



REVIEW ARTICLE OPEN

Trends in insulin resistance: insights into mechanisms and therapeutic strategy

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The centenary of insulin discovery represents an important opportunity to transform diabetes from a fatal diagnosis into a medically manageable chronic condition. Insulin is a key peptide hormone and mediates the systemic glucose metabolism in different tissues. Insulin resistance (IR) is a disordered biological response for insulin stimulation through the disruption of different molecular pathways in target tissues. Acquired conditions and genetic factors have been implicated in IR. Recent genetic and biochemical studies suggest that the dysregulated metabolic mediators released by adipose tissue including adipokines, cytokines, chemokines, excess lipids and toxic lipid metabolites promote IR in other tissues. IR is associated with several groups of abnormal syndromes that include obesity, diabetes, metabolic dysfunction-associated fatty liver disease (MAFLD), cardiovascular disease, polycystic ovary syndrome (PCOS), and other abnormalities. Although no medication is specifically approved to treat IR, we summarized the lifestyle changes and pharmacological medications that have been used as efficient intervention to improve insulin sensitivity. Ultimately, the systematic discussion of complex mechanism will help to identify potential new targets and treat the closely associated metabolic syndrome of IR.

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INTRODUCTION

The discovery of insulin in 1921 was a milestone event¹ that introduced the possibility of systematic research of insulin action (Fig. 1). Frederick Sanger (1918–2013) sequenced bovine insulin in 1955, identifying its exact amino-acids composition,^{2,3} and was awarded with the Nobel Prize for Chemistry in 1958. In 1965, a large team in the People's Republic of China successfully synthesized the crystalline bovine insulin with full biological activity, immunogenicity and chemical property for the first time in the world.⁴ Subsequently, human insulin was produced using recombinant DNA methods and genetic modification of bacteria.⁵ Insulin therapy and understanding its mechanisms of action become important later research targets. Insulin is a peptide hormone that is produced and released by islets pancreatic β cells, that finely regulates the glucose uptake from blood into liver, fat, and skeletal muscle cells.⁶ Insulin also promotes several other cellular processes, in addition to glucose homeostasis, including regulation of glycogen synthesis, lipid metabolism, DNA synthesis, gene transcription, amino acid transport, protein synthesis and degradation.⁷

Under normal physiological conditions, increased plasma glucose levels lead to increased insulin secretion and circulating insulin levels, thereby stimulating glucose transfer into peripheral tissues and inhibiting hepatic gluconeogenesis. Individuals with defected insulin-stimulated glucose uptake into muscle and adipocytes tissues, in addition to impaired insulin suppression of hepatic glucose output, are described as having 'insulin resistance'(IR).⁸ Several diseases are clinically associated with IR includes obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome, cardiovascular disease, MAFLD, PCOS, and cancer.^{9–13} Thus, there is an urgent need to identify the

mechanisms of IR and effective interventions for treating these metabolic diseases. A relatively safe and well accepted approach in the prevention and treatment of IR is via lifestyle interventions. Nutritional intervention is an important first step that emphasizes a low-calorie and low-fat diet that stimulates excessive insulin demands. In addition, increased physical activity is recommended to help increase energy expenditures and improve muscle insulin sensitivity, this two approach represent the fundamental treatment for IR.^{14,15} The second step is the use of pharmacologic medications, including metformin, oral sulphonylureas, oral sodium-glucose cotransporter 2 (SGLT2) inhibitors, oral dipeptidyl peptidase 4 (DPP-4) inhibitors, oral α -Glucosidase, injectable glucagon-like peptide 1 (GLP1) receptor agonists, or injectable insulin.^{16,17}

In this review, the mechanism of insulin action and IR are first described to promote the development of new therapeutic strategies. Further, the direct and indirect effects of insulin on target tissues are discussed to better understand the pivotal role of tissue crosstalk in systemic insulin action. Lastly, diseases associated with IR are discussed and summarized. Many methods and multiple surrogate markers have been developed to calculate the IR. We then summarize the current measurements and potential biomarkers of IR to facilitate the clinical diagnosis. Finally, we provide the general approaches including lifestyle intervention, specific pharmacologic interventions and clinical trials to reduce IR.

THE INSULIN SIGNALING AND IR

Insulin is an endocrine peptide hormone with 51 amino acids and composed of an α and a β chain linked together as a dimer by two

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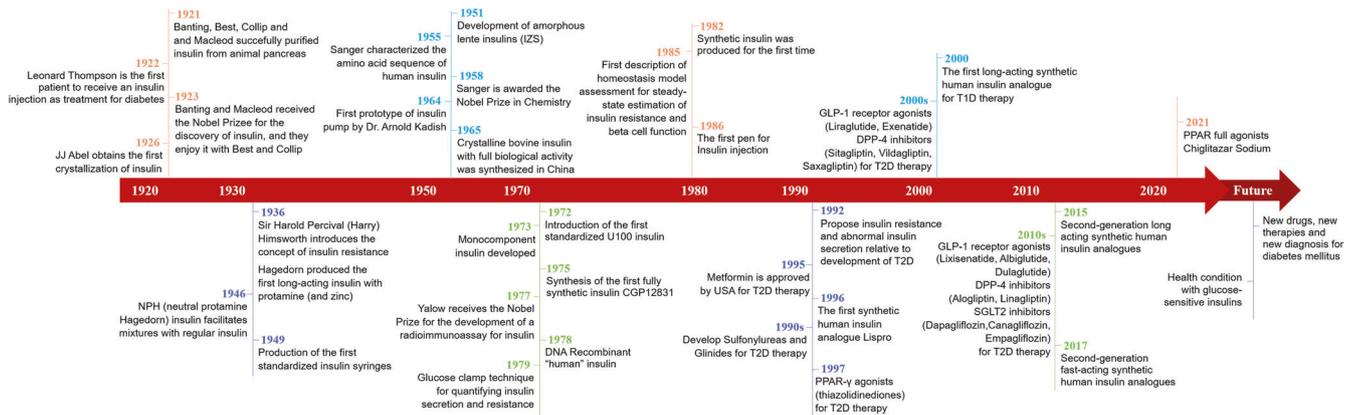


Fig. 1 A timeline of key discoveries in our understanding of insulin and insulin resistance

disulfide bridges¹⁸ along with a third intrachain disulfide bridge in the α chain.^{19,20} Insulin is released by pancreatic beta cells and is essential for glucose and lipid homeostasis.²¹ Insulin binds the insulin receptor (INSR) on the plasma membrane of target cells, leading to the recruitment/phosphorylation of downstream proteins, that primarily including insulin receptor substrate (IRS), PI3-kinase(PI3K), and AKT isoforms, that are largely conserved among insulin target tissues and that initiate the insulin response.^{22,23} The pathway diversification of insulin signaling downstream targets of Akt activation leads to different distal signaling in target tissues response to insulin (Fig. 2). (1) AKT substrates include the inactive glycogen synthase kinase 3 (GSK3) and active protein phosphatase 1 (PP1) that promote glycogen synthesis.^{24–26} In addition, the transcription factor forkhead box O1 (FOXO1) is phosphorylated by AKT and is transported from the nucleus, thereby disabling its transcription factor activity and inhibiting gluconeogenesis.^{27,28} (2) Tuberous sclerosis complex 1/2 (TSC1/2) and proline-rich Akt substrate 40 (PRAS40) are inhibitors of mTORC1, thereby inducing protein synthesis and adipogenesis.^{29,30} (3) The upregulation of sterol regulatory element binding protein 1c (SREBP-1c), de-phosphorylation of ACC1/2 through inhibition of AMP-dependent protein kinase (AMPK), and the phosphorylation of ATP citrate lyase (ACLY) lead to constitutive increases in *de novo* lipogenesis (DNL);^{31–33} (4) Phosphodiesterase 3B (PDE3B) and the abhydrolase domain containing 15 (ABHD15) protein are involved in suppression of lipolysis in adipocytes by inhibiting adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL).^{34,35}

Accumulation of reports have demonstrated that IR is a complex metabolic disorder with integrated pathophysiology. The exact causes of IR has not been fully determined,^{36–38} but ongoing research seeks to better understand how IR develops. Here, we focus on the underlying mechanism of IR including direct defective of insulin signaling, epidemiological factors, interorgan metabolic crosstalk, metabolic mediators, genetic mutation, epigenetic dysregulation, non-coding RNAs, and gut microbiota dysbiosis.

The direct defective of insulin signaling

As has been mentioned, the proper modulators acting on different steps of the signaling pathway ensure appropriate biological responses to insulin in different tissues. Thus, the diverse defect in signal transduction contributes to IR.

Proximal insulin receptor signaling and IR. Insulin exerts its biological effects by binding to its cell-surface receptors, thereby activating specific adapter proteins, such as the insulin receptor substrate (IRS) proteins (principally IRS1 and IRS2), Src-homology 2 (SH2) and protein-tyrosine phosphatase 1B (PTP1B), eventually

promoting downstream insulin signaling involving glucose homeostasis.³⁹ Thus, changes in insulin receptor expression, ligand binding, phosphorylation states, and/or kinase activity accounts for many IR phenotypes.

Most individuals that are obese or diabetic exhibit decreased surface INSR content and INSR kinase (IRK) activity.⁴⁰ Defective IRK activity is also implicated by decreased IRS1 tyrosine phosphorylation which is consistently observed in insulin-resistant skeletal muscles.⁴¹ In addition, the specific knockout or ablation of *INSR* in livers prevents insulin suppression of hepatic glucose production (HGP),^{42,43} suggesting a direct role for INSR in hepatic IR. Second, decreased expression or serine phosphorylation of IRS proteins^{44,45} can reduce their binding to PI3K, thereby down-regulating PI3K activation and inducing apparent IR. Third, homozygous mice of *IRS1* or *IRS2* gene leading to peripheral IR and diabetes, and impaired insulin secretion through restrained PI3K/AKT signal transduction.⁴⁶ Thus, pharmacological inhibitors, blocking antibodies and knockdown of PI3K abolishes the insulin stimulation of glucose transport, GLUT4 translocation and DNA synthesis.^{47–49} Additionally, deletion of *Pik3r1* and *Pik3r2* that encode PI3K subunit isoforms in skeletal muscle inhibits insulin-stimulated glucose transport.⁵⁰ Similarly, interfering Akt mutant suppresses insulin-stimulated GLUT4 translocation,⁵¹ and inhibition of AKT expression, or impairment in AKT Ser473 phosphorylation are certainly detected in both muscle and liver IR.^{52,53} Further, there are three known isoforms of Akt1/2/3 in insulin sensitive tissues, the present study showed that the Akt2 and Akt3 defects impaired insulin-stimulated glucose transport in IR.⁵⁴ In addition, elevated plasma nonesterified fatty acid (NEFA) levels impaired the insulin-induced increase in IRS-1-associated PI3K activity, but no defect in Akt phosphorylation was observed.⁵⁵ Together, the combined actions of various disorders in the proximal signaling components leads to impaired glucose metabolism and IR, and a major challenge remains for understanding IR mechanisms regarding how to distinguish the causes from insulin effects or primary defects from their consequences.

Distal downstream signaling and IR. It is generally accepted that diverse downstream targets of Akt activation lead to different distal signaling in target tissues response to insulin. As mentioned above, there are more than 100 Akt substrates mainly including GLUT4, FOXO1, GSK3, mTORC1, SREBP-1c, TSC1/2, PRAS40, ABHD15, PDE3B.⁵⁶ Among them, GLUT4 is the best characterized and mediates glucose uptake in skeletal muscle and white adipose cells after insulin stimulation.^{57,58} Impaired translocation of intracellular GSV (GLUT4 storage vesicles) caused decreased insulin-stimulated glucose uptake which are associated with IR in muscle and adipose tissues.⁵⁹ This proved that heterozygous deletion of *Glut4* mice reduce glucose uptake and develop

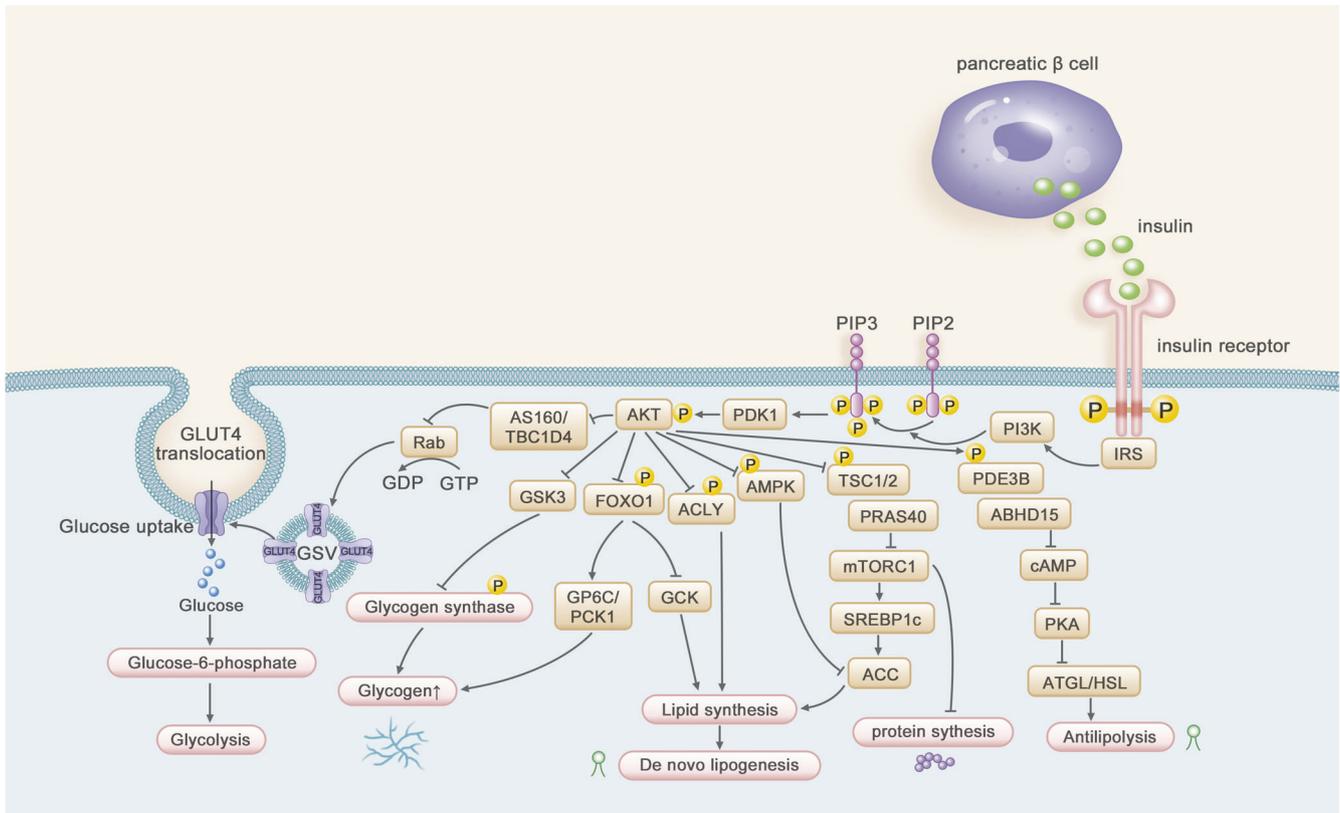


Fig. 2 The insulin signaling pathway including proximal and distal segments

metabolic disease in adipocytes.⁶⁰ Similarly, defection of insulin-stimulated GLUT4 translocation to the cell surface occurs in skeletal muscle in various IR mice models^{61,62} and humans with T2DM.^{63,64} In addition, loss of *Tbc1d4* in mice that phosphorylated by Akt leads to the attenuation of downstream target activation of Rab-GTPase proteins associated with GLUT4 vesicles, and completely abolishes insulin-stimulated adipocyte glucose uptake.⁶⁵ Mice homozygous for the physiologically important AKT substrate TBC1D4 Thr⁶⁴⁹ knock-in exhibit impaired insulin-stimulated myocellular GLUT4 translocation and induction of glucose intolerance.⁶⁶ In summary, continued discovery of novel AKT substrates involved in GLUT4 translocation indicates that many but not all of the same effectors are involved in the glucose uptake of different tissues, and further studies should be conducted to identify the molecular mediators in all phases of insulin-stimulated glucose uptake.

Epidemiology studies of IR

Sex difference. Different investigations have indicated that premenopausal women exhibit many less metabolic disorders than men, including lower incidence of IR, although this effect diminishes severely when women reach the postmenopausal situation.^{67,68} Specifically, female sex hormones including estradiol (e.g., 17 β -oestradiol)⁶⁹ protect female proopiomelanocortin (POMC) neurons from IR by enhancing POMC neuronal excitability and coupling insulin receptors to transient receptor potential (TRPC) channel activation. Concomitantly, clinical and experimental observations^{70,71} have revealed that endogenous estrogens can protect against IR primarily through ER- α activation in multiple tissues, including in the brain, liver, skeletal muscle, and adipose tissue, in addition to pancreatic β cells. Further, female hormone estrogens are determinants that mediate body adiposity levels and body fat distribution in addition to glucose metabolism and insulin sensitivity. Specifically, insulin sensitivity and capacities for

insulin responses in women is significantly higher than men.⁷² Women younger than 51 had a significantly lower fasting glucose and triglyceride concentration compared with men.⁷³ Furthermore, sex differences are associated with genetic polymorphisms in the development of IR and diabetes. Male homozygous for the polymorphism of *PPP1R3A* gene that involved in glycogen synthase activity are significantly younger at diagnosis than female.⁷⁴ The difference in regard to visceral, hepatic adiposity, hypoadiponectinemia, the insulin-sensitizing hormone-adiponectin, resting energy expenditure,^{67,75,76} lipid metabolism⁷⁷ may also contribute to higher IR in male compared with female. Thus, additional studies are required to understand mechanisms underlying sex differences and IR development.

Ethnicity difference. T2DM is anticipated to impact nearly 600 million people globally by 2035,⁷⁸ much of the investigations have recognized that the prevalence of T2DM are affected by different race/ethnicity, partly because of the differences in insulin sensitivity which affects plasma triglyceride levels.^{79–81} For example, Singapore Adults Metabolism Study (SAMS) performed a sub-group analysis and observed that Chinese and Malays exhibit greater insulin-sensitivity compared with Asian Indians among lean and young Singaporean males,⁸² the previous reports was further confirmed that the prevalence of T2DM in Chinese (9.7%) and Malays (16.6%) are lower than Asian Indians (17.2%).⁸³ In the United Kingdom. South Asian children exhibit greater IR compared with white European children, while girls are more insulin resistant than boys, with sex and ethnicity differences related to insulin sensitivity and body composition.^{84,85} In addition, individuals of Aboriginal or South Asian descent (among Aboriginal, Chinese, and Indian populations) that also exhibit increased levels of body fat and visceral fat deposition appear to have a greater propensity for IR and type 2 diabetes.⁸⁶ These interesting findings force us to reconsider the effect of the ethnic

differences in IR, which is important to reduce the morbidity and mortality related to diabetes mellitus and metabolic syndrome.

Modifiable lifestyle factors. Despite the above objective factors, some modifiable lifestyle factors including diet, exercise, smoking, sleep and stress are also considered to contribute to IR.^{87–89} For instance, irregular daily eating habits or poor sleep are connected to elevated risk for both obesity and IR. Further, circadian clocks disruption might also be an important factor to IR development via various factors including clock gene mutations, disturbed sleep cycles, shift work and jet lag.^{90,91} Moreover, epidemiologic studies of different institutions showed that individuals with regular exercise, healthy diet (including more soluble fiber, colorful fruit, vegetables, green tea, or less intake of added sugars, carbs, trans-fats), limiting alcohol intake, avoiding smoking cigarettes and reduced levels of stress, which indeed increase insulin sensitivity.^{92–94} Collectively, there are many relatively simple things we can do to naturally increase insulin sensitivity but ensure professional healthcare consultant first before adding medication regimen.

Different investigations suggest that vitamin D supplementation might reduce IR in some people due to increasing insulin receptor genes transcription and anti-inflammatory properties,⁹⁵ while some researchers found that Vitamin D has no effect on IR.⁹⁶ Thus, further studies should be performed to discover more about the mechanism and the effect of vitamin D on insulin resistance. Both experimental (animals) and clinical studies have shown that many hormones can induce IR including glucocorticoids (GCs),⁹⁷ cortisol,⁹⁸ growth hormone,⁹⁹ and human placental lactogen,¹⁰⁰ which may decrease the insulin-suppressive effects on glucose production and reduce the insulin-stimulated glucose uptake. Several other clinical medications including anti-adrenergic (such as salbutamol, salmeterol, and formoterol),¹⁰¹ HIV protease inhibitors,^{102,103} atypical antipsychotics¹⁰⁴ and some exogenous insulin¹⁰⁵ that may improve IR because of the disordered insulin signaling. All together, there may have synergistic effects of different risk factors on insulin resistance, scientific researchers should cooperate with medical experts to reduce the chances of becoming insulin resistant.

The interorgan metabolic crosstalk in IR

As described above, insulin signaling calibrates glucose homeostasis by limiting hepatic glucose output via decreased gluconeogenesis and glycogenolysis activities. These processes consequently increase the glucose uptake rates in muscle and adipose tissues. In addition, insulin profoundly affects lipid metabolism by increasing lipid synthesis in liver and fat cells (Fig. 3), in addition to switching-off fatty acid release from triglycerides (TG) in fat and muscle tissues.¹⁰⁶

Despite stimulated glucose uptake, insulin rapidly reduces hepatic glucose output and hepatic glucose production (HGP) by activating glycogen synthesis, and suppressing glycogenolysis and gluconeogenesis in liver.^{107,108} Further, gluconeogenesis suppression by insulin is mediated by inhibition of FOXO1 transcription factors.¹⁰⁹ For example, some mouse models of profound hepatic IR exhibit increased *G6pc* (glucose-6-phosphatase) expression suggesting increased FOXO1 activity.¹¹⁰ Moreover, correct hepatocellular insulin action also carries suppression of hepatic glycogenolysis and stimulation of glycogenesis.¹¹¹ A remaining question is whether potential controlling factors including allosteric control of glycogen synthase (GS) and phosphorylases via glucose-6-phosphate (G6P),¹¹² in addition to the insulin-independent transport of glucose across the hepatocellular plasma membrane by GLUT2 (as a glucose-sensitive protein in liver cells), can impact hepatic glycogen metabolism.^{113,114} Loss of GLUT2 leads to a typical combination of hepatic glycogen accumulation, glucose intolerance, and fasting hypoglycemia.¹¹⁵ In addition, liver insulin also effectively regulates lipid metabolism

primarily by promoting cleavage and nuclear translocation of the sterol regulatory element binding protein 1c (SREBP-1c)^{116,117} that activates lipogenesis in hepatocytes. Insulin induces SREBP-1c maturation via a proteolytic mechanism started in the endoplasmic reticulum (ER), wherein hepatic IR is highly associated with hepatic steatosis.¹¹⁸ Over-expression of liver SREBP-1c has been described in several IR models of including IRS2 knockout,¹¹⁹ lipodystrophic and ob/ob mice.¹²⁰ In addition, hepatic glucose lead to a deficiency in the transcription factor carbohydrate responsive element binding protein (ChREBP) resulting in reduced mRNA levels of glycolytic and lipogenic enzymes, as well as SREBP-1c levels. Accordingly, restoration of nuclear SREBP-1c expression in liver-specific *ChREBP* defective mice normalized expression of some lipogenic genes, while not affecting glycolytic genes expressing. In contrast, ChREBP overexpression alone failed to promote the expression of lipogenic genes in the livers of mice lacking active SREBPs. Together, these data demonstrate that SREBP-1c mediates the induction of insulin lipogenic genes, but that SREBP-1c and ChREBP are both necessary for harmonious induction of glycolytic and lipogenic genes.¹²¹ ChREBP inhibition markedly decreased the expression of L-PK, ACC, and FAS, but restored liver insulin sensitivity by restoring protein kinase B (Akt), and Foxo1 phosphorylation activity with insulin.¹²² This paradoxical response of SREBP-1c and ChREBP in hepatic IR is probably due to many branch effectors (including PI3K/AKT, AMPK, mTORC1, and FOXO1) involved in the hepatic glucose and lipid metabolism. Altogether, these above pathways and components can be used to clarify the popular pathophysiology of hepatic IR.

The lipid metabolisms including increased *de novo* lipogenesis and attenuation of lipolysis in the adipose tissue largely coordinate with glucose homeostasis response to insulin stimulation. *De novo* lipogenesis regulation in adipose is similar to that in livers, wherein adipose-ChREBP is a major determinant of adipose tissue fatty acid production and systemic insulin sensitivity, that is induced by GLUT4-mediated glucose uptake, and genetically ablating ChREBP impairs insulin sensitivity in adipose tissue.¹²³ In addition, lipogenic gene *FASN* and *DGAT* mRNA expression in adipose tissue have been shown to correlate strongly and positively with insulin sensitivity, which were may reduced by larger adipocytes in adipose tissue of obese individuals. The lipogenesis stimulation of insulin is also reduced in larger, more insulin-resistant cells. Insulin suppression of lipolysis includes the hydrolytic cleavage of triglycerides, resulting in the generation of fatty acids and glycerol. The best understood effectors for this process are PDE3B and ABHD15 that operated by the suppression of cAMP to attenuate pro-lipolytic PKA signaling toward adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and perilipin (PLIN).^{34,124,125} Phosphorylation and activation of PDE3B at Ser²⁷³ and Ser²⁹⁶ by AKT is a key event in antilipolysis after insulin stimulation. Further, inhibition of PDE3B inhibits insulin-induced glucose uptake and antilipolysis.¹²⁶ *Pde3b* knockout mice exhibit impaired suppression of plasma NEFA levels and corresponding impairment in hepatic glucose production suppression during glucose tolerance tests.¹²⁷ Further, mice homozygous for the *Plin1* deletion exhibit high basal lipolysis and are unresponsive to adrenergic stimulation, confirming that PLIN is actively controls lipolysis.^{128,129} Taken together, all the evidence supports that white adipose tissue (WAT) lipolysis suppression of insulin also associated with hepatic gluconeogenesis, and the mechanisms of IR include complex mediators and metabolic networks.

Insulin stimulated protein synthesis is mediated by activation of the protein kinases Akt and mTOR (specifically mTORC1 and mTORC2) in numerous insulin-responsive cell types, such as hepatocytes, adipocytes, and myocytes.¹³⁰ AKT also phosphorylates the TSC1-TSC2 complex to relieve its inhibition on the mTORC1. Inhibition of mTOR by rapamycin obviously impairs insulin-activated protein synthesis.¹³¹ Recent studies have

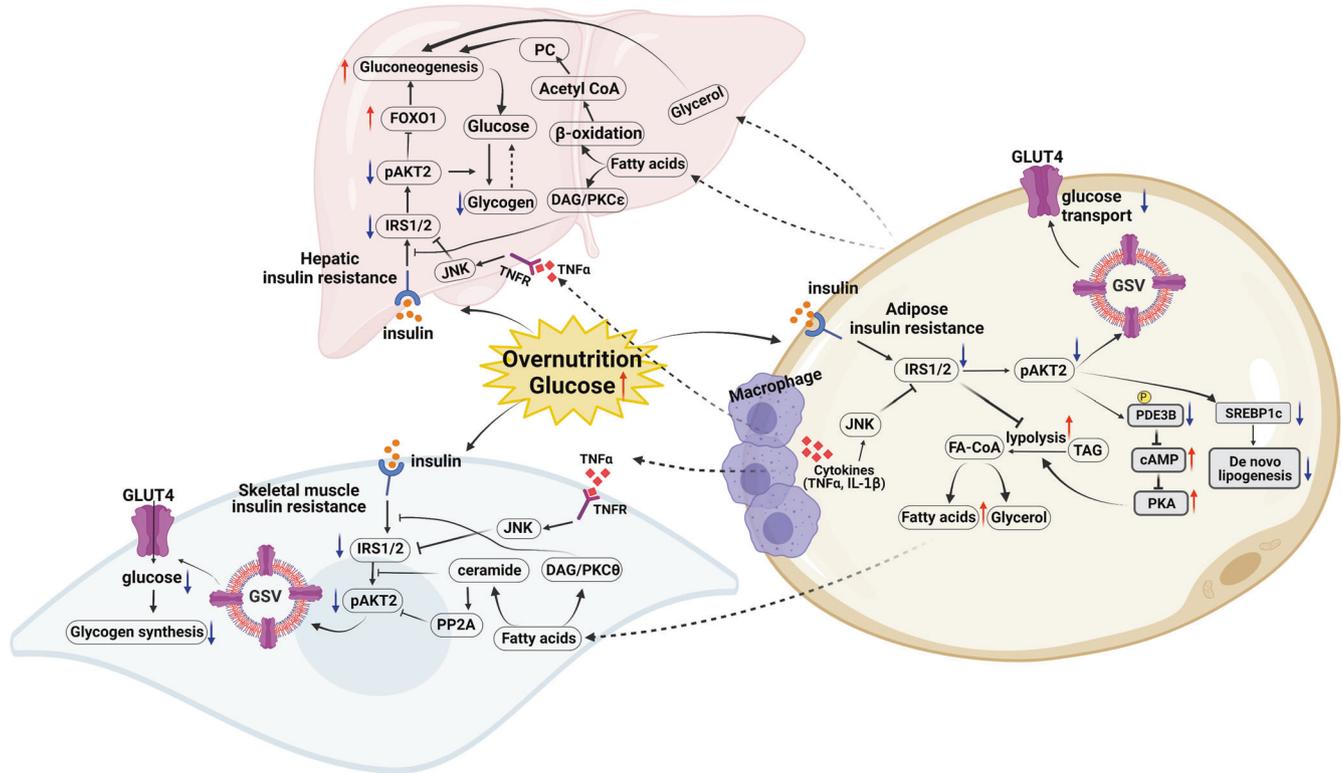


Fig. 3 An integrated physiological signaling on different target tissues insulin resistance

identified that Akt phosphorylates and inactivates the PRAS40 (proline-rich Akt/PKB substrate 40), which abates its binding inhibition of mTOR signaling and promotes protein synthesis. Amino acids metabolic substrates enhance insulin sensitivity and responsiveness of the protein synthesis system by increasing mTOR activity and inhibiting protein degradation in liver, muscle, and heart tissues.^{132,133} Skeletal muscle tissue is the largest protein/amino acid (AA) reservoir in the human body, and lower muscle protein synthesis (MPS) induces fed-state anabolic resistance,¹³⁴ in addition to exercise training of the Akt/mTOR pathway. These processes, in turn, promote protein synthesis and antagonize protein degradation.¹³⁵ Further, PRAS40 and mTOR also exerts negative feedbacks on proximal insulin signaling, PRAS40 knockdown significantly decreases Akt phosphorylation, mTORC1 binds and inhibits INSR by inducing destabilization of IRS1.²⁸ The signaling system of IR are multifactorial including different metabolic pathways, such as glucose, lipid, and protein, identifying new molecules will be crucial to unraveling more effective treatment of IR and associated metabolic diseases.

The contribution of metabolic mediators to IR

Adipokines dysregulation and IR. Adipose tissue can secrete various bioactive circulating mediators referred as 'adipokines', like adiponectin, leptin, chemerin, resistin, visfatin and vaspin, in addition to cytokines and chemokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-1 β , and monocyte chemoattractant protein-1.¹³⁶ Dysregulation of these adipokines has been implicated in the onset of obesity, IR, type 2 diabetes, cardiovascular disease, hypertension and metabolic syndromes.¹³⁷

Adiponectin is the most abundant protein secreted by adipose tissue and exhibits potent anti-inflammatory properties.¹³⁸ In contrast to other adipokines, plasma adiponectin levels were reduced by pro-inflammatory factors like TNF- α , IL-6, ROS, and hypoxia in animal models of obesity and IR.^{139,140} Adiponectin activates the AMPK and PPAR- α signaling pathways through adiponectin receptor 1 (AdipoR1) and AdipoR2 respectively,

leading to enhanced fatty acid oxidation and glucose uptake in muscle, along with suppressed gluconeogenesis in liver tissues. Moreover, targeted disruption of AdipoR1 results in halted adiponectin-induced AMPK activation, increased endogenous glucose production and increased IR. Similarly, AdipoR2 deletion results in decreased PPAR- α signaling pathway activity and IR. In addition, chemerin is a chemokine highly expressed in liver and white adipose tissue that regulates the expression of adipocyte genes involved in glucose and lipid homeostasis like IRS-1 tyrosine phosphorylation activity, GLUT4, fatty acid synthase and adiponectin. Thus, chemerin may increase insulin sensitivity in adipose tissue.^{141,142} Adipocytes also produce apelin that is increased in insulin resistant individuals¹⁴³ and morbidly obese subjects with T2DM.¹⁴⁴ However, apelin enhances glucose uptake and Akt phosphorylation through AMPK pathway to improve glucose homeostasis and insulin sensitivity,¹⁴⁵ apelin deficient mice are insulin resistant and have decreased skeletal muscle Akt phosphorylation.¹⁴⁶ The precise role of anti-inflammatory adipokine in regulating IR require further investigation.

Leptin is a cytokine encoded by *ob* gene and produced by the adipocytes.¹⁴⁷ Leptin binds to the leptin receptor (LepRb) and activates JAK2/STAT3 pathway to decrease body weight and normalizes blood glucose concentration, meanwhile, JAK2 stimulates the phosphorylation of insulin receptor substrate (IRS1/2), then activates PI3K/Akt pathway and directly affect insulin action.¹⁴⁸ Leptin limits insulin synthesis and secretion from pancreatic β -cells, resulting in increased insulin sensitivity, reduced hepatic glucose production and decreased glucagon levels.^{149,150} In turn, insulin also plays a role in leptin production stimulation and secretion in adipose tissues.¹⁵¹ Leptin-deficient mice (*ob/ob*) causes both obesity and diabetes due to hyperphagia and blunted metabolic rate, and treatment with exogenous leptin could prevent and reverse the obese phenotype.¹⁵² Moreover, the decreased permeability of the brain-blood barrier (BBB) to leptin and impaired leptin signal transduction in neurons will lead to leptin resistance in obesity.^{148,153} Specifically, high

serum leptin levels connected to IR likely promote the release of pro-inflammatory compounds that include IL-6, TNF- α , and IL-12 by monocytes and macrophages.¹⁵⁴ Together, leptin and insulin share pivotal roles in the regulating glucose metabolism, and additional studies are needed to understand the effects of leptin on glucose-insulin homeostasis. In summary, adipose tissue is a central node for distinct adipokines and bioactive mediators in IR pathophysiology. Consequently, identifying the effects of new adipokines will help in the development of new therapeutic strategies for obesity-induced diseases.

Fatty acid/lipid metabolism in IR. The specific insulin actions in adipose tissue include activation of glucose uptake and triglyceride synthesis, suppression of triglyceride hydrolysis and free fatty acids (FFA) and glycerol release into the blood circulation.^{155–157} Among these actions, an extremely important function of adipose tissue is via the switches that favor lipids storage in adipocytes over their release into circulation upon need. Once the adipose tissue expandability exceeded limit under overnutrition, excess lipids and toxic lipid metabolites (FFA, diacylglycerol, ceramide) accumulated in non-adipose tissues, thus leading to lipid-induced toxicity (lipotoxicity) and developed IR in liver and muscle.^{158,159} The early mechanism of lipid accumulation pouch plasma fatty acid to induce the IR as detailed in glucose-fatty acid cycle proposed by Randle and colleagues.^{160,161} Their hypothesis suggested that available fatty acids promote fatty acid oxidation and cause the accumulation of mitochondrial acetyl coenzyme A (CoA) and NADH, with subsequent inactivation of pyruvate dehydrogenase. This process would in turn induce increased intracellular citrate levels, thereby inhibiting glucose-6-phosphate (G6P) accumulation. Increased G6P levels then result in decreased hexokinase activity, increased glucose accumulation, and reduced glucose uptake.

Other studies have demonstrated the relevance of the glucose-fatty acid cycle to lipid-induced IR. For example, lipid infusions combined with heparin can be used to activate lipoprotein lipase, thereby increasing plasma concentrations of fatty acids. Further, these infusions promote muscle lipid accumulation and effectively induce IR.^{162,163} In addition, contrary to predicted increases based on the Randle hypothesis, elevated free fatty acid levels were associated with reduced intracellular G6P levels in acute lipid-induced IR and type 2 diabetics.^{158,164} In parallel, lipid-induced IR in skeletal muscle leads to defected insulin signaling and decreased insulin-stimulated glucose transport mediated by GLUT4 translocation, and not by glycolysis inhibition as Randle hypothesized.¹⁶⁵ Together, these researches suggest that lipid ectopic accumulation is directly correlated with IR.

Diacylglycerol and ceramide accumulation. Consistent with the above studies, elevated plasma fatty acid concentrations can result in increased intracellular diacylglycerol (DAG) levels, leading to the activation of protein kinase C isoform (PKC- θ) and PKC- ϵ isoforms in skeletal muscles and liver respectively. These processes, in turn, decrease insulin-stimulated IRS-1/IRS-2 tyrosine phosphorylation, PI3K activation and downstream insulin signaling that then induces IR in muscles and livers.^{166–168} Deletion of PKC θ in mice inhibits muscle IR induced by lipid infusion.¹⁶⁹ Moreover, knockdown of PKC- ϵ in rats leads to protection from fat-induced hepatic IR,¹⁷⁰ these results confirm the distinct roles of PKC- θ and PKC- ϵ in fat-induced IR in skeletal muscles and livers, respectively. Since diacylglycerol acyltransferase 1 (DGAT1) can increase the conversion of DAG into triacylglycerol (TAG),¹⁷¹ DGAT1 overexpression could decrease DAG levels and improve insulin sensitivity partially attenuating the fat-induced activation of DAG-responsive PKCs.^{172,173} Conversely, DGAT1 ablation may result in elevated DAG levels and lipid-induced IR. Taken together, these studies strongly support that DAG as a key intermediate of TAG synthesis from fatty acids has central modulation and potential therapeutic values in IR.

Ceramide is another specific lipid metabolite that increases in concentration, along with DAG, in association with IR in obese mice.¹⁷⁴ Accumulated ceramide mediates the activation of protein phosphatase 2A (PP2A) and impairs insulin-dependent activation, in addition to signaling of PI3K/Akt by PKC ζ ,¹⁷⁵ thereby also disrupting lipid metabolism by inhibiting oxidation and stimulating fatty acid uptake.¹⁷⁶ In particular, C18- and C16-long-chain ceramides that are produced by ceramide synthase isoforms (CerS1, CerS5, and CerS6) have higher concentrations in insulin-resistant tissues.^{177,178} Consistently, C18-derived ceramides play an important role in fat-induced skeletal muscle IR.¹⁷⁹ Likewise, C16-ceramides exist in higher concentrations in obese adipose tissue and are associated with hepatic IR.^{180,181} Several lines of evidence suggest that circulating adiponectin is closely related to ceramide concentrations, wherein adiponectin increases ceramidase activity associated with its two receptors AdipoR1/AdipoR2, while lower concentrations of circulating adiponectin increases ceramide concentrations in different tissues.^{182,183} Furthermore, inhibited ceramide synthesis or stimulation of ceramide degradation¹⁷⁶ can prevent the effects of ceramide on Akt/PKB activation and improve insulin signaling. Thus, ectopic lipid metabolite concentrations (e.g., diacylglycerols and ceramides) may be mechanistic factors underlying liver and muscle IR. Consequently, concerted efforts to decrease lipid components in these organs are the most efficacious therapeutic targets for treating IR and metabolic diseases.

Genetic mutations in IR

Some human genetic studies indicated that different genomic loci were associated with fasting insulin levels, higher triglyceride and lower HDL cholesterol levels,^{184,185} which are different hallmarks of IR.¹⁸⁶ Epidemiological and family genetic studies have provided considerable evidence for the genetic basis of both IR and the individual components of the metabolic syndromes.^{187,188} Since 2007, genome-wide association studies (GWAS) and next-generation sequencing (NGS) have identified different genetic variants associated with IR, including *PPAR γ* , *IRS1*, *IGF1*, *NAT2*, *KLF14*, *GCKR*, *FTO*, *TCF7L2*, *TMEM163*, *MC4R*, *SC4MOL*, *TCERG1L*, and *ARL15*^{184,189,190} by influencing insulin action via different regulatory mechanisms.

The peroxisome proliferator-activated receptor gamma (*PPAR γ*) variant Pro12Ala was one of the first genetic variants identified that is involved in fatty acid and energy metabolism and that is associated with a low risk of developing T2DM.¹⁹¹ Variants (A allele to G allele) within IGF-1 (insulin-like growth factor 1) contribute to its low plasma levels, and cause a reduction in insulin sensitivity.¹⁹² A variant in N-acetyltransferase 2 (*NAT2*) was recently identified as an insulin sensitivity gene.¹⁹³ Adiponectin is an adipokine involved in improving insulin sensitivity, variants within ADP ribosylation factor like GTPase 15 (*ARL15*) are associated with decreased adiponectin levels and nominally associated with IR.¹⁹⁴

Despite these potential genetic correlates, variants account for only 25% to 44% of the heritability of IR.¹⁹⁵ Consequently, the contributions of low-frequency and rare genetic variants towards the heritability of IR have also been explored through a combination of both genome and exome sequencing.^{196,197} Such studies have identified a low-frequency variant of *CD300LG* that is associated with fasting high-density lipoprotein cholesterol (HDL-C),¹⁹⁸ and a *TBC1D4* variant that together are connected to higher fasting glucose levels and reduced insulin sensitivity.¹⁹⁹ The rapid development of genomics methods has enabled considerable progress towards the identification of genetic loci associated with IR by direct or indirect effects. Nevertheless, additional studies are needed to assess the functional relationships between the genetic variants and IR, that are also influenced by various lifestyle and environmental factors.

Epigenetic dysregulation in IR

Recent studies have suggested that epigenetic modifications such as DNA methylation (DNAm) and histone post-translational modifications (PTM) are implicated in the development of systemic IR.^{200,201}

DNA methylation. Global and site-specific DNA methylation is generally mediated by DNA methyltransferases (DNMTs). These processes mainly occur in the context of CG dinucleotides (CpGs) and promoter region, while also involving covalent addition or removal of methyl groups as a means to repress or stimulate transcription, respectively.²⁰²

Firstly, DNA methylation regulates different insulin signaling genes, such as insulin (*INS*), insulin receptor substrate 1 (*IRS1*), Insulin-like growth factor-1/2 (*IGF-1/2*), Insulin-like growth factor-binding protein 1/2 (*IGFBP-1/2*), phosphatidylinositol 3-kinase regulatory subunit (*PIK3R1*), and Glucagon-like peptide-1 receptor (*GLP-1R*).^{203–206} The methylation status of these genes was found to be altered in obesity and IR. For example, increased *INS* promoter methylation levels and *INS* mRNA suppression were observed under over-nutrition conditions and obese T2DM patients.²⁰⁷ Similarly, a research with blood samples of T2DM found that increased *IGF-1* DNA methylation were associated with reduced IGF-I serum levels in T2DM patients.²⁰⁸ Insulin-like growth factor binding proteins 1 and 2 (*IGFBP1* and *IGFBP2*, respectively) are the most abundant circulating IGFBPs and have lower concentrations in adipose tissue in obese patients, in addition to being negatively associated with hyperinsulinemia and IR.²⁰⁹ Several studies have indicated that increased *IGFBP-1* DNA methylation levels and decreased IGFBP-1 serum levels are associated with newly diagnosed T2DM. Another study demonstrated²¹⁰ that increased *IGFBP2* DNA methylation levels were associated with lower mRNA expression levels in Visceral Adipose tissue (VAT) of abdominal obesity. Moreover, the first global genome-wide epigenetic analysis in VAT²¹¹ from IR and insulin-sensitive (IS) morbidly obese patients identified a novel IR-related gene, the zinc finger protein 714 (*ZNF714*) exhibited the highest DNA methylation difference, and its methylation levels is lower in IR patient than in IS patient, consistent with increased transcription levels, such studies provide potential epigenetic biomarkers related to IR in addition to novel treatment targets for the prevention and treatment of metabolic disorders.

Some DNA methylation in the promoter regions of specific genes related to lipid metabolism (*PPARG*, *PPARA*),²¹² low-density lipoprotein receptor-related protein 1 (*LRP1*),²¹³ lipoprotein lipase (*LPL*),²¹⁴ *SREBF1/2*,²¹⁵ and inflammation (stearoyl-CoA desaturase 1 (*SCD1*), chemokine C-C motif chemokine ligand 2 (*CCL2*), *TNF- α* , and *TGF- β 1*)^{216,217} are associated with adipose tissue dysfunction and could lead to metabolic disorders. For example, peroxisome proliferator-activated receptor- α and - γ (*PPAR- α* and *PPAR- γ* , respectively) are encoded by *PPARA* and *PPARG*, respectively, and they are the two primary nuclear peroxisome proliferator-activated receptors involved in lipid metabolism. Higher *PPARA* and *PPARG* methylation levels were observed in association with obesity, consistent with decreased *PPAR- α* and *PPAR- γ* protein expression levels,²¹⁸ that lead to dyslipidemia and IR. *SLC19A1*, a gene encoding a membrane folate carrier, was reduced in obese WAT and induced global DNA hypermethylation of chemokine C-C motif chemokine ligand 2 (*CCL2*) that is a key factor in WAT inflammation,²¹⁹ resulting in increased *CCL2* protein secretion and the development of IR in obese.

In addition, several genes methylation involved in hypoxia stress and endoplasmic reticulum stress were regulated in obesity related metabolic diseases.²²⁰ Hypoxia-inducible factor-3 α (*HIF3A*) belongs to the hypoxia-inducible factors family (*HIFs*) that play important roles in the pathogenesis of obesity-induced IR, adipose tissue-inflammation and the etiology of obesity related disease. Recent epigenetic genome-wide analysis identified low *HIF3A*

methylation levels upregulates *HIF3A* expression in adipose tissue, thereby leading to adipose tissue dysfunction and adiposity.²²¹ Further, reduced *HIF3A* methylation and increased *HIF3A* levels in blood are associated with IR and higher body mass index (BMI) values in T2DM patients.²²² The major function of the endoplasmic reticulum (ER) is the synthesis and folding of secreted and transmembrane proteins, increasing evidence suggests that persistent ER stress is associated with the onset and progress of chronic metabolic disorders like obesity and IR. Ramos-Lopez et al.²²⁰ found that the methylation levels of four ER genes including *ERO1LB*, *NFE2L2*, *MBTPS1* and *EIF2AK4* which encoded ER oxidoreductin-1 β (*ERO1 β*), nuclear factor-erythroid 2-related factor (*Nrf2*), site-1 protease (*S1P*) and eIF-2-alpha kinase (*GCN2*) respectively, were strongly correlated with total body fat levels. Specifically, increased insulin concentrations and HOMA-IR index were accompanied by lower *ERO1LB* and *NFE2L2* methylation levels.²²³ Hence, related to hypoxia and ER stress genes could be considered as precise therapeutics to control the IR.

Histone modifications. The histone modification effect on gene expression mainly includes histones methylation and acetylation. Histone methylation could either activate gene transcription (*H3K4*, *H3K36*, and *H3K79*) or silence gene expression (*H3K9* and *H3K27*), which depends on the modification site.²²⁴ Several studies have reported various histone epigenetic modifications of metabolic and mitogenic cascade-related genes of insulin signaling during IR.²²⁵ *PPARG* is a key transcription factors that regulates insulin sensitivity, lower histone *H3* acetylation and methylation of the *PPARG* gene are associated with reduced *PPARG* expression that is associated with IR.²¹² Further, increased expression and low methylation of *CDKN1A* and *PDE7B* genes in T2DM can lead to impaired insulin release that is stimulated by glucose in T2DM patients.²²⁶ The high level of *H3K4* trimethyl (*H3K4me3*) of *Fxyd3* gene negatively regulates the glucose capacity of insulin-secreting cells in mice.²²⁷

Histone acetylation increases the accessibility and gene expression of various transcription factors by reducing the positive charge and histone affinity for DNA.²²⁸ Histone deacetylation is considered to be the inhibition of DNA assembly by chromatin condensation and transcription factors, resulting in transcriptional inhibition. Increasing evidence indicates^{229,230} that *IGFR*, *InsR*, *IRS1*, *Akt*, *GLUT4*, and *PPAR* are more deacetylated in association with IR than in normal physiological conditions. In contrast, *IRS2*, *FoxO*, *JNK*, and *AMPK* are usually acetylated in association with IR. Castellano-Castillo, D., et al.²³¹ utilized a chromatin immunoprecipitation (ChIP) assay to determine that the human adipose tissue *H3K4me3* histone mark site in adipogenic, lipid metabolism, and inflammatory genes (such as *LEP*, *LPL*, *SREBF2*, *SCD1*, *PPARG*, *IL6*, *TNF*, and *E2F1*) is positively associated with BMI and HOMA-IR. Further, global proteomic analyses have revealed 15 histone modifications that are differentially abundant in hepatic IR.²³² These observations provide evidence for diabetes-related histone modification and related impaired insulin release.

non-coding RNA regulation in IR

Non-coding RNAs (ncRNAs) comprise approximately 98% of human genome transcripts and are generally not translated into proteins.²³³ The rising studies^{234,235} have shown that ncRNAs include microRNAs (miRNAs), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) are key mediators in the pathogenesis and progression of metabolic homeostasis.

MiRNAs and IR. MicroRNAs (miRNAs) are small ncRNAs (18-22 nucleotides) incorporated into Argonaute (Ago) protein to form miRISCs, which can inhibit the expression of partially or completely complementary target mRNAs.²³⁶ Dysregulation of various miRNAs within different fluids (e.g., blood, saliva, and urine) is associated with obesity and IR development, including

β -cell dysfunction, glucose and lipid homeostasis, and chronic inflammation.²³⁷

Firstly, pancreatic β cell mass due to dysfunction and/or death are the major cause of insufficient insulin secretion, and the main common mechanisms of T1DM and T2DM. Several miRNAs are involved in β cell differentiation and mature β cell functioning. For example, islet-specific miR-375 overexpression represses glucose-stimulated insulin secretion (GSIS) and insulin gene transcription, that is then reversed upon miR-375 inhibition.²³⁸ Further, deregulated plasma levels of miR-375 together with miR-150, miR-30a-5p, and miR-15a are observed before T2DM and pre-diabetes onset. Thus, these markers may improve disease prediction and prevention in individuals at high risk for T2DM.²³⁹ Other miRNAs have been implicated in β -cell proliferation and insulin secretion regulation during IR including miR-124a2, miR-204, miR-184 and miR-24. Further, miR-124a2 targets the gene encoding cAMP-response-element binding protein (creb-1) and forkhead/winged helix transcription factor boxa2 (foxA2) mRNA.²⁴⁰ FoxA2 is an upstream regulator of pancreatic duodenal homeobox 1 (pdx1) that is essential for pancreatic development and glucose homeostasis. Furthermore, pdx1, neurogenin-3 (ngn3), and a transcriptional factor essential for insulin transcription (MafA) are essential transcription factors for β -cell differentiation. Thus, miR-124a2 can directly modulate insulin expression through foxA2 and then pdx1. miR-204 expression is induced by the cellular redox regulator thioredoxin-interacting protein (TXNIP) that then represses MafA, thereby inhibiting insulin production.²⁴¹ In addition, miR-185-5p overexpression enhances insulin secretion and promotes pancreatic β -cell proliferation by targeting SOCS3 and regulating the Stat3 pathway.²⁴²

Numerous studies suggest that miRNAs have pivotal roles in glucose and lipid metabolism.^{243,244} As we mentioned above, glucose metabolism contains different processes including glucose transport, glucose uptake, gluconeogenesis and glycogenolysis. miR-93 was first reported to directly regulate GLUT4 expression in adipocytes.²⁴⁵ Further, miR-29 and miR-31 regulate GLUT4 expression in skeletal muscle and adipose tissues of T2DM patients,²⁴⁶ respectively. In addition, miR-27a/b levels are higher in the sera of patients with type 2 diabetes, while miR-27a/b overexpression suppresses hepatic glucose output and alleviates hyperglycemia by targeting FOXO1.²⁴⁷ Moreover, elevated miR-338-3p levels are responsible for decreased glycogenolysis and subsequent glycogen accumulation by directly targeting the glycogen phosphorylase brain form (PYGB).²⁴⁸ miR-185-5p overexpression in db/db mice that represent genetic diabetes models leads to alleviated blood hyperglycemia and decreased gluconeogenesis by directly targeting glucose-6-phosphatase (G6Pase). In addition, the anti-diabetic drug metformin can up-regulate miR-185-5p expression to suppress G6Pase and inhibit hepatic gluconeogenesis.²⁴⁹

The balance of low-density lipoprotein (LDL) and high-density lipoprotein (HDL) molecules that are synthesized in hepatocytes is critical for lipid homeostasis. Many miRNAs have been identified as critical regulators of HDL and LDL biogenesis. For example, miR-33, miR-128-1, miR-144, and miR-148a repress expression of the ATP-binding cassette transporter ABCA1 that mediates hepatic HDL generation.^{250–252} Thus, inhibition of these miRNAs increases circulating HDL levels. In addition, miR-30c targets the gene encoding microsomal triglyceride transfer protein (MTP) that is required for the lipidation of newly synthesized APOB in the liver for LD lipoprotein production. miR-30c overexpression reduces the assembly and secretion of these APOB-containing lipoproteins, resulting in decreased plasma LDL levels.²⁵³ The LDL receptor (LDLR) of hepatocytes is highly expressed and binds LDLs, clearing them from circulation. miR-148a and miR-128-1 repress LDLR expression and inhibition of these miRNAs results in enhanced LDLR expression and clearance of circulating LDL. Further, miR-224 and miR-520d target the LDLR chaperonin PCSK9 and IDOL in addition to the rate-limiting enzyme in cholesterol biosynthesis,

HMGCR.²⁵⁴ In addition, inhibition of PCSK9, IDOL, and HMGCR by miR-224 and miR-520d was associated with increased LDLR protein levels and increased LDL binding, resulting in decreased plasma LDL cholesterol levels.

Chronic inflammation in insulin-reactive tissues is one of the most important causes of IR and increasing evidence suggests that miRNAs has a pivotal role in the inflammatory process. Obesity inhibited miR-30 expression in adipose tissue macrophages (ATMs), and miR-30 was shown to target Delta-like-4 (DLL4), a Notch1 ligand is associated with ATM inflammation.²⁵⁵ miR-30 inhibition triggers Notch1 signaling, pro-inflammatory cytokine (TNF α and CCL2) production, and M1 macrophage polarization, indicating that miR-30 manipulation could be a therapeutic approach for reducing obesity-induced inflammation. Conversely, Wang et al. discovered that miR-1249-3p is significantly upregulated in Natural killer (NK) cells-derived exosomes from lean mice, which directly targets SKI family transcriptional corepressor 1 (SKOR1), subsequently down-regulated the expression levels of pro-inflammatory cytokine factors (including IL-1 β , IL-6, and TNF- α) levels and attenuated IR. Therefore, it might be that metabolism-regulating miRNAs play a vital role in the dynamics of metabolic homeostasis.²⁵⁶

LncRNAs and IR. Long non-coding RNAs (lncRNAs)²⁵⁷ are non-coding transcripts more than 200 nucleotides, and the subcellular localization of lncRNAs determines their function. lncRNAs located in the nucleus could affect chromosomal biology or interact with transcription factors to regulate gene transcription; lncRNAs located in cytosol could modulate mRNA stability and translational efficiency by acting as sponges for miRNAs or direct pairing with mRNA. Recent advances have shown that lncRNAs play crucial roles in the pathologies of IR and diabetes.^{213,258,259}

Glucose and lipid metabolism disorders are the primary causes for the pathophysiological development of IR. The lncRNA SRA promotes insulin-stimulated glucose uptake by co-activating PPAR γ , leading to increased phosphorylation of the downstream targets Akt and FOXO1 in adipocytes.²⁶⁰ Furthermore, glucagon-stimulated upregulation of H19 via the AMP/PKA pathway induces nuclear translocation of HNF4A and activates the transcription of *G6PC* and *PCK* that are involved in gluconeogenesis, resulting in hepatic glucose production.²⁶¹ In addition, the H19 sponge cell miR-130a induces PPAR γ nuclear translocation, thereby activating the transcription of adipogenic genes like those encoding acetyl coenzyme a carboxylase 1 (ACC1), fatty acid synthase (FAS), and cytochrome c oxidase (SCO1), thereby promoting intracellular lipid accumulation.²⁶² H19 is downregulated in skeletal muscles of db/db mice and interacts with heterogeneous nuclear ribonucleoprotein (hnRNP1) that then increases fatty acid oxidation (FFA) protein translation. These processes are closely related to the genes *PGC1a* and *CPT1b* that reverse FFA-induced lipid accumulation and improve IR.²⁶³ This suggests the complex effect of lncRNAs on the IR progression.

In addition the insulin target tissues,²⁶⁴ transcriptome profiling and different studies have identified several β -cell specific lncRNAs that contribute to obesity-mediated β -cell dysfunction and apoptosis. lncRNA MALAT1 downregulation may lead to pancreatic β -cell dysfunction and T2DM development by direct interaction and regulation of polypyrimidine bundle binding protein 1 (PTBP1). The lncRNA-p3134 positively regulates GSIS by promoting PI3K/Akt/mTOR signaling and the key regulators (Pdx-1, MafA, GLUT2, and Tcf7l2) in pancreatic β cells. Further, lncRNA-p3134 overexpression can decrease the β cell apoptosis ratio and partially reverse the glucotoxicity effects on GSIS function.^{265,266} Similarly, the newly identified lncRNA β -cell function and apoptosis regulator (β Faar) ameliorates obesity-associated β -cell dysfunction and apoptosis by upregulating the islet-specific genes (*Ins2*, *NeuroD1*, and *Creb1*) by sponging miR-138-5p.²⁶⁷ In addition, a novel micropeptide TUNAR encoded by lncRNA transcripts play a critical role in pancreatic β cell functions and insulin

homeostasis.²⁶⁸ Collectively, these studies provide new insights into the use of lncRNAs as possible biomarkers or therapeutic targets for obesity-associated IR and metabolic diseases.

Circular RNAs (circRNAs) and IR. Contrary to conventional linear RNA, circRNAs are noncoding RNAs that generated from precursor mRNAs by back-splicing circularization, which is derived from exonic circRNAs, intronic circRNAs, exonic-intronic circRNAs and ntergenic circRNAs.²⁶⁹ CircRNAs can affect gene transcription, splicing and translation by acting as a miRNA sponges, binding to RNA binding proteins or transcription factors (TFs). Recent studies have suggested that newly identified circRNAs are novel factors in the initiation and development of IR.²⁷⁰ For example, ci-Ins2 is a conserved intronic circRNA derived from insulin pre-mRNA that exhibits lower levels in the islets of rodents and humans with type 2 diabetes.²⁷¹ ci-Ins2 silencing in pancreatic islets leads to decreased expression of several genes important for insulin secretion (*Raggef4*, *Pld1*, *Pclo*, and *Cacna1c*) by interacting with the TAR DNA-binding protein 43 (TDP43), thereby contributing to β -cell dysfunction during diabetes. CircHIPK3 is one of the most abundant circRNAs in β -cells and regulates hyperglycemia and IR by sequestering miR-124-3p and miR-338-3p, thereby increasing mRNA expression of key β -cell genes (e.g., *Slc2a2*, *Akt1*, and *Mtpn*), insulin secretion, and β -cell proliferation.²⁷² A similar effect of circHIPK3 on hyperglycemia and IR has been observed by sponging miR-192-5p and increasing FOXO1 expression.²⁷³ In addition, Hsa_circ_0054633 suppression promotes β -cell proliferation and facilitates insulin secretion through inhibiting caspase-8 expression by sponging miR-409-3p.²⁷⁴ These recent results point to circRNAs as novel regulators of β -cell dysfunction under diabetic conditions.

Similar to the miRNAs and lncRNAs, several circRNAs also contribute to the regulation of glucose and lipid homeostasis.²⁷⁵ Li et al.²⁷⁶ first demonstrated that circRNA-1897 is highly down-regulated in the subcutaneous tissues of two pig breeds, and that it directly targets miR-27a and miR-27b-3p that are negative regulators of adipocyte differentiation by suppressing PPAR γ expression. Deep sequencing analysis of adipose circRNA revealed that circArhgap5-2 is highly upregulated during differentiation of human white adipocytes.²⁷⁷ circArhgap5-2 silencing results in inhibited lipid accumulation and adipose marker (PPAR γ , AdipoQ, Cebpa, and FABP4) downregulation. Thus, circRNAs likely serve as important regulators of adipocyte differentiation and lipid metabolism. Another circRNA deep sequencing analysis of sera from patients with metabolic syndrome (MetS) identified the presence of a novel circRNA, circRNF111, involved in MetS progression.²⁷⁸ CircRNF111 inhibition enhances IR and lipid deposition in MetS by regulating the miR-143-3p/IGF2R pathway.

AMPK is a critical factor in energy homeostasis including glycolysis, lipolysis, and fatty acid oxidation (FAO). CircACC1 is a circRNA derived from the human acetyl-CoA carboxylase 1 (*ACC1*) gene and directly binds to the β and γ subunits of AMPK, facilitating its activity,²⁷⁹ and promoting glycolysis and fatty acid β -oxidation during metabolic stress. circMAP3K4 is another potentially important circRNA involved in glucose metabolism that is highly expressed in the placentas of patients with gestational diabetes mellitus (GDM) and the IR model.²⁸⁰ circMAP3K4 can suppress the insulin-PI3K/Akt signaling pathway via the miR-6795-5p/PTPN1 axis, thereby contributing to GDM-associated IR. Nevertheless, the exact roles and regulatory mechanisms of circRNAs in IR require additional clarity.

Involvement of the gut microbiota in IR

Influencing factors of Gut microbiome composition. The microbes living in the human gut are key contributors to host metabolism and immune function through mediating the interaction between the host and environment, or releasing metabolites and cytokines.²⁸¹ In 2012, the Human Microbiome Project Consortium began to show that the gut microbial phyla in humans mainly consist of the gram-positive Firmicutes, gram-negative Bacteroidetes and

Proteobacteria.²⁸² Although the composition of human gut microbiota remains relatively stable from around age 3, gut microbiota undergoes the increase in diversity and altered proportions of composition.²⁸³

Different factors influencing these alterations of gut microbiome composition have been explored including diet, exercise, circadian disruption, antibiotics treatments, and genetics.²⁸⁴ (1) Regarding diet: David et al.²⁸⁵ conducted a study of human wherein volunteers were placed on either a plant-based diet (i.e., with grains, legumes, fruits, and vegetables) or an animal product-based diet (i.e., with meats, eggs, and cheeses) for five consecutive days. The gut microbial communities of the groups significantly diverged over time, with participants on animal diets experiencing proliferation of bile-tolerant microorganisms (e.g., *Alistipes*, *Bifidobila*, and *Bacteroides*) and decreased abundances of fiber-fermenting bacteria. Furthermore, differences in gut microbiota exists between humans with western diets rich in lipids and animal proteins in comparison to African diets rich in millet/sorghum and local vegetables, with little contribution of lipids and animal proteins to diets.²⁸⁶ (2) With regards to exercise, recent studies have highlighted the capacity of exercise to increase the abundances of beneficial gut microbial species, increasing gut microflora diversity, improving the proliferation of commensal bacteria, and reducing inflammation in addition to intestinal permeability.^{287,288} (3) Circadian disruption: both human and non-human models examination indicate that insufficient sleep (less than 7 h sleep per night) and circadian misalignment (such as workforce with shift workers or social jetlag) may lead to modifications in gut microbial diversity, structure and function.^{289,290} (4) Antibiotics: In deed, short-time antibiotic exposures can directly perturb the gut microbiota, reduce bacterial diversity and metabolic activity, disrupt intestinal integrity,²⁹¹ which is a major cause for concern in human health. (5) Host genetics also shape the composition of the gut microbiome. For example, microbiome genome-wide association studies (mGWAS) have identified that variants of different genes (for example, *VDR*, *LCT*, *NOD2*, *FUT2*, and *APOA5*) that are associated with distinct gut microbiome compositions.²⁹² Furthermore, 16 S ribosomal RNA (16 S rRNA) sequencing with microbiome analysis revealed that some species (especially from the phyla Firmicutes and Verrucomicrobia) in the gut microbiome are heritable.²⁹³ Thus, how to modulate the gut microbiota based on internal and external factors is important to maintain the public health.

Gut microbiome dysbiosis involved in IR. Growing evidence in the last two decades has suggested that gut microbial dysbiosis contributes to increased risks of metabolic defects like obesity, IR, and diabetes.²⁹⁴ For example, several studies have shown that obese adults harbor reduced gut microbial diversity and altered microbiota compositions compared with adults exhibiting normal weight.²⁹⁵ Another study of sixty-eight obese young patients revealed reduced fecal bacterial richness in patients with IR and high diastolic blood pressure (BP).²⁹⁶ Moreover, distinct microbial population markers were associated with impaired glucose tolerance, high BP, and low high-density lipoprotein cholesterol.²⁹⁷ A whole-genome sequencing investigation of the intestinal microbiota from 49 obese adults revealed that low bacterial gene counts were associated with unhealthy phenotypes like higher IR, dyslipidemia, and inflammation compared to adults with higher bacterial gene counts.²⁹⁸ While the exact roles of gut microbiomes in IR remain incompletely understood, many studies have nevertheless begun to elucidate the mechanisms by which gut microbiome dysbiosis produces different signaling activation.²⁹⁹ For example, gut microbiota can influence host glucose metabolism and hormone production via the production of several metabolites like short-chain fatty acids (SCFAs, mainly including acetate, propionate, and butyrate) and bile acids.³⁰⁰ Hyperglycemia then increases gut permeability and subsequent translocation of bacterial lipopolysaccharide (LPS) into systemic circulation. LPS circulation then contributes to the chronic

inflammation of liver and adipose tissue that is associated with the development of IR, in addition to other conditions associated with metabolic syndromes.³⁰¹ The potential mechanisms related to gut microbiome activities and IR are very complex, and numerous studies with contradictory results render it difficult to identify clear mechanistic pathways. Nevertheless, some strategies have been developed to modulate microbiota, such as fecal microbiota transplants, probiotics or prebiotics supplementation, in combination with medications and/or healthy lifestyle, in hope to ameliorate microbiota composition and IR.^{302,303}

IR RELATED DISEASES IN HUMAN

As we all know, IR is a state in which higher than normal concentrations of insulin are needed for a normal response, leading directly to hyperinsulinaemia and impaired glucose tolerance.³⁰⁴ As mentioned above, the primary characteristics of IR are inhibited lipolysis in adipose tissue, impaired glucose uptake by muscle and inhibited gluconeogenesis in liver.³⁰⁵ Nevertheless, IR can be linked to a cluster of abnormal syndrome (Fig. 4), which include obesity, diabetes, Nonalcoholic fatty liver disease, cardiovascular disease, polycystic ovary syndrome, and other abnormalities.^{306–308} Since obesity and diabetes have been discussed in the previous content, this part we mainly summary other related metabolic syndrome in human.

Metabolic (dysfunction)-associated fatty liver disease (MAFLD) and IR Non-alcoholic fatty liver disease (NAFLD) is one of the most common liver diseases worldwide.³⁰⁹ It includes a series of diseases, such as simple fatty liver (hepatic steatosis), non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma.³¹⁰ Recently, a consensus of international experts proposed that the disease acronym have to be changed from NAFLD to metabolic (dysfunction) associated fatty liver disease (MAFLD). Adipose tissue is a physiologic reservoir of fatty acids,³¹¹ when the storage capacity is exceeded, the accumulation of heterotopic lipids leads to lipotoxicity, thereby promoting low-grade inflammation and IR in the liver.³¹² Lipotoxicity impairs insulin signaling, induces oxidative damage, promotes inflammation and fibrosis,³¹³ which is thought to be related to the progression of MAFLD patients from simple steatosis to NASH, hepatic fibrosis, and hepatocellular carcinoma. Lipotoxic injury appears to occur in response to excessive levels of serum free fatty acids (FFAs) in hepatocytes.³¹⁴ Circulating FFAs are the primary causes of liver fat accumulation in MAFLD that mainly occur due to lipolysis of adipose tissues, and partially from excess lipoprotein.³¹⁵ Patients with MAFLD have increased levels of FFAs owing to a failure of insulin-mediated suppression of lipolysis, and excess FFAs excretion into the bloodstream.³¹⁴ In addition to the influence of abnormalities on lipid metabolism, IR also indirectly contributes to MAFLD through inflammation.^{316,317} The transcription factor NF- κ B exhibits higher levels in liver and adipose tissues during IR.³¹⁸ Further, activation of NF- κ B translocation to the nucleus leads to upregulated of the expression of target genes that encoded inflammatory mediators, like TNF- α and IL-6, which are released by hypertrophic adipocytes and elevated during MAFLD.³¹⁹ In addition, adiponectin is an anti-inflammatory adipokine that mediates fatty acid β -oxidation (FAO), glucose use, and suppression of fatty acid synthesis.^{320,321} Hepatic adiponectin expression is lower in NASH patients, while the levels of adiponectin and its receptors increase after weight loss.³²² Moreover, adiponectin overexpression increases subcutaneous fat levels and protects against diet-induced IR.³²³ Isotopic tracer studies have shown that MAFLD patients exhibit increased de novo lipogenesis (DNL), that is respectively mediated by two transcription factors SREBP-1c and ChREBP.^{324,325} SREBP-1c is a major regulator of fatty acid synthesis and hepatic SREBP-1c overexpression increases DAG content and PKC ϵ translocation that in turn impairs INSR tyrosine kinase activity, thereby inducing hepatic IR.^{326,327} While ChREBP deficiency improves IR and hepatic steatosis by inhibiting

the entire lipogenic and esterification process.^{328,329} Thus, inhibition of DNL and related pathway may effectively alleviate MAFLD and IR.

Polycystic ovary syndrome (PCOS) and IR

Polycystic ovary syndrome (PCOS) is an endocrine and metabolic disorder characterized by imbalances of multiple hormones that reflect the clinical manifestations of hyperandrogenism and affects 5%–10% of women of childbearing age.^{330,331} It is believed that IR and obesity play prevalent roles in causing PCOS, and PCOS women show an higher increased comorbidities of IR, including obesity, dyslipidemia, hypertension, and T2DM than healthy women.^{332–334} Specifically, IR leads to compensatory hyperinsulinemia, which stimulates GnRH gene transcription through MAPK pathway in PCOS and increases LH pulse secretion, thereby significantly increasing ovarian androgen synthesis.^{12,332} In addition to directly interfering with insulin signaling, androgens may also trigger lipolysis and increase circulating FFA, thereby leading to IR.³³⁵ Moreover, androgens decrease the type I muscle fibers (TIMF) with highly oxidative and insulin-sensitive properties, while increase type II muscle fibers (TIIMF) that are glycolytic and less insulin-sensitive, further decreasing glycogen synthase expression and favoring the development of IR in PCOS.^{336–338} This evidence supports that IR and hyperandrogenemia continuously stimulates each other in a vicious cycle under the condition of PCOS. At present, the molecular mechanism of insulin in PCOS has been well described. First of all, the defects downstream of insulin receptor phosphorylation, such as activation of phosphorylated IRS-1 through PKC or GLUT-4 translocation through PI3K/Akt signaling pathway, are the causes of IR in some PCOS women.^{41,339} Second, certain proinflammatory mediators including TNF, C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1) and IL-18 levels are elevated in PCOS women independently of obesity.^{340–342} Furthermore, hyperglycemia may contribute to inflammation in PCOS, possibly by inducing oxidative stress via increased ROS production. Such modifications then activate NF- κ B that is involved in the expression of proinflammatory mediators such as TNF and IL-6,^{343,344} and that induces key steroidogenic molecules, like CYP11A1, CYP17A1 and StAR, leading to further aggravation of hyperandrogenemia.^{345–347} Altogether, obesity and IR play pivotal role in women with PCOS and subsequent metabolic complications, and targeting these areas may become an important therapeutic approach for effectively reducing incidence of this pathology.

Cardiovascular disease and IR

Cardiovascular diseases (CVDs) are the leading causes of death globally. The World Health Organization estimates that 17.9 million people live with CVDs each year, and CVD-related deaths accounted for 32% of all global deaths in 2019. Moreover, over 23 million people are estimated to die from CVDs each year by 2030.^{348–350} CVDs represent a general compromising abnormal conditions including any disorders of heart and blood vessels. However, the most common types of CVDs include high blood pressure, coronary artery disease (CAD), stroke, cerebrovascular disease and rheumatic heart disease (RHD).^{351,352} Currently, the mechanisms of IR contribute to cardiovascular diseases mainly include chronic hyperglycemia, dyslipidemia, endothelial dysfunction and inflammation.^{9,353–355} Specifically, the increased gluconeogenesis and decreased glycogen synthesis in hepatic IR results in fasting hyperglycemia that increases total TG levels and blood pressure (BP), reduces HDL-C levels, and increases the risk of thrombosis formation.^{43,356} Moreover, long-term follow up data from patients with type 1 and type 2 diabetes have confirmed that hyperglycemia is a risk factor for CVD.³⁵⁷ IR in adipose tissue leads to high FFAs levels,³⁵⁸ visceral fat accumulation that is associated with elevated levels of plasminogen activator inhibitor 1 (PAI-1) and BP,³⁵⁹ and ectopic lipid and toxic lipid metabolite accumulation (lipotoxicity) in blood vessels that alters cellular signaling and cardiac structure, thereby contributing to the increased

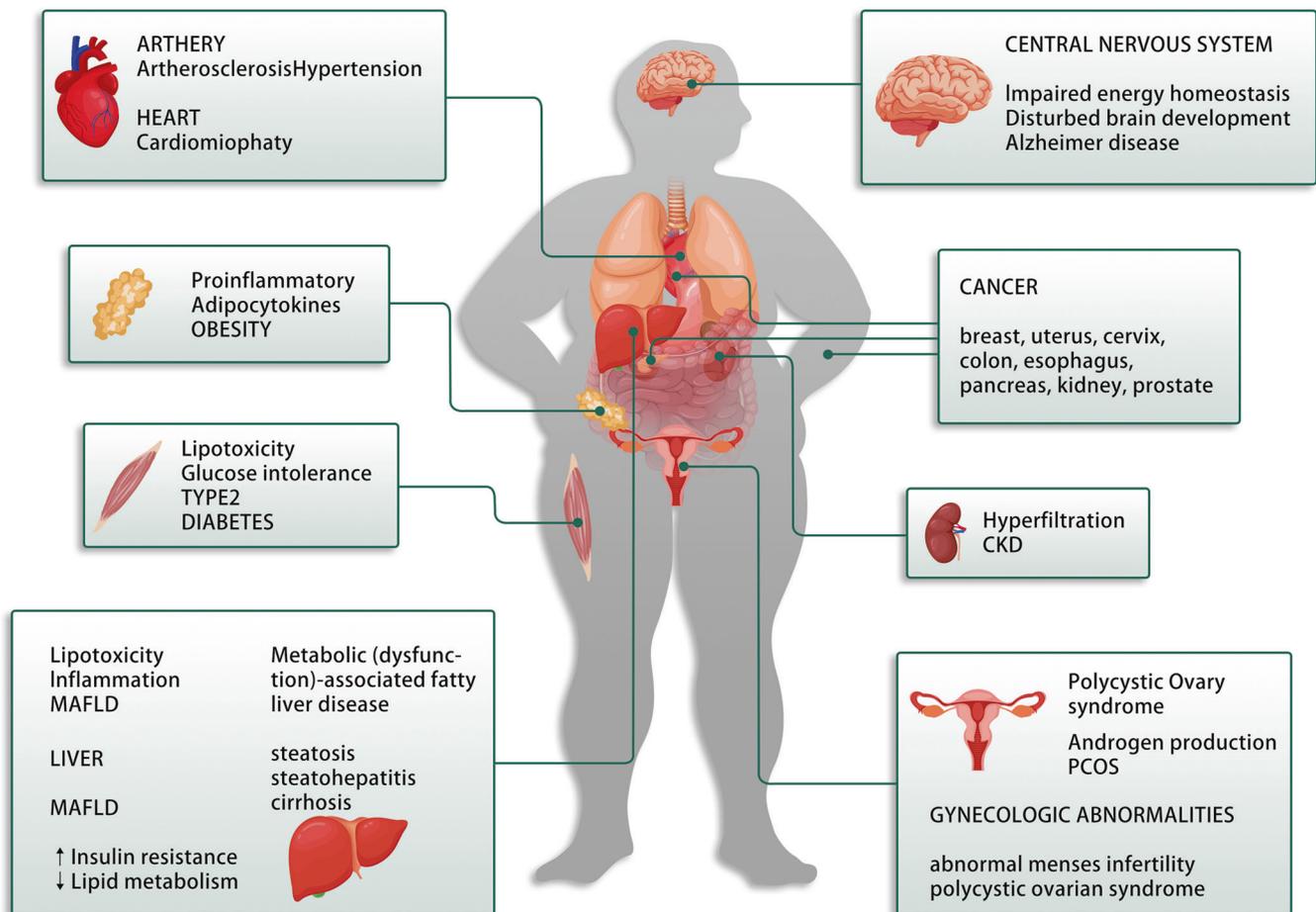


Fig. 4 Insulin resistance related diseases in human

prevalence of cardiovascular diseases.^{360,361} Furthermore, IR induces dyslipidemia characterized by elevated serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), or triglycerides (TG) along with reduced HDL-C concentrations, together which enhance the incidence of CVD by 32% in men and 76% in women.^{362,363} IR contributes to endothelial dysfunction by decreasing nitric oxide (NO) production via PI3K/Akt pathway from endothelial cells,³⁶⁴ and increasing reactive oxygen species (ROS) production, prothrombotic factors and proinflammatory markers mediated by MAPK/ERK activity,³⁶⁵ that both increases the cardiovascular risk, increased ROS levels in turn leads to the inhibition of insulin-PI3K signaling pathway through IRS-1 phosphorylation, which may aggravate IR.³⁶⁶ Overall, IR is a complex syndrome, which can significantly increase the risk of cardiovascular diseases. Identifying new therapies to reduce IR may contribute to the reduced prevalence of CVDs.

Alzheimer's disease and IR

Alzheimer's disease (AD) is a progressive neurodegenerative disease and is considered the sixth leading cause of death in the United States, with most patients aged 65 or older.³⁶⁷ Recent epidemiological studies have suggested that IR increases the risk for AD and related dementias.³⁶⁸ AD is actually a brain-specific form of diabetes that exhibit increased A β accumulation, tau hyperphosphorylation and impaired glucose transportation, energy metabolism, hippocampus and inflammatory pathways.³⁶⁹ Additional research has identified that insulin receptor is expressed on almost all cell types in the brain, with expression highest in the olfactory bulb, followed by the cerebral cortex, hippocampus, hypothalamus, and cerebellum.³⁷⁰⁻³⁷² Thus, insulin signaling likely also carries important and

diverse roles in brain functioning and AD pathogenesis. Insulin primarily enters the brain via selective, saturable transport across the blood-brain barrier (BBB)³⁷³⁻³⁷⁵ Peripherally produced insulin can also be actively transported into the brain via an endocytic-exocytic mechanism.³⁷⁶ Similar to systemic IR, brain IR can be defined as failed response to insulin by brain cells,^{377,378} primarily due to down-regulated insulin receptors, an inability of insulin receptors to bind insulin, or dysfunction of the insulin signaling cascade.³⁷⁹

Current researches have demonstrated that the mechanisms of systemic IR and brain-specific IR have close links with AD pathogenesis. For example, major abnormalities in AD brains include decreased mRNA and protein expression levels of insulin, insulin receptors, insulin receptor substrate 1 (IRS1) and IGF1/2, in addition to reduced protein indicators of downstream insulin signaling activity (including phosphorylated AKT (pAKT) and phosphorylated GSK3 β), tau mRNA, and increased amyloid precursor protein levels.^{380,381} Furthermore, recent evidence indicated that inflammation and lipid metabolism might contribute to the development of AD.³⁸² Potential targets include PPAR γ , Apolipoprotein E (ApoE), Apolipoprotein E receptor (LRP1), and leptin.³⁸³⁻³⁸⁵ Chronic inflammation exacerbates IR signaling that contributes to AD by provoking proinflammatory mediators including TNF- α , IL-6, and IL-1 β .³⁸⁶⁻³⁸⁹ Among these mediators, IL-6 can stimulate amyloid precursor protein (APP) formation, and is often co-localized with A β plaques in AD patients.³⁹⁰⁻³⁹² Thus, rosiglitazone has anti-inflammatory effect by decreasing levels of NF κ B and inhibiting the A β 42 production in mice, is considering as therapeutic agent for AD. Pioglitazone acts similarly as Rosiglitazone by reducing tau and A β deposits in the hippocampus, and improving neuronal plasticity and learning in AD.³⁹³⁻³⁹⁵ These

studies collectively suggest that IR contributes to AD pathogenesis through multiple pathways. Moreover, overlapping pathological features exist for diabetes, IR, and AD. Thus, the development of additional therapeutic drugs including antidiabetics or IR interventions with beneficial effects against cognitive impairment and Alzheimer's disease carry promising future application potentials.

Chronic kidney disease and IR

Chronic kidney disease (CKD) involves a gradual loss of kidney function and inability to filter blood^{396,397} and is a major risk factor for end-stage kidney failure (ESKF) and CVDs.^{398,399} And the inflammatory and glycometabolic abnormalities that closely related to IR are common features in CKD and CVD, which explain the strong relationship between them.⁴⁰⁰ In the last decades, researchers have showed that IR is an early metabolic alteration in CKD, because IR plays a primary role in metabolic syndromes characterized by abdominal obesity, high fasting glucose levels, hypertriglyceridemia, depressed serum HDL-C, and high blood pressure, that are commonly observed in CKD patients.^{401–403} Furthermore, CKD patients demonstrate systemic inflammation and elevated levels of pro-inflammatory cytokines like C-reactive protein (CRP), TNF- α , IL-6 and IL-1 β .^{404,405} In particular, reduced renal excretion leads to abnormal plasma adipokines levels including leptin and adiponectin in CKD patients.⁴⁰⁶ Leptin may also be considered as a uremia toxin through proinflammatory effects,^{407,408} while adiponectin mediates insulin-sensitizing and anti-inflammatory responses.^{409–411} Indeed, an accumulation of leptin is larger than adiponectin in CKD, and this abnormal ratio may further promote IR and metabolic disorders.⁴¹² Despite these above factors, evidences persist that endothelial dysfunction, oxidative stress, and vitamin D deficiency are important in the glucose intolerance pathogenesis and IR in patients with CKD.^{413–415} Thus, newly developed methods for improving IR could lead to potential strategies for preventing excess mortality of CKD patients.

Cancer and IR

Numerous recent epidemiological studies have suggested that IR increases the risks for different cancers including colon, liver, pancreas, breast, endometrium, thyroid and gastric cancer.^{416–418} Diverse cellular and molecular mechanisms are involved in the relationship between IR and cancer. Further, a growing body of evidence suggests that increased insulin, in addition to IGF1 and IGF2 levels critically influence tumor initiation and progression in IR patients.⁴¹⁹ Specifically, the three ligands (insulin, IGF1, and IGF2) binds the receptors (IGF-IR and INSR) and activate the insulin receptor substrates. This in turn, first activates the PI3K/Akt/mTOR, PI3K/Akt/FoxO, or Ras/MAPK/ERK-1/2 pathways that have important roles in cancer cell growth and carcinogenesis.^{420–422} Second, these processes inactivate GSK3 β through the PI3K/Akt signaling pathway, resulting in oncogenic β -catenin signaling activation, that has been associated with cancer stemness and chemoresistance.^{423,424} In addition, insulin and IGF1 inhibit sex-hormone binding globulin (SHBG) synthesis, although both hormones stimulate ovarian synthesis of sex steroids that can promote cellular proliferation and inhibit apoptosis in breast epithelium and endometrium.^{416,425} Furthermore, the increased risk of cancer in IR patients may be due to excessive ROS production that then impairs the contribution of DNA to mutation and carcinogenesis.^{426–428} With the elucidation of more new molecular mechanisms of IR and cancer, the relationship of IR with different tumors will be more complicated, and novel diagnostic and therapeutic strategies may provide a new approach for preventing cancer other related diseases.

THE DIAGNOSIS AND THERAPEUTIC STRATEGY OF IR

Diagnosis methods of IR

As we all know, IR is related to several metabolic abnormalities including obesity, glucose tolerance, dyslipidemia, type 2

diabetes and other metabolic syndrome.^{429,430} Several methods are used to measure blood insulin levels that primarily include glucose tolerance tests (GTTs), insulin tolerance tests (ITTs), hyperinsulinemic-euglycemic clamp (HEC), continuous infusion of glucose with model assessment (CIGMA), the minimal model technique (MMT), insulin suppression test (IST), and insulin release tests (IRTs) (Fig. 5), and their differences are the sensitivity, limitation, and complexity of technical procedures.^{35,431} Glucose tolerance test (GTT) is given to determine how quickly exogenous glucose delivered via oral, intraperitoneal, or intravenous administration is cleared from the blood.⁴³² The GTT method is used to diagnose diabetes mellitus including T1DM, T2DM and GDM.⁴³³ The Insulin Tolerance Test (ITT) is designed to examine the systemic sensitivity of insulin receptors by measuring blood glucose levels changes before and after intravenous insulin administration.⁴³⁴ This method is used to assess the insulin-sensitizing efficacy of test compounds and pharmacological agents that can modify insulin sensitivity.⁴³⁵ However, ITT often induces adequate hypoglycemia, severe hypokalaemia, it may as the systemic counter regulatory responses following the intravenous insulin.^{436,437} Despite these limitations, GTT and ITT are the most widely tests for assessing insulin sensitivity, largely because they are inexpensive and easy to perform.⁴³⁸ The HEC has been considered as the gold-standard method to assess insulin sensitivity in vivo. Actually, IR precedes the occurrence of T2DM, so how to increase the accurate assessment of insulin sensitivity is very important to predict the risk and evaluate the management of impaired insulin sensitivity and metabolic syndrome in research and clinical practice.

As the same time, some other evaluation indices have been developed and tested the insulin sensitivity/resistance. HOMA2 (updated HOMA model which took account of variations in hepatic and peripheral glucose resistance), homeostatic Model Assessment for IR (HOMA-IR), the oral glucose insulin sensitivity index (OGSI), fasting Insulin (FINS), and fasting plasma glucose (FPG) based on fasting glucose and insulin levels^{439–443} are widely utilized IR measurements in clinical research. Other indices based on fasting insulin include the glucose to insulin ratio (GIR), the quantitative insulin sensitivity check index (QUICKI),^{444–446} triglycerides (McAuley Index) alone or in accordance with HDL cholesterol (HDL-C),⁴⁴⁷ whole-body insulin sensitivity index (WBISI), Matsuda Index to evaluate whole body physiological insulin sensitivity by the above methods. Indeed, the early symptoms of IR in different individuals are not obvious, and the related symptoms are very complex, combining with screening indicators may provide more precise diagnosis for IR in the general population.

Therapeutic strategy of IR

Clinical approved treatment to IR. No medications exist currently that are specifically approved to treat IR, but IR management^{91,448,449} is possible through lifestyle changes like dietary, increased exercise, and disease prevention in addition to alternative medications (Fig. 6). Among these treatments, lifestyle changes should be the main focus for IR treatment, with nutritional intervention to decrease calories, avoidance of carbohydrates, and focusing on aliments with low glycemic index (including vegetables, fruits, whole-grain products, nuts, lean meats or beans) to provide higher fiber, vitamins, healthy fats and protein are particularly helpful for people trying to improve insulin sensitivity.^{450–452} A healthy diet and regular physical exercise including approximately 30 minutes of exercise at least five days a week leads to activation of muscle cells that increase AMPK activity, thereby inactivating TCB1D1 and promoting GLUT4 translocation to cellular membrane, in addition to increasing glucose uptake that increases insulin reactivity.^{453,454} Moreover, losing just 5%–7% of body weight can prevent or delay 60% of diabetes and ameliorate insulin sensitivity in obese and

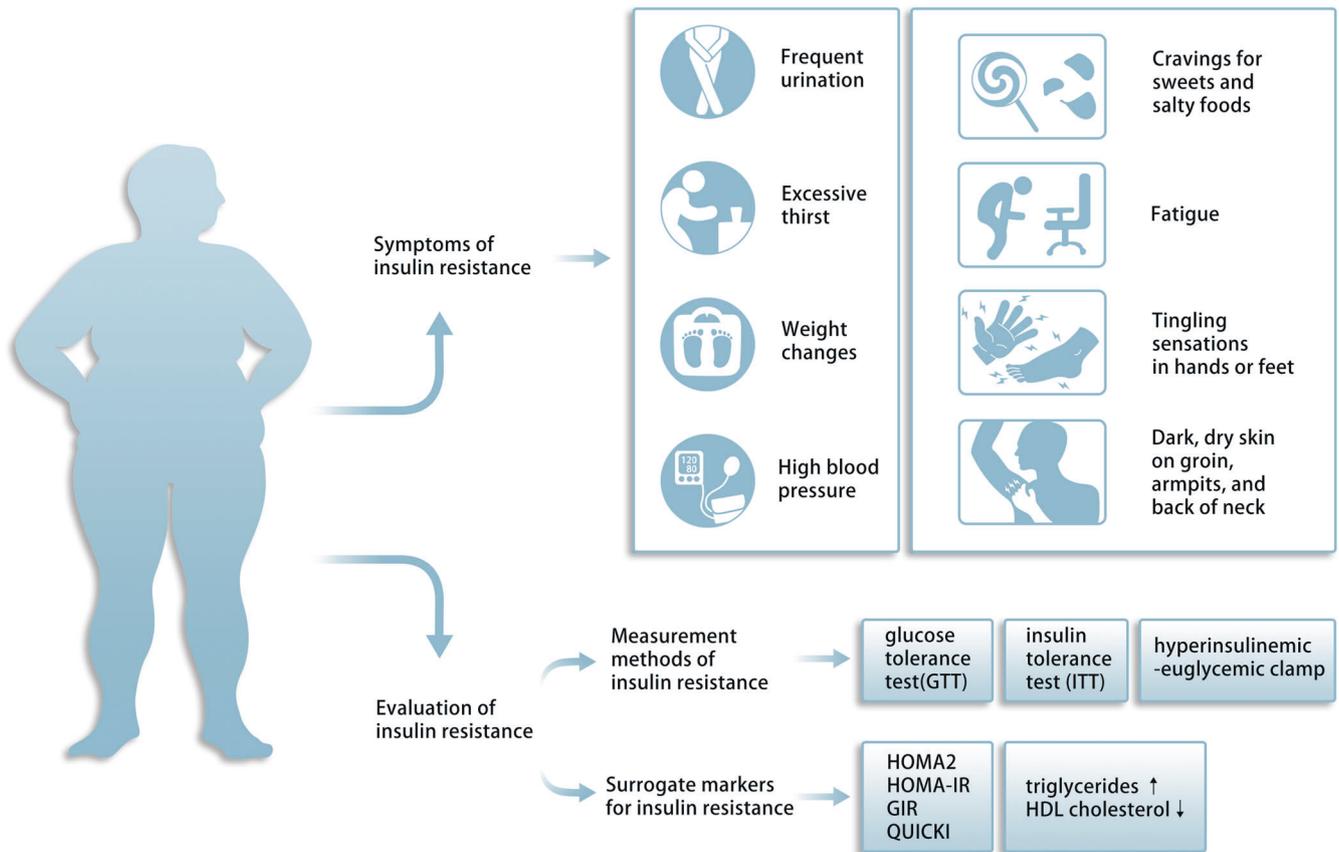


Fig. 5 Ex vivo diagnosis methods for insulin resistance

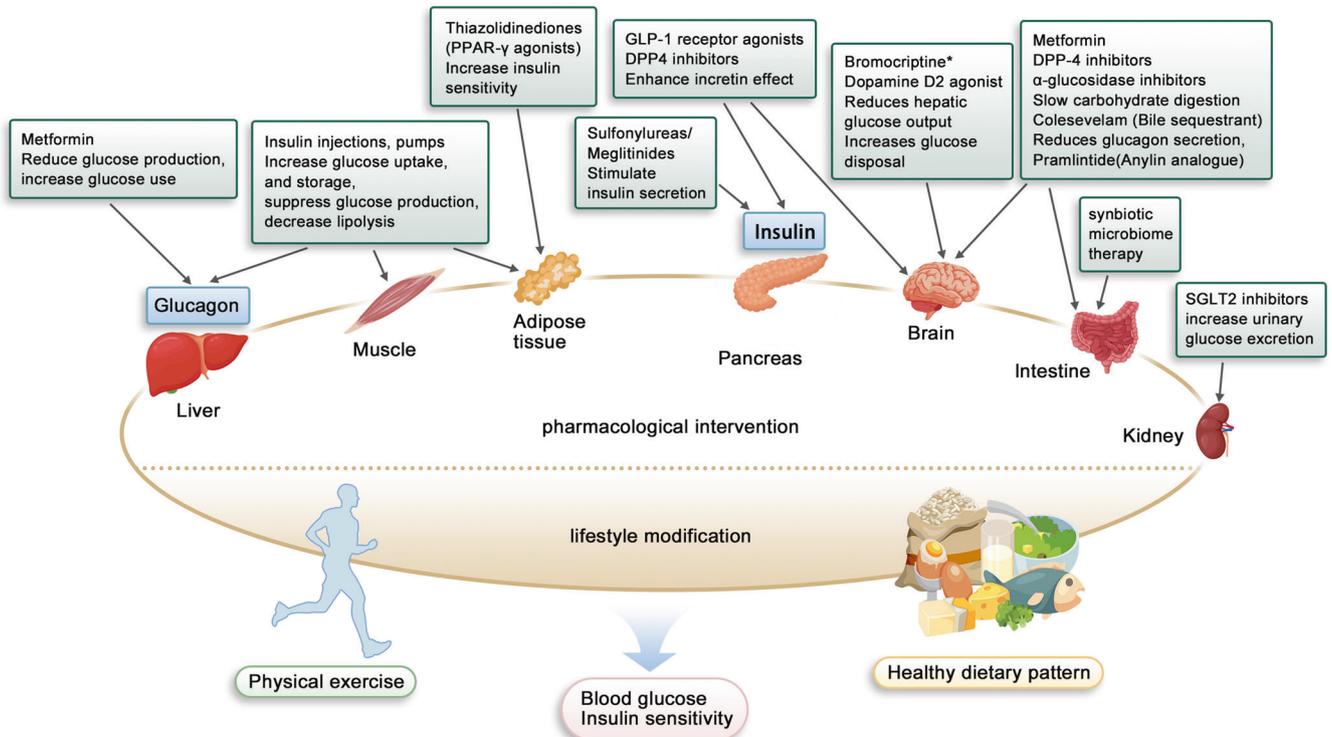


Fig. 6 Therapeutic strategy of insulin resistance

Table 1. Clinical medication for improving insulin resistance

| Type | Listed drugs | Mechanism |
|-------------------------|--|---|
| Biguanides | Metformin | The exact mechanism of metformin is still unclear and may be related to increased insulin receptor tyrosine kinase activity, enhanced glycogen synthesis, and recruitment of the GLUT4 glucose transporter. |
| Thiazolidinediones | Pioglitazone Rosiglitazone | It mainly activates peroxisome proliferator-activated receptor γ (PPAR- γ) to enhance the sensitivity of adipose muscle and liver to insulin. |
| GLP-1 receptor agonists | Liraglutide Exenatide Semaglutide Dulaglutide | GLP1 receptor agonists (GLP-1RAs) can affect IR by increasing the expression of glucose transporters in insulin-dependent tissues, reducing inflammation and oxidative stress, and regulating lipid metabolism. |
| DPP-4 inhibitors | Saxagliptin Vildagliptin Alogliptin Linagliptin Gemigliptin Teneligliptin Trelagliptin | It can decrease the degradation of GLP-1 by inhibiting the activity of DPP4, thereby exerting a role in the treatment of type 2 diabetes. |
| Sulfonylureas | Glimepiride | It promotes insulin receptor activation, thereby increasing the amount of glucose transporters, which in turn increases insulin sensitivity and improves insulin resistance. |
| PPAR full agonists | Chiglitazar Sodium | Chiglitazar Sodium is a peroxisome proliferator-activated receptor (PPAR) full agonist that simultaneously activates three subtypes of PPAR receptors (α , γ , and δ). It can induce the expression of downstream target genes related to insulin sensitivity, fatty acid oxidation, energy conversion and lipid transport, and inhibit the phosphorylation of PPAR γ receptors associated with insulin resistance. |

overweight individuals.⁴⁵⁵ Some specific pharmacological medications have been used as a preventives in type 2 diabetes by improving insulin sensitivity, primarily including Biguanides, Thiazolidinediones and GLP-1 receptor agonists, etc. (Table 1). Metformin is a first-line medication and the most widely prescribed insulin-sensitizing agent in T2DM and PCOS patients.^{456,457} Metformin mediates improved insulin sensitivity by increasing insulin receptor tyrosine kinase activity, enhancing glycogen synthesis, and increasing the recruitment and activity of the glucose transporter GLUT4.^{458–460} Metformin also promotes re-esterification of free fatty acids and inhibits lipolysis, which may then indirectly increase insulin sensitivity by reducing lipotoxicity in adipose tissues.⁴⁶¹ In addition to metformin, other targeted drugs exist for T2DM treatment and improving insulin sensitivity. For example, (1) Glucagon-like peptide 1 (GLP1) is an intestinal hormone that can enhance insulin secretion in a glucose-dependent manner by activating the GLP-1 receptor (GLP-1R) that is highly expressed on islet β cells.^{462,463} GLP-1 receptor agonists including Liraglutide, Semaglutide, Dulaglutide and Exenatide have now been approved for treating T2DM.^{464–468} These GLP-1 receptor agonists can suppress the inflammatory response of macrophages, thereby inhibiting IR.⁴⁶⁹ (2) Dipeptidyl peptidase-4 (DPP-4) also referred as the T-cell antigen CD26 can degrade GLP-1.⁴⁷⁰ DPP is a local mediator of inflammation and IR in adipose and hepatic tissue that interacts with the integral membrane protein, caveolin-1, then impairing the activation of down-stream AKT signaling.^{471–473} DPP-4 inhibitors (such as gemigliptin, saxagliptin, sitagliptin, teneligliptin, trelagliptin, vildagliptin, et al.) are now world-wide therapy of T2DM since 2006 and could improve insulin sensitivity.^{474–476} (3) A third targeted drug is sodium-glucose cotransporter (SGLT2) that is the major cotransporter involved in glucose reabsorption in kidneys.⁴⁷⁷ SGLT2 inhibitors include canagliflozin, dapagliflozin, and empagliflozin that have been approved by the FDA for combined use with diet and exercise to lower blood sugar and excessive insulin secretion in adults with type 2 diabetes.^{478–480} In addition, SGLT2 inhibitors exhibit beneficial effects for reducing IR and protecting pancreatic β cell functioning.^{481,482} (4) A fourth targeted drug approach is the activation of PPAR- γ in mature adipocytes that then induces altered expression of several genes (increased GLUT4 and adiponectin expression, along with decreased TNF- α , IL-6 and

leptin expression) involved in the insulin signaling cascade, thereby improving insulin sensitivity.^{483–485} PPAR- γ agonists (thiazolidinediones and TZDs) used for diabetes mellitus, which enhance adipocyte lipid storage, decrease ectopic lipid accumulation and improve insulin sensitivity in liver and skeletal muscles.^{486,487} Unfortunately, these agents have produced limited success due to reduced efficacy, low tolerability, and significant side effects including hypoglycemia, weight gain, bone fractures, and vomiting.^{488,489} Thus, it is urgent to find new approaches to treat T2DM and modify insulin sensitivity.

Clinical trials for insulin sensitivity management. In clinical research, scientists and physicians have explored different strategies to prevent and treat diabetes mellitus and IR. We have searched the complete clinical trials (<https://clinicaltrials.gov>) to reduce IR and summarized them mainly include: (1) Diet intervention, such as Low-fat vegetarian Food, high-protein food, calorie restriction, vitamin D supplementation to reduce the IR in human obesity.^{490,491} (2) Pharmacological Intervention, such as BFKB8488A, the anti-fgfr1/KLB agonist antibody mimics the effect of FGF21, and causes short-term weight loss and increases insulin sensitivity.^{492,493} Several studies have also demonstrated that chromium picolinate administration lowers glucose and insulin levels in patients with type 2 diabetes.^{494,495} Salsalate inhibits IKK/NF- κ B and may improve insulin sensitivity.⁴⁹⁶ Besides the above drugs, structural and functional dysbiosis of intestinal microbiome are induced in obese rodents,^{497,498} and they may cause IR and systemic inflammation.^{294,499,500} Thus, synbiotic therapy on intestinal microbiota has been developed as a new treatment strategy in clinical study (NCT04642482). We present some clinical trials of IR intervention in Table 2. Over the past years, our knowledge of the pathogenesis of IR and T2DM has improved, the development of new treatments of IR and metabolic syndrome have gained certain success, while the complexity of IR and the presence of multiple feedback loops make a challenge to the specific intervention.

Preclinical studies for IR intervention. In recent years, accumulating preclinical studies on the intervention of IR have been reported, which have important reference significance for the development of new drugs. We present the related studies on IR reported in recent years in Table 3, including animal models,

Table 2. Selected clinical trials for insulin resistance in recent ten years (2013–2022)

| Conditions | Starting time | Phase | Interventions | Treatment schedule | Outcome measures | NCT Number |
|---|---------------|----------------|---|--|---|-------------|
| HIV Related Insulin Resistance Protease Inhibitor Related Insulin Resistance Endoplasmic Reticulum Stress | 2013 | Not Applicable | Drug: Tauroursodeoxycholic acid | The intervention group will receive 1.75 g of tauroursodeoxycholic acid daily for 30 days. | Glucose Uptake Body Composition Liver Fat Liver Function Tests | NCT01877551 |
| Metabolic Syndrome X | 2013 | Phase 3 | Drug: Ezetimibe | Ezetimibe 10 mg/d for 12 weeks | Change in Intestinal mRNA Expression Levels of LDL Receptor Change in Intestinal mRNA Expression Levels of SREBP-2, NPC1L1, ABCG5/8, PCSK9 and HMG CoA | NCT01849068 |
| Type 2 Diabetes Mellitus | 2015 | Phase 4 | Drug: Dapagliflozin | Dapagliflozin 10 mg Tablets, Oral, Once Daily, 8 weeks | Adjusted Change From Baseline in Skeletal Muscle Insulin-stimulated Glucose Uptake Adjusted Change in Adipose Tissue Insulin-stimulated Glucose Uptake Adjusted Change in Liver Insulin-stimulated Glucose Uptake From Baseline to Week 8 | NCT02426541 |
| Diabetes Metabolic Syndrome Insulin Resistance | 2013 | Phase 2 | Drug: Pegvisomant | Pegvisomant 20 mg subcutaneously Qday will be administered by the study subject for 28 days during this study. | Insulin Sensitivity Lipolysis | NCT02023918 |
| Insulin Resistance Obesity Sedentary Lifestyle | 2013 | Not Applicable | Behavioral: Exercise training | Study participants in the intervention arm of the study will be asked to exercise on 3 days per week for 48 weeks. | Change From Baseline in Volume of Exercise (Total Time) Insulin Resistance Exercise Fitness Body Composition | NCT01848553 |
| Diabetes Mellitus, Type 2 Skeletal Muscle Insulin Sensitivity | 2017 | Phase 4 | Drug: Dapagliflozin | Patients will receive dapagliflozin 10 mg in tablet for a maximum of 40 days | Corrected Glucose Disposal Rate (GGDR) Measured as Change in Rate of Disposal (Delta RD) Basal vs High Insulin After 5 Weeks of Treatment | NCT03338855 |
| Glucose Intolerance | 2015 | Not Applicable | Dietary Supplement: Artemisia dracunculus | Artemisia Dracunculus extract, 2 capsules of 500 mg, two times per day before breakfast and dinner during 90 days | Postprandial Glucose Levels Fasting Glucose Levels Glycosylated Hemoglobin First Phase of Insulin Secretion Total Insulin Secretion Insulin Sensitivity | NCT02330341 |
| Diabetes Mellitus, Type 2 | 2018 | Not Applicable | Behavioral: SLEEP-Extend intervention | The subjects extended their sleep time by at least 1 hour but not more than 2 hours for 4 weeks. | Homeostatic Model Assessment for IR (HOMA-IR) Scores | NCT03616171 |
| Metabolic Syndrome | 2014 | Phase 4 | Drug: Sildenafil citrate | Subjects took Sildenafil citrate 25 mg three times a day for three months. | Insulin-stimulated AKT Phosphorylation | NCT02129725 |
| Obesity Fatty Liver, Nonalcoholic | 2014 | Not Applicable | Drug: pitavastatin | Pitavastatin 4 mg daily by mouth for 6 months | Insulin-stimulated Glucose Uptake Liver Fat Alanine Aminotransferase Aspartate Aminotransferase Hepatic Insulin Sensitivity Hemoglobin A1c Quantitative Insulin Sensitivity Check Index | NCT02290106 |
| Diabetes Mellitus Type 2 | 2015 | Phase 2 | Drug: Momordica charantia | Two 500 mg capsules of Momordica Charantia twice daily before breakfast and dinner for 90 days | Total and First Phase of Insulin Secretion (Insulinogenic Index and Stumvoll Index) After 90 Days Insulin Sensitivity (Matsuda Index) After 90 Days | NCT02397447 |

Table 3. Selected preclinical studies for insulin resistance

| Intervention | Model | Method | Results | Reference |
|--|---|---|---|-----------|
| Propranolol and L-D-ISOPROT | Swiss albino mice of high fructose and high fat diet (HFrHFD) model | The mice were injected with propranolol (30 mg/kg/d, i.p.) and low-dose isoproterenol (5 mg/kg/day, i.p.) for 4 weeks after the 13th week of HFrHFD feeding. | Propranolol and L-d- Isopropionic acid can reduce IR of HFrHFD mice by up-regulating β -inhibin 2 signaling activity. | 505 |
| Sarsasapogenin (ZGY) | High-fat diet (HFD) C57BL/6 J mice; LPS-induced acute adipose tissue inflammation model | ZGY treatment (80 mg/kg/d, ig, lasting for 18 days) can significantly inhibit the acute adipose tissue inflammation of LPS-treated mice. In obese mice fed with high-fat diet, taking ZGY orally (80 mg/kg/d for 6 weeks) can reduce the infiltration of macrophages, improve IFand reduce the inflammation of adipose tissue. | ZGY can improve IR and reduce fat inflammation in HFD mice, which may be related to the inhibition of IKK/NF- κ B and JNK inflammatory signaling pathway. | 506 |
| PPAR α / dual Agonist: Propane-2-sulfonic acid octadec-9-enyl-amide (N15) Valdecoxib (VAL) | High fat diet and streptozotocin (STZ)-induced diabetic mice HFD-fed mice | The mice were received single daily oral treatment with N15 (50 or 100 mg/kg, respectively) for 6 weeks. HFD - fed mice were orally administered VAL (5 mg/kg, once every 2 days) for 8 weeks. | The anti-IR effect of N15 may be depended on PPAR γ pathway. | 507 |
| D-chiro-Inositol (DCI) | HFD-fed mice | HFD-fed mice were intragastrically administered with 50 mg of DCI/(kg of body weight (bw))/day for 8 weeks. | VAL can inhibit inflammation and endoplasmic reticulum stress through AMPK-regulated HSPB1 pathway, thus improving skeletal muscle IR under hyperlipidemia. | 508 |
| Stagliptin | HFD-fed SD rat | Rats were given Sitagliptin(100 mg/kg/d) by gavage for 8 consecutive days. | DCI decreased the hepatic glucose output and the expression levels of PEPCK and G6Pase through PKC ϵ -IRS/PI3K/AKT signaling pathway in insulin-resistant mice. | 509 |
| Muscular resistance, hypertrophy and strength training | HFD-fed Swiss mice | Weight-bearing stair climbing training;Muscle resistance exercise; hypertrophy training and strength training. | Sitagliptin can significantly inhibit lipid accumulation in blood and liver of rats and improve insulin resistance. | 510 |
| Sodium-glucose cotransporter (SGLT) 2 inhibitor: empagliflozin | HFD induced obese mice. | HFD with 0.003% empagliflozin (3 mg/kg bodyweight). And HFD with 0.01% empagliflozin (10 mg/kg bodyweight). | Muscle resistance training program can reduce weight, obesity index, adipocyte area and low-grade chronic inflammation, and improve insulin resistance. | 511 |
| Heat shock protein (HSP) 70 | HFD-fed C57BL/6 mice | Mice were administered intranasally under isoflurane anesthesia, 10 μ l (10 and 40 μ g) of the appropriate solution was injected into one nostril, and the mice were supine for 1–2 min, three times a week for 26 days. | SGLT2 inhibitor empagliflozin enhances fat utilization and browning by M2 or replacing macrophages activation, and reduces obesity-induced inflammation and insulin resistance. | 512 |
| Insulin and exenatide | Male Tg2576 mice | Daily treatment of 0.43 $\times 10^{-3}$ IU NovoRapid insulin + 0.075 μ g exenatide + 5 μ g BSA per mouse was used. The treatment was given 6 days a week for 8 months | 4 μ g HSP70 significantly improved insulin sensitivity, and 10 μ g HSP70 showed a trend of improvement. | 513 |
| GLP-1 receptor agonists: exendin-4 | Senescence-accelerated mouse (SAMP8) | A proper amount of exendin-4 and L-form of peneracin were respectively dissolved in PBS solution containing 0.001% methylcellulose (MC), and an equal amount of peptide drug and osmin solution were gently mixed. The mice were injected with sodium pentobarbital intraperitoneally and then with exendin-4 and L-peneratin intranasally. | Compared with the control mice, the expression of insulin receptor cascade related genes in AD-like mice treated with insulin and exenatide was normalized. | 502 |
| Lactobacillus reuteri strain | HFD-fed mice | The mice were gavaged daily with 10 ⁹ CFU of L. reuteri CNCM I-5022 for 12 weeks. | After intranasal administration with L-form of peneracin, the distribution of exendin-4 in the whole brain increased significantly. Through intranasal injection of L-form of peneracin, the delivery of exendin-4 and insulin to the brain may contribute to insulin signal transduction in hippocampus. | 503 |
| Sterilized bifidobacteria | HFD-fed mice | Mice were orally administered with bifidobacteria (200 mg/kg ⁻¹ , 400 mg kg ⁻¹) daily for 4 weeks. | Lactobacillus reuteri improved HOMA-IR and glucose clearance and exhibited better insulin sensitivity in HFD-fed mice. | 514 |
| Exercise training combined with Bifidobacterium longum OLP-01 | Male C57BL/6 J db/db mice | The mice administered orally at a dosage of 1.03 g per kg per day (1.03 $\times 10^{10}$ CFU per kg per day) using a stomach tube. The mice were supplemented with strength training. | Oral glucose tolerance and IR test showed that Bifidobacterium sterilization could improve glucose tolerance and reduce insulin resistance. | 504 |

treatment methods and results. Pre-clinical IR intervention mainly includes drug intervention, probiotic therapy and exercise supplement. Drug therapy to improve IR is the main research direction at present. Researchers found that Valdecoxib (VAL) can inhibit inflammation and endoplasmic reticulum (ER) stress through AMPK-regulated HSPB1 pathway, thus improving skeletal muscle IR under hyperlipidemia.⁵⁰¹ The insulin signal described above has a key role in AD pathogenesis. The researchers found that the mixed nasal administration of GLP-1 receptor agonist and L-form of peneracin can effectively alleviate the cognitive dysfunction of SAMP8 mice.⁵⁰² In addition, studies have found that probiotics can adjust the changes of intestinal flora to reduce inflammation and IR, so probiotics may be an important supplement for the treatment of IR. Natividad et al.⁵⁰³ found that HFD-fed mice intervention by *Lactobacillus reuteri* strain could effectively alleviate IR. Regular exercise is an alternative intervention measure to maintain the blood sugar level in the normal range and reduce the risk factors. Hsu and colleagues⁵⁰⁴ found that exercise combined with probiotics intervention can have a positive effect on blood sugar and increase insulin sensitivity in mice. The above results show that drug intervention, probiotic supplementation and intensive exercise can improve IR but more clinical data are still needed.

Overall, the increased incidence of IR and the key roles of IR plays in many diseases, urgently require a better understanding of IR pathogenesis in addition to how IR interacts with genetics and different environments. A deeper understanding of IR can be achieved with a more systematic approach involving large-scale omics to study the molecular landscape is of major importance in addition to exploring new intervention strategies to prevent abnormal IR syndrome.

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AUTHOR CONTRIBUTIONS

M.W.L., X.W.C., and Y.W. wrote the article and researched data for the article. H.M.X., S.S., and W.W.X reviewed and revised the article. All authors contributed to reviewing and editing the manuscript before submission.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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