Insulin resistance and non-alcoholic fatty liver disease: a review of the pathophysiology and the potential targets for drug actions

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ABSTRACT

Insulin resistance refers to the reduced physiological effects of insulin on various tissues. Insulin resistance has been implicated in the pathophysiology of non-alcoholic fatty liver disease (NAFLD), which is a spectrum of diseases ranging from hepatic steatosis on one end to steatohepatitis, liver cirrhosis and hepatocellular carcinoma on the other end. In most parts of the developed world, it is now the most common cause of chronic liver disease and the most common indication for liver transplantation. A similar finding is emerging in the developing world due to the rising prevalence of obesity and widespread adoption of Western lifestyles. Despite these epidemiological data, there are no universally approved medications for the treatment of NAFLD. The pathophysiological mechanisms of NAFLD essentially include adipose tissue insulin resistance, hepatic insulin resistance, inflammation and fibrosis. At the subcellular level, mitochondrial dysfunction, oxidative changes and endoplasmic reticulum dysfunction have been documented. Several drugs have been tested in vitro and in animal studies to target these pathophysiological mechanisms. Some are presently going through clinical trials, while others have already gone through clinical trials with variable results. Other potential target sites of drug development for the treatment of NAFLD are based on the complex pathophysiology of the disease. Insulin resistance plays an important role in the development of NAFLD. There are potential targets in the pathophysiology of NAFLD that can be explored in the development of medications for the disease.

INTRODUCTION

Insulin resistance is defined as a reduced physiological response to a given amount of insulin. It is the attenuated sensitivity of tissues to the biological effects of insulin.¹ Insulin is a peptide hormone secreted by the β-cells of the pancreatic islets of Langerhans.² Its physiological roles are broadly divided into two, namely, metabolic and mitogenic effects. The metabolic effects include glucose disposal into the cells and the regulation of carbohydrate, lipid and protein metabolism, while the mitogenic effect refers to cell growth. The metabolic effects of insulin are most pronounced in the liver, muscle and adipose tissue. Whenever there is reduced sensitivity of peripheral tissues to insulin, pancreatic β-cells secrete supra-physiological amounts of insulin to overcome insulin resistance, causing hyperinsulinaemia.²

Insulin resistance is associated with a number of laboratory and clinical abnormalities that tend to cluster together in a clinical syndrome termed metabolic syndrome. These abnormalities include variable degrees of glucose intolerance, dyslipidemia, visceral obesity and elevated blood pressure.¹

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Other documented disorders include cardiovascular disease, fatty liver disease, hyperuricaemia, cancers, polycystic ovarian syndrome and obstructive sleep apnoea. The pathophysiological mechanisms underlying metabolic syndrome include chronic subclinical inflammation, enhanced coagulability, haemodynamic dysfunction and endothelial dysfunction. Insulin resistance in the peripheral tissue, especially the adipose tissue, leads to enhanced lipolysis and increased flux of free fatty acids from the adipose tissue into the liver. The liver uses free fatty acids to synthesize triglycerides, which are incorporated into very low density lipoproteins (VLDL). When the physiological machinery for these synthetic processes is overwhelmed, fatty liver disease develops. With increasing levels of liver damage, non-alcoholic fatty liver disease may progress to steatohepatitis, fibrosis, liver cirrhosis and sometimes liver cancer.

Nonalcoholic fatty liver disease (NAFLD) is characterized by macrovascular steatosis in at least 5% of hepatocytes, and other causes, such as alcohol and drugs, must have been ruled out. It is now the leading cause of liver cirrhosis globally, yet the pharmacotherapeutic options for the disease are limited. The global prevalence of NAFLD is approximately 25%. The prevalence is highest in developed countries and is rising in developing countries due to the increasing prevalence of obesity and adoption of Western lifestyles. The presence of fibrosis on histology is associated with increased clinical morbidity and mortality. Approximately 1-5% of this cohort with fibrosis tends to develop liver cancer. Ultrasonography is the noninvasive diagnostic approach of choice, while liver biopsy for histologic examination remains a valuable tool in confirming fibrosis.

The central approach in the management of NAFLD involves lifestyle and cardiovascular risk factor modifications such as dietary management, weight loss, control of dyslipidemia, stopping smoking and engaging in adequate physical activity. However, studies have shown that lifestyle modifications alone are often not enough to regress or prevent the progression of the disease. Drugs such as vitamin E and pioglitazone, a peroxisome proliferator-activator receptor gamma (PPAR-γ) agonist, have been studied in clinical trials on pharmacotherapy of NAFLD, but the results are conflicting, and most guidelines have refrained from recommending these medications.

Therefore, pharmacotherapy for NAFLD is a rapidly evolving field, and many studies are ongoing to identify potential target sites for drug action. Some drugs, such as asabetcholic acid (a farnesoid X receptor agonist), are already undergoing clinical trials, but there is still a need to develop more drugs, and a deep understanding of the disease will play an important role in achieving this. This review article aimed to expatiate the pathophysiological relationships between insulin resistance and NAFLD and to highlight the potential sites of target for pharmacotherapy to alleviate the burden of the disease.

DISCUSSION

Insulin Physiology

Overview of insulin as a biomolecule

Insulin is a polypeptide hormone made up of 51 amino acids and is coded in the short arm of chromosome 11. It is made up of two chains, an alpha chain comprising 21 amino acids and a beta chain comprising 30 amino acids. The two chains are connected by two disulfide bonds. Messenger ribonucleic acid (mRNA) transcribed from the insulin gene is translated as preproinsulin on
the ribosomes of rough endoplasmic reticulum. Pre-proinsulin is made up of the α chain, β chain, signal peptide and connecting peptide (C-peptide). Removal of the signal peptide from preproinsulin leads to the formation of proinsulin in the endoplasmic reticulum. Proinsulin is converted into insulin and C-peptide and is later packaged into secretory granules, where they are secreted into the circulation in equimolar amounts.

**Insulin secretion**

Glucose-mediated insulin release is biphasic. It is made up of an initial rapid release followed by a less intense and more sustained release. Glucose enters pancreatic β-cells via a non-insulin-mediated mechanism through glucose transporter-2 (GLUT-2). It is then phosphorylated to glucose-6-phosphate by glucokinase. This process progresses to the synthesis of adenosine triphosphate (ATP). Increased ATP/ADP ratio causes opening of membrane-bound ATP-sensitive potassium (K\text{ATP}) channels and influx of potassium ions. Ultimately, this leads to depolarization of the membrane and the opening of voltage-gated calcium channels. Influx of calcium ions leads to pulsatile insulin secretion.

There are other documented mechanisms by which insulin is secreted, especially the second phase of insulin secretion. These include activation of protein kinase A and protein kinase C as well as the stimulation of adenylyl cyclase and phospholipase. These mechanisms are regulated by incretin hormones such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP).

Factors that stimulate the release of insulin are termed insulin secretagogues. They are divided into nutrient and non-nutrient secretagogues. Examples of nutrient secretagogues are glucose and fructose, and examples of non-nutrient secretagogues are neurohormonal factors such as the cholinergic and adrenergic neural pathways and peptide hormones such as incretin hormones.

Cholinergic stimulation of the muscarinic receptors of pancreatic β-cells leads to the activation of phospholipase C and protein kinase C and eventual mobilization of intracellular calcium to enhance insulin secretion. This sometimes occurs on sighting, tasting or chewing food, a phenomenon referred to as the cephalic phase of insulin secretion. During stress and exercise, activation of α-2 adrenergic receptors leads to inhibition of insulin release. The amino acids arginine, L-ornithine and leucine are also known to behave like insulin secretagogues.

**Insulin action**

The physiological effects of insulin occur when it binds to its specific receptor. The insulin receptor is a heterotetramer made up of 2α and 2β glycoprotein subunits and is coded by a gene on the short arm of chromosome 19. The α subunit is extracellular, whereas the β subunit is intracellular. Insulin binds to the extracellular subunit, producing a conformational change in the receptor, thereby allowing ATP to bind to the β intracellular subunit. The β subunit is phosphorylated, and it acquires tyrosine kinase ability. This phosphorylates some protein molecules called insulin responsive substrate (IRS).

The phosphorylated IRS proteins bind other proteins generally called src-homology-2 domain proteins (SH2). Functionally, SH2 proteins are divided into two, namely, enzymatic SH2 (examples are phosphatidylinositol-3-kinase and phosphotyrosine phosphatase) and non-enzymatic SH2 (an example is an adaptor protein called Grb 2). Phosphatidylinositol-3-kinase phosphorylates some serine and threonine kinases, such as Akt/protein kinase B and protein kinase C, which promote the metabolic actions of...
insulin. These metabolic actions include translocation of glucose transporter (GLUT) to the cell membrane, glycogenesis, lipogenesis, protein synthesis and anti-lipolysis. Grb2, on the other hand, connects IRS-1 to the RAS (rat sarcoma protein) pathway, which mediates the mitogenic effects of insulin.

**Pathophysiology of Insulin Resistance**

Insulin action is influenced by other hormones. Hormones, often referred to as counterregulatory hormones, such as glucagon, glucocorticoids, catecholamines and growth hormone, dictate metabolic activities in the fasting state and oppose the physiological actions of insulin. Any pathology leading to excessive production of these hormones can lead to insulin resistance. However, most individuals with insulin resistance do not have abnormalities with counterregulatory hormones. Insulin resistance is mostly a cellular problem that occurs as a result of defects in normal signaling. The pathophysiological impact of insulin resistance varies across different tissues.

**Hepatic insulin resistance**

A large portion of glucose absorbed from digested carbohydrates is disposed into hepatocytes, and the process is independent of insulin. In the fed state, carbohydrate metabolism in the liver rapidly changes from glucose synthesis (gluconeogenesis) to glycogen synthesis (glycogenesis) and storage. This metabolic transition is under the regulation of neurohormonal mechanisms. For this transition to occur, there is a need to activate the enzyme glycogen synthase. Insulin activates this enzyme and makes hepatic glycogenesis possible in the postprandial state. Evidence for this role of insulin is seen in type 1 diabetes patients in whom glycogenesis is markedly reduced compared with controls after eating a mixed meal due to insulin deficiency. In the presence of insulin, ketogenesis is inhibited. The mitogenic effects of insulin on hepatocytes are mediated by increased synthesis of insulin-like growth factor-1 (IGF-1).

Hepatic insulin resistance is characterized by enhanced hepatic glucose output through gluconeogenesis. There is also pronounced abnormality of lipoprotein metabolism in insulin-resistant states. Insulin resistance in adipose tissue causes increased lipolysis and the release of large amounts of free fatty acids into circulation, which are trafficked to the liver. This increased fatty acid flux to the liver increases triglyceride synthesis in hepatocytes. Physiologically, triglycerides are supposed to be incorporated into VLDL, which are released into the circulation. However, hepatic insulin resistance reduces VLDL production, and triglycerides accumulate in the liver, causing steatosis. Moreover, insulin-resistant adipose tissue releases proinflammatory cytokines such as tumor necrosis factor-1 (TNF-1), which act on hepatocytes to produce C-reactive protein, fibrinogen and plasminogen activator inhibitor-1 (PAI-1), which can be assayed as markers of hepatic insulin resistance.

**Adipose insulin resistance**

The main roles of insulin in adipose tissue are glucose influx into adipocytes, stimulation of lipogenesis and inhibition of lipolysis. In the postprandial state, glucose enters adipocytes via GLUT 4. This process is dependent on insulin. In patients with type 2 diabetes, where insulin resistance is significantly high, downstream signaling following the action of insulin on its receptor is impaired. There is reduced gene expression of IRS-1, and the AKt/PKB pathway is dysregulated. This implies
reduced translocation of GLUT4 into the adipocyte membrane, which leads to reduced glucose disposal into adipose tissue.

The entry of glucose into adipocytes affects lipid metabolism by activating the carbohydrate response element binding protein (ChREBP).\(^{16}\) ChREBP is involved in the synthesis of fatty acid esters that enhance glucose tolerance. Adipose tissue secretes certain humoral molecules with varying physiological roles. These adipocytokines, namely, leptin, resistin and adiponectin, affect insulin sensitivity. Adiponectin enhances insulin sensitivity, whereas resistin and leptin are associated with insulin resistance.\(^{17}\) Mice with adiponectin gene knockout are more prone to hepatic steatosis.\(^{18}\)

**Pathophysiology of Non-alcoholic Fatty Liver Disease.**

NAFLD is currently the most common cause of end-stage liver disease and the most common common reason for liver transplantation.\(^{19}\) It is a diagnosis of exclusion. There are two types of hepatic steatosis, namely, microsteatosis and macrosteatosis. Other causes of macrosteatosis, apart from NAFLD, are alcohol, drugs such as steroids, autoimmune hepatitis, hepatitis C and parenteral nutrition. Common causes of microsteatosis include Reye syndrome, acute fatty liver of pregnancy, inborn errors of metabolism and drugs such as valproate. All these causes of hepatic steatosis need to be excluded before a diagnosis of NAFLD is made.

NAFLD is a metabolic disorder and is a result of the interplay between hormonal, dietary and genetic factors.\(^{19}\) Researchers working on genome-wide association studies (GWAS) have documented some promising candidate genes that serve as the underlying genetic factors for NAFLD.\(^{20}\) These genetic factors appear to predispose obese populations with insulin resistance to NAFLD. Obesity is the link between insulin resistance and NAFLD. Insulin resistance and consequent hyperinsulinaemia are central to the pathophysiology of NAFLD. In fact, some authors have documented that NAFLD is essentially a hepatic component of insulin resistance syndrome.\(^{21}\)

Hepatic steatosis must be present before a diagnosis of NAFLD is considered. The pathogenic mechanisms of hepatic steatosis include consumption of food high in fats, increased lipolysis due to adipose tissue insulin resistance, reduced \(\beta\)-oxidation of fatty acids, increased hepatic lipogenesis and decreased VLDL export from the liver due to hepatic insulin resistance.\(^{20}\) The molecular biology behind these mechanisms is still not fully understood, but research is ongoing to elucidate the biochemical processes underlying hepatic steatosis.\(^{21}\)

A proposed hypothesis is that inflammatory cytokines from adipose tissue as a result of adipose tissue insulin resistance initiate the processes leading to NAFLD.\(^{22}\) However, the factors that initiate inflammation in adipose tissue are not known. Hypoxia and necrosis of rapidly enlarging adipocytes have been suggested by some researchers.\(^{22}\) Inflammatory cytokines such as tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interleukin-6 (IL-6) released by adipose tissue macrophages have been reported to regulate insulin resistance in the liver.\(^{23}\) Additionally, adipocytes secrete adipokines such as adiponectin, which has been reported to regulate \(\beta\)-oxidation of fatty acids in the liver through AMP-activated protein kinase and acetyl-CoA carboxylase signaling.\(^{24}\)

Simple deposition of fats in hepatocytes progresses to a varying degree of the NAFLD spectrum due to the cellular mechanisms of oxidative stress, lipotoxicity, endoplasmic reticulum stress and mitochondrial
dysfunction. Increased flux of fatty acids to hepatocytes is due to adipose tissue insulin resistance and increased lipolysis in adipocytes. The metabolism of fatty acids in the hepatic mitochondria and peroxisomes leads to increased generation of reactive oxygen species, reactive nitrogen species and lipid peroxidation, hence enhancing oxidative stress in hepatocytes. Accumulation of saturated fatty acids, free cholesterol and lipid intermediates in hepatocytes has been found to produce lipotoxic stress to the mitochondria and endoplasmic reticulum of hepatocytes, leading to cellular death and tissue inflammation. There is also reduced activity of the enzyme complex of the electron transport chain causing mitochondrial dysfunction and hence reduced β-oxidation and accumulation of fatty acids. Endoplasmic reticulum stress is indicated by the reduced capacity and efficiency in protein folding and repair despite the increased demand for it due to the cellular stress of NAFLD. The summary of the pathophysiology of NAFLD is illustrated in FIGURE 1.

FIGURE 1. Pathophysiology of non-alcoholic fatty liver disease

**Target Sites of Drug Action in NAFLD.**

Presently, there are no universally accepted pharmacotherapeutic protocols for NAFLD. Several clinical trials have been performed, yet no approved drug for the treatment of NAFLD is universally accepted. Although lifestyle modifications such as weight loss, dietary management and exercise have been shown to be helpful, they are difficult to adopt for a long time. Therapeutic failure is therefore not uncommon due to the inability to sustain weight loss for a long period. There is therefore a need to develop drugs that will target strategic points in the pathophysiology of NAFLD. A summary of the drugs that can be used in NAFLD is summarized in TABLE 1 and 2.
Targeting absorption of fat

A high-fat diet has been implicated in the pathophysiology of NAFLD. Pancreatic lipase inhibitors such as orlistat inhibit the absorption of fats. In a randomized controlled trial, however, the use of orlistat was not associated with any histological improvement. Indeed, a high-fat diet has been implicated in the pathophysiology of NAFLD. Pancreatic lipase inhibitors such as orlistat inhibit the absorption of fats. In a randomized controlled trial, however, the use of orlistat was not associated with any histological improvement.29

Targeting intrahepatic lipogenesis may be more beneficial than merely inhibiting ingested fat absorption.

Targeting hepatic fat storage

Activation of endocannabinoid receptors (CB1) widely expressed in hepatocytes has been associated with hepatic steatosis. It is therefore thought that CB1 antagonists will reduce hepatic steatosis. Truly, a CB-1 antagonist, Rimonabant, was approved for the treatment of hepatic steatosis as well as obesity.30 However, it was withdrawn due to its neuropsychiatric side effects. Developing drugs that can specifically target hepatic CB-1 receptors without activating the central nervous endocannabinoid system may be extremely useful in treating NAFLD.

Thyroid hormone analogues have also been suggested to have the capability of reducing hepatic storage and hence may play a role in the treatment of NAFLD. Animal studies have shown that there are specific nuclear receptors in hepatocytes that thyroid hormones interact with, and it is through that this interaction is able to inhibit hepatocyte lipogenesis.
Targeting hepatic insulin resistance

Metformin improves insulin sensitivity in the liver. It reduces hepatic glucose output, hepatic expression of TNF-α and intrahepatic lipogenesis. Metformin is well tolerated, but studies have not shown its effectiveness in treating NAFLD. A randomized controlled trial comparing metformin with vitamin E in the treatment of NAFLD did not show a significant reduction in transaminases or improvement in histology in the metformin arm.

Incretin-based antidiabetic medications, dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) agonists, have been shown to improve hepatic insulin sensitivity. They have been tried in animal studies and non-randomized human studies to treat NAFLD and have been found to be effective, but randomized clinical trials are being awaited to substantiate the clinical benefits of these drugs in the treatment of NAFLD.

There are some receptors in hepatocytes, and most other tissues in the body are called farnesoid X receptors. Activation of these receptors is known to improve insulin sensitivity in the liver. Agonists of these receptors have been demonstrated in animal studies to reduce transaminases and improve histology in animal models of NAFLD. Human studies on these pharmacologic agents are still being awaited. An example of farnesoid X receptor agonists is chenodeoxycholic acid.

Targeting adipose tissue insulin resistance

Thiazolidinediones (glitazones) are peroxisome proliferator-activator receptor-γ (PPAR-γ) agonists. Pioglitazone has been demonstrated to be effective for treating NAFLD in randomized clinical trials; however, the side effects of drugs such as weight gain, heart failure and osteoporosis are of grave concern. The beneficial effects of pioglitazone were reversed by stopping it, implying that it has to be taken for a long period of time. Using it for this long, however, may predispose the patients to the side effects of pioglitazone.

Targeting serum lipids

The rationale for targeting serum lipids is that it is believed that lowering serum lipids will reduce hepatic accumulation of fatty acids and triglycerides. Studies have shown that lipid-lowering agents, namely, statins, fibrates and ezetimibe, are well tolerated and efficacious in the treatment of hepatic steatosis. Despite these findings, multicentric randomized controlled trials for a long duration are lacking to substantiate the effects of lipid lowering agents on hepatic steatosis. In addition to its lipid-lowering effects, probucol also has a significant antioxidant effect, but studies are insufficient to support its beneficial roles in the treatment of NAFLD.

Targeting oxidation pathways

In the pathogenesis of fatty liver, oxidation of fatty acids leads to cellular damage and activation of pro-inflammatory cytokines. This is the main mechanism by which hepatic steatosis progresses to steatohepatitis, then to liver cirrhosis and finally hepatocellular carcinoma. Vitamin C and vitamin E have been used as antioxidants in the treatment of NAFLD. Vitamin E has been shown to improve histology and reduce aminotransferases in many trials. Silymarin, which is an extract of milk thistle (Silybummarianum), has been shown to possess antioxidative effects and anti-inflammatory properties, and some studies have demonstrated its usefulness in NAFLD, especially when combined with vitamin E.
Other antioxidants that have been tried in NAFLD with variable effects include quercetin and betaine. In addition, pentoxifylline has been found to protect against lipid oxidation, one of the mechanisms underlying the pathogenesis of NAFLD. Randomized controlled trials have reported the efficacy of pentoxifylline in improving the histology seen in NAFLD.

**Targeting gut microbiota**

Interfering with the gut microbiota using probiotics has been found to be beneficial in reducing insulin resistance, especially in animal studies. Some studies have shown beneficial effects of probiotics in the treatment of NAFLD, but these studies are scant.

**Targeting inflammatory cytokines**

Clearly, inflammation plays a crucial role in the development and progression of NAFLD. In animal studies, the use of antibodies against TNF-α (infliximab) has been reported to improve the histologic features of NAFLD. These findings will still need to be replicated in humans through randomized clinical trials before they are considered for the treatment of NAFLD.

**Targeting hepatic fibrosis**

The renin-angiotensin-aldosterone system (RAAS) has been implicated in hepatic fibrosis that characterizes the progression of NAFLD. Animal studies have demonstrated that the use of valsartan, which is an angiotensin receptor blocker, can improve the histological changes seen in NAFLD. The role of RAAS inhibition against hepatic fibrosis in humans is still a potential area for future research.

**Targeting apoptosis and necro-inflammation.**

Apoptosis and necro-inflammation are involved in the progression of hepatic steatosis to steatohepatitis, cirrhosis and hepatocellular cancer. Caspases are proteases that break down essential proteins in the cell during programmed cell death, and their roles in NAFLD have been reported in the literature. Drugs inhibiting caspases, designed for the treatment of NAFLD, are in various stages of drug development trials.

**CONCLUSION**

Insulin resistance is the hallmark of many chronic diseases with high cardiovascular risk. Insulin resistance syndrome refers to a constellation of cardiovascular risk factors, and non-alcoholic liver disease is considered the hepatic manifestation of the syndrome. Nonalcoholic liver disease is a diagnosis of exclusion, and it is a spectrum of hepatic diseases ranging from simple hepatic steatosis on one end and hepatocellular carcinoma on the other end. The prevalence is very high globally and is the most common cause of cirrhosis in developed nations. The prevalence is also rapidly rising in low- and middle-income countries due to the obesity epidemic and westernization in those settings.

The review has emphasized the limitations of the presently available therapy to diseases that are now of public health significance. In view of this, potential targets for the development of new drugs based on the pathophysiology of the disease have been identified. There is a need to carry out more in vitro studies to design these drugs and clinical trials to test the clinical safety and acceptability of such drugs.
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