Immune regulation of glucose and lipid metabolism

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Abstract The immune response and metabolic regulation are highly integrated, and their interface maintains a homeostatic system. Their dysfunction can cause obesity and its comorbidities, including insulin resistance, type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD). Endoplasmic reticulum (ER) stress is a central abnormality linking obesity, insulin resistance, and NAFLD. ER stress in response to increased hepatic lipids may decrease the ability of the liver to secrete triglyceride by limiting apolipoprotein B secretion, thereby worsening fatty liver. Overnutrition or obesity activates the innate immune system, with the subsequent recruitment of immune cells that contributes to the development of insulin resistance. A significant advance in our understanding of obesity-induced inflammation and insulin resistance has been a recognition of the critical role of adipose tissue macrophages. A role for chemokines, small proteins that direct the trafficking of immune cells to sites of inflammation, has also been demonstrated. Chemokines activate the production of inflammatory cytokines through specific chemokine receptors. This review highlights the chemokine systems linking obesity to inflammation and insulin resistance. Treatment options that target immune cells with the aim of halting the development of insulin resistance and type 2 diabetes remain limited. DPP-4 inhibitors or micronutrients may contribute to the immune regulation of glucose and lipid metabolism by regulating macrophage polarization, thereby reducing insulin resistance and preventing the

progression of NAFLD. A detailed understanding of the immune regulation of glucose and lipid homeostasis can lead to the development of a novel therapy for insulin resistance, type 2 diabetes, and NAFLD.

Keywords Insulin resistance · Macrophage · Nonalcoholic fatty liver disease · Endoplasmic reticulum stress · Chemokine · Dipeptidyl peptidase-4

Introduction

Obesity is a state of low-grade sustained inflammation [1, 2]. Caloric excess or obesity activates the innate immune system, with the subsequent recruitment of immune cells such as macrophages and T cells into metabolic tissues, including adipose tissue and liver, leading to the development of insulin resistance. This low-grade inflammation and the subsequent development of insulin resistance are the conditions underlying type 2 diabetes, cardiovascular disease, and nonalcoholic fatty liver disease (NAFLD). A significant advance in our understanding of obesity-associated inflammation and insulin resistance has been a recognition of the critical role of adipose tissue macrophages (ATMs) [3, 4].

Macrophage-mediated inflammation also plays a role in the pathogenesis of diabetic complications. Diabetic nephropathy is characterized by albuminuria, which is caused by a loss of the size and charge barrier, mesangial expansion, and macrophage-derived cytokine production [5]. Previously, we found that the administration of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (or statin) decreases oxidative stress, the expression of transforming growth factor- β (TGF β), and macrophage recruitment and activation, thereby reducing albuminuria

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and preventing the progression of diabetic nephropathy through its pleiotropic effects (Fig. 1) [6]. This finding led us to explore the mechanism of immune-cell-mediated metabolic inflammation in the pathogenesis of insulin resistance, type 2 diabetes, and NAFLD. This review focuses on the immune regulation of glucose and lipid metabolism, linking obesity to insulin resistance, NAFLD, and type 2 diabetes.

A causal link between insulin resistance and nonalcoholic steatohepatitis

NAFLD comprises a disease spectrum ranging from simple steatosis in the absence of inflammation to nonalcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC) [7]. Lipotoxicity, insulin resistance, and inflammation are involved in the disease process. Although an association between NASH and insulin resistance is well known, questions remain as to whether insulin resistance per se is a cause of NASH [8]. Some patients with NASH do not manifest insulin resistance or other features of metabolic syndrome [9]. Furthermore, weight loss from dieting and exercise improves insulin sensitivity but only modestly decreases fatty liver and plasma transaminase levels; it does not reverse NASH [9]. Nonetheless, insulin sensitizers (e.g., metformin and thiazolidinediones)

attenuate fatty liver, inflammation, and fibrosis in NASH, suggesting a causal role for insulin resistance [10, 11].

We explored the connection between insulin resistance and NASH by feeding rats a methionine-choline-deficient diet (MCD) [12]. To test the hypothesis that obesity, insulin resistance, and steatosis precede the onset of NASH, we studied two strains of rats, Otsuka Long-Evans Tokushima Fatty (OLETF) and Long-Evans Tokushima Otsuka (LETO), bred for divergent metabolic features. OLETF rats are prone to obesity, hyperinsulinemia, diabetes, and steatosis, similar to patients with metabolic syndrome [13]. LETO rats are lean, insulin-sensitive, and less susceptible to steatosis [13]. MCD-fed OLETF rats developed hepatic steatosis after 2 weeks, inflammation by 4 weeks, and fibrosis by 8 weeks. A high-fat diet (HFD) enhanced insulin resistance and accelerated NASH in MCD-OLETF rats. MCD-induced NASH led to a rapid increase in hepatic triglycerides (TGs) associated with the expression of lipogenic genes (SREBP-1c, fatty acid synthase, and stearoyl CoA desaturase), an increase in plasma interleukin-6 (IL-6), IL-1 β , hepatic tumor necrosis factor- α (TNF- α), and transforming growth factor- β (TGF β), stellate cell activation, and fibrogenesis. By contrast, LETO rats exhibited greater insulin sensitivity and a milder response to MCD and HFD feeding [12]. The link between the insulin response and MCD-induced NASH was explored further by measuring adiponectin levels and testing the effect of pioglitazone treatment. Adiponectin is secreted by adipocytes and regulates glucose and lipid metabolism by suppressing gluconeogenesis and stimulating glucose utilization and fatty acid oxidation [14]. We observed a decrease in adiponectin in MCD-OLETF rats. Pioglitazone treatment increased adiponectin, consistent with its insulin-sensitizing action, and reduced the severity of NASH in MCD-OLETF rats. By contrast, pioglitazone did not affect adiponectin or NASH in MCD-LETO rats [14]. The lack of insulin sensitization suggested a causal link between insulin resistance and NASH [15].

This study provided new information on the natural history of NASH in an animal model and supported an association among obesity, insulin resistance, and NASH [12]. The MCD model, although imperfect, is relevant to human NASH and should assist in the investigation and treatment of this condition. The study clearly demonstrated that rats genetically predisposed to metabolic syndrome are more susceptible to NASH. An important finding was that an improvement in insulin sensitivity via thiazolidinedione treatment attenuates NASH in susceptible rats. This model may prove useful for delineating the molecular mechanisms by which insulin resistance results in key features of NASH, including steatosis, inflammation, and fibrosis (Fig. 2).

Fig. 1 Pleiotropic effects of statin on preventing the progression of diabetic nephropathy. Diabetic nephropathy is characterized by albuminuria caused by glomerular hyperfiltration, loss of the size and charge barrier, mesangial expansion, and macrophage-mediated cytokine production. Statin decreases oxidative stress, the expression of transforming growth factor- β (TGF β), and macrophage recruitment and activation. Thus, through its pleiotropic effects, it reduces albuminuria and prevents the progression of diabetic nephropathy

Fig. 2 Insulin resistance accelerates nonalcoholic steatohepatitis (NASH). The study by Ota et al. provided new information on the natural history of NASH in an animal model and supports an association among obesity, insulin resistance, and NASH. Obesity is associated with insulin resistance, the increased expression of proinflammatory cytokines, and a reduction in adiponectin levels. The rate of adipocyte lipolysis is increased in obesity, which increases the influx of fatty acids into the liver. Hyperinsulinemia associated with obesity promotes hepatic lipogenesis. Small amounts of

triglycerides are exported in the form of very low-density lipoprotein, but large amounts are retained, resulting in fatty liver. This eventually overwhelms the oxidative capacity, worsens hepatic steatosis, and generates reactive oxygen species, which increase lipid peroxidation, induces the release of cytokines such as interleukin-6 (IL-6), IL-1 β , and tumor necrosis factor- α (TNF- α) and increases the chemoattraction of inflammatory cells, stellate cell activation, and collagen synthesis

A significant role of endoplasmic reticulum stress in hepatic lipid homeostasis

Hepatic steatosis and the increased secretion of apolipoprotein B (apoB)-containing lipoproteins, particularly very low-density lipoprotein (VLDL), are characteristic concomitants of insulin resistance, metabolic syndrome, and type 2 diabetes [16]. In these states of excess energy status and/or obesity, plasma levels of TG-carrying VLDL are increased, indicating an increase in lipid secretion in the liver [17]. Nevertheless, the liver also stores excess amounts of TG, leading to steatosis. An important question in this context is as follows: Why does steatosis develop despite increased VLDL secretion?

Endoplasmic reticulum (ER) stress is a coordinated program of molecular and biochemical events that occur when the ER accumulates excess unfolded proteins. It is associated with several human diseases [18]. To maintain ER function, cells initiate an adaptive response called the unfolded protein response (UPR) [19]. In mammals, the UPR is mediated through three distinct pathways under the control of PERK, ATF6, and IRE1 α (Fig. 3a). To decrease the accumulation of proteins in the ER, the UPR attenuates translation, particularly of secretory proteins, via the

activation of PERK and the subsequent phosphorylation of eIF2 α . Additionally, the UPR increases the folding capacity of the ER by upregulating ER chaperones, such as Grp78, via the transcription factors XBP1 and ATF6. Studies suggest that hepatic ER stress is a central abnormality linking obesity, hepatic insulin resistance, and hepatic steatosis [20] (Fig. 3a). However, it is not known whether the increased delivery of fatty acids (FAs) directly to the liver and/or the accumulation of liver TG produce ER stress or if ER stress induced in this way affects the secretion of apoB. We and other groups have demonstrated the increased secretion of apoB-lipoproteins in response to increased hepatic FA and/or TG [17]. However, we also provided strong evidence that excessive loading of the liver with FA and/or TG can cause ER stress, thereby inhibiting apoB secretion [21].

The prolonged incubation of cultured liver cells with either FA or lipid (Intralipid) induces steatosis and ER stress, with increases in Grp78 and phosphorylated eIF2 α , and the activation of XBP1 (Fig. 3b). Although moderate steatosis and ER stress were associated with the increased secretion of apoB100, greater lipid loading and further ER stress selectively inhibited apoB100 secretion (Fig. 3c). There was both a slower rate of translation and a higher

Fig. 3 Endoplasmic reticulum (ER) stress plays a significant role in hepatic lipid homeostasis. To maintain ER function, cells initiate adaptive responses called the unfolded protein response (UPR). In mammals, the UPR is mediated by three distinct pathways under the control of PERK, ATF6, and IRE1 α (a). To decrease the accumulation of proteins in the ER, the UPR attenuates translation, particularly of secretory proteins, via the activation of PERK and the subsequent phosphorylation of eIF2 α . Ozcan et al. showed that obesity activates UPR in adipose tissue, and ER stress links obesity to insulin resistance and type 2 diabetes (a). Ota et al. found that an increased, prolonged influx of fatty acids (FAs) into the liver causes hepatic ER stress and the subsequent inhibition of apolipoprotein B

(apoB) expression. This in turn results in decreased very low-density lipoprotein (VLDL) secretion and the development of hepatic steatosis (b). Additionally, docosahexaenoic acid (DHA) induces autophagy, which also degrades apoB, thereby limiting VLDL secretion and lowering plasma triglyceride (TG) levels. We demonstrated that (1) the increased delivery of FAs and/or the accumulation of liver TG caused ER stress; (2) although mild ER stress was associated with the increased secretion of apoB, greater ER stress resulted in reduced apoB secretion (c). Excessive ER stress in response to increased hepatic lipids may decrease the ability of the liver to secrete TG by limiting apoB secretion, thereby worsening steatosis (c)

rate of the intracellular degradation of apoB100. The inhibition of ER stress by phenylbutyric acid (PBA), a low-molecular-weight chaperone that stabilizes protein confirmation [22], blocked FA-induced ER stress and reversed the inhibition of apoB100 secretion. In parallel experiments, the intravenous infusion of FA into mice for 6 or 9 h also caused ER stress. Importantly, at 9 h, there was a greater increase in Grp78 and phospho-eIF2 α levels and in XBP1 splicing than at 6 h. After 9 h of FA infusion, liver TG content was higher than following saline infusion. ApoB secretion was stimulated by 6-h infusions of FA, but this effect was lost after 9 h. However, treatment of mice with PBA for 7 days blocked FA-induced ER stress and restored FA-stimulated apoB secretion after 9-h infusions of FA.

Thus, we demonstrated, both in vitro and in vivo, that (1) increased FA delivery and/or the accumulation of liver TG caused ER stress and (2) although mild ER stress was associated with the increased secretion of apoB, greater ER stress resulted in reduced apoB secretion [21]. Excessive

ER stress in response to increased hepatic lipids may decrease the ability of the liver to secrete TG, by limiting apoB secretion, thereby worsening steatosis (Fig. 3b). Furthermore, we found that docosahexaenoic acid (DHA), an *n*-3 polyunsaturated FA, induced autophagy and caused the degradation of apoB independently of ER stress. This in turn resulted in decreased VLDL secretion and a lowering of plasma TG levels [23]. Overall, hepatic ER stress could be a therapeutic target for steatosis, albeit at the expense of increased secretion of atherogenic apoB-lipoproteins.

Chemokine systems link obesity to insulin resistance

Obesity involves a state of chronic low-grade systemic inflammation [1] that develops in response to an excess nutrient flux into metabolic tissues, including adipose tissue, liver, and skeletal muscle. Indeed, inflammation is currently recognized as an important link between obesity

and insulin resistance. Obesity-associated systemic inflammation is characterized by increased circulating concentrations of proinflammatory cytokines and chemokines as well as the activation of several kinases that regulate inflammation, including c-Jun NH2 terminal kinase (JNK), I κ B-kinase β (IKK β)/nuclear factor κ B (NF- κ B), and the mammalian target of rapamycin (mTOR)/S6 kinase, all of which interfere with insulin action in adipocytes and hepatocytes.

Increasing evidence supports the mediation by obesity-induced inflammation primarily by immune cells, such as the macrophages and T lymphocytes in metabolic tissues. These cells are a prominent source of proinflammatory cytokines, including TNF- α and IL-6, that block insulin action in adipocytes via autocrine/paracrine signaling and cause systemic insulin resistance via endocrine signaling, suggesting a link between inflammation and insulin resistance [2, 24]. In both humans and rodents, ATMs accumulate in adipose tissue with increasing body weight, and their number correlates positively with insulin resistance [3, 4, 25].

Tissue macrophages are phenotypically heterogeneous and are characterized according to their activation/polarization state as M1 (or “classically activated” proinflammatory macrophages) or M2 (or “alternatively activated” noninflammatory macrophages) [26–28]. M2 ATMs predominate in lean mice, whereas obesity induces the accumulation of M1 ATMs with high-level expression of TNF- α , IL-6, and iNOS, leading to a proinflammatory environment in white adipose tissue (WAT). Thus, both the recruitment and the proinflammatory activation of ATMs are required for the development of insulin resistance in obese mice.

Chemokines, a family of cytokines that induce leukocyte chemotaxis, are deeply involved in the development of allergic diseases and autoimmune diseases. More than 50 chemokines exhibiting various physiological and pathological properties have been discovered to date [29, 30]. Recent studies have shown that, in addition to their expression in preadipocytes and adipocytes, chemokines infiltrate the bone-marrow-derived macrophages of obese adipose tissue, thus contributing to the development of insulin resistance.

It has become increasingly evident that the expression of chemokines and their receptors (chemokine systems) is associated with chronic subacute inflammation, which is the common condition underlying obesity, insulin resistance, and type 2 diabetes. The interaction of MCP-1 with its receptor CCR2 is a pivotal step in obesity-induced insulin resistance [31, 32]. Therefore, the MCP-1-CCR2 axis is of central importance in promoting ATM recruitment and insulin resistance in mice. Several studies, however, have produced conflicting results, demonstrating greater complexity than suggested by earlier reports. They

showed that a loss of MCP-1 neither attenuates obesity-associated macrophage recruitment to WAT nor improves metabolic function, suggesting that MCP-1 is not critical for obesity-induced ATM recruitment and systemic insulin resistance [33, 34]. Furthermore, although the WAT of HFD-fed Ccr2^{-/-} mice has fewer macrophages than that of WT mice [32], CCR2 deficiency does not normalize ATM content and insulin resistance to the levels in lean animals. This indicates that ATM recruitment and subsequent insulin resistance are also regulated by MCP-1-CCR2-independent signals. Thus, additional as-yet-unidentified chemokine/chemokine receptor pathways may play significant roles in ATM recruitment and insulin sensitivity [35, 36].

CCR5 regulates adipose tissue inflammation and insulin resistance

Do the two CC chemokine receptors, CCR2 and CCR5, play common or unique roles in obesity-induced adipose tissue inflammation and insulin resistance? [37]. No significant compensatory increase or decrease in the expression of CCR2 or CCR5 has been found. Rather, CCR5, independently from and/or cooperatively with CCR2, plays a role in the maintenance of ATM dysfunction and insulin resistance once obesity and its metabolic consequences have been established. CCR5 is preferentially expressed on Th1 cells [38]. Several studies have demonstrated that obesity is associated with the increased accumulation of not only macrophages but also T cells in adipose tissue. Wu et al. showed that RANTES/CCL5 mRNA levels are highly correlated with the T cell marker CD3 in human visceral adipose tissue [39].

Our study provided new information linking CCR5 to obesity, adipose tissue inflammation, and insulin resistance [40] (Fig. 4). In identifying and characterizing this receptor, we observed that: (1) in the WAT of obese mice, the expression of CCR5 and its ligands is significantly increased and is equal to that of CCR2 and its ligands; (2) CCR5⁺ ATMs accumulate in the WAT of obese mice; (3) Ccr5^{-/-} mice are protected from insulin resistance and diabetes through both a reduction in ATM accumulation and an induction of an alternatively activated, M2-dominant shift in ATM [40].

DPP-4 plays a role in immune-cell-mediated inflammation and insulin resistance

Dipeptidyl peptidase-4 (DPP-4) inhibitors are effective in the treatment of type 2 diabetes as they maintain blood glucose levels by inhibiting the degradation of incretin

Fig. 4 CCR5 promotes obesity-induced inflammation and insulin resistance independently of the MCP1-CCR2 axis. Kitade et al. identified and characterized CCR5, a CC chemokine receptor, and made several important observations. First, the expression of CCR5 and its ligands is significantly increased and is equal to that of CCR2 and its ligands in the white adipose tissue (WAT) of obese mice. Second, CCR5⁺ adipose tissue macrophages (ATMs) accumulate in

the WAT of obese mice. Third, Ccr5^{-/-} mice are protected from insulin resistance and diabetes through a reduction in ATM accumulation and the induction of an alternatively activated, M2-dominant shift in ATM. Taken together, these findings indicate that CCR5 provides a novel link among obesity, adipose tissue functionality, inflammation, and insulin resistance

peptides, glucagon-like peptide 1, and glucose-dependent insulinotropic polypeptide [41]. Numerous investigations have detailed the effects of DPP-4 inhibitors on insulin and/or glucagon secretion, but there is little evidence that DPP-4 inhibitors directly improve inflammation. DPP-4, originally known as T-cell surface marker CD26, is widely expressed in many cells, including immune cells [42]. It cleaves many of the chemokine and peptide hormones involved in the regulation of the immune system, which implies its role in the pathogenesis of inflammation [41, 43]. Earlier work focused on the function of DPP-4 as a surface protease involved in T-cell activation [44]. Recent studies showing that DPP-4 release correlates with adipocyte size or visceral adiposity have led to the recognition of DPP-4 as an adipokine with the potential to impair insulin sensitivity [45, 46]. In addition, DPP-4 expressed on dendritic cells/macrophages contributes to potentiating inflammation in adipose tissue in obesity [47]. However, the role of DPP-4 in macrophage-mediated inflammation and insulin resistance remains largely unknown.

We recently showed that DPP-4 plays a crucial role in regulating macrophage-mediated adipose and liver inflammation as well as obesity-associated insulin resistance [48] (Fig. 5). First, DPP-4 activity in both plasma and WAT was significantly higher in HFD-fed obese mice than in normal chow (NC)-fed lean mice. Second, DPP-4 was

expressed in crown-like structures by F4/80⁺ macrophages but it was poorly expressed by adipocytes. Interestingly, most CD11c⁺ M1 macrophages but few CD206⁺ M2 macrophages expressed DPP-4, indicating that, in obesity, DPP-4 is predominantly expressed by macrophages, particularly M1 ATMs, rather than by adipocytes. Finally, flow cytometry analysis revealed a significantly higher percentage of DPP-4⁺ cells within the M1 ATMs of HFD-fed mice than those of NC-fed mice, whereas the percentage of DPP-4⁺ cells within M2 ATMs was unaffected by an HFD. Collectively, these results indicate that DPP-4⁺ M1 ATMs accumulate in response to obesity [48].

DPP-4 inhibition attenuates obesity-related inflammation and insulin resistance

We also obtained evidence that DPP-4 inhibition attenuates obesity-related insulin resistance and inflammation by regulating both macrophage recruitment and M1/M2 status in obese mice [48]. C57BL/6J mice were fed an HFD with or without the DPP-4 inhibitor linagliptin for 8 weeks. Linagliptin administration improved HFD-induced glucose intolerance, hyperinsulinemia, and hepatic steatosis. It also enhanced insulin signaling in both the WAT and the liver of these mice. The migration of F4/80⁺ macrophages into

Fig. 5 The inhibition of dipeptidyl peptidase-4 (DPP-4) attenuates obesity-related adipose tissue inflammation and insulin resistance by regulating M1/M2 macrophage polarization. Zhuge et al. demonstrated that DPP-4 is predominantly expressed in the M1-polarized macrophages in the WAT of obese mice and that DPP-4⁺ M1 ATMs accumulate in response to obesity. They also presented evidence that DPP-4 inhibition attenuates obesity-related insulin resistance and inflammation by regulating both macrophage recruitment and M1/M2

status in diet-induced obese mice. Much like incretin peptides, chemokines act as DPP-4 substrates. Among the chemokines, macrophage inflammatory protein (MIP)-1α becomes the most efficient monocyte chemoattractant after its cleavage by DPP-4. The apparently protective effects of linagliptin, a DPP-4 inhibitor, are abrogated in MIP-1α-deficient mice, suggesting that the inhibitor alleviates obesity-associated inflammation partly via its actions in a chemokine MIP-1α-dependent mechanism

the WAT and liver as well as the formation of crown-like structures in the WAT was lower in HFD + linagliptin mice than in HFD mice, although the levels of adiposity were similar. These findings were associated with decreased expression of mRNAs from chemokine systems (MCP-1-CCR2 and RANTES-CCR5) and proinflammatory cytokines (IL-1β, IL-6, and TNF-α) in the WAT and liver of the HFD + linagliptin mice. Furthermore, flow cytometry analysis revealed that these mice had fewer M1 ATMs and LMs, respectively, and more M2 ATMs and LMs, respectively, than HFD mice, demonstrating the predominance of the M2 over the M1 ATM and LM populations. Additionally, while the total numbers of CD3⁺, CD4⁺, and CD8⁺ T cells in the WAT and liver increased in HFD mice, the effect was significantly diminished by linagliptin [48].

In isolated macrophages, linagliptin (50–200 nM) decreased the expression of M1 marker mRNAs and the generation of reactive oxidative species (ROS) in response to lipopolysaccharide (LPS). By contrast, linagliptin augmented IL-4-induced M2 marker expression and decreased ROS production in a dose-dependent manner. These results suggested that DPP-4 directly regulates M1/M2 macrophage polarization following LPS or IL-4 stimulation and that linagliptin suppresses M1-polarized activation and induces M2-polarized activation, at least in part, by regulating intracellular ROS generation.

Much like incretin peptides, chemokines act as DPP-4 substrates. After its cleavage by DPP-4, macrophage inflammatory protein (MIP)-1α is the most efficient monocyte chemoattractant among the chemokines. In MIP-1α-deficient mice, the apparently protective effects of linagliptin are abrogated, suggesting that DPP-4 inhibitor alleviates obesity-associated inflammation partly because of its MIP-1α-dependent actions. Overall, our investigation and studies by other groups [47, 48] highlight the potential clinical utility of DPP-4 inhibition in both the attenuation of macrophage- or immune-cell-mediated inflammation and the prevention of insulin resistance and type 2 diabetes. Very recently, we found that a sodium-glucose cotransporter 2 (SGLT2) inhibitor also reduces the accumulation of M1-polarized macrophages [49]. In parallel, SGLT2 induces the antiinflammatory M2 phenotype of macrophages in the WAT and liver, thereby lowering plasma TNF-α levels and attenuating both obesity-related chronic inflammation and insulin resistance [49].

Potential new treatments for insulin resistance, NAFLD, and type 2 diabetes

Despite the pivotal role of insulin resistance and immune cell infiltration in NAFLD and NASH, there is no consensus regarding the most effective pharmacological agents

for the treatment of either condition because their pathologies are not fully understood. In our previous study, we demonstrated that a diet high in fat and cholesterol led to the development of pathophysiological NASH in humans [50]. We also found that excessive hepatic lipid accumulation promoted the M1 activation of macrophages/Kupffer cells, leading to the exacerbation of insulin resistance, hepatic inflammation, and fibrogenesis [50].

Micronutrient antioxidants, such as vitamins and carotenoids, are mainly found in fruit and vegetables and protect against ROS formation [51]. Low antioxidant levels are present in the serum and liver tissue of patients with chronic liver diseases [52] and are associated with liver dysfunction, particularly in the case of carotenoids [53]. Thus, micronutrient antioxidant deficiencies may contribute to the development of NASH [54–56]. Although carotenoids are as potent as vitamin E in inhibiting lipid peroxidation [57, 58], carotenoid supplementation (β -cryptoxanthin and astaxanthin) has not been widely used as an antioxidant treatment for patients with NASH. In two studies, we showed that the carotenoids β -cryptoxanthin and astaxanthin exhibit antioxidant and antiinflammatory effects in addition to regulating M1/M2 macrophage polarization in NASH [59, 60].

We also demonstrated that β -cryptoxanthin and astaxanthin prevent NASH development by suppressing lipid accumulation, lipid peroxidation, and insulin resistance [59, 60] (Fig. 6). As common mechanisms, these two carotenoids prevent the accumulation of T cells and the activation of stellate cells in addition to regulating the M1/M2 status of Kupffer cells in the liver, in part by down-regulating the MCP-1-CCR2 and RANTES/CCL5-CCR5 pathways [59, 60]. The expression of several genes associated with cell death, free radical scavenging, and the recruitment and activation of macrophages/Kupffer cells, leukocytes, and T cells is also inhibited by β -cryptoxanthin [61] and astaxanthin [62]. Both β -cryptoxanthin and astaxanthin directly decrease LPS-induced M1 macrophage activation and increase IL-4-induced M2 macrophage activation in vitro, suggesting the direct targeting of macrophages by either of these compounds [59, 60]. These findings support the investigation of therapeutic strategies aimed at inhibiting M1 polarization and/or driving alternative M2 macrophage/Kupffer cell activation to protect against inflammation and thereby halt NASH progression [63–65]. By reducing the recruitment of proinflammatory immune cells and the M2-dominant shift in macrophages/Kupffer cells, β -cryptoxanthin and astaxanthin hold therapeutic promise in NASH patients [51, 54].

Low-grade sustained inflammation links obesity to insulin resistance, type 2 diabetes, and NAFLD. Macrophage recruitment and polarization are pivotal as mechanisms underlying obesity-induced inflammation and insulin

resistance [3, 4, 28]. However, therapeutic approaches to improve the systemic energy balance and chronic inflammation in obesity are limited. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a basic leucine zipper transcription factor, is widely expressed in human and mouse tissues, where it serves as a defense response against extrinsic and intrinsic stressors [66]. Several synthetic Nrf2 inducers, such as triterpenoid 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO)-imidazolidine [67], CDDO-methyl ester (known as bardoxolone methyl) [68], and dithiolethione analog, oltipraz [69], were shown to ameliorate HFD-induced obesity and diabetes. Although enhanced Nrf2 signaling has shown promising results in several animal studies, synthetic Nrf2 inducers are not clinically available owing to safety concerns [70, 71]. These observations prompted us to explore a safer Nrf2 inducer for the treatment of obesity, insulin resistance, and NAFLD.

Sulforaphane, an isothiocyanate derived from cruciferous vegetables, is one of the most potent naturally occurring Nrf2 inducers; this compound exhibits anticancer activity in cancer cell lines and in rodent models of carcinogen-induced cancer [72]. Among the cruciferous vegetables, broccoli sprouts are the best source of glucoraphanin, a stable glucosinolate precursor of sulforaphane [72]. We recently demonstrated that glucoraphanin mitigated the weight gain, insulin resistance, hepatic steatosis, oxidative stress, and chronic inflammation induced by feeding on an HFD in mice [73]. The weight-reducing and insulin-sensitizing effects of glucoraphanin were abolished in Nrf2^{-/-} mice. Additionally, glucoraphanin lowered plasma LPS levels in HFD-fed mice and decreased the relative abundance of members of the gram-negative bacterial family Desulfotribionaceae in their gut microbiomes. In HFD-fed mice, glucoraphanin increased energy expenditure and the protein expression of uncoupling protein 1 (Ucp1) in WAT. Additionally, in these animals, glucoraphanin attenuated hepatic lipogenic gene expression, lipid peroxidation, classically activated M1-like macrophage accumulation, and inflammatory signaling pathways. By promoting fat browning, limiting metabolic endotoxemia-related chronic inflammation, and modulating redox stress, glucoraphanin may mitigate obesity, insulin resistance, and NAFLD [73] (Fig. 6).

Conclusions and future directions

It has become increasingly evident that immune cells such as macrophages and T cells play a major role in insulin resistance and its comorbidities, including type 2 diabetes and NAFLD/NASH. In particular, macrophage recruitment and polarization are pivotal in obesity-induced inflammation and insulin resistance. Vigorous research has shown

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