of experiments to elucidate complex pathways and mechanisms.



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# Hoofbeats, zebras, and insights into insulin resistance

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**In this issue of the *JCI*, Semple and colleagues report phenotypic evaluation of patients with a germline mutation in the gene encoding serine/threonine kinase AKT2 (see the related article beginning on page 315). Their findings support the idea that the postreceptor actions of insulin in the liver — suppression of gluconeogenesis and stimulation of lipogenesis — are mediated through divergent pathways that can be uncoupled. The results appear to refine the arrangement of crucial steps along these pathways and show how comprehensive study of the phenotype, "deep phenotyping," of patients who carry rare mutations might complement other types of experiments to elucidate complex pathways and mechanisms.**

"When you hear hoofbeats, think horses, not zebras" is the quintessential maxim of clinical medicine. But in clinical investigation, the "zebras" — rare conditions that recapitulate, often to an extreme, the components of a common disease — can help to understand the "horse" or common complex phenotype. Extending the metaphor, the current pandemic of obesity and insulin resistance is a veritable stampede that threatens to flatten global medical care infrastructures. A multifaceted approach is required to understand the mechanisms underlying this pandemic, ranging from the strategic use of model systems to population studies and clinical trials. Within this methodological spectrum is the evolving discipline of clinical phenomics, which uses objective and systematic acquisition of phenotypic data (i.e., deep phenotyping) of selected informative patients (1). Phenomic evaluation of patients with rare genetic disorders is a potential tool to help solve the puzzle of insulin resistance and its downstream metabolic consequences, such as hyperglycemia, hepatosteatosis and dyslipidemia, elevated triglyceride (TG) levels, and depressed HDL cholesterol levels.

## Insulin-resistant diabetes: a disease of abnormal glucose and lipid metabolism

The complex web of interactions between glucose and lipid metabolism in diabetes has long been appreciated, if incompletely understood mechanistically (2–5). Induced-mutant animal models have steadily advanced our understanding of insulin resistance. For instance, insulin-resistant mice with a liver-specific deletion of the insulin receptor (INSR) develop hyperglycemia but not dyslipidemia (6). This suggests an uncoupling or divergence (see Figure 1) between the post-INSR pathways, with loss of insulin-mediated suppression of gluconeogenesis normally driven by phosphoenolpyruvate decarboxylase and glucose-6-phosphatase, but retention of insulin's stimulatory effect on hepatic lipogenesis catalyzed by fatty acid synthases (7). Signaling intermediates along the gluconeogenesis limb include serine/threonine kinase AKT2 (also called phosphokinase B) and forkhead box O transcription factor 1 (FOXO1), while intermediates along the de novo hepatic lipogenesis limb include PKCλ and SREBP-1c (8, 9). To clarify the basis of such "asymmetric" or partial insulin resistance, deep phenotyping of patients carrying naturally occurring loss-of-function mutations in these intermediate signaling molecules might be instructive.

In their current study in this issue of the *JCI*, Semple and colleagues (10) have attempted to probe these pathways by studying patients with extremely rare mutations in either *INSR* or *AKT2* genes together with subjects who had either idiopathic insulin resistance or inherited lipodystrophies. The study took advantage of a valuable archive of phenotypically and molecularly characterized patients who were carefully collected over many years. Among patients with *INSR* mutations and anti-INSR antibodies, Semple and colleagues first confirmed earlier work that showed severe hyperglycemia and hyperinsulinemia but normal plasma lipids in these patients (11). These findings are

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**Nonstandard abbreviations used:** FOXO1, forkhead box O transcription factor 1; INSR, insulin receptor; TG, triglyceride.

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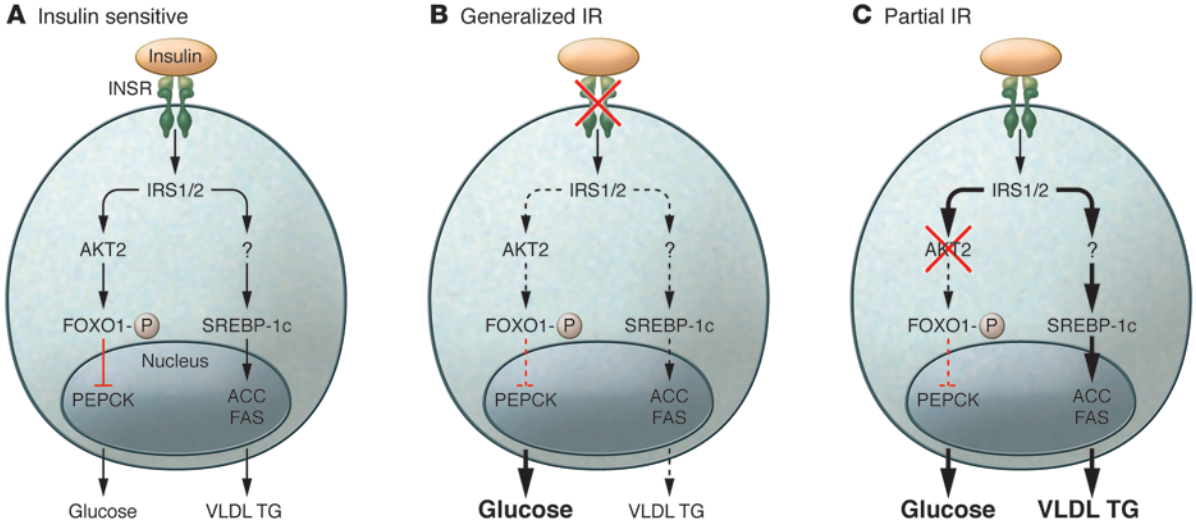


**Figure 1**
Proposed model for distinct hepatic insulin resistance (IR) phenotypes resulting from *INSR* and *AKT2* gene mutations. (**A**) Under normal circumstances, insulin stimulation of the INSR activates apparently independent arms of the signaling pathway: (i) AKT2 activation, phosphorylation of FOXO1, and inhibition of gluconeogenic gene transcription, leading to decreased glucose output, and (ii) activation of SREBP-1c target lipogenic genes, leading to secretion of triglyceride-rich VLDLs (VLDL TG). (**B**) Mutation in *INSR* prevents activation of both arms of the pathway, resulting in increased glucose levels without increased TG levels. (**C**) Mutation in the *AKT2* gene impairs inhibition of gluconeogenesis without abolishing the lipogenic effect of insulin, resulting in increased glucose and TG levels. ACC, acetyl-coenzyme A carboxylase; FAS, fatty acid synthase; PEPCK, phosphoenolpyruvate carboxykinase.

consistent with the idea that at least at the hepatic level, loss of INSR function leads to loss of suppression of hepatic glucose output without enhanced hepatic lipogenesis. This was confirmed in a subgroup of four patients using stable isotope methodology to directly measure hepatic lipogenesis.

## New insights from two patients with very rare mutations

A new observation in the report by Semple and colleagues (10) derives from the evaluation of two patients with a dominant loss-of-function missense mutation — namely R274H — in *AKT2*. Affected family members were previously shown to have a unique distribution of lipoatrophy with severe insulin resistance leading to diabetes (12). Now, deeper phenotyping of two *AKT2* R274H heterozygotes revealed a syndrome of insulin resistance that was associated with hepatosteatosis, increased de novo lipogenesis, and metabolic dyslipidemia, with elevated plasma TG and depressed HDL cholesterol levels. Because of where AKT2 has been thought to reside in the canonical cascade of insulin-mediated suppression of gluconeogenesis, the observations in the patients with the germline *AKT2* mutations are consistent with the idea that AKT2 is distal to the branch point of pathways that modulate hepatic glucose and lipid production, especially considered together with the hyperglycemia plus normolipemia observed in patients with *INSR* mutations. The observations indicate that hepatic lipogenesis and dyslipidemia persist among patients with functional impairment in AKT2, with the caveat that other background genetic or environmental factors were uncontrolled and might have been substantial given the heterogeneity of many clinical and biochemical attributes between the two individuals, including variable lipoatrophy.

These results (10), while interesting and potentially important, must be interpreted cautiously. For instance, surrogate biochemical measures, as opposed to more direct measures, were used to infer the presence of insulin resistance (13). Also, the sample of patients who received deeper phenotyping (four with *INSR* and two with *AKT2* mutations), while it provides interesting anecdotal data, is too small for meaningful statistical comparisons, thus preventing firmer conclusions. Furthermore, there is the possible confounding influence of pleiotropic effects of the mutations, interactions with unmeasured background oligogenic or polygenic factors, or long-term gene-environment interactions. In addition, more recent data from mice with hepatic-specific deletion of INSR substrates 1 and 2 (IRS1/2) (14) and from mice with inactivated hepatic *Foxo1* (15) suggest that the working model (7) for downstream consequences of insulin signaling (at least for the gluconeogenesis limb) might require some revision (16). Finally, the data derived from the study of *INSR* and *AKT2* mutations cannot rule out a model in which the gluconeogenic and lipogenic arms of the insulin-signaling pathway diverge prior to the action of IRS1/2. Further work with mutations in humans or mouse models may allow refinement of the model.

## Shifting from the local to the global

While it is tempting to extrapolate the potential mechanism of partial insulin resistance to "garden-variety" obesity-related insulin resistance, the investigators (10) did not directly study individuals with this highly prevalent phenotype. The common acquired and rare genetic forms of insulin resistance are each subject to complex and likely distinct physiological background and long-term compensatory mechanisms. For instance, alterations related to diet-induced obesity have been found in insulin-resistant adipose tissue and muscle (17). Emerging evidence also implicates intestinal insulin resistance as a major contributor to metabolic dyslipidemia (18). Signaling pathways in adipose tissue can modulate hepatic insulin sen-

sitivity (19). Furthermore, incretins (20) and inflammatory molecules (21), among many others, contribute to an increasingly complex network of mechanisms in insulin resistance. Thus, any interpretation derived from two patients with a rare genetic mutation affecting insulin signaling would seem to require further validation by independent experiments, with only a consistent totality of evidence eventually permitting extrapolation to the general population.

The caveats of the phenomic analysis of small human cohorts notwithstanding, the findings of Semple and colleagues are intriguing and encourage the generation of hypotheses (10). Future clinical investigations might mirror the current report but instead evaluate a larger number and wider range of subjects, including a subgroup with garden-variety obesity and insulin resistance and control subjects with normal insulin sensitivity. Also, higher-sensitivity tools could be used to evaluate such metabolic parameters as (a) endogenous glucose production determination using tracer glucose techniques, (b) peripheral glucose disposal using the euglycemic hyperinsulinemic clamp, (c) plasma fatty acid, lipid, and lipoprotein kinetics using tracers such as stable isotopes, (d) morphology and function of fat stores, using spectroscopy of muscle and whole-body MRI imaging of visceral and subcutaneous adipose depots, and (e) response to interventions such as diet, exercise, or medications. In addition, complementary studies would include phenotypic evaluation of induced-mutant animal models with strategic crosses of specific informative strains, accompanied by appropriate real-time knockdown, gene replacement, or pharmacologic inhibition of key molecules. Finally, high-throughput resequencing of *AKT2*, *FOXO1*, *SREBP1C*, or *PCK* in large patient cohorts or even the looming possibility of "agnostic" whole-genome sequence analysis of patients with insulin resistance (an approach that ignores prior knowledge of a gene product's function) will greatly increase the detection of rare mutations or low-frequency functional polymorphisms in known candidate genes and in previously unknown genes.

## Redefining insulin resistance

In addition to fleshing out the steps, branch points, and redundancies in pathways of glucose and lipid metabolism, the results of future experiments might eventually permit an expanded, perhaps molecularly based classification of insulin resistance, defined for instance on the primary mechanistic disturbance or the identity of the disrupted signaling molecule. This in turn might permit more rational drug design and tailored interventions. Additional deep phenotyping of patients with naturally occurring mutations — "gift horses" — will increasingly become part of a comprehensive strategy to understand complex traits and biological pathways such as insulin resistance. As far as patients with common insulin resistance and metabolic syndrome are concerned, horses of a different color found within the general population may actually be zebras with different stripes.

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