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Review Article

TLR4 and Insulin Resistance

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Chronic inflammation is a key feature of insulin resistance and obesity. Toll-Like Receptor 4 (TLR4), involved in modulating innate immunity, is an important mediator of insulin resistance and its comorbidities. TLR4 contributes to the development of insulin resistance and inflammation through its activation by elevated exogenous ligands (e.g., dietary fatty acids and enteric lipopolysaccharide) and endogenous ligands (e.g., free fatty acids) which are elevated in obese states. TLR4, expressed in insulin target tissues, activates proinflammatory kinases JNK, IKK, and p38 that impair insulin signal transduction directly through inhibitory phosphorylation of insulin receptor substrate (IRS) on serine residues. TLR4 activation also leads to increased transcription of pro-inflammatory genes, resulting in elevation of cytokine, chemokine, reactive oxygen species, and eicosanoid levels that promote further insulin-desensitization within the target cell itself and in other cells via paracrine and systemic effects. Increased understanding of cell type-specific TLR4-mediated effects on insulin action present the opportunity and challenge of developing related therapeutic approaches for improving insulin sensitivity while preserving innate immunity.

1. Introduction

1.1. Insulin Resistance. Insulin resistance is a primary defect leading to and a characteristic feature of type 2 diabetes [1, 2]. The state of insulin resistance leads to increased insulin secretion by pancreatic β-cells and compensatory hyperinsulinemia. As long as compensatory hyperinsulinemia is sufficient to overcome the insulin resistance, fasting glycemia and glucose tolerance remain relatively normal. In patients destined to develop type 2 diabetes, β-cell compensation efficiency declines and relative insulin insufficiency develops leading to impaired glucose tolerance and eventually frank type 2 diabetes. Although there is still some debate as to whether the insulin resistance or the β-cell defect comes first, most epidemiologic studies indicate that in the early, prediabetic state, insulin resistance is the initiating abnormality.

Type 2 diabetes only develops in insulin resistant patients with a concomitant β-cell defect. As such, many subject groups with insulin resistance who do not have diabetes. These include patients with simple obesity, polycystic ovarian syndrome, and advanced age. There are a number of other abnormalities associated with insulin resistance that are included in the state of metabolic syndrome. Patients with metabolic syndrome are insulin resistant, hyperinsulinemic, and dyslipidemic (usually elevated triglyceride and decreased HDL levels) and frequently also have hypertension, nonalcoholic fatty liver disease, albuminuria, and increased plasminogen activator inhibitor 1 (PAI-1) levels. Epidemiologic evidence demonstrates that patients with metabolic syndrome have a high likelihood of developing type 2 diabetes and cardiovascular disease. Thus, while it is well established that treatment of insulin resistance has beneficial effects in patients with type 2 diabetes, it is becoming increasingly clear that enhanced insulin sensitivity is also therapeutically important in nondiabetic individuals with insulin resistance.

1.2. TLR4 Activity and Insulin Resistance-Associated Inflammation. Activation of the pro-inflammatory pathway has been described in a variety of insulin resistant states [3–6]. Chronic inflammation inhibits insulin sensitivity through

the activation of signaling pathways that directly interfere with the normal function of key components of the insulin signaling pathway [5, 6]. Inflammation impairs insulin sensitivity in part via the activation of the Toll-Like Receptor (TLR) family of pattern recognition receptors, specifically TLR2 and TLR4. For the purposes of this review, we will focus on the role of TLR4 in insulin resistance. TLR4 is a cell surface receptor that generates innate immune responses to pathogens by inducing signaling cascades of kinase and transcription factor activation (Figure 1). These cascades lead to the generation of pro-inflammatory cytokines, chemokines, eicosanoids, and reactive oxygen species (ROS), all effectors of innate immunity. Notably, TLR4 is expressed in many cell components of insulin target tissues, including liver, adipose tissue, skeletal muscle, vasculature, pancreatic β cells, and brain (Figure 2). Thus, activation of TLR4 can dampen insulin action directly, through activation of pro-inflammatory kinases and ROS, and indirectly, via activation of cytokine signaling cascades and systemic release of pro-inflammatory, insulin-desensitizing factors (Figure 1).

Lipopolysaccharide (LPS) and its endotoxic moiety Lipid A are potent agonists of TLR4. LPS is an outer membrane component of gram-negative bacteria and is composed of oligosaccharides and acylated saturated fatty acids (SFA). Free SFA are also reported to bind and activate TLR4. However, there are conflicting interpretations of these data which are discussed in more detail below. Endogenous activators of TLR4 include S100A8/S100A9 (calprotectin) [7], high-mobility group 1 (HMBG1) [8], fibronectin [9], and minimally modified low-density lipoprotein (mmLDL) [10]. LPS binding protein (LBP), CD14 and MD-2 serve as TLR4 accessory proteins that facilitate ligand delivery in the circulation and receptor binding. Two signaling pathways are initiated by TLR4 activation (Figure 1). One, modulated by MyD88 and TIRAP, activates IKK, p38, JNK, CREB, AP2, and NFκB and leads to the induction of pro-inflammatory genes. The other pathway, modulated by TRAM and TRIF, requires internalization of TLR4 (not depicted in Figure 1), activates IKK, NFκB, and IRF3, and leads to induction of type 1 interferon genes. Transcriptional activation by these pathways induces robust expression of thousands of genes, depending on the cell type, that propagate the defense mechanisms of innate immunity. These signaling cascades induce feed forward signaling cascades (e.g., via IL-6 and TNFα receptor activation) and negative feedback loops (e.g., via transcriptional activation of the IκB gene) [3]. In addition, TLR4-activated pathways inhibit the components of other signaling systems, for example, insulin signaling via IRS1 serine phosphorylation (Figure 1).

1.3. Regulation of TLR4. TLR4 expression and signaling are regulated by a variety of inputs, explained briefly here. Adiponectin impairs LPS activation of TLR4 signaling pathways in hepatic Kupffer cells and macrophages through mechanisms involving AMPK, IL-10, and heme oxygenase-1 [11–15]. AMPK also impairs LPS-induced IκB degradation and CREB activation in macrophages [16]. Activated nuclear receptor transcription factors glucocorticoid receptor (GR), peroxisome proliferators-activated receptor gamma (PPARγ), and liver X receptor alpha (LXRα) each transrepress TLR4-activated gene transcription and impair TLR4-mediated inflammation [17]. PPARγ activation also inhibits expression of TLR4 [18, 19] and, conversely, TLR4 activation inhibits expression of PPARγ [20]. Sex hormones can also affect TLR4 expression. Progesterone impairs LPS/TLR4 signaling efficacy via GR and progesterone receptor [21, 22]. Estrogen treatment of ovariectomized mice increases cell surface localization of TLR4 but does not change total cellular protein levels [23]. Testosterone downregulates TLR4 expression in macrophages both *in vitro* and *in vivo* [24]. Long-chain polyunsaturated omega-3 fatty acids (ω-3 FA) including DHA and EPA are antagonists of TLR4 activation by LPS and SFA in humans and mice [25–27]. One mechanism by which ω-3 FA interfere with TLR4 signaling is by altering plasma membrane lipid raft composition and function. For example, ω-3 FA block the ability of LPS and SFA treatments to stimulate assembly of TLR4 homodimers and signaling component complexes within lipid rafts, preventing subsequent signal transduction [28]. Stearoyl CoA desaturase 1 (SCD1) deficient mice, although protected from high-fat diet-induced insulin resistance, accumulate macrophage plasma membrane SFA, exhibit greater susceptibility to atherosclerosis, and are hypersensitive to inflammatory stimuli including those signaling through TLR4 [29, 30]. Supplementation with ω-3 FA completely protects SCD1 deficient mice from diet-induced atherosclerosis and metabolic syndrome [31]. These SCD1 deficient phenotypes may, in part, relate to lipid raft compositional changes that alter TLR4 signaling efficiency. SFA are conducive to formation and function of lipid rafts, essential sites of particular signal transduction pathways including TLR4, but lipid raft function and signaling component assembly are disrupted by ω-3 FA [32–34].

1.4. Role of Saturated Fatty Acids and Gut-Generated LPS as TLR4 Ligands. Are saturated fatty acids actual ligands of TLR4? SFA activation of TLR4 is an attractive link between obesity, insulin resistance, and inflammation, as cellular exposure to SFA greatly increases in the obese state. SFA are acyl components of LPS, activate TLR4 *in vitro*, and bind directly to TLR4/MD2/LPS crystal structures, although in an orientation that would probably rely on their presentation in an acylated form [35, 36]. Recent studies document endotoxin contamination of experimental reagents (such as bovine serum albumin, BSA) which would generate false-positive experimental results regarding the TLR4 agonistic effects of SFA [37–39]. LPS contamination is pervasive and LPS levels can only be assayed indirectly [31, 40]. Nonetheless, many publications document activation of TLR4 via SFA and many of these include samples that control for possible endotoxin contamination, for example, studies wherein BSA-complexed SFA treatments activate TLR4 effects but BSA alone and BSA-complexed monounsaturated fatty acid treatments do not. Extensive literature suggests that high-fat diet-augmented postprandial endotoxemia is a possible mode by which dietary SFAs induce inflammation through TLR4 in diet-induced obesity (DIO) models.

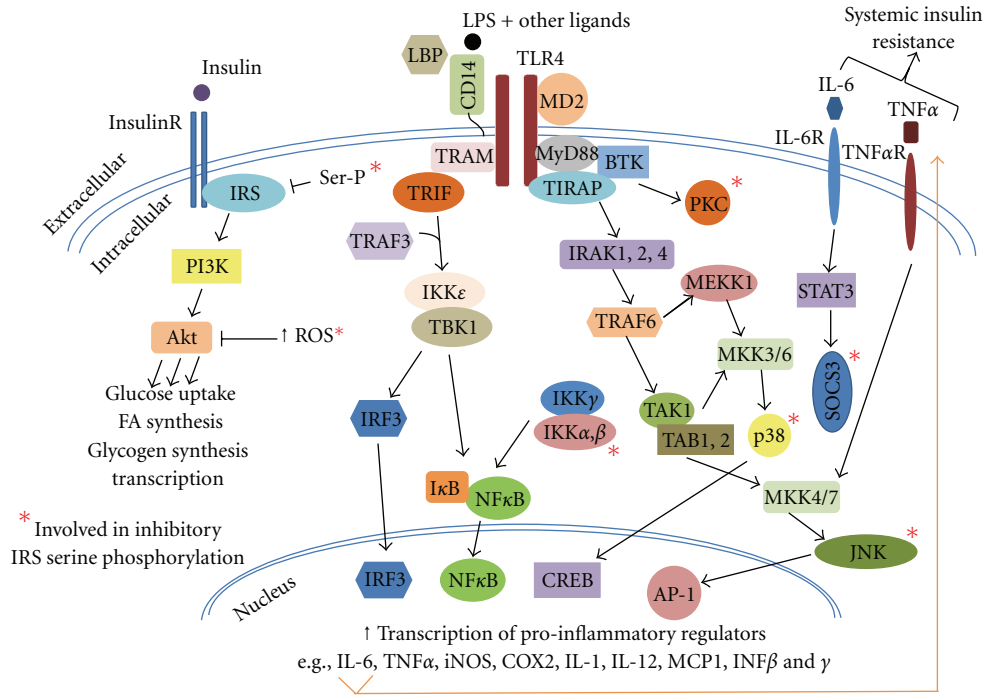

Figure 1: Schematic of TLR4 signaling cascades. Activation of TLR4 signal transduction through MyD88/TIRAP and TRAM/TRIF pathways leads to activation of innate immune responses and inhibition of insulin signal transduction, primarily through IRS serine phosphorylation. Additional cellular responses to TLR4 activation not shown include activation of NADPH oxidase, cytoskeletal rearrangement, and internalization of TLR4 complexes to endosomal compartments.

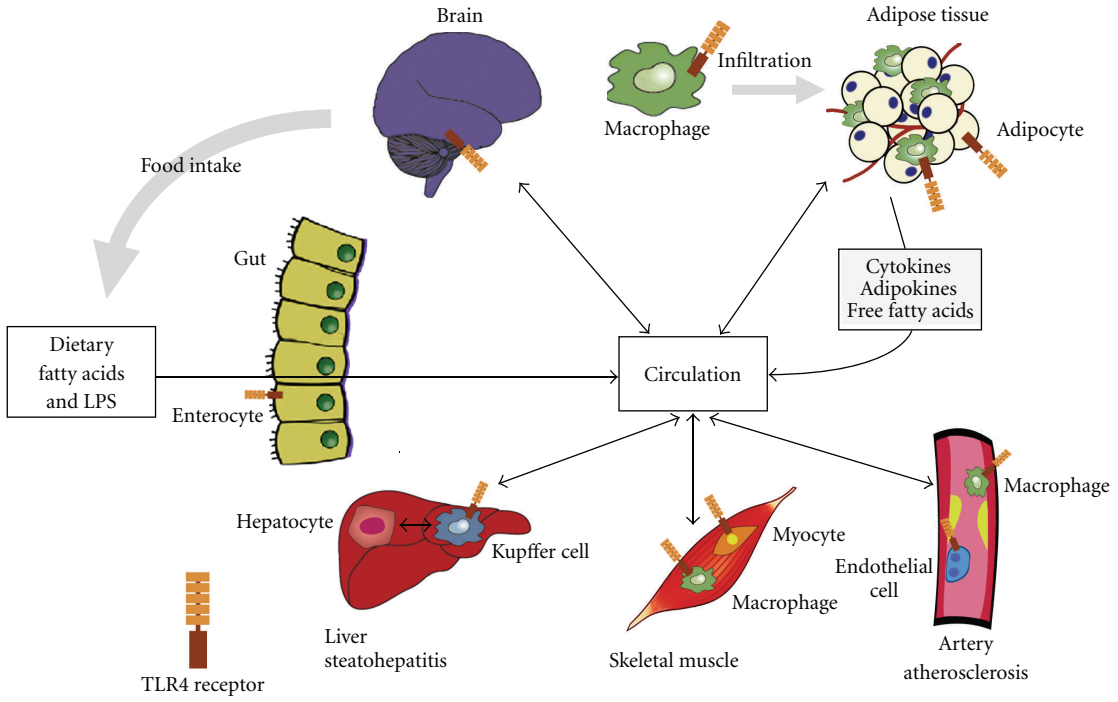

Figure 2: Distribution of TLR4 expression in the integrated organ/tissue systems that modulate energy homeostasis and insulin sensitivity. Schematic representation of the cross-talk between tissues. Adipose tissue shown in the macrophage-enriched, inflamed state.

Both high-fat diets and high-fructose diets influence enterobacterial bacterial production and circulating levels of LPS/endotoxin by altering gut flora growth and composition and gut permeability [41–44]. Dietary lipids facilitate LPS incorporation into chylomicrons [45] and TLR4 is responsible for phagocytosis of gram-negative bacteria by gut enterocytes [46], each contributing to postprandial endotoxemia. Insulin resistant DIO and genetically obese mice and type 2 diabetic humans [41, 46–48] all exhibit elevated plasma LPS levels and endotoxemia is correlated with insulin resistance and atherogenic markers. Thus, chronic elevation of circulating gut-generated LPS or "metabolic endotoxemia" [49] would result in sustained, systemic pro-inflammatory stimulation of TLR4. Interestingly, germ-free mice or mice treated with antibiotics specific for gram-negative bacteria do not acquire high-fat diet-induced insulin resistance or other associated metabolic abnormalities [50–52]. Genetically obese *ob/ob* mice treated with an LPS inhibitor or in a CD14 KO background have reduced inflammation and metabolic abnormalities compared to normal *ob/ob* mice [51, 52] which suggests that these *ob/ob* phenotypes are partly mediated by gut LPS and TLR4 signaling.

1.5. Mouse Model Overview. Several mouse model studies have demonstrated the importance of TLR4 and its signaling components in diet-induced insulin resistance, inflammation, and atherosclerosis. These studies include those conducted in whole body TLR4 knockout (KO) or loss-of-function mutations [26, 53–55], hematopoetic TLR4 KO [56], and whole body KOs of MyD88 [57] or CD14 [58, 59]. Some discrepancies in phenotypic reports with regard to food intake, weight gain, and adipose tissue macrophage accumulation will be discussed in the sections below. There are two nonsynonymous polymorphisms (SNPs) in the human TLR4 gene that result in changes in the TLR4 extracellular domain. These polymorphisms are reported to alter responsiveness to TLR4 activation and correlate with protection from atherosclerosis, CVD and the metabolic syndrome in some populations [60–62].

In the next sections, we will highlight tissue-specific effects of TLR4 activation and its role in insulin resistance. As many of the mouse models are whole body KOs of TLR4 signaling, it is unclear which exact cell type(s) mediate the phenotypes observed in these models. TLR4 expression in tissues, the mediate glucose homeostasis, and insulin sensitivity is shown in Figure 2 along with a schematic representation of the intra- and intertissue crosstalk that exists *in vivo*.

1.6. Adipose Tissue. Adipose tissue acts not only as a storage depot for excess calories, but also secretes large numbers of hormones, cytokines, and chemokines that influence energy homeostasis and metabolism (Figure 2). Adipose tissue consists of a variety of cell types, including adipocytes, immune cells (macrophages and lymphocytes), preadipocytes, and endothelial cells. Among these cell types, adipocytes and macrophages release cytokines and chemokines such as MCP-1, IL-1β, IL-6, and TNF-α [5] that promote inflammation. In addition, adipocytes are the unique source of hormones termed "adipokines" such as leptin [63, 64] and adiponectin [65], which can promote insulin sensitivity as well as resistin and retinol-binding protein 4 (RBP4), which can impair insulin sensitivity [6].

In states of chronic nutrient excess and obesity, insulin resistance manifests through several mechanisms such as increased free fatty acid (FFA) flux, ER stress, and microhypoxia in adipocytes, all of which are associated with increased inflammation [6]. In recent years, much interest has been focused on this association between obesity, chronic inflammation and insulin resistance. In 2003, two landmark studies showed bone-marrow derived macrophages invade adipose tissue in obese states [66, 67]. Recruited macrophages may initially remove dying cells and contribute to adipose tissue vascularization, but their activation towards an inflammatory phenotype soon results in cytokine production [68]. These macrophages contribute to the development of inflammation in adipose tissue and are believed to be a key contributor to insulin resistance [69]. This relationship between increased macrophage infiltrates and obesity has since been verified in human studies [70]. A growing body of literature is focused on the role of these adipose tissue macrophages (ATMs) in insulin resistance, characterizing their activation, recruitment, and function.

Attenuating the inflammatory signaling pathway by gene knockout experiments has been shown to reduce obesity-related insulin resistance in mice [69, 71–74]. For example, mice with myeloid cell-specific deletion of IKKβ or JNK1 have significantly improved glucose tolerance and insulin sensitivity despite high-fat diet-induced obesity. Several lines of evidence point to the involvement of TLR signaling in this paradigm. For example, TLR4 expression is highly abundant in pro-inflammatory macrophages [75, 76] and differentiated adipocytes [77, 78]. Moreover, TLR4 expression in adipocytes increases with obesity [26] and it can be activated by LPS to induce NFκB activation and cytokine production in both rodent and human tissues [79–81]. SFA acylated in the lipid A moiety of LPS is essential for the biological activity of LPS [82]. LPS and SFA have also been shown to attenuate insulin signaling in adipocytes with decreased phosphorylation of Akt and GSK3β [78].

Most intriguingly, TLRs in adipocytes [26, 83] and pro-inflammatory macrophages [76] may be directly activated by nutrients, particularly SFA. Although it is not yet clear if LPS contamination may contribute to any of these findings, dietary SFA appear to differentially modify the risk of developing many chronic inflammatory diseases in both human and animal studies [84]. In contrast, fish oil-derived ω-3 FA can mitigate the effect of LPS and free SFA-induced inflammation in macrophages [25] and adipocytes [26] and prevent high-fat diet-induced insulin resistance in rodents [85]. Taken together, this evidence suggests that elevated FFAs in obesity activate TLR signaling and impair insulin action, providing yet another link between obesity, inflammation, and insulin resistance.

Toll-like receptors in adipose tissue play a key role in initiating the inflammatory response, thereby promoting insulin resistance. Several studies to date have shown that disruption of the TLR4 gene in mice confers protection

from obesity-induced inflammation and insulin resistance [26, 54, 86–88]. Several models of TLR4 deficiency have been studied in this context. Two LPS-resistant naturally occurring mouse strains have been identified with loss-of-function (C3H/HeJ mice) or deletion (C57BL/10ScN mice) mutations in the TLR4 gene [55, 81]. TLR4 null mice have also been generated using homologous recombination [80]. Body composition differs between mouse strains following high-fat feeding, with unaltered [88] or decreased [55, 86, 87] weight in C3H/HeJ and C57BL/10ScN mice, and unaltered or increased weight in *TLR4*$^{-/-}$ mice [26]. It is unknown whether this divergence results from differences in TLR4 mutations, mouse strains, or other factors. However, in both models, TLR4 signaling in macrophages and adipose tissue appears to regulate whole body glucose homeostasis via effects on adipose, muscle, and liver tissues.

Since body composition strongly influences insulin sensitivity, it is challenging to ascertain whether improved glucose metabolism results from altered TLR4 expression or decreased body weight. Therefore, models in which body weight is unaltered or increased are advantageous. There are two papers published to date showing increased adiposity following high-fat feeding in mice with disrupted TLR4 gene expression [26, 88]. In these studies, adipose inflammation was greatly attenuated with reduced expression of pro-inflammatory genes, despite their greater adiposity. TLR4 deletion also improved insulin sensitivity, with higher rates of glucose disposal into skeletal muscle and adipose tissue, and reduced inflammation and insulin resistance induced by either LPS or FFAs in isolated primary adipocytes [26, 88]. Moreover, while *in vivo* lipid infusion promotes insulin resistance and NFκB activation in adipose tissue of control mice, similar effects were not observed in TLR4 knockout mice [26].

Additional work indicates that TLR4-mediated alterations in macrophage activity underlie the adipose-specific improvements in inflammation and insulin. Saturated fatty acids such as palmitate, lauric acid, and oleate fail to elicit TNFα production or IKKβ degradation in elicited peritoneal macrophages from TLR4 mutant mice [26, 89]. Furthermore, mice with myeloid-specific ablation of TLR4, generated by transplanting bone marrow from *TLR4*$^{-/-}$ animals into wild-type mice, demonstrate significantly improved insulin sensitivity in adipose tissue with reduced ATMs and adipose pro-inflammatory gene expression [54]. Because these chimeric mice possess TLR4 deficiency only in bone marrow-derived cells, it is possible to determine the contribution of hematopoietic TLR4 to insulin sensitivity. In addition, body weight and adipose depot sizes are equal between chimeric and control mice, such that differences in adiposity are not a confounding factor in the comparison between groups. Similar conclusions were drawn from studies in mice lacking the TLR4/TLR2 coreceptor CD14. Adipose tissue from obese CD14 null mice had fewer macrophages, with reduced glucose intolerance despite increased body weight [59]. Interestingly, TLR4 expression in peripheral blood mononuclear cells is reduced in overweight individuals with metabolic syndrome that undergo weight loss [90]. Although our understanding of macrophage function is incomplete, it appears that their absence in adipose tissue confers protection against insulin resistance during nutrient overload.

1.7. Liver. The liver is comprised of heterogeneous cell types including hepatocytes, stellate cells, endothelial cells, and immune cells. Liver-resident macrophages called Kupffer cells make up 10% of cells in the liver and 80%–90% of all tissue macrophages in the body [91]. They are localized at sinusoids where they are in close contact with circulating factors (hormones, cytokines, lipids, danger signals, post-prandial LPS, etc.). Kupffer cells, able to migrate and cross-talk with other cell types within the liver, are important mediators of liver inflammation and non-alcoholic fatty liver disease (NAFLD), reviewed in more detail within this volume [92]. TLR4 is expressed on Kupffer cells and other liver cell components and modulates liver pro-inflammatory activity induced by high-fat diet- and fructose-induced hepatic steatosis and insulin resistance in mouse models [26, 44, 54, 93], although a direct role for liver tissue TLR4 in these processes is unclear. In obese patients with nonalcoholic steatohepatitis (NASH), liver tissue levels of activated pro-inflammatory NFκB and AP-1 were correlated with oxidative stress and insulin resistance [94]. Both whole body and myeloid-specific TLR4 signaling deficiency result in reduced lipid accumulation, inflammation, JNK and IRS1 serine phosphorylation, and insulin resistance in liver [26, 54, 59, 86–88]. Interestingly, acute LPS treatment inhibits hepatic glucose production *in vivo* and *in vitro* via a TLR4 signaling pathway and induces insulin resistance 48 hours post-treatment *in vivo*, suggesting possible cross-talk between TLR4 and insulin receptor signaling pathways in this system [95]. The hypoglycemic effects of acute LPS are PI3K- and TNFα-independent and additive when combined with insulin. There are several models of how steatosis could facilitate activation of TLR4 in Kupffer cells [93]. Increased lipid content and exposure can affect TLR4 signaling complex assembly, endosomal sorting, and signaling cascade flow by altering lipid raft composition and membrane fluidity. Liver hypertrophy and elevated lipid content can impair sinusoidal perfusion efficacy and constrict circulatory flow. Circulating leukocytes, stuck within the sinusoids, would be more likely to activate liver resident cells, including Kupffer cells. Liver tissue infiltration of circulating monocytes might also be facilitated in the inflamed, hypertrophic, and steatotic state. In the insulin resistant state, liver-expressed TLR4 would be exposed to elevated circulating ligands including adipose tissue FFA and, in obese/high-fat diet states, LPS and dietary SFA.

1.8. Muscle. Skeletal muscle is the primary site for insulin-stimulated glucose utilization, accounting for over 75% of this process under normal physiological conditions [96, 97]. Glucose disposal into muscle is markedly reduced in obese, hyperinsulinemic subjects [98], underscoring the importance of skeletal muscle in normal glucose homeostasis and in the development of insulin resistance. Putative mechanisms of insulin resistance in skeletal muscle include direct effects of intramyocellular FFA metabolites, paracrine effects

of adipocytes, and macrophages (interspersed between muscle fiber bundles) in muscle tissue as well as endocrine effects from adipocytes and macrophages present in adipose tissue. As an example of the latter, the production of the pro-inflammatory cytokine TNFα from adipose tissue can impair insulin signaling in muscle through inhibitory serine phosphorylation of IRS-1 [99].

TLR4 has been shown to regulate substrate metabolism in muscle, favoring glucose oxidation rather than fatty acid oxidation in the absence of insulin [100]. Emerging data indicates that TLR signaling may also underlie the development of chronic inflammation and insulin resistance in skeletal muscle. Skeletal muscle cells and intact whole muscle express multiple TLRs, including TLR2 and TLR4 [101], that are responsive to LPS [102]. Moreover, skeletal muscle TLR4 gene and protein expression are significantly elevated in muscle from obese subjects with type 2 diabetes [103]. In these individuals, TLR4 protein expression in muscle correlates with the severity of insulin resistance. TLR4 contributes to skeletal muscle metabolism. Activation of TLR4 has also been shown to regulate substrate utilization in muscle, favoring glucose oxidation rather than fatty acid oxidation in the basal state.

Much of the current data implicates saturated FFAs as ligands for TLRs in muscle tissue. For example, in human myotubes and in muscle from lean human subjects, acute palmitate treatment induces robust NFκB-activation via TLR4 [103]. In C2C12 mouse myotubes, palmitate has been also shown to activate NFκB, as well as JNK1/2 and novel PKC pro-inflammatory pathways via TLR2 [104]. Rodent studies also show that disrupted expression of TLR4 protects against saturated fatty acid-induced insulin resistance in muscle resulting in improved insulin-stimulated glucose uptake, improved IRS1 tyrosine phosphorylation, reduced IRS1 serine phosphorylation, and decreased JNK1 phosphorylation in TLR mutant mice [26, 87]. More recent work indicates that conditioned media from macrophages treated with palmitate, but not LPS, can impair glucose uptake in muscle cells, suggesting that saturated fatty acids mediate their effects on muscle via macrophage cells [68].

1.9. Other Tissues

1.9.1. Brain. In recent years, much interest has been focused on the role of the brain in regulating glucose metabolism. Several investigators have identified the hypothalamus and mesolimbic area as important sites in the regulation of food intake, energy expenditure, peripheral insulin resistance, and pancreatic β-cell function. For example, neural influences on the liver and muscle directly influence glucose output and uptake in these tissues [105]. Inflammatory signaling pathways in adipose tissue have been shown to regulate energy balance by increasing thermogenesis [106]. Studies in TLR4 mutant mice suggest that TLR signaling in the CNS may also contribute to obesity phenotypes by affecting nutrient intake. For example, TLR4 null females demonstrate increased adiposity secondary to increased food intake on both normal chow and high-fat diets [26]. In addition, a study of C3H/HeJ mice showed decreased food intake when fed ad libitum, and developed obesity when pair-fed with control mice [88]. It is difficult to ascertain direct effects of TLR signaling in the brain since TLR4 expression is disrupted in the whole body in both models. However, high-fat diet has been shown to increase JNK1 and NFκB activation in the hypothalamus with impaired insulin signaling and apoptosis that may result in dysregulated feeding control [107]. TLR4 in the hypothalamus may activate pro-inflammatory pathways that contribute to the development of insulin and leptin resistance. For example, mice deficient for the TLR adaptor molecule MyD88 in the CNS are protected from hypothalamic inflammation and leptin resistance induced by acute central application of palmitate as well as from impairment of peripheral glucose metabolism induced by either centrally administered palmitate or by HFD [108]. However, the role of TLR4 is still unclear as some work suggests that TLR4 may in fact play dual roles in the hypothalamus by both activating pro-inflammatory pathways and restraining apoptosis [109].

1.9.2. Pancreatic Islet. Several TLRs (TLR2, 3 and 4) are highly expressed in human and rodent islets [110]. Although types 1 and 2 diabetes differ in etiology, β-cell destruction eventually occurs in both cases, leading to clinical manifestations of absolute or relative insulin deficiency. Islet inflammation is a well-established observation in autoimmune-mediated type 1 diabetes. However, it has been more recently described in type 2 diabetes and is thought to be secondary to toxicity induced by high glucose, FFAs, cytokine signaling, or ER stress. TLR signaling in β-cells has been primarily implicated in autoimmune type 1 diabetes [111–114]. Nevertheless, recent studies also implicate TLR2/4 and MyD88 in insufficient β-cell compensation in type 2 diabetes. For example, the chemokine CXCL10 is highly expressed in islets isolated from individuals with type 2 diabetes and has been shown to impair insulin secretion and promote β-cell apoptosis via TLR4 [115]. In addition, TLR activation by FFAs can induce the expression of proinflammatory cytokines in purified mouse and human islets [116], suggesting that the dyslipidemia associated with insulin resistance promotes islet inflammation.

1.9.3. Artery and Endothelial Cells. TLR4 activity impairs endothelial cell function and contributes to atherosclerosis. TLR4 is required for LPS-stimulated NFκB activation in endothelial cells [117]. Kim et al. report that mice with whole body deletion of TLR4 were protected against high-fat diet-induced vascular inflammation (aorta) *in vivo* [53]. The mice were also protected from high-fat diet-induced insulin resistance while exhibiting the same body weight, adiposity, and plasma insulin and FFA levels as wild-type controls. These authors also demonstrated that the SFA palmitate stimulated TLR4-dependent IKK and NFκB activation and impairment of insulin signaling and NO production in wild type aortic explants and cultured human endothelial cells. Whole body disruption of TLR4 signaling prevents atherosclerosis in proatherogenic genetic mouse models [57, 58]. Interestingly, in hematopoetic cell TLR4 KO/whole body LDLR KO agouti mice, only females fed normal chow

plus cholesterol diet exhibited less ATM, inflammation, and atherosclerotic lesion size than TLR4 wild-type controls [56]. In these studies, metabolic and atherogenic sequelae were the same in hematopoetic cell TLR4 KO versus wild type females fed three different high-fat diet plus cholesterol formulae. The authors state that no significant phenotypic differences were observed between the male genotypes. Direct action of TLR4 signaling in vascular inflammation and atherosclerosis in vivo is unclear as the phenotypes described above could be mediated by TLR4 indirectly via reduced inflammation elsewhere (e.g., adipose tissue).

1.9.4. Sex-Specific Dimorphism. Numerous observations in the literature reveal sexual dimorphism in the regulation of TLR4 and TLR4 signaling. Sex hormones differentially regulate TLR4 localization, gene expression, and sensitivity, described above. Males and females exhibit dramatic differences in their immune responses and susceptibility to sepsis [118]. Innate immunity modulation and insulin resistance are observed in pregnancy during which estrogen and progesterone levels are dramatically elevated [119–121]. Two of the TLR4 knockout mouse studies described above report TLR4-dependent phenotypic effects in females only [26, 56]. Sex differences in acute lipid infusion-induced insulin resistance have been reported in both human [122] and rat [123] studies where only males become insulin resistant during lipid infusion. Future studies should provide more clarity about the mechanisms by which TLR4 signaling is modulated by sex hormones.

2. Conclusion

TLR4 activation promotes insulin resistance. Given the wide-spread expression pattern of TLR4 in tissues and cell types that modulate energy homeostasis and insulin action (Figure 2), the direct, cell type-specific relationship between TLR4 activation and insulin resistance is unclear. TLR4 plays an important insulin-desensitizing role in myeloid-derived cells and future studies of nonmyeloid, cell type-specific transgenic and knockout models are of great interest. Our increasing understanding of TLR4 and insulin resistance will facilitate the design of novel therapeutic approaches that can derail the negative metabolic effects of TLR4 activation from the important functions of innate immunity.

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References

[1] C. de Luca and J. M. Olefsky, "Inflammation and insulin resistance," *FEBS Letters*, vol. 582, no. 1, pp. 97–105, 2008.

[2] S. M. Grundy, H. B. Brewer Jr., J. I. Cleeman, S. C. Smith Jr., and C. Lenfant, "Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition," *Circulation*, vol. 109, no. 3, pp. 433–438, 2004.

[3] J. M. Olefsky and C. K. Glass, "Macrophages, inflammation, and insulin resistance," *Annual Review of Physiology*, vol. 72, pp. 219–246, 2010.

[4] P. K. Shah, "Innate immune pathway links obesity to insulin resistance," *Circulation Research*, vol. 100, no. 11, pp. 1531–1533, 2007.

[5] G. S. Hotamisligil and E. Erbay, "Nutrient sensing and inflammation in metabolic diseases," *Nature Reviews Immunology*, vol. 8, no. 12, pp. 923–934, 2008.

[6] S. Schenk, M. Saberi, and J. M. Olefsky, "Insulin sensitivity: modulation by nutrients and inflammation," *Journal of Clinical Investigation*, vol. 118, no. 9, pp. 2992–3002, 2008.

[7] J. M. Ehrchen, C. Sunderkötter, D. Foell, T. Vogl, and J. Roth, "The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer," *Journal of Leukocyte Biology*, vol. 86, no. 3, pp. 557–566, 2009.

[8] M. E. Bianchi, "HMGB1 loves company," *Journal of Leukocyte Biology*, vol. 86, no. 3, pp. 573–576, 2009.

[9] S. P. Gondokaryono, H. Ushio, F. Niyonsaba et al., "The extra domain A of fibronectin stimulates murine mast cells via Toll-like receptor 4," *Journal of Leukocyte Biology*, vol. 82, no. 3, pp. 657–665, 2007.

[10] Y. I. Miller, et al., "Toll-like receptor-4 and lipoprotein accumulation in macrophages," *Trends in Cardiovascular Medicine*, vol. 19, no. 7, pp. 227–232, 2009.

[11] P. Mandal, P.-H. Park, M. R. McMullen, B. T. Pratt, and L. E. Nagy, "The anti-inflammatory effects of adiponectin are mediated via a heme oxygenase-1-dependent pathway in rat kupffer cells," *Hepatology*, vol. 51, no. 4, pp. 1420–1429, 2010.

[12] H. Huang, P.-H. Park, M. R. McMullen, and L. E. Nagy, "Mechanisms for the anti-inflammatory effects of adiponectin in macrophages," *Journal of Gastroenterology and Hepatology*, vol. 23, supplement 1, pp. S50–S53, 2008.

[13] N. Kamio, S. Akifusa, N. Yamaguchi, K. Nonaka, and Y. Yamashita, "Anti-inflammatory activity of a globular adiponectin function on RAW 264 cells stimulated by lipopolysaccharide from Aggregatibacter actinomycetemcomitans," *FEMS Immunology and Medical Microbiology*, vol. 56, no. 3, pp. 241–247, 2009.

[14] M. Neumeier, J. Weigert, A. Schäffler et al., "Different effects of adiponectin isoforms in human monocytic cells," *Journal of Leukocyte Biology*, vol. 79, no. 4, pp. 803–808, 2006.

[15] N. Yamaguchi, J. G. M. Argueta, Y. Masuhiro et al., "Adiponectin inhibits Toll-like receptor family-induced signaling," *FEBS Letters*, vol. 579, no. 30, pp. 6821–6826, 2005.

[16] D. Sag, D. Carling, R. D. Stout, and J. Suttles, "Adenosine 5'-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype," *Journal of Immunology*, vol. 181, no. 12, pp. 8633–8641, 2008.

[17] C. K. Glass and S. Ogawa, "Combinatorial roles of nuclear receptors in inflammation and immunity," *Nature Reviews Immunology*, vol. 6, no. 1, pp. 44–55, 2006.

[18] M. R. Dasu, S. Park, S. Devaraj, and I. Jialal, "Pioglitazone inhibits toll-like receptor expression and activity in human monocytes and db/db mice," *Endocrinology*, vol. 150, no. 8, pp. 3457–3464, 2009.

[19] C. M. Reynolds, E. Draper, B. Keogh et al., "A conjugated linoleic acid-enriched beef diet attenuates lipopolysaccharide-induced inflammation in mice in part through PPARγ-mediated suppression of toll-like receptor 4," *Journal of Nutrition*, vol. 139, no. 12, pp. 2351–2357, 2009.

[20] B. M. Necela, W. Su, and E. A. Thompson, "Toll-like receptor 4 mediates cross-talk between peroxisome proliferator-activated receptor γ and nuclear factor-κB in macrophages," *Immunology*, vol. 125, no. 3, pp. 344–358, 2008.

[21] L. A. Jones, J.-P. Anthony, F. L. Henriquez et al., "Toll-like receptor-4-mediated macrophage activation is differentially regulated by progesterone via the glucocorticoid and progesterone receptors," *Immunology*, vol. 125, no. 1, pp. 59–69, 2008.

[22] L. Miller, E. W. Alley, W. J. Murphy, S. W. Russell, and J. S. Hunt, "Progesterone inhibits inducible nitric oxide synthase gene expression and nitric oxide production in murine macrophages," *Journal of Leukocyte Biology*, vol. 59, no. 3, pp. 442–450, 1996.

[23] J. A. Rettew, Y. M. Huet, and I. Marriott, "Estrogens augment cell surface TLR4 expression on murine macrophages and regulate sepsis susceptibility in vivo," *Endocrinology*, vol. 150, no. 8, pp. 3877–3884, 2009.

[24] J. A. Rettew, Y. M. Huet-Hudson, and I. Marriott, "Testosterone reduces macrophage expression in the mouse of toll-like receptor 4, a trigger for inflammation and innate immunity," *Biology of Reproduction*, vol. 78, no. 3, pp. 432–437, 2008.

[25] J. Y. Lee, K. H. Sohn, S. H. Rhee, and D. Hwang, "Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4," *Journal of Biological Chemistry*, vol. 276, no. 20, pp. 16683–16689, 2001.

[26] H. Shi, M. V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, and J. S. Flier, "TLR4 links innate immunity and fatty acid-induced insulin resistance," *Journal of Clinical Investigation*, vol. 116, no. 11, pp. 3015–3025, 2006.

[27] J. Y. Lee, A. Plakidas, W. H. Lee et al., "Differential modulation of Toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids," *Journal of Lipid Research*, vol. 44, no. 3, pp. 479–486, 2003.

[28] S. W. Wong, M.-J. Kwon, A. M. K. Choi, H.-P. Kim, K. Nakahira, and D. H. Hwang, "Fatty acids modulate toll-like receptor 4 activation through regulation of receptor dimerization and recruitment into lipid rafts in a reactive oxygen species-dependent manner," *Journal of Biological Chemistry*, vol. 284, no. 40, pp. 27384–27392, 2009.

[29] J. M. Brown, S. Chung, J. K. Sawyer et al., "Inhibition of stearoyl-coenzyme A desaturase 1 dissociates insulin resistance and obesity from atherosclerosis," *Circulation*, vol. 118, no. 14, pp. 1467–1475, 2008.

[30] M. L. E. MacDonald, M. Van Eck, R. B. Hildebrand et al., "Despite antiatherogenic metabolic characteristics, SCD1-deficient mice have increased inflammation and atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 3, pp. 341–347, 2009.

[31] J. M. Brown, S. Chung, J. K. Sawyer et al., "Combined therapy of dietary fish oil and stearoyl-CoA desaturase 1 inhibition prevents the metabolic syndrome and atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, no. 1, pp. 24–30, 2010.

[32] R. S. Chapkin, N. Wang, Y.-Y. Fan, J. R. Lupton, and I. A. Prior, "Docosahexaenoic acid alters the size and distribution of cell surface microdomains," *Biochimica et Biophysica Acta*, vol. 1778, no. 2, pp. 466–471, 2008.

[33] Q. Li, M. Wang, L. Tan et al., "Docosahexaenoic acid changes lipid composition and interleukin-2 receptor signaling in membrane rafts," *Journal of Lipid Research*, vol. 46, no. 9, pp. 1904–1913, 2005.

[34] T. M. Stulnig, J. Huber, N. Leitinger et al., "Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition," *Journal of Biological Chemistry*, vol. 276, no. 40, pp. 37335–37340, 2001.

[35] M. B. Fessler, L. L. Rudel, and J. M. Brown, "Toll-like receptor signaling links dietary fatty acids to the metabolic syndrome," *Current Opinion in Lipidology*, vol. 20, no. 5, pp. 379–385, 2009.

[36] B. S. Park, D. H. Song, H. M. Kim, B.-S. Choi, H. Lee, and J.-O. Lee, "The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex," *Nature*, vol. 458, no. 7242, pp. 1191–1195, 2009.

[37] C. Erridge and N. J. Samani, "Saturated fatty acids do not directly stimulate toll-like receptor signaling," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 11, pp. 1944–1949, 2009.

[38] B. Gao and M.-F. Tsan, "Recombinant human heat shock protein 60 does not induce the release of tumor necrosis factor α from murine macrophages," *Journal of Biological Chemistry*, vol. 278, no. 25, pp. 22523–22529, 2003.

[39] K. E. Taylor, J. C. Giddings, and C. W. Van Den Berg, "C-reactive protein-induced in vitro endothelial cell activation is an artefact caused by azide and lipopolysaccharide," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 6, pp. 1225–1230, 2005.

[40] J. Cohen, "The detection and interpretation of endotoxaemia," *Intensive Care Medicine, Supplement*, vol. 26, supplement 1, pp. S51–S56, 2000.

[41] P. Brun, I. Castagliuolo, V. Di Leo et al., "Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis," *American Journal of Physiology*, vol. 292, no. 2, pp. G518–G525, 2007.

[42] P. D. Cani and N. M. Delzenne, "The role of the gut microbiota in energy metabolism and metabolic disease," *Current Pharmaceutical Design*, vol. 15, no. 13, pp. 1546–1558, 2009.

[43] C. Erridge, T. Attina, C. M. Spickett, and D. J. Webb, "A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation," *American Journal of Clinical Nutrition*, vol. 86, no. 5, pp. 1286–1292, 2007.

[44] A. Spruss, G. Kanuri, S. Wagnerberger, S. Haub, S. C. Bischoff, and I. Bergheim, "Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice," *Hepatology*, vol. 50, no. 4, pp. 1094–1104, 2009.

[45] S. Ghoshal, J. Witta, J. Zhong, W. de Villiers, and E. Eckhardt, "Chylomicrons promote intestinal absorption of lipopolysaccharides," *Journal of Lipid Research*, vol. 50, no. 1, pp. 90–97, 2009.

[46] M. D. Neal, C. Leaphart, R. Levy et al., "Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier," *Journal of Immunology*, vol. 176, no. 5, pp. 3070–3079, 2006.

[47] S. J. Creely, P. G. McTernan, C. M. Kusminski et al., "Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes," *American Journal of Physiology*, vol. 292, no. 3, pp. E740–E747, 2007.

[48] M. R. Dasu, S. Devaraj, S. Park, and I. Jialal, "Increased Toll-Like Receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects," *Diabetes Care*, vol. 33, no. 4, pp. 861–868, 2010.

[49] P. D. Cani, J. Amar, M. A. Iglesias et al., "Metabolic endotoxemia initiates obesity and insulin resistance," *Diabetes*, vol. 56, no. 7, pp. 1761–1772, 2007.

[50] F. Bäckhed, J. K. Manchester, C. F. Semenkovich, and J. I. Gordon, "Mechanisms underlying the resistance to diet-induced obesity in germ-free mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 3, pp. 979–984, 2007.

[51] P. D. Cani, R. Bibiloni, C. Knauf et al., "Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice," *Diabetes*, vol. 57, no. 6, pp. 1470–1481, 2008.

[52] M. Membrez, F. Blancher, M. Jaquet et al., "Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice," *FASEB Journal*, vol. 22, no. 7, pp. 2416–2426, 2008.

[53] F. Kim, M. Pham, I. Luttrell et al., "Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity," *Circulation Research*, vol. 100, no. 11, pp. 1589–1596, 2007.

[54] M. Saberi, N.-B. Woods, C. de Luca et al., "Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice," *Cell Metabolism*, vol. 10, no. 5, pp. 419–429, 2009.

[55] J. E. Davis, N. K. Gabler, J. Walker-Daniels, and M. E. Spurlock, "Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat," *Obesity*, vol. 16, no. 6, pp. 1248–1255, 2008.

[56] K. R. Coenen, M. L. Gruen, R. S. Lee-Young, M. J. Puglisi, D. H. Wasserman, and A. H. Hasty, "Impact of macrophage toll-like receptor 4 deficiency on macrophage infiltration into adipose tissue and the artery wall in mice," *Diabetologia*, vol. 52, no. 2, pp. 318–328, 2009.

[57] K. S. Michelsen, M. H. Wong, P. K. Shah et al., "Lack of toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 29, pp. 10679–10684, 2004.

[58] H. Björkbacka, V. V. Kunjathoor, K. J. Moore et al., "Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways," *Nature Medicine*, vol. 10, no. 4, pp. 416–421, 2004.

[59] R. Roncon-Albuquerque Jr., M. Moreira-Rodrigues, B. Faria et al., "Attenuation of the cardiovascular and metabolic complications of obesity in CD14 knockout mice," *Life Sciences*, vol. 83, no. 13-14, pp. 502–510, 2008.

[60] R. A. Bagarolli, M. J. A. Saad, and S. T. O. Saad, "Toll-like receptor 4 and inducible nitric oxide synthase gene polymorphisms are associated with type 2 diabetes," *Journal of Diabetes and Its Complications*, vol. 24, no. 3, pp. 192–198, 2010.

[61] B. Ferwerda, M. B. B. McCall, K. Verheijen et al., "Functional consequences of toll-like receptor 4 polymorphisms," *Molecular Medicine*, vol. 14, no. 5-6, pp. 346–352, 2008.

[62] A. P. Steinhardt, F. Aranguren, M. L. Tellechea et al., "A functional nonsynonymous toll-like receptor 4 gene polymorphism is associated with metabolic syndrome, surrogates of insulin resistance, and syndromes of lipid accumulation," *Metabolism*, vol. 59, no. 5, pp. 711–717, 2010.

[63] R. Coppari, M. Ichinose, C. E. Lee et al., "The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity," *Cell Metabolism*, vol. 1, no. 1, pp. 63–72, 2005.

[64] A. Pocai, K. Morgan, C. Buettner, R. Gutierrez-Juarez, S. Obici, and L. Rossetti, "Central leptin acutely reverses diet-induced hepatic insulin resistance," *Diabetes*, vol. 54, no. 11, pp. 3182–3189, 2005.

[65] S. Galic, J. S. Oakhill, and G. R. Steinberg, "Adipose tissue as an endocrine organ," *Molecular and Cellular Endocrinology*, vol. 316, no. 2, pp. 129–139, 2009.

[66] S. P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante Jr., "Obesity is associated with macrophage accumulation in adipose tissue," *Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1796–1808, 2003.

[67] H. Xu, G. T. Barnes, Q. Yang et al., "Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance," *Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1821–1830, 2003.

[68] P. J. Bilan, V. Samokhvalov, A. Koshkina, J. D. Schertzer, M. C. Samaan, and A. Klip, "Direct and macrophage-mediated actions of fatty acids causing insulin resistance in muscle cells," *Archives of Physiology and Biochemistry*, vol. 115, no. 4, pp. 176–190, 2009.

[69] D. Patsouris, P.-P. Li, D. Thapar, J. Chapman, J. M. Olefsky, and J. G. Neels, "Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals," *Cell Metabolism*, vol. 8, no. 4, pp. 301–309, 2008.

[70] S. Cinti, G. Mitchell, G. Barbatelli et al., "Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans," *Journal of Lipid Research*, vol. 46, no. 11, pp. 2347–2355, 2005.

[71] M. Yuan, N. Konstantopoulos, J. Lee et al., "Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ," *Science*, vol. 293, no. 5535, pp. 1673–1677, 2001.

[72] M. C. Arkan, A. L. Hevener, F. R. Greten et al., "IKK-β links inflammation to obesity-induced insulin resistance," *Nature Medicine*, vol. 11, no. 2, pp. 191–198, 2005.

[73] G. Solinas, C. Vilcu, J. G. Neels et al., "JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity," *Cell Metabolism*, vol. 6, no. 5, pp. 386–397, 2007.

[74] J. K. Kim, J. J. Fillmore, M. J. Sunshine et al., "PKC-θ knockout mice are protected from fat-induced insulin resistance," *Journal of Clinical Investigation*, vol. 114, no. 6, pp. 823–827, 2004.

[75] H.-M. Zhang, L.-L. Chen, L. Wang et al., "Macrophage infiltrates with high levels of Toll-like receptor 4 expression in white adipose tissues of male Chinese," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 19, no. 10, pp. 736–743, 2009.

[76] M. T. A. Nguyen, S. Favelyukis, A.-K. Nguyen et al., "A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways," *Journal of Biological Chemistry*, vol. 282, no. 48, pp. 35279–35292, 2007.

[77] S. Bès-Houtmann, R. Roche, L. Hoareau et al., "Presence of functional TLR2 and TLR4 on human adipocytes," *Histochemistry and Cell Biology*, vol. 127, no. 2, pp. 131–137, 2007.

[78] M. J. Song, K. H. Kim, J. M. Yoon, and J. B. Kim, "Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes," *Biochemical and Biophysical Research Communications*, vol. 346, no. 3, pp. 739–745, 2006.

[79] O. I. Vitseva, K. Tanriverdi, T. T. Tchkonia et al., "Inducible Toll-like receptor and NF-κB regulatory pathway expression in human adipose tissue," *Obesity*, vol. 16, no. 5, pp. 932–937, 2008.

[80] K. Hoshino, O. Takeuchi, T. Kawai et al., "Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide evidence for TLR4 as the Lps gene

product," *Journal of Immunology*, vol. 162, no. 7, pp. 3749–3752, 1999.

[81] A. Poltorak, X. He, I. Smirnova et al., "Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene," *Science*, vol. 282, no. 5396, pp. 2085–2088, 1998.

[82] C. R. H. Raetz, "Biochemistry of endotoxins," *Annual Review of Biochemistry*, vol. 59, pp. 129–170, 1990.

[83] Y. Lin, H. Lee, A. H. Berg, M. P. Lisanti, L. Shapiro, and P. E. Scherer, "The lipopolysaccharide-activated Toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes," *Journal of Biological Chemistry*, vol. 275, no. 32, pp. 24255–24263, 2000.

[84] J. Y. Lee and D. H. Hwang, "The modulation of inflammatory gene expression by lipids: mediation through Toll-like receptors," *Molecules and Cells*, vol. 21, no. 2, pp. 174–185, 2006.

[85] L. H. Storlien, E. W. Kraegen, and D. J. Chisholm, "Fish oil prevents insulin resistance induced by high-fat feeding in rats," *Science*, vol. 237, no. 4817, pp. 885–888, 1987.

[86] T. Suganami, T. Mieda, M. Itoh, Y. Shimoda, Y. Kamei, and Y. Ogawa, "Attenuation of obesity-induced adipose tissue inflammation in C3H/HeJ mice carrying a Toll-like receptor 4 mutation," *Biochemical and Biophysical Research Communications*, vol. 354, no. 1, pp. 45–49, 2007.

[87] D. M. L. Tsukumo, M. A. Carvalho-Filho, J. B. C. Carvalheira et al., "Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance," *Diabetes*, vol. 56, no. 8, pp. 1986–1998, 2007.

[88] M. Poggi, D. Bastelica, P. Gual et al., "C3H/HeJ mice carrying a Toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet," *Diabetologia*, vol. 50, no. 6, pp. 1267–1276, 2007.

[89] T. Suganami, K. Tanimoto-Koyama, J. Nishida et al., "Role of the Toll-like receptor 4/NF-κB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 1, pp. 84–91, 2007.

[90] V. D. F. De Mello, M. Kolehmainen, L. Pulkkinen et al., "Downregulation of genes involved in NFκB activation in peripheral blood mononuclear cells after weight loss is associated with the improvement of insulin sensitivity in individuals with the metabolic syndrome: the GENOBIN study," *Diabetologia*, vol. 51, no. 11, pp. 2060–2067, 2008.

[91] L. Bouwens, M. Baekeland, R. De Zanger, and E. Wisse, "Quantitation, tissue distribution and proliferation kinetics of Kupffer cells in normal rat liver," *Hepatology*, vol. 6, no. 4, pp. 718–722, 1986.

[92] K. Miura, et al., "Role of Toll-like receptors and their downstream molecules in the development of non-alcoholic fatty liver disease," *Gastroenterology Research and Practice*. In press.

[93] G. Baffy, "Kupffer cells in non-alcoholic fatty liver disease: the emerging view," *Journal of Hepatology*, vol. 51, no. 1, pp. 212–223, 2009.

[94] L. A. Videla, G. Tapia, R. Rodrigo et al., "Liver NF-κB and AP-1 DNA binding in obese patients," *Obesity*, vol. 17, no. 5, pp. 973–979, 2009.

[95] C. F. Raetzsch, N. L. Brooks, J. M. Alderman et al., "Lipopolysaccharide inhibition of glucose production through the Toll-like receptor-4, myeloid differentiation factor 88, and nuclear factor κB pathway," *Hepatology*, vol. 50, no. 2, pp. 592–600, 2009.

[96] R. A. DeFronzo, R. Gunnarsson, and O. Bjorkman, "Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus," *Journal of Clinical Investigation*, vol. 76, no. 1, pp. 149–155, 1985.

[97] A. D. Baron, G. Brechtel, P. Wallace, and S. V. Edelman, "Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans," *American Journal of Physiology*, vol. 255, no. 6, part 1, pp. E769–E774, 1988.

[98] O. G. Kolterman, J. Insel, M. Saekow, and J. M. Olefsky, "Mechanisms of insulin resistance in human obesity. Evidence for receptor and postreceptor defects," *Journal of Clinical Investigation*, vol. 65, no. 6, pp. 1272–1284, 1980.

[99] G. S. Hotamisligil, P. Peraldi, A. Budavari, R. Ellis, M. F. White, and B. M. Spiegelman, "IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance," *Science*, vol. 271, no. 5249, pp. 665–668, 1996.

[100] M. I. Frisard, R. P. McMillan, J. Marchand et al., "Toll-like receptor 4 modulates skeletal muscle substrate metabolism," *American Journal of Physiology*, vol. 298, no. 5, pp. E988–E998, 2010.

[101] Y. Wei, K. Chen, A. T. Whaley-Connell, C. S. Stump, J. A. Ibdah, and J. R. Sowers, "Skeletal muscle insulin resistance: role of inflammatory cytokines and reactive oxygen species," *American Journal of Physiology*, vol. 294, no. 3, pp. R673–R680, 2008.

[102] J. H. Boyd, M. Divangahi, L. Yahiaoui, D. Gvozdic, S. Qureshi, and B. J. Petrof, "Toll-like receptors differentially regulate CC and CXC chemokines in skeletal muscle via NF-κB and calcineurin," *Infection and Immunity*, vol. 74, no. 12, pp. 6829–6838, 2006.

[103] S. M. Reyna, S. Ghosh, P. Tantiwong et al., "Elevated Toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects," *Diabetes*, vol. 57, no. 10, pp. 2595–2602, 2008.

[104] J. J. Senn, "Toll-like receptor-2 is essential for the development of palmitate-induced insulin resistance in myotubes," *Journal of Biological Chemistry*, vol. 281, no. 37, pp. 26865–26875, 2006.

[105] D. A. Sandoval, S. Obici, and R. J. Seeley, "Targeting the CNS to treat type 2 diabetes," *Nature Reviews Drug Discovery*, vol. 8, no. 5, pp. 386–398, 2009.

[106] S.-H. Chiang, M. Bazuine, C. N. Lumeng et al., "The protein kinase IKKε regulates energy balance in obese mice," *Cell*, vol. 138, no. 5, pp. 961–975, 2009.

[107] C. T. De Souza, E. P. Araujo, S. Bordin et al., "Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus," *Endocrinology*, vol. 146, no. 10, pp. 4192–4199, 2005.

[108] A. Kleinridders, D. Schenten, A. C. Könner et al., "MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity," *Cell Metabolism*, vol. 10, no. 4, pp. 249–259, 2009.

[109] J. C. Moraes, A. Coope, J. Morari et al., "High-fat diet induces apoptosis of hypothalamic neurons," *PLoS ONE*, vol. 4, no. 4, article e5045, 2009.

[110] L. Wen, J. Peng, Z. Li, and F. S. Wong, "The effect of innate immunity on autoimmune diabetes and the expression of Toll-like receptors on pancreatic islets," *Journal of Immunology*, vol. 172, no. 5, pp. 3173–3180, 2004.

[111] A. Goldberg, M. Parolini, B. Y. Chin et al., "Toll-like receptor 4 suppression leads to islet allograft survival," *FASEB Journal*, vol. 21, no. 11, pp. 2840–2848, 2007.

[112] H. S. Kim, M. S. Han, K. W. Chung et al., "Toll-like receptor 2 senses β-cell death and contributes to the initiation of autoimmune diabetes," *Immunity*, vol. 27, no. 2, pp. 321–333, 2007.

[113] L. Wen, R. E. Ley, P. Y. Volchkov et al., "Innate immunity and intestinal microbiota in the development of type 1 diabetes," *Nature*, vol. 455, no. 7216, pp. 1109–1113, 2008.

[114] F. S. Wong, C. Hu, L. Zhang et al., "The role of Toll-like receptors 3 and 9 in the development of autoimmune diabetes in NOD mice," *Annals of the New York Academy of Sciences*, vol. 1150, pp. 146–148, 2008.

[115] F. T. Schulthess, F. Paroni, N. S. Sauter et al., "CXCL10 impairs β cell function and viability in diabetes through TLR4 signaling," *Cell Metabolism*, vol. 9, no. 2, pp. 125–139, 2009.

[116] M. Böni-Schnetzler, S. Boller, S. Debray et al., "Free fatty acids induce a proinflammatory response in islets via the abundantly expressed interleukin-1 receptor I," *Endocrinology*, vol. 150, no. 12, pp. 5218–5229, 2009.

[117] X. Li, J. C. Tupper, D. D. Bannerman, R. K. Winn, C. J. Rhodes, and J. M. Harlan, "Phosphoinositide 3 kinase mediates Toll-like receptor 4-induced activation of NF-κB in endothelial cells," *Infection and Immunity*, vol. 71, no. 8, pp. 4414–4420, 2003.

[118] I. Marriott and Y. M. Huet-Hudson, "Sexual dimorphism in innate immune responses to infectious organisms," *Immunologic Research*, vol. 34, no. 3, pp. 177–192, 2006.

[119] M.-P. Piccinni, E. Maggi, and S. Romagnani, "Role of hormone-controlled T-cell cytokines in the maintenance of pregnancy," *Biochemical Society Transactions*, vol. 28, no. 2, pp. 212–215, 2000.

[120] R. Raghupathy, "Th1-type immunity is incompatible with successful pregnancy," *Immunology Today*, vol. 18, no. 10, pp. 478–482, 1997.

[121] T. G. Wegmann, H. Lin, L. Guilbert, and T. R. Mosmann, "Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon?" *Immunology Today*, vol. 14, no. 7, pp. 353–356, 1993.

[122] J. P. Frias, G. B. Macaraeg, J. Ofrecio, J. G. Yu, J. M. Olefsky, and Y. T. Kruszynska, "Decreased susceptibility to fatty acid-induced peripheral tissue insulin resistance in women," *Diabetes*, vol. 50, no. 6, pp. 1344–1350, 2001.

[123] A. Hevener, D. Reichart, A. Janez, and J. Olefsky, "Female rats do not exhibit free fatty acid-induced insulin resistance," *Diabetes*, vol. 51, no. 6, pp. 1907–1912, 2002.