Insulin action and resistance in obesity and type 2 diabetes

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Nutritional excess is a major forerunner of type 2 diabetes. It enhances the secretion of insulin, but attenuates insulin’s metabolic actions in the liver, skeletal muscle and adipose tissue. However, conflicting evidence indicates a lack of knowledge of the timing of these events during the development of obesity and diabetes, pointing to a key gap in our understanding of metabolic disease. This Perspective reviews alternate viewpoints and recent results on the temporal and mechanism connections between hyperinsulinemia, obesity and insulin resistance. Although much attention has addressed early steps in the insulin signaling cascade, insulin resistance in obesity seems to be largely elicited downstream of these steps. New findings also connect insulin resistance to extensive metabolic cross-talk between the liver, adipose tissue, pancreas and skeletal muscle. These and other advances over the past 5 years offer exciting opportunities and daunting challenges for the development of new therapeutic strategies for the treatment of type 2 diabetes.

The term ‘insulin resistance’ refers to a decrease in a target cell’s metabolic response to insulin, or, at the whole-organism level, an impaired lowering effect of circulating or injected insulin on blood glucose (see Box 1 for an overview of insulin signaling)1. It is a hallmark of obesity and sedentary behavior and a forerunner of type 2 diabetes, which affects a remarkable 9% of the US population2. A substantial number of comorbidities are associated with diabetes, including kidney failure, neuropathy, retinopathy and vascular morbidities that lead to ischemic heart disease and to nearly 75,000 amputations per year2.

The factors involved in the development of metabolic disease are complex, however, because many individuals with obesity who have a preponderance of subcutaneous, rather than visceral, adipose tissue seem to be protected from insulin resistance and adverse metabolic responses3. Nonetheless, numerous findings over many decades of work have solidified a strong overall paradigm4,5 in which overnutrition causes insulin resistance in the liver, skeletal muscle and adipose tissue. A decrease in GLUT4 levels at the surface membrane in muscle would reduce glucose uptake from the circulation. In the liver, insulin normally causes phosphorylation and suppression of FOXO1 in the liver12 and disruption of GLUT4 glucose-transporter translocation to the surface membrane in skeletal muscle13,14. FOXO1 increases the expression of key enzymes of gluconeogenesis; hence, its phosphorylation results in the increased conversion of incoming substrates to the liver to glucose. A decrease in GLUT4 levels at the surface membrane in muscle would reduce glucose uptake from the circulation. In the liver, insulin normally causes phosphorylation and suppression of FOXO1 function through the action of the protein kinase Akt, which causes FOXO1 to be retained in the cytoplasm, where it is inactive15,16 (Fig. 2). However, in mice with obesity, Foxo1 expression is upregulated, and the protein is apparently modified to become insensitive to insulin regulation17,18. How overnutrition causes the disruption of FOXO1 regulation is still under investigation19,20, but recent studies

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Glucose homeostasis is maintained by coordinating the production of glucose in the liver through the pathways of glycogenolysis and gluconeogenesis in times of fasting with the disposal during feeding of glucose into skeletal muscle through glycogen synthesis and glucose metabolism, and to a much lesser extent, with adipose tissue (lower box in illustration). The hormone insulin, secreted by the beta cells of the pancreas in times of nutrient uptake, inhibits hepatic glucose output while enhancing glucose uptake into muscle and adipose tissue. Glucose is released through the glucose transporter GLUT2 in the liver, whereas the insulin-sensitive GLUT4 mediates glucose uptake in muscle and fat. The major canonical insulin signaling cascade required for this maintenance of blood glucose concentrations activates a key protein kinase Akt (upper box in the illustration). This Akt protein kinase (three isoforms are known) is required for insulin regulation of the pathways that control systemic glucose homeostasis, including glucose transport in adipocytes and muscle, and the inhibition of hepatic gluconeogenesis and cell-autonomous activation of hepatic lipogenesis.

The binding of insulin to its receptor protein activates its intrinsic tyrosine kinase activity, which phosphorylates insulin-receptor substrate (IRS) proteins on tyrosine residues that then serve as anchoring sites for the p85 regulatory subunits of p85/p110 PI3K kinase at the cell membrane. This generates the formation of the phospholipid phosphatidylinositol 3,4,5-phosphate (PtdIns(3,4,5)P3) from PtdIns(4,5)P2 in the membrane, which facilitates recruitment and interaction between the protein kinases PDK1 and Akt. This in turn leads to the phosphorylation and activation of Akt on threonine 308. Additional activation of Akt occurs upon its phosphorylation by a second protein kinase, mTORC2. Interestingly, through an Akt-mediated pathway, the activation of Akt protein kinases triggers insulin sensitivity in mice with insulin resistance in obesity.

This Akt protein kinase (three isoforms are known) is required for insulin signaling to Akt (red elements on right side of upper box in the illustration). Such mechanisms are often claimed to be the cause of systemic insulin resistance in obesity. A major thesis of this Perspective is that the attenuated systemic metabolic responses to insulin observed in obesity and under HFD conditions in rodents and humans largely occur either downstream or independently of insulin signaling to Akt. PKCε, protein kinase Cε; JNK, c-jun N-terminal kinases; IKKβ, inhibitor of nuclear factor kappa-B kinase subunit β; PKC, protein kinase C; PP2A, protein phosphatase 2A.

In mice, pinpointing it as a key step in a feed-forward loop of unrestrained gluconeogenesis in obesity, hyperglycemia caused by the uncoupling of FOXO1 from suppression by insulin, in conjunction with the resulting chronic hyperinsulinemia, might dampen insulin’s inhibitory action on adipose tissue lipolysis. This unrestrained lipolysis in visceral adipocytes in turn increases delivery to the liver of its products—free fatty acids, which promote gluconeogenesis through allosteric mechanisms during their metabolism, and the gluconeogenesis substrate glycerol. This unrestrained lipolysis concept was proposed in earlier studies in dogs, as well as in more recent work showing that in mice lacking the adipocyte lipase ATGL, hepatic gluconeogenesis is attenuated and glucose intolerance is attenuated. Thus, under high-fat diet (HFD) conditions, the hepatic glucose output stimulated by upregulated Foxo1 is further enhanced by unrestrained adipocyte lipolysis.

In addition to impaired insulin responsiveness in adipocytes, obesity might also promote lipolysis, through the decreased expression of adipocyte lipid-droplet proteins such as perilipin and Cide proteins. These molecules promote triglyceride retention in unilocular droplets in mature adipocytes through the inhibition of lipolysis; humans or mice lacking perilipin and Cide have lipodystrophy and insulin resistance.

The decreased capacity of adipocytes to store and retain triglyceride in obesity, causing ectopic fat accumulation and lipotoxicity, in the liver and muscle, has received much support as a potential cause of insulin resistance. Experiments also show that transplants of relatively small amounts of adipose tissue from lean mice can induce weight loss and correct insulin resistance in mice with obesity. Such small transplants would not seem to have the capacity to store much triglyceride, which suggests that secreted factors might also offer therapeutic value. In any case, the resultant blood-glucose increase in response to the primary insulin resistance caused by either lipotoxicity or the disruption of beneficial factors secreted from adipocytes is postulated to trigger insulin secretion, and thereby cause hyperinsulinemia.

The above scenario also explains how hypertriglyceridemia may occur in obesity. Insulin signaling through Akt in the liver activates fatty acid synthesis from glucose and amino acids, a pathway termed de novo lipogenesis (DNL), which culminates in the packaging of triglycerides into very-low-density lipoproteins (VLDLs) for export and uptake into peripheral tissues. Thus, under conditions of nutrition excess, hyperinsulinemia might amplify the usual stimulation of this lipogenic pathway that occurs under normal feeding conditions, thus sustaining the obese state and leading to overproduction of lipids. How insulin resistance could be selectively imposed on gluconeogenesis while leaving its actions on lipogenesis intact is likely explained by the divergence of insulin signaling downstream of Akt. Although FOXO1 inactivation by Akt controls gluconeogenesis,
Akt activation of the mTORC1 protein-kinase complex and transcription factor SREBP-1c enhances lipid synthesis. Under HFD feeding conditions, the Akt activation blunted by insulin is unable to suppress the modified, dysregulated hepatic FOXO1 and adipocyte lipolysis, but remains sufficient to activate mTORC1 and the lipogenic pathway. The availability of additional substrate for triglyceride synthesis in the liver also accompanies overnutrition, and amino acids might further activate mTORC1 (ref. 37). Thus, lipogenesis and VLDL synthesis and export are brisk in obesity.

The model described above would be exaggerated in type 2 diabetes, wherein hyperglycemia develops even during fasting, and beta cell deficiency fails to secrete enough insulin to overcome the insulin insensitivity of FOXO1 (refs. 38,39). But whether the deregulation of FOXO1 is mediated by dietary or gut factors or by chronic high circulating insulin is extremely difficult to decisively validate experimentally, because insulin resistance and hyperinsulinemia are so tightly linked. As noted, inducing insulin resistance experimentally does indeed cause hyperinsulinemia, but induced hyperinsulinemia in turn causes insulin resistance and, perhaps, other maladies.

**Viewpoint: hyperinsulinemia causes insulin resistance.** In individuals with obesity who are mildly glucose intolerant but do not have diabetes, fasting hyperinsulinemia occurs without the detectable increases in blood glucose that would theoretically be required to stimulate beta cells to secrete additional insulin. This is also true of the apparently identical increases in blood glucose concentrations that occur in people with hyperinsulinemia upon the ingestion of glucose. Such apparent uncoupling of circulating insulin levels from glucose levels is also observed after bariatric surgery in individuals with obesity.

The above confounding considerations gave rise to the hypothesis (Fig. 3) that hyperinsulinemia is the initial, primary effect of HFD feeding and obesity, induced by the stimulation of beta cell insulin secretion and hepatic lipogenesis, thereby resulting in hyperglycemia and hyperlipidemia. HFDs and overfeeding, either directly or indirectly, deregulate hepatocyte regulators of gluconeogenesis (such as FOXO1), which causes increased hepatic glucose output, and deregulate the glucose transporter GLUT4 response to insulin in muscle, which results in decreased glucose uptake by muscle. These disruptions—in addition to a decreased responsiveness of adipose tissue to insulin (not shown)—cause hyperglycemia, which stimulates islet beta cells to secrete insulin. This leads to hyperinsulinemia, which in turn activates hepatic lipogenesis and increased secretion of VLDL (hyperlipidemia).

**Figure 2** Effect of a HFD and/or obesity on liver and adipose metabolism. (a) Under normal fed conditions, insulin suppresses gluconeogenesis by activating Akt, which in turn inhibits the FOXO1, a transcription factor that regulates enzymes important for gluconeogenesis. Insulin also suppresses adipocyte lipolysis, thereby limiting the availability of gluconeogenesis substrates. (b) Under HFD obese conditions FOXO1 is activated because it is no longer susceptible to suppression by insulin signaling to protein kinase Akt. This causes increased glucose output. The resultant hyperglycemia and chronic hyperinsulinemia are hypothesized to disrupt insulin suppression of adipocyte lipolysis. The resultant glycerol is a substrate for gluconeogenesis and the fatty acids are oxidized in the liver, and the products promote gluconeogenesis through allosteric regulation. Insulin is also required for lipogenesis in the liver through an Akt-dependent pathway that activates mTOR1 and stimulates the expression of enzymes in the de novo lipogenesis pathway. Despite findings from previous models, this suggests that Akt is active even under HFD-feeding and obesity conditions.

**Figure 1** A model for how HFD feeding and obesity induce insulin resistance, thereby resulting in hyperglycemia and hyperlipidemia. Hyperinsulinemia? Adipocyte

insulin secretion and the suppression of insulin degradation. According to this viewpoint, primary hyperinsulinemia is what initially causes insulin resistance in target tissues such as liver, at least under conditions of nutrient excess. The mechanisms involved may include downregulation of insulin signaling to Akt, but other, indirect pathways are probably even more important. For example, in people with obesity and mild diabetes, enhanced conversion of glucose to lactate in skeletal muscle in response to hyperinsulinemia is predicted to provide increased substrate for gluconeogenesis and hepatic glucose output. Bariatric surgery in such individuals...
markedly reduces circulating lactate, in conjunction with bringing insulin levels to within the normal range through decreased lactate-driven gluconeogenesis. Additionally, hyperinsulinemia in both rats and humans enhances the activation of inflammatory pathways, which can, in turn, impair insulin responsiveness in target tissues. Even relatively acute infusions of insulin in humans cause elevated circulating cytokines. Moreover, the attenuation of hyperinsulinemia in genetically obese mice through treatment with streptozotocin or diazoxide reduces adipose tissue inflammation and increases insulin responsiveness. Similar improvement in glucose tolerance is seen by reducing hyperinsulinemia in a knockout mouse model in which beta cell insulin secretion is impaired.

HFD feeding can cause primary hyperinsulinemia by directly stimulatig islet beta cells to produce insulin in the absence of insulin resistance or increased blood glucose levels. Potential mediators of increased insulin secretion are the elevated circulating free fatty acids that sometimes occur in obesity. Experimentally raising circulating free fatty acid levels in humans under hyperglycemic conditions increases insulin secretion rates, as confirmed by assessing concentrations of C-peptide, which is also released into the circulation upon its cleavage from proinsulin in beta cells to produce insulin. Such direct effects on the pancreas are supported by data from studies of other species. Preservatives, such as monoacylglycerides, or other substances in the food supply might also be a cause of heightened insulin secretion. Intracellular mediators that may potentiate glucose-induced insulin secretion include reactive oxygen species and long chain acyl-CoA, which are increased in beta cells exposed to fatty acids. Thus, insulin secretion in response to glucose may be amplified directly by agents supplied through overnutrition.

The effects of blocking hyperinsulinemia. Genetic manipulation of one or both of the mouse insulin-encoding genes (Ins1 and Ins2) have produced important insights into the effects of hyperinsulinemia under HFD conditions. Ins2 is most highly expressed in pancreatic beta cells, but is also expressed at low levels in other tissues, including the brain, similarly to the single human gene INS. The expression of Ins1 in mice seems to be restricted to beta cells, and it also contributes to secreted insulin. Mice lacking only Ins2 show normal insulin levels on control diets and respond to HFD with beta cell expansion and fasting hyperinsulinemia at all ages, as do wild-type mice. Deletion of a single Ins1 allele in mice lacking Ins2 and fed a HFD results in initial hyperinsulinemia at 5–8 weeks, but insulin levels return to normal at 50 weeks on a HFD. Surprisingly, at this later time, mice lacking Ins2 and carrying only one copy of Ins1 maintain the same level of glucose tolerance as hyperinsulinemic mice lacking only Ins2 and fed a HFD, which indicates that high insulin levels in these mice do not enhance glucose tolerance. Importantly, the hyperinsulinemic mice lacking only Ins2 gain more weight when fed a HFD, relative to control-diet-fed mice, as expected, whereas Ins1-deficient mice that are also missing Ins2 do not gain weight on a HFD, despite there being no difference in food intake between the two types of mice.

A second mouse model of genetic insulin deficiency, in which mice are missing both alleles of Ins1 and one allele of Ins2, also displayed less weight gain on a HFD than did Ins1-deficient mice with both alleles of Ins2 intact. Thus, hyperinsulinemia in response to a HFD regimen is a requirement for the increased weight gain seen as a result of adipose-tissue expansion in these mice. Taken together, these results are reminiscent of the remarkable weight gains of human subjects with untreated type 1 diabetes upon receiving insulin, and the oft-observed cases of people with type 2 diabetes who gain weight on insulin therapy.

Increased energy expenditure would explain the reduced fat deposition in insulin-deficient mice, assuming no increased calorie loss...
Insulin resistance is the initiating factor in the development of diabetes. In one study, HFD feeding to mice caused increased adipose mass and fasting hyperinsulinemia after only 1 d without a change in fasting blood-glucose levels. In five out of six studies, rodents fed a HFD for 3 or 4 d exhibited no change in fasting blood glucose, and fasting insulin levels were already elevated in four of these studies. At this 3- to 4-d point of HFD feeding in rats and mice, most studies also revealed an increase in body weight or adipose tissue mass and glucose intolerance or hepatic or systemic insulin resistance. At 7 d of HFD feeding, most studies also failed to detect a change in fasting blood glucose, and all studies showed a statistically significant or strong trend toward fasting hyperinsulinemia.

A timeline of metabolic changes upon overfeeding. A major technical problem in assessing the roles of hyperinsulinemia and insulin resistance in established obesity is that measurements of blood glucose and insulin concentrations might not be sufficiently precise to detect cause and effect, in a manner analogous to the difficulty of measuring temperature changes within the limits set by a thermostat. Therefore, it should be noted that the two hypotheses illustrated in Figures 1 and 3 are not mutually exclusive and probably act in parallel, given that hyperinsulinemia initially induced by insulin resistance, as shown in Figure 1, further exaggerates insulin resistance through the mechanisms depicted in Figure 3. Other important complications are the heterogeneity of insulin resistance in various mouse strains studied, and not knowing whether liver, skeletal muscle or both are affected by insulin resistance.

One approach to the question of whether hyperinsulinemia or insulin resistance is the initiating factor in the development of diabetes is to dissect the sequence of events that occur at very early time points after the start of a HFD or overfeeding. Results from many such studies in mice, rats and humans are summarized in Table 1. In one study, HFD feeding to mice caused increased adipose mass and fasting hyperinsulinemia after only 1 d without a change in fasting blood-glucose levels. In five out of six studies, rodents fed a HFD for 3 or 4 d exhibited no change in fasting blood glucose, and fasting insulin levels were already elevated in four of these studies. At this 3- to 4-d point of HFD feeding in rats and mice, most studies also revealed an increase in body weight or adipose tissue mass and glucose intolerance or hepatic or systemic insulin resistance. At 7 d of HFD feeding, most studies also failed to detect a change in fasting blood glucose, and all studies showed a statistically significant or strong trend toward fasting hyperinsulinemia. Of seven reports on humans presented in Table 1 (refs. 77–83), all but one demonstrated fasting hyperinsulinemia at the earliest stages of overfeeding or a HFD in study participants, whereas most did not detect increases in fasting blood-glucose concentrations. Although a few reports described in Table 1 indicated either no change or an increase in both parameters at early times after overfeeding, none found a case in which fasting hyperglycemia occurred first.

Taken together, the experimental findings summarized in Table 1 indicate that the first measurable change that occurs in HFD feeding regimens in both murine and human subjects is usually an elevated fasting level of circulating insulin—no glucose—which is consistent with hyperinsulinemia being a key initiating cause of insulin resistance. It is also generally recognized that some people with long-established obesity display fasting hyperinsulinemia without detectable elevations in blood-glucose concentrations that would theoretically be required to stimulate insulin secretion. A caveat to these conclusions is the difficulty in measuring the minute changes in blood-glucose concentrations that may be sufficient to be sensed by beta cells. It is also possible that postprandial increases in blood-glucose concentrations may influence insulin secretion even during subsequent fasting periods, or that portal-vein glucose concentrations are higher than peripheral levels. Nonetheless, it will be important in future studies to identify and characterize the signals either in the diet or emanating from the gut or brain or peripheral tissues that may stimulate or potentiate beta cells to chronically secrete insulin in the early stages of HFD feeding.

Interestingly, impaired insulin responsiveness of hepatocyte glucose output occurs before defective insulin-stimulated glucose uptake by muscle during the initial course of HFD feeding in mice and rats. Perhaps this is because portal-vein insulin levels are much higher than circulating levels and thereby affect the liver more than muscle. This is an important difference when compared to people with insulin-resistant prediabetes who present with skeletal-muscle insulin resistance as the earliest abnormality. In any case, chronic hyperinsulinemia may be a factor in the HFD-mediated disruption of FOXO1 depicted in Figure 2, or the deregulated TBC1D4 (and the related TBC1D1) required for full GLUT4 translocation in skeletal muscle (Fig. 1), which could be tested in future experiments.

**Cellular and molecular causes of impaired insulin responsiveness**

Whether hyperinsulinemia or dietary factors cause insulin resistance in HFD feeding can also be addressed by defining the molecular mechanisms that cause defective intracellular signaling and metabolic pathways.

**Akt-independent mechanisms of insulin resistance.** Much elegant work has decisively demonstrated that human monogenic mutations in insulin receptor, PI3-kinase and Akt cause severe insulin resistance. Most studies on common forms of obesity have therefore...
also concentrated on deficiencies in insulin-receptor signaling to Akt, which is required for the major metabolic effects of insulin (see Box 1). Much of this work has attributed the cause of insulin resistance to inhibitory serine/threonine phosphorylations of the insulin-receptor tyrosine kinase or its obligatory substrate IRS proteins mediated by diacylglycerol, or dephosphorylation of Akt by phosphatase activity in response to ceramides. These concepts continue to be explored and debated, and conflicting data are common among different laboratory groups. However, careful examination of the available data indicates that upstream and downstream pathways of insulin responsiveness, including modulation of metabolic flux, transcriptional regulation, and other pathways, could be even more important than the phosphorylation mentioned above in the majority of people with obesity and type 2 diabetes.

For example, provocative findings in mice show that skeletal-muscle resistance to insulin in obesity is likely due to a defect downstream of the insulin receptor and IRS proteins. In these studies, mice with ectopic expression of PDGF receptors in their skeletal muscle were able to respond to the growth factor PDGF in a manner analogous to insulin, such that PDGF signaling causes increased glucose transport in the muscle. HFD feeding of these PDGF-receptor-expressing transgenic mice caused resistance to both PDGF and insulin action on glucose transport, even though PDGF receptor signaling does not involve IRS proteins. Even more strikingly, at 17 d of HFD feeding, the impaired glucose-transport responsiveness was not accompanied by decreased phosphorylation of Akt or its substrate TBC1D4, which is linked to GLUT4 glucose-transporter regulation. Similarly, marked glucose intolerance at 7 d of HFD feeding of wild-type mice can be observed without changes in insulin-stimulated Akt phosphorylation in liver, adipose tissue and skeletal muscle, despite marked impaired insulin responsiveness in the former two tissues. Only at longer times of HFD feeding do decreases in phospho-Akt become detectable, even though insulin resistance has not further increased. These data reinforce the point that resistance to the actions of insulin on metabolism can be strongly promoted by pathways downstream of insulin signaling to Akt.

### Upstream of Akt

Even when Akt activity is compromised in obesity models, the primary sites of signaling disruption may be far removed from insulin-receptor signaling to this protein kinase. Factors

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Table 1 Progression of metabolic parameters upon initiating HFD or overfeeding

<table>
<thead>
<tr>
<th>Metabolic parameter</th>
<th>Mouse</th>
<th>Time after feeding (d)</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>3–4</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>NC (68)</td>
<td>NC (69)</td>
<td>NC (70)</td>
</tr>
<tr>
<td>Fasting plasma insulin</td>
<td>Increase 2× (68)</td>
<td>Increase 2× (69)</td>
<td>Increase 3× (69)</td>
</tr>
<tr>
<td>Fasting NEFA</td>
<td>NC (69)</td>
<td>Increase (70)</td>
<td>Increase (72)</td>
</tr>
<tr>
<td>GTT</td>
<td>Intolerance (69)</td>
<td>Intolerance (73)</td>
<td>Intolerance (69)</td>
</tr>
<tr>
<td>ITT</td>
<td>Decrease (73)</td>
<td>Decrease (71)</td>
<td>Decrease (73)</td>
</tr>
<tr>
<td>HOMAR-IR</td>
<td>Increase (69)</td>
<td>Increase (71)</td>
<td>Increase (74)</td>
</tr>
<tr>
<td>Hepatic glucose output</td>
<td>Increase (69)</td>
<td>Increase (71)</td>
<td>Increase (74)</td>
</tr>
</tbody>
</table>

WATg, gonadal white adipose tissue; NEFA, non-esterified fatty acids; GTT, glucose-tolerance test results; ITT, insulin tolerance test results; HOMAR-IR, homeostatic assessment model–insulin resistance index. NC, no change; 2×, approximately two-fold increase; pound sign (#) denotes strongly increased values that did not reach statistical significance. References to the relevant studies are provided in parentheses.
upstream of the insulin receptor that might impair insulin action on adipocytes, skeletal muscle and liver in obesity include extracellular matrix signaling and reduced capillary recruitment and blood flow that could limit the access of insulin and glucose to the myotubes and perhaps other tissues. Enhanced expression of collagens and other extracellular matrix proteins and their integrin receptors that are in direct contact with skeletal muscle capillaries promote insulin resistance in mice. The pseudokinase integrin-linked kinase (ILK), which binds within a complex to the intracellular domain of β-integrins, is required for optimal HFD-induced glucose intolerance and insulin resistance of skeletal-muscle glucose disposal. Mice without ILK in skeletal muscle have increased capillarization and, presumably, blood flow to the muscle, owing to the lack of negative regulation from stress kinases such as JNK, P38 and ERK. Interestingly, the accumulation of extracellular matrix proteins and fibrosis together promote insulin resistance in adipose tissue, where capillary formation and expansion are critical for normal adipose function. A fragment of collagen VI has also been reported to confer metabolic dysfunction in adipose tissue. Given that insulin-like growth factor 1 (IGF1) is a potent stimulator of collagen expression, perhaps high insulin levels stimulate the IGF1 receptor or cause degradation of the IGF-binding protein to strongly promote collagen synthesis in fibroblasts of adipose tissue. Thus, this pathway could represent another mechanism through which hyperinsulinemia causes insulin resistance.

**Downstream of Akt.** Downstream of insulin signaling to Akt, GLUT4-mediated glucose transport is relatively rate limiting for glucose utilization under normal glucose and insulin concentrations in skeletal muscle and insulin-stimulated GLUT4 glucose transporter translocation to the plasma membrane is impaired in obesity. However, conflicting data have been reported on whether the amount of free intracellular glucose increases or not in the skeletal muscle of people who are insulin resistant. Increased intracellular glucose would reflect decreased activity of glucose metabolism, as is predicted to be the case in response to observed decreased glycogen synthesis in muscle. Especially at the high concentrations of circulating glucose and insulin observed in both HFD-fed mice and people with obesity, both glucose transport and metabolism may be impaired in skeletal muscle. The utilization of glucose and fatty acids can be increased in mice with obesity by uncoupling electron transport from ATP production to increase mitochondrial respiration. This has the beneficial effect of ameliorating fatty liver and insulin resistance, although a causative role for mitochondrial dysfunction in insulin resistance is still debated. The extent to which chronic hyperinsulinemia might play a part in inducing these skeletal-muscle abnormalities in glucose metabolism is unknown.

In some studies of mouse adipocytes during short-term HFD feeding, downstream pathways of glucose metabolism, GLUT4 protein expression and insulin signaling to Akt are already impaired, as they are in long-term obesity. However, a much less than maximal activation of Akt by insulin is needed to obtain a maximal stimulation of adipocyte glucose transport. Thus, even marked inhibition of Akt would not diminish the ability of a high insulin concentration to maximally stimulate glucose metabolism, but in fact, adipocytes from rats with obesity are resistant to even very high insulin concentrations. Perhaps this insulin resistance is partially a reflection of some specificity in the disruption of Akt-mediated phosphorylation, for example, at the level of TBC1D4 or downstream in the insulin signaling pathway. Remarkably, small interfering RNA (siRNA)-based depletion of Akt protein levels by 80% does not affect TBC1D4 phosphorylation, despite the fact that glucose transport is markedly reduced, which indicates that TBC1D4 might not be the major driver of GLUT4 translocation. Furthermore, when adipocytes from rats with obesity are stimulated with insulin at very low glucose concentrations, in which intracellular enzymes are not saturated, insulin stimulation is robust, although these adipocytes are considered to be insulin resistant. In addition, glucose uptake into adipose tissue is markedly reduced without a decrease in insulin-stimulated Akt phosphorylation at early times after HFD feeding. These results suggest that modest inhibitions of insulin signaling to Akt, even in long-term obesity, are not the major cause of insulin resistance in adipocytes that result in decreased glucose utilization. Rather, decreased activity in the pathways of glucose uptake and metabolism are the primary cause of decreased utilization.

On the other hand, overexpression of adipocyte GLUT4 rescues the systemic insulin resistance of mice on a HFD, which indicates that increasing the numbers of glucose transporters can still enhance glucose uptake under insulin-resistant conditions. Activating insulin signaling to Akt in adipocytes in mice by deleting the negative regulator Pten exclusively in adipocytes also enhances glucose tolerance and greatly lowers circulating insulin levels in such lean and obese mice. Thus, even though disruptions occur downstream of Akt in obesity, experimentally enhancing insulin signaling and glucose uptake in adipocytes can overcome these downstream defects, providing multiple opportunities for therapeutic approaches. Interestingly, chronic insulin stimulation of cultured mouse adipocytes in vitro also decreases GLUT4 expression, indicating that hyperinsulinemia may indeed drive this major adipocyte dysfunction to cause insulin resistance.

The pathway of adipocyte glucose metabolism downstream of Akt that is very rapidly and most dramatically depressed by obesity is de novo fatty acid synthesis (DNL), reflecting greatly decreased expression of the enzymes acetyl-CoA carboxylase, fatty acid synthase and ATP citrate lyase (not shown). These effects derive from decreased activity of the lipogenic transcription factors ChREBP-α and ChREBP-β, potentially caused by the depressed levels of GLUT4, given that they are responsive to intermediates of glucose metabolism. Fatty acid–synthase deletion in adipose tissue can prevent insulin resistance in mice, possibly through the generation of bioactive lipids, although other work suggests that beneficial lipids are actually derived from DNL. DNL may regulate adipocyte biology through the multiple signaling pathways that it controls (Fig. 5, in rectangle at right), including potentially regulating neuronal innervation and sympathetic-nerve activity in adipose tissue. Acetyl-CoA is a substrate for protein acetylation reactions—most notably, the acetylation of histones that modulate their DNA-binding activities to regulate transcription. Many of the adipocyte genes that are downregulated in obesity are specifically upregulated during adipocyte differentiation and controlled by the major regulator of adipogenesis PPAR γ, which is also regulated by acetylation. These include genes encoding components of insulin signaling pathways, lipid-droplet and lipolytic regulators and mitochondrial proteins. Thus, adipocytes become less capable during the onset of obesity in their crucial functions, such as lipid storage, that indirectly maintain normal hepatocyte and glucose handling by skeletal muscle. Recent results exploring the effects of a HFD in mice on the global DNA-site-binding and transcriptional activity of PPAR γ also show how environmental cues can modulate the epigenome and alter adipocyte function.
Akt-independent regulation of adipocyte lipolysis. Finally, insulin's control of adipocyte lipolysis is a critical mode by which adipocytes influence hepatic gluconeogenesis and overall systemic glucose tolerance in HFD conditions and obesity (Fig. 2).26,152–154. Much is known about adipocyte lipid droplets and the components that mediate the activation of the lipases that cause hydrolysis of triglycerides in response to activation of the cAMP pathway.26,152–154. Circumstantial data initially suggested that phosphorylation and activation of cAMP phosphodiesterase by Akt could explain insulin's inhibition of lipolysis155–157. However, recent results unexpectedly undermine this concept, demonstrating that inhibition of Akt phosphorylation of phosphodiesterase does not eliminate this action of insulin158–160. The mechanism of insulin action on adipocyte lipolysis thus remains a premier unsolved question in the field, and is further complicated by an indirect action of insulin on lipolysis, mediated through the brain.161. How these anti-lipolytic actions of insulin may be blunted by hyperglycemia, hyperinsulinemia or other factors under certain HFD conditions also remains a mystery.17. Taken together, the disruptions in obesity that occur in many of the pathways of adipocyte metabolism downstream of insulin-activated Akt (Fig. 5) mirror the situation in the liver. The influences of these downstream pathways in adipocytes and the liver on systemic glucose and lipid metabolism, and the extent to which chronic stimulation by insulin itself modulates these pathways, offer fertile territory for future research in this field.

Conclusions and perspectives for future studies

The deterioration of systemic insulin responses related to glucose handling, referred to as insulin resistance, is a serious syndrome associated with obesity and sedentary behavior. It promotes glucose intolerance and type 2 diabetes with associated comorbidities, and also increases the risk of cancer.162 Yet, the etiology of insulin resistance is complicated and multifaceted, involving both cell-autonomous mechanisms and inter-organ communications (Fig. 2). Careful investigation has revealed that many disruptions responsible for systemic insulin resistance actually occur downstream or independently of insulin signaling to the protein kinase Akt69,122,126,127,130,139, even though the Akt pathway is often also affected. We are still unable to precisely define the mechanisms that cause most of these basic disruptions, partly because there is considerable disagreement among the many laboratories in the field. For example, what goes awry with FOXO1 downstream of Akt in obesity? How is adipocyte GLUT4 expression decreased, and how is the translocation of adipocyte and skeletal muscle GLUT4 to the plasma membrane attenuated in obesity? What mediates the blockade of adipocyte fatty acid synthesis under HFD and obesity conditions, and does this metabolic pathway in adipocytes control systemic glucose tolerance? How does insulin suppress adipocyte lipolysis, and what disconnects insulin signaling from adipocyte lipolysis under HFD feeding conditions? It is striking that such fundamental questions remain elusive.

Have we learned enough over the past few years to suggest novel therapeutic strategies for approaching type 2 diabetes? The striking beneficial effects of implanting relatively small amounts of mouse subcutaneous 811 or human beige adipocytes into insulin-resistant mouse models offer the possibility that as-yet-undiscovered factors in adipocytes are potent enhancers of systemic glucose tolerance. Such adipocyte factors might also connect to neuronal control of metabolism.33,139 Enhanced inhibition of adipocyte lipolysis under feeding conditions,
or the potentiation of insulin’s anti-lipolytic action in obesity, would also seem useful (Fig. 2). But one huge challenge to these ideas might be the need to make such a therapeutic selective for adipocytes, because the inhibition of lipolysis in other tissues, such as heart, might lead to toxicity. The issue of tissue selectivity is a major hurdle for exploiting many potential targets that have been uncovered in recent years. For example, enhancing adipose DNL might prove beneficial, but not if hepatic lipogenesis is also activated to produce hyperlipidemia and fatty liver. These considerations suggest that a steep challenge for future success in diabetes therapies (and therapeutics in general) will be the development of tissue-specific delivery modalities for therapeutic agents.

The fact that insulin resistance triggers hyperinsulinemia, and that hyperinsulinemia in turn causes insulin resistance, makes the above conundrums even more interesting. Mechanisms whereby insulin secretion is enhanced in obesity need further exploration. Adipocytes may signal directly to beta cells to regulate insulin secretion and could thereby drive hyperinsulinemia independently of blood glucose levels. Experimental blockade of hyperinsulinemia in mice prevents obesity while increasing energy expenditure and adipose browning, showing that insulin itself has both beneficial and deleterious roles in the obese, insulin-resistant syndrome and possibly, in the promotion of type 2 diabetes onset. These insights raise the possibility that pancreatic islets are the direct or indirect target of HFD feeding, and that hyperinsulinemia is the primary driving force eliciting insulin resistance. More likely, hyperinsulinemia is one of a combination of factors in HFD feeding and obesity that markedly contributes to the malady. Thus, rather than searching for therapeutic modalities that enhance insulin secretion, perhaps the discovery of mild suppressors of insulin secretion specifically in response to overfeeding may prove to be of value in certain cases of diabetes. In any case, opportunities abound for further exploration of the molecular mechanisms whereby chronic hyperinsulinemia modulates pathways that may lead to insulin resistance, such as adipose whitening and inflammation.

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