Trends in Endocrinology & Metabolism

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REVIEWS

Review

Adipocyte Metabolism and Insulin Signaling Perturbations: Insights from Genetics

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Insulin resistance (IR) is a rapidly growing pandemic. It poses an enormous health burden given its comorbidity with obesity, type 2 diabetes (T2D), and other metabolic and cardiovascular diseases (CVDs). Adipose tissue has been established as a key regulator of whole-body metabolic homeostasis, with interest growing rapidly. Emerging evidence suggests that adipocytes play an important role in these afflictions and contribute to IR. Genome-wide association studies (GWAS) have begun to illuminate the genetics underlying obesity, T2D, and IR, and this will allow further study into the disease mechanisms of the genes implicated in these metabolic diseases. Progress towards understanding the molecular mechanisms underlying diseased adipocytes will be discussed here, with an eye towards the future in developing novel therapeutics to combat metabolic disease.

Highlights

Insulin resistance has emerged as a main driver behind type 2 diabetes, obesity and cardiovascular disease. This has become an epidemic creating a significant burden on the healthcare system and economy around the world.

The genetic underpinnings of insulin resistance are heavily under investigation and have been partly elucidated over the past few years. Many GWAS have been undertaken to identify variants associated with BMI, obesity, insulin response, cardiovascular disease and glycemic traits.

Metabolic signatures of insulin resistance have been discovered, spurring more research into identifiable biomarkers for early diagnosis. Endocrine hormones and circulating factors can be measured and studied for their role in IR progression.

Therapeutics targeting IR and metabolic disease are starting to be developed with the prospect of helping millions of people.

Metabolic Disease and Genetics

The genetics of metabolic disease has been studied for decades. Candidate gene-based approaches have led to the identification of genes regulating energy balance, and variation in **body mass index** (**BMI**; see Glossary) has been shown to be affected by variants in *LEP*, *LEPR*, *PPARG*, *POMC*, *NPY,* and *MC4R* [1]. The pre-**genome-wide association studies (GWAS**) era from 1970 to 2005 uncovered 26 genes that were contributors to obesity, many of which are implied in adipocyte biology. Metabolic disease is polygenic, thus complicating gene-based approaches and causing research to focus on identifying genetic variants through GWAS. Large-scale sequencing has found that common variants represent the overwhelming majority of genetic contribution to **type 2 diabetes** (**T2D**) [2]. Only a few rare variants affect metabolic disease, emphasizing the importance of GWAS in the identification of common **single-nucleotide polymorphisms** (**SNPs**). One must assess the genetics of all pathologies under the umbrella of metabolic disease to connect them and demonstrate a relationship with **insulin resistance** (**IR**; Box 1). Although a large amount of the genetic variation contributing to these diseases has already been explained, there is an opportunity to explore the mechanisms behind the genetics. We focus here on adipocyte biology and on what we have learned about the influence of adipocytes on whole-body metabolism.

Modeling Adipocyte Metabolic Pathways

Finding a variant associated with altered risk of metabolism or IR opens up the possibility of detailed study of the mechanism underlying the action of the variant. Adipocyte insulin signaling research started decades ago, uncovering several molecular signaling components of the pathway [3]. Computational and network approaches have recently provided additional insight. A comprehensive *Drosophila* InsR/PI3K/Akt network was built by combining computational, genetic, and biochemical approaches [4], and 566 proteins were found to interact, of which 47% affect the activity of the pathway, and 10% are phosphorylated after insulin stimulation. Two other groups examining the phosphoproteome in murine white and brown

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adipocytes showed that 15% of all phosphorylation sites on proteins respond to insulin. These data further revealed how Akt regulates mTORC2, and identified seven novel insulin-induced effectors [5].

Another recently developed method was used to interrogate Akt isoform-specific signaling in adipocytes. By creating Akt mutants resistant to the inhibitor MK-2206, Kajno and colleagues found that both Akt1 and Akt2 mediate insulin regulation of FOXO1 and GLUT4, whereas Akt1 was uniquely required for adipogenesis [6]. Thus, both conventional and more recent methods have successfully been used to elucidate the molecular elements involved in transducing metabolic signals in adipocytes.

Organismal Models of Impaired Adipocytes

The mouse model has been the mainstay for understanding metabolic disease, and has proved to be extremely useful given the complex multitissue and multiorgan involvement in metabolic homeostasis. The first study examining abrogated insulin signaling in adipocytes led to a flurry of research because it was observed that knocking out the insulin receptor in adipocytes led to protection from obesity and glucose intolerance [7]. Moreover, further studies showed that impaired insulin signaling in adipocytes was linked to increased lifespan in mice [8]. Although extremely exciting, these studies suffered from several technical deficiencies, chief among them being the use of a relatively promiscuous *FABP4* promoter to delete *Insr* in adipose tissue [9]. This promoter leads to expression in a range of tissues that could lead to unclear results, notably the intestine, brain, and macrophages. Thus, recent work has painted a more refined picture, whereby adipose deletion of *Insr* almost entirely ablates white adipose tissue in the mouse, leading to severe diabetes [10]. With an inducible promoter, adipose can be depleted on demand, leading to IR, glucose intolerance, hyperinsulinemia, and hepatosteatosis [11]. After a recovery period, these mice regenerate adipocytes and metabolically normalize. This confers resistance to high-fat diet-induced obesity owing to the absence of adipose tissue, and the mice therefore do not display glucose intolerance or insulin sensitivity. However, the benefits are offset by increased liver weight and lipid content, as well as reduced lifespan [12]. These recent findings are in line with *Insr* knockout in other tissues. In the following we touch upon several models which are relevant to adipocyte insulin signaling, but for a more thorough review of insulin signaling mouse models please refer to [13]. Instead of modulating insulin receptor signaling, adipocyte metabolism can also be studied by altering glucose sensitivity. Knockout of the carbohydrate sensor CHREBP causes IR and adipose inflammation, as well as a cell-autonomous decrease in GLUT4 translocation [14].

The retinol transporter RBP4 has been found to contribute to diabetes risk, further linking inflammation with IR. When mice overexpress RBP4 they become glucose-intolerant and display IR [15]. The RBP4-overexpressing adipose tissue macrophages express proinflammatory markers, and when transferred into healthy mice these cells are sufficient to drive adipose inflammation and IR.

More recent studies have sought to connect GWAS findings with IR. For example, *Nat1*-deficient mice exhibit elevated blood glucose, insulin, and triglycerides as well as IR [16]. *In vitro* silencing of *Nat1* decreased insulin-mediated glucose uptake and stimulated lipolysis. These studies appear to begin connecting *NAT2* GWAS findings, which we will discuss later, with a deeper mechanistic understanding of IR. Similarly, a *PIK3R1* variant, that was found in humans to cause SHORT (Short stature, Hyperextensbility, Hernia, Ocular depression, Rieger anomaly, and Teething delay) syndrome, recapitulated many of the phenotypes of the patients when

Glossary

Body mass index (BMI): derived from the height and weight of an individual expressed in kg/m^2, BMI categorizes people into underweight, normal, overweight, and obese.
Cardiovascular disease (CVD): a range of conditions including blood vessel disease, heart rhythm and structure problems, and blood clotting. This leads up to a potentially terminal event such as a heart attack or stroke.
Genome-wide association study (GWAS): a study that attempts to correlate genetic variants with traits, often focusing on human diseases.
Hemoglobin HbA1c: glycated Hemoglobin a measure of the 3 month average plasma glucose of an individual. It is used for monitoring blood sugar control in (pre-) diabetic patients.
Induced pluripotent stem cells (iPSCs): pluripotent stem cells generated directly from adult cells by reprogramming. They have limitless expansion and differentiation potential.
Insulin resistance (IR): the inability of insulin to increase glucose clearance or reduce glucose production in an organism to the extent that it does in a healthy individual.
Single-nucleotide polymorphism (SNP): the most common genetic variant in humans, representing a difference of only one nucleotide. These are normal occurrences and can act as biological markers or play a direct role in disease.
Type 2 diabetes (T2D): a chronic disease affecting glucose metabolism. The body has an inadequate insulin response, and thus fails to maintain an appropriate glucose level. T2D can eventually lead to other long-term complications.

Box 1. The IR Phenomenon

IR denotes an attenuated or abrogated response of cells to insulin. Insulin signaling maintains metabolic homeostasis through pleiotropic mechanisms such as clearance of blood glucose and suppression of glucose production, as well as modulating growth and lipid and amino acid metabolism [65]. IR is conventionally defined around the inability of insulin to control glucose homeostasis normally [25]. Clearance of blood glucose is achieved by rapid translocation of GLUT4 to the cell surface upon insulin stimulation [66]. A decreased insulin response in this pathway can ultimately progress to hyperglycemia as a result of decreased glucose uptake in peripheral tissues, most notably muscle, liver, and fat [67]. Hyperglycemia, even transiently, in conjunction with other mechanisms, causes IR to pose an increased risk for T2D and **cardiovascular disease** (**CVD**) [68]. Obesity often accompanies IR, and its incidence has doubled over the past 30 years. As the complete opposite of obesity, lipodystrophy also has a severe effect on whole-body insulin signaling [69]. Figure I shows the steady increase in research into IR. T2D, CVD, and obesity together have often been called metabolic disease.

Studies into the etiology of obesity began over 50 years ago, focusing on adipose tissue [13]. Adipocytes in fat tissue are important players in long-term glucose metabolism because they are tasked with storage of energy in the form of lipids. These cells are exquisitely insulin-sensitive, and upregulate glucose uptake in response to insulin. Lipogenesis and fatty acid uptake are increased, and lipolysis is decreased. Adipocytes have also been recognized as a major endocrine organ, releasing adipokines that regulate whole-body metabolic homeostasis [28]. Their acknowledged importance in regulating metabolism has led to increased research into their overall physiological functions, with an emphasis on insulin signaling in adipocytes [21]. There has been an explosion of recent findings concerning the genetics of metabolic disease and the role of the insulin pathway in adipocytes as a main driver for IR, and several tools and models have been generated to study this disease [70]. We are only now beginning to understand the pivotal role that adipose plays in regulating metabolic health and to explore methods for exploiting these insights to develop new therapies.

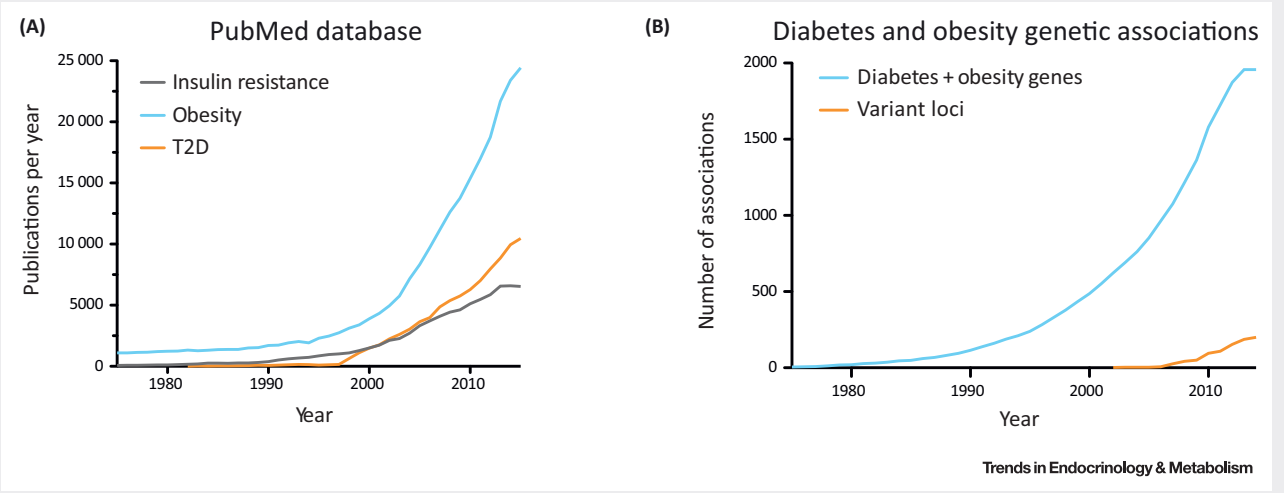

Figure I. The Explosion of Research on Metabolic Disease. (A) Total publications per year (PubMed) for the indicated search terms. (B) Associations with diabetes and obesity. Genes associated with these diseases as identified by T-HOD (Text-Mined Hypertension, Obesity, and Diabetes Candidate Gene Database) [62]. Variant loci associated with type 2 diabetes (T2D) or glycemic traits are aggregated from [63].

introduced in mice. There was a reduction in bodyweight and length, partial lipodystrophy, and IR [17]. This could be attributed to the reduced capacity of insulin to activate PI3K. GWAS analysis has also pinpointed an association between *SORL1* and obesity, which was validated by a gene-dosage effect linking human SORLA expression to obesity and glucose tolerance [18]. This link was confirmed by mouse studies showing that SORLA acts as a sorting factor enhancing INSR surface expression. *FTO* has long been known as a genetic factor that increases the risk of diabetes and obesity, but the molecular mechanism has only recently been elucidated. The variant in this region disrupts a ARID5B repressor motif, doubling *IRX3* and *IRX5* expression, and the repressor directly interacts with the *IRX3* promoter [19,20]. IRX3-deficient mice, which present with reduced bodyweight and increased basal metabolic rate, reproduce this phenotype.

Of potential therapeutic interest is the finding that increasing insulin sensitivity in adipocytes is sufficient for metabolic improvements. Mice with loss-of-function of PTEN, which normally

stops the insulin signaling transduction cascade, gain more weight but retain enhanced insulin sensitivity [21]. This can even rescue metabolic phenotypes in animals by inducing PTEN loss after 8 weeks of a high-fat diet.

Human *In Vitro* Adipocyte Modeling

Advances in **induced pluripotent stem cell (iPSC)** technology and differentiation protocols have made it feasible to study human adipocytes *in vitro* [22]. Combined with genome editing, this allows unprecedented freedom to study patient variants. An example of this is the modeling of a mutation in *AKT2*, reported in three patients, which was found to increase lipid accumulation, glucose uptake, and inflammatory signals in adipocytes, resolving the cellular basis of several pathophysiological phenotypes [23]. Further studies into this unique activating mutation in the insulin signaling pathway in human cells and tissues would likely improve our understanding of insulin dysregulation and disease. Other patient mutations are not as directly linked to the insulin signaling pathway, but iPSC-derived adipocytes from two patients with familial partial lipodystrophy appeared to have impaired insulin signaling and aberrant lipolysis [24]. Lipolysis has been shown to affect insulin signaling in multiple ways [25]. For example, fat-specific protein 27 (FSP27) has been shown to interact with adipose triglyceride lipase (ATGL) and play a role in free fatty acid-induced adipocyte IR [26].

IR is often associated with inflammation, and it is possible to model the cell-autonomous effect of this *in vitro*. In human primary obese adipocytes, *IRF1*, encoding a major inflammatory transcription factor, is upregulated [27]. Overexpression of *IRF1* leads to abrogated insulin response, and increased lipolysis. After implanting *IRF1*-overexpressing adipocytes in a mouse, these fat pads show increased inflammation and macrophage recruitment. Although the relationship between inflammation and metabolic disease in adipose remains enigmatic, these studies provide a roadmap for how we might begin to unravel the mechanisms underlying these relationships. Overall, *in vitro* experiments such as these will ultimately allow us to accurately dissect the causality of mutations found in patients, either through GWAS or rare variants. The confidence derived from these studies will give us mechanistic insights into idiopathic metabolic disease.

Adipocyte Endocrine Signaling Affecting Metabolic Pathways

Secreted factors influencing whole-body metabolic homeostasis have been a topic of intense investigation, especially when involved in IR. Several hormones regulating nutritional state have been discovered, leading to a complex interplay of regulatory factors that affect insulin signaling. A short overview of various known adipokines is presented in Table 1. Two main hormones which have been studied for a long time are adiponectin and leptin, and both greatly affect metabolism [28]. This has spurred the field into research concerning other adipokines. One example, adipocyte protein 2 (aP2), has long been known to be a carrier protein for fatty acids, and is secreted and regulated by fasting and lipolysis-related signals [29]. Interestingly, aP2 is also upregulated in obesity, and controls hepatic gluconeogenesis by increasing obesity-related hyperglycemia. Many adipokines are insulin- or nutrient-regulated, including asprosin, which is induced by fasting and increased to pathological levels by IR [30]. Reduction of asprosin can protect against hyperinsulinemia because it acts on the cAMP–PKA pathway in the liver to release glucose into circulation.

WISP1 is another insulin-regulated adipokine [31]. In overweight patients undergoing hyperinsulinemic clamps, WISP1 induction was absent, suggesting abrogated insulin signaling and IR in overweight subjects. The effect of WISP1 on macrophages is proinflammatory, linking IR in adipocytes directly to inflammation.

Table 1. Functions of Adipocyte-Secreted Factors[a]

Adipokine	Primary functions
Adiponectin	Improves insulin sensitivity; antidiabetic, antiatherogenic, and anti-inflammatory
Adipsin	Activates the alternative complement pathway
Angiopoietin-like protein 8	Promotes pancreatic β cell proliferation; improves glucose tolerance
Apelin	Inhibits insulin secretion
Bone morphogenetic protein (BMP)-4	Regulates adipogenic precursor cell commitment and differentiation
BMP-7	Stimulates brown adipogenesis; reduces food intake; increases energy expenditure
Cathepsins S, L, K	Regulate glucose metabolism and adipose tissue mass
Chemerin	Chemoattractant protein; regulates adipogenesis
Clusterin	Promotes tumor progression and angiogenesis
Dipeptidyl peptidase (DPP)-4	Degrades GIP and GLP-1; inhibitors in clinical use for T2D
Fatty-acid-binding protein (FABP)-4	Associated with increased T2D risk and impaired myocardial contractility
Fetuin-A	Reflects liver fat content; associated with lipid-induced inflammation and IR; promotes cancer progression
FGF21	Stimulates glucose uptake into adipocytes; increases thermogenesis, energy expenditure, and fat utilization; improves glucose and lipid metabolism
Gremlin-1	Inhibits BMP-4 and BMP-7
IL-1β	Proinflammatory
IL-6	Proinflammatory
Leptin	Satiety signal; regulates appetite, food intake, locomotor activity, energy expenditure, fertility, and other processes
Lipocalin 2	Related to IR and inflammation
Monocyte chemoattractant protein (MCP)-1	Chemoattractant protein; adipose tissue inflammation
Nesfatin-1	Direct glucose-dependent insulinotropic effect on β cells
Omentin	Anti-inflammatory; insulin-sensitizing
Progranulin	Chemoattractant protein; neurodegenerative diseases; adipose tissue inflammation
RBP4	Related to IR, visceral fat distribution, and dyslipidemia
Resistin	Related to obesity, IR, and inflammation
Transforming growth factor (TGF)-β	Regulates cell proliferation, differentiation, and apoptosis
Tissue inhibitor of matrix metalloproteinase (TIMP)-1	Decreases adipogenesis; impairs glucose tolerance
Tumor necrosis factor (TNF)-α	Proinflammatory
Vaspin	Serine protease inhibitor; decreases food intake; improves hyperglycemia
Vascular endothelial growth factor (VEGF)	Stimulates angiogenesis in adipose tissue
Visfatin/PBEF/Nampt	Nampt-mediated systemic NAD biosynthesis is crucial for β cell function
WISP-1	Regulates adipogenesis and adipose tissue inflammation

[a]Modified from [64].

Not all secreted factors affecting insulin signaling from adipocytes are proteins. One major mechanism by which insulin suppresses hepatic glucose production is the reduction of hepatic acetyl-CoA through decrease of adipose lipolysis [32]. Obese rats respond to insulin suppression of lipolysis only when treated with an interleukin (IL)-6-neutralizing antibody, again linking IR with inflammation. In addition, a signaling circuit between white fat, liver, and brown fat has

been described. Cold-induced lipolysis in fat promotes hepatic acylcarnitine production that is subsequently taken up by brown adipocytes as fuel for thermogenesis [33]. Similarly, the aforementioned CHREBP knockout mice have a defect in palmitic-acid-9-hydroxy-stearic-acid (9-PAHSA) plasma levels, and supplementation of this factor rescues the IR and inflammation seen in this model [14].

Other factors, despite not being directly secreted, still confer a whole-body metabolic effect. RAB10 is required for insulin-stimulated GLUT4 translocation in adipose [34]. Knockout of *Rab10* causes hyperglycemia owing to abrogated adipose blood glucose clearance, causing near-complete failure of insulin to suppress hepatic glucose production. Conversely, adipose-specific *Nprc* knockout mice increase browning and energy expenditure by upregulating energy metabolism after stimulation with natriuretic peptides [35]. This decreases blood glucose, protecting mice from diet-induced hepatic steatosis and visceral fat inflammation.

Growth hormone (GH) injection leads to hepatic IR. However, when adipose *Jak2a* is deleted, an obligate GH signaling transducer, this effect is absent [36]. This proves that adipose JAK2 signaling governs the effect of GH on whole-body glucose and insulin homeostasis, as well as liver insulin sensitivity. Taken together, these data underscore the importance of adipose tissue in whole metabolic homeostasis.

The Genetic Etiology of IR

Common Genetics Affecting Adiposity

In 2007, a locus was robustly associated with BMI for the first time. The causal gene turned out to be *FTO*, an often-replicated finding. Since then, many more associations have been established, with significant overlap between studies.

Early GWAS utilizing small cohorts only found variants that were strongly associated with metabolic disease. One such locus was *GRB10*, encoding an adaptor protein that regulates insulin receptor signaling [37]. A European cohort, combined with the Diabetes Genetics Initiative and compared to the Genetic Investigation of Anthropometric Traits (GIANT) Consortium data, identified seven loci not previously connected to obesity [38]. In a follow-up study it was ascertained these loci increased total body weight by increasing the mass of adipose tissue, but not of lean tissue [39]. Five of these loci were also found to be associated with food intake, hinting at a possible cause for their correlation with obesity [40]. A network analysis including new T2D loci also implicated fat by displaying a strongly enriched effect of adipocytokine signaling in diabetes pathogenesis [41].

In a massive cohort of 339 224 individuals, 97 loci were associated with BMI, 56 of which were novel [42]. This has been the most thorough description so far, explaining almost 3% of BMI variation. Pathway analyses strongly implicated the brain and neuronal pathways in regulating weight. A meta-analysis following this finding examined body fat (BF) percentage. Four loci were novel associations with BF%, whereas seven loci showed a stronger association with BF % than with BMI, suggesting a primary relationship to adiposity [43]. Strikingly, two of the 12 loci (*IRS1*, *GRB14*) influence insulin receptor signaling directly because both proteins bind to the insulin receptor to mediate downstream phosphorylation and signaling events. Two other genes, *IGFBP2* and *PICK1*, encode factors that are involved in the insulin-like growth factor 1 (IGF1) pathway and both modulate insulin-like growth factor signaling.

A combination of three large-scale analyses showed that, although neural processes dominate in the BMI dataset as mentioned before, increased statistical power also implicated pathways

involved in fat-cell size, body fat amount, insulin signaling, and cholesterol levels [1]. Based on all these studies, computational imputation estimated that 27% of variance for BMI could currently be explained, out of a predicted 30–40% heritability contribution [44]. This suggests the field is drawing closer to fully explaining the genetics of BMI.

To understand the differential contributions of adipose depots, one can measure visceral and subcutaneous fat using computed tomography. In this study only the *FTO* locus was associated with subcutaneous fat, and no SNPs were found that associated with visceral adipose [45]. The drawback of this work was a relative lack of power, and employed prefiltering for T2D-associated SNPs, meaning that other loci could be involved in fat area distribution, as evidenced by a study implying limited peripheral adipose storage as a major cause for IR [46]. Three previously unexplained loci (*CCDC92*, *DNAH3*, *L3MBTL3*) showed an adipogenesis phenotype in follow-up *in vitro* experiments. When adjusted for BMI, examining waist–hip ratio (WHR) can also provide a good indication of adipose distribution. Loci identified to be associated with waist and hip circumference or WHR adjusted for BMI were strongly enriched for adipose tissue genes and regulatory elements [47].

Aberrant insulin signaling not only affects BMI or adiposity but can also prompt hyperglycemia or other glycemic traits. We will not go into detail here because 73 variants underlying the genetics of glycemic traits have recently been reviewed by [48]. Similarly, for a more general overview on T2D genetics, not focused on adipocytes, please refer to [49].

WHR is heavily affected by gender, and even the genetics of adiposity distribution demonstrates sexual dimorphism. At least seven loci exhibit a stronger effect on WHR in women [50]. There are also racial differences because, in a cohort of African ancestry, less than half of the regions previously found to associate with WHR in European ancestry were confirmed, and two novel loci were identified [51]. The racial impact has also been seen in diabetes, and several new SNPs were discovered in a high-risk South Asian ancestry cohort [52]. Astonishingly, a single *SLC16A11* variant was found to contribute to nearly 20% of diabetes risk in Mexicans [53]. The variant causes changes in fatty acid and lipid metabolism, thereby increasing acylcarnitines and triglycerides.

Treatment of IR Progression

Secreted factors regulating glucose or insulin homeostasis are prime targets for therapeutic intervention. aP2, for instance, regulates hepatic gluconeogenesis, and if depleted in circulation rescues the diabetic phenotype of obese mice [29]. Thus, targeted therapeutics designed to lower the circulating levels or activity of this protein might confer therapeutic benefit. Fibroblast growth factor 21 (FGF21) is another secreted factor that has been repeatedly shown to be beneficial for metabolic homeostasis. A long-acting analog administered to obese T2D patients caused a decrease in bodyweight and serum triglycerides, and an increase in adiponectin secretion [48]. Another family member, FGF1, can also lead to antidiabetic effects. Interestingly, FGF1 does not cause a risk of hypoglycemia, and is not secondary to weight loss. The mechanism is not fully understood but is thought to involve an FGF1-responsive brain circuit that is able to mediate diabetes remission [54]. More recently identified factors such as asprosin require further validation before therapeutic development.

Although not as immediately tractable as secreted factors, directly manipulating intracellular regulators of insulin signaling is also an avenue for potential treatment of metabolic disease. For example, PI3K is a well-established mediator of insulin signaling, and pharmacological inhibition decreases adiposity, serum glucose, and liver steatosis in both obese mice and rhesus monkeys [55]. Targeting this pathway in some severe metabolic conditions may be a possible

treatment option. In addition, peroxisome proliferator-activated receptor (PPAR) agonists constitute a well-studied group of therapeutics. However, these often have significant drawbacks, and a range of literature on PPARs has been reviewed in [56].

Modulating a single factor is often not enough for complex diseases such as T2D. Thus, combinatorial therapies have become increasingly attractive as treatment modalities for metabolic disease. Combinatorial agonism of gastric inhibitory polypeptide receptor (GIPR) and glucagon-like peptide-1 receptor (GLP-1R) improves glycemic control, as well as reducing plasma cholesterol and body weight [57]. Another application of combined therapeutics is to target one specific subset of cells for pharmacological intervention. Targeting GLP-1R-expressing cells by fusing GLP1 to dexamethasone bypasses the deleterious effects of chronic dexamethasone treatment [58]. This strategy decreases body weight and IR, while increasing energy expenditure and glucose tolerance. It also appears to reverse IR-linked systemic inflammation.

Not every diabetic will respond equally to a given therapeutic. A clinical study described using an IKK1ε and TBK1 inhibitor to reduce **hemoglobin (HbA1c)** and plasma free fatty acids [59]. Only one-third of subjects showed a decrease in hepatic steatosis and IR. Responders exhibited much higher baseline inflammation, with an inflammatory gene expression signature in adipocytes. These data suggest that better genetic and physiological segregation of patient populations would allow more refined and effective therapeutic intervention. This is a prime example of how therapies could be informed by large-scale genetic studies, which has led to the burgeoning field of pharmacogenomics.

Concluding Remarks and Future Perspectives

Recent years have seen a frenzy of research on IR, now established as an indicator of progression towards obesity and diabetes. We are identifying many genetic variants and metabolic markers associated with IR, but there is still a dearth of mechanistic explanation. Many mouse and cell models have shed light on the players in the insulin signaling pathway but do not recapitulate the effect of genetic variants found in human populations. iPSC and genome-editing technology will allow many more patient mutations to be modeled *in vitro* as well as in animal models. Hopefully these studies will elucidate driver mutations leading to IR and illuminate which tissues cause the systemic dysregulation of metabolism. Diagnosis currently often occurs when patients present with T2D, which has been less tractable to pharmacological reversal. Earlier intervention would appear prudent because it may ultimately be more effective in re-establishing metabolic homeostasis, although as seen in the Outstanding Questions the extent to which we can treat IR remains to be evaluated, as do the targets or tissues to which potential therapeutics should be aimed. More knowledge about the metabolites involved in IR may aid in detecting this condition and provide additional mechanistic insight. One attractive idea with some limited experimental support is that lipid species might communicate IR from adipose to various other tissues and thus affect their function [25]. Further metabolomic research should help to clarify the roles that lipids and other metabolites play in IR.

Although human genetic studies have led to numerous findings, current cohorts suffer from a lack of racial and ethnic diversity, although efforts to recruit people of more mixed backgrounds are increasing [51,52]. Racial disparity as well as sexual dimorphism in both adipose function and size are apparent, with distinct genetic underpinnings [41,50,51,60]. Human genetic studies promise to continue to expand our knowledge of the genes and variants involved in IR. Given that the tools for mining these variants are constantly improving and are becoming ever more refined, an era of greater mechanistic insights into IR draws near. Increasingly,

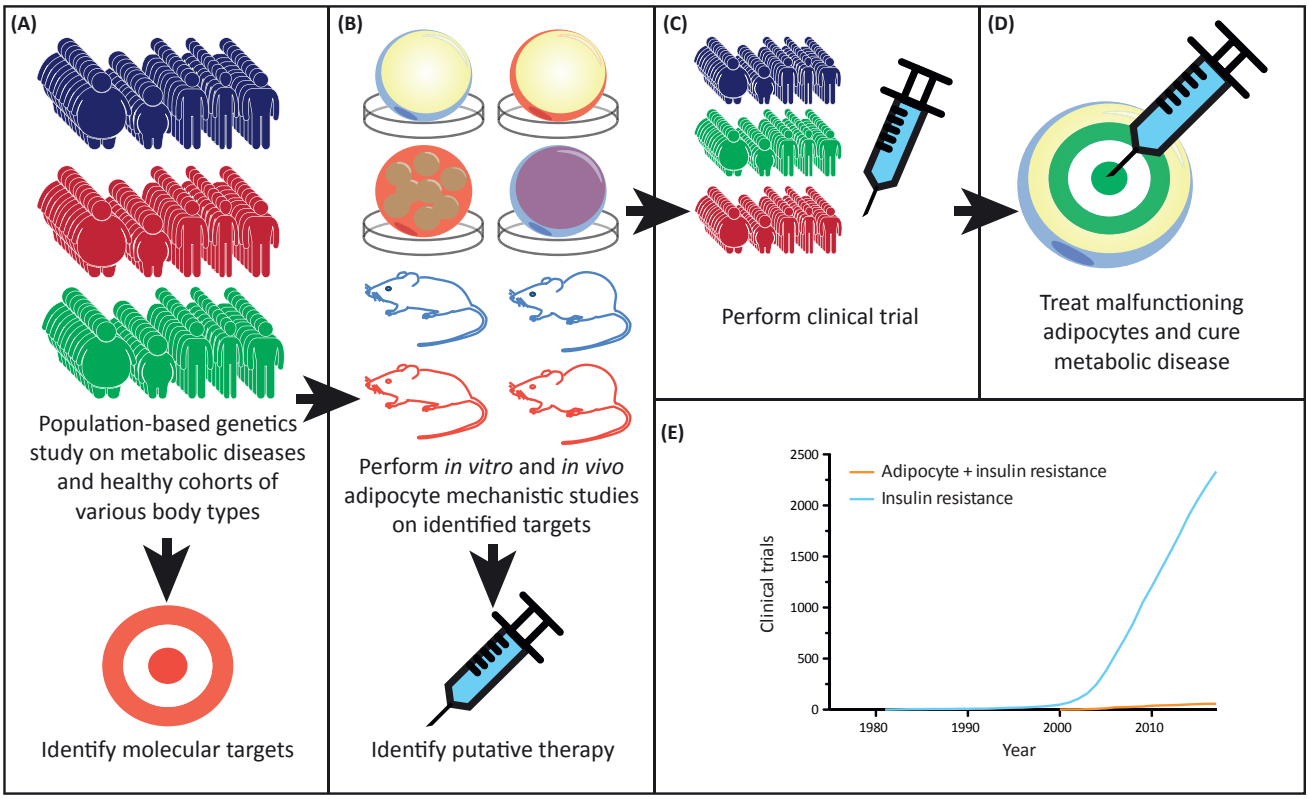

Figure 1. A Genetics-Based Approach to Curing Malfunctioning Adipocytes. (A) Population-based genetic studies such as GWAS are used to identify disease-affecting variants. (B) Mechanistic studies *in vitro* and *in vivo* can elucidate the mechanism of these genetic variants. Based on the mechanism putative therapeutics can be developed. (C) Establishing the efficacy of a therapeutic by conducting a clinical trial. (D) Curing diseased adipocytes to mitigate metabolic disease. (E) Cumulative number of clinical trials being conducted on insulin resistance or on insulin-resistant adipocytes. Data from ClinicalTrials.gov.

adipose tissue has been recognized as a central player in metabolic homeostasis, and combining genetic insights with cellular and mechanistic discoveries should further propel the search for improved therapeutics. In the Outstanding Questions we discuss these issues further. Studies in animal models of GWAS, where study conditions are more malleable and one can investigate phenomena that are unethical or impossible in humans, have given further insights. One study subjected mice to ketogenic or nutrient-deficient diets, and evaluated the response to food intake conditions that no person would willingly undergo [61]. The genetics underlying the reaction to particular stimuli such as diet or lifestyle remain opaque. Although we have learned a tremendous amount over the past years about adipose IR, most of this knowledge is descriptive. In the future, mechanistic studies should allow us to pinpoint therapeutic targets and alleviate the burden of people suffering from metabolic disease (Figure 1).

References

1. Ghosh, S. and Bouchard, C. (2017) Convergence between biological, behavioural and genetic determinants of obesity. *Nat. Rev. Genet.* 18, 731–748
2. Fuchsberger, C. *et al.* (2016) The genetic architecture of type 2 diabetes. *Nature*, 536, 41–47
3. Haeusler, R.A. *et al.* (2018) Biochemical and cellular properties of insulin receptor signalling. *Nat. Rev. Mol. Cell. Biol.* 19, 31–44
4. Vinayagam, A. *et al.* (2016) An integrative analysis of the InR/PI3K/Akt network identifies the dynamic response to insulin signaling. *Cell Rep.* 16, 3062–3074

5. Humphrey, S.J. *et al.* (2013) Dynamic adipocyte phosphoproteome reveals that Akt directly regulates mTORC2. *Cell Metab.* 17, 1009–1020
6. Kajno, E. *et al.* (2015) Development of a new model system to dissect isoform specific Akt signalling in adipocytes. *Biochem. J.* 468, 425–434
7. Blüher, M. *et al.* (2002) Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. *Dev. Cell*, 3, 25–38
8. Blüher, M. *et al.* (2003) Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science*, 299, 572–574
9. Jeffery, E. *et al.* (2014) Characterization of Cre recombinase models for the study of adipose tissue. *Adipocyte*, 3.3, 206–211
10. Boucher, J. *et al.* (2016) Differential roles of insulin and IGF-1 receptors in adipose tissue development and function. *Diabetes*, 65, 2201–2213
11. Sakaguchi, M. *et al.* (2017) Adipocyte dynamics and reversible metabolic syndrome in mice with an inducible adipocyte-specific deletion of the insulin receptor. *Cell Metab.* 25, 448–462
12. Friesen, M. *et al.* (2016) Adipocyte insulin receptor activity maintains adipose tissue mass and lifespan. *Biochem. Biophys. Res. Commun.* 476, 487–492
13. Kubota, T. *et al.* (2017) Imbalanced insulin actions in obesity and type 2 diabetes: key mouse models of insulin signaling pathway. *Cell Metab.* 25, 797–810
14. Vijayakumar, A. *et al.* (2017) Absence of carbohydrate response element binding protein in adipocytes causes systemic insulin resistance and impairs glucose transport. *Cell Rep.* 21, 1021–1035
15. Moraes-Vieira, P.M. *et al.* (2014) RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. *Cell Metab.* 19, 512–526
16. Knowles, J.W. *et al.* (2015) Identification and validation of N-acetyltransferase 2 as an insulin sensitivity gene. *J. Clin. Invest.* 125, 1739–1751
17. Winnay, J.N. *et al.* (2016) PI3-kinase mutation linked to insulin and growth factor resistance *in vivo*. *J. Clin. Invest.* 126, 1401–1412
18. Schmidt, V. *et al.* (2016) SORLA facilitates insulin receptor signaling in adipocytes and exacerbates obesity. *J. Clin. Invest.* 126, 2706–2720
19. Claussnitzer, M. *et al.* (2015) FTO obesity variant circuitry and adipocyte browning in humans. *N. Engl. J. Med.* 373, 895–907
20. Smemo, S. *et al.* (2014) Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature*, 507, 371–375
21. Morley, T.S. *et al.* (2015) Selective enhancement of insulin sensitivity in the mature adipocyte is sufficient for systemic metabolic improvements. *Nat. Commun.* 6, 7906
22. Ahfeldt, T. *et al.* (2012) Programming human pluripotent stem cells into white and brown adipocytes. *Nat. Cell Biol.* 14, 209–219
23. Ding, Q. *et al.* (2013) A TALEN Genome-editing system for generating human stem cell-based disease models. *Stem Cell*, 12, 238–251
24. Friesen, M. and Cowan, C.A. (2017) FPLD2 LMNA mutation R482W dysregulates iPSC-derived adipocyte function and lipid metabolism. *Biochem. Biophys. Res. Commun.* 495, 254–260
25. Samuel, V.T. and Shulman, G.I. (2012) Mechanisms for insulin resistance: common threads and missing links. *Cell*, 148, 852–871
26. Grahn, T.H.M. *et al.* (2014) Fat-specific protein 27 (FSP27) interacts with adipose triglyceride lipase (ATGL) to regulate lipolysis and insulin sensitivity in human adipocytes. *J. Biol. Chem.* 289, 12029–12039
27. Friesen, M. *et al.* (2017) Activation of IRF1 in human adipocytes leads to phenotypes associated with metabolic disease. *Stem Cell Rep.* 8, 1164–1173
28. Wozniak, S.E. *et al.* (2008) Adipose tissue: the new endocrine organ? A review article. *Dig. Dis. Sci.* 54, 1847–1856
29. Cao, H. *et al.* (2013) Adipocyte lipid chaperone aP2 is a secreted adipokine regulating hepatic glucose production. *Cell Metab.* 17, 768–778
30. Romere, C. *et al.* (2016) Asprosin, a fasting-induced glucogenic protein hormone. *Cell*, 165, 566–579
31. Murahovschi, V. *et al.* (2015) WISP1 is a novel adipokine linked to inflammation in obesity. *Diabetes*, 64.3, 856–866
32. Perry, R.J. *et al.* (2015) Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. *Cell*, 160, 745–758
33. Simcox, J. *et al.* (2017) Global analysis of plasma lipids identifies liver-derived acylcarnitines as a fuel source for brown fat thermogenesis. *Cell Metab.* 26, 509–522.e6
34. Vazirani, R.P. *et al.* (2016) Disruption of adipose Rab10-dependent insulin signaling causes hepatic insulin resistance. *Diabetes*, 65, 1577–1589
35. Wu, W. *et al.* (2017) Enhancing natriuretic peptide signaling in adipose tissue, but not in muscle, protects against diet-induced obesity and insulin resistance. *Sci. Signal.* 10, eaam6870
36. Corbit, K.C. *et al.* (2017) Adipocyte JAK2 mediates growth hormone-induced hepatic insulin resistance. *JCI Insight*, 2, 752–814
37. Rampersaud, E. *et al.* (2007) Identification of novel candidate genes for type 2 diabetes from a genome-wide association scan in the Old Order Amish: evidence for replication from diabetes-related quantitative traits and from independent populations. *Diabetes*, 56, 3053–3062
38. Thorleifsson, G. *et al.* (2008) Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat. Genet.* 41, 18–24
39. Renström, F. *et al.* (2009) Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. *Hum. Mol. Genet.* 18, 1489–1496
40. Bauer, F. *et al.* (2009) Obesity genes identified in genome-wide association studies are associated with adiposity measures and potentially with nutrient-specific food preference. *Am. J. Clin. Nutr.* 90, 951–959
41. Morris, A.P. (2012) Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. *Nat. Genet.* 44, 981–990
42. Locke, A.E. *et al.* (2015) Genetic studies of body mass index yield new insights for obesity biology. *Nature*, 518, 197–206
43. Lu, Y. *et al.* (2016) New loci for body fat percentage reveal link between adiposity and cardiometabolic disease risk. *Nat. Commun.* 7, 10495
44. Yang, J. *et al.* (2015) Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. *Nat. Genet.* 47, 1114–1120
45. Hotta, K. *et al.* (2012) Association between type 2 diabetes genetic susceptibility loci and visceral and subcutaneous fat area as determined by computed tomography. *J. Hum. Genet.* 57, 305–310
46. Lotta, L.A. *et al.* (2016) Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. *Nat. Genet.* 49, 17–26
47. Shungin, D. *et al.* (2015) New genetic loci link adipose and insulin biology to body fat distribution. *Nature*, 518, 187–196
48. Talukdar, S. *et al.* (2016) A long-acting FGF21 molecule, PF-05231023, decreases body weight and improves lipid profile in non-human primates and type 2 diabetic subjects. *Cell Metab.* 23, 427–440
49. Bonnefond, A. and Froguel, P. (2015) Rare and common genetic events in type 2 diabetes: what should biologists know? *Cell Metab.* 21, 357–368
50. Heid, I.M. *et al.* (2010) Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat. Genet.* 42, 949–960
51. Liu, C.-T. *et al.* (2013) Genome-wide association of body fat distribution in African ancestry populations suggests new loci. *PLoS Genet.* 9, e1003681

52. Kooner, J.S. *et al.* (2011) Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. *Nat. Genet.* 43, 984–989
53. Rusu, V. *et al.* (2017) Type 2 diabetes variants disrupt function of SLC16A11 through two distinct mechanisms. *Cell*, 170, 199–202
54. Scarlett, J.M. *et al.* (2016) Central injection of fibroblast growth factor 1 induces sustained remission of diabetic hyperglycemia in rodents. *Nat. Med.* 22, 800–806
55. Ortega-Molina, A. *et al.* (2015) Pharmacological inhibition of PI3K reduces adiposity and metabolic syndrome in obese mice and rhesus monkeys. *Cell Metab.* 21, 558–570
56. Gross, B. *et al.* (2016) PPARs in obesity-induced T2DM, dyslipidaemia and NAFLD. *Nat. Rev. Endocrinol.* 13, 36–49
57. Frias, J.P. *et al.* (2017) The sustained effects of a dual GIP/GLP-1 receptor agonist, NNC0090-2746, in patients with type 2 diabetes. *Cell Metab.* 26, 343–352
58. Quarta, C. *et al.* (2017) Molecular integration of incretin and glucocorticoid action reverses immunometabolic dysfunction and obesity. *Cell Metab.* 26, 620–632
59. Oral, E.A. *et al.* (2017) Inhibition of IKKε and TBK1 improves glucose control in a subset of patients with type 2 diabetes. *Cell Metab.* 26, 157–170
60. Randall, J.C. (2013) Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet.* 9, e1003500
61. Parks, B.W. *et al.* (2015) Genetic architecture of insulin resistance in the mouse. *Cell Metab.* 21, 334–346
62. Dai, H. *et al.* (2013) T-HOD: a literature-based candidate gene database for hypertension, obesity and diabetes. *Database*,
63. Prasad, R. and Groop, L. (2015) Genetics of type 2 diabetes – pitfalls and possibilities. *Genes*, 6, 87–123
64. Fasshauer, M. and Blüher, M. (2015) Adipokines in health and disease. *Trends Pharmacol. Sci.* 36, 461–470
65. Saltiel, A.R. and Kahn, C.R. (2001) Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*, 414, 799–806
66. Klip, A. *et al.* (2014) Signal transduction meets vesicle traffic: the software and hardware of GLUT4 translocation. *Am. J. Physiol. Cell Physiol.* 306, C879–C886
67. Boucher, J. *et al.* (2014) Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb. Perspect. Biol.* 6, a009191
68. Rask-Madsen, C. and Kahn, C.R. (2012) Tissue-specific insulin Signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler. Thromb. Vasc. Biol.* 32, 2052–2059
69. Hegele, R.A. *et al.* (2007) Lipodystrophies: windows on adipose biology and metabolism. *J. Lipid Res.* 48, 1433–1444
70. Johnson, A.M.F. and Olefsky, J.M. (2013) The origins and drivers of insulin resistance. *Cell*, 152, 673–684