Review

Toll-like Receptor Status in Obesity and Metabolic Syndrome: A Translational Perspective

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Context: The prevalence of both obesity and metabolic syndrome (MetS) is increasing at alarming rates globally. Both predispose to diabetes, cardiovascular disease, fatty liver disease, obstructive sleep apnea, and certain cancers. Understanding the mechanisms contributing to increased cardiometabolic risk in obesity and MetS is of utmost importance.

Evidence Acquisition: For this review, we performed a detailed literature search on PubMed of all publications related to Toll-like receptors (TLRs) and obesity and MetS for the last 20 years.

Evidence Synthesis: The TLRs are well-characterized immune receptors that enhance inflammation. The recognition of pathogen-associated molecular patterns and endogenous (host-derived) ligands released by various cell types triggers activation and expression of TLRs. TLRs, especially TLR2 and TLR4, induce insulin resistance, which is pivotal in the pathogenesis of obesity and MetS. Both obesity and MetS are characterized by low-grade chronic inflammation, possibly triggered by activation of TLR2 and TLR4. TLRs, especially TLR4, are activated by fatty acids and endotoxinemia (a marker of gut permeability), features of both obesity and MetS, resulting in activation of nuclear factor-κB and increased release of inflammatory biomediators such as IL-6, IL-1β, TNF-α, and monocyte chemotactic protein-1, which play a role in the pathophysiology of obesity and MetS. Reduction of calories, exercise, and nutraceutical and pharmacological agents can modulate TLRs.

Conclusions: In this review, we present evidence for a pivotal role of TLR-induced inflammation in both obesity and MetS and speculate that targeting these TLRs can forestall their adverse sequelae of diabetes and cardiovascular disease. **(*J Clin Endocrinol Metab* 99: 39–48, 2014)**

Obesity is a worldwide epidemic, and approximately 700 million adults will be obese by 2015 (1). More than one-third of US adults (35.7%) and approximately 17% (or 12.5 million) of children and adolescents are obese in the United States (1, 2). Obesity is a major risk factor for the development of metabolic syndrome (MetS) and other associated health complications (3–6). MetS, which afflicts 35% of American adults over 20 years of age, constitutes a constellation of cardiometabolic risk factors including abdominal obesity, hyperglycemia, hypertension, and dyslipidemia comprising high triglycerides and/or low levels of high-density lipoprotein-cholesterol (7). It is important to emphasize that the diagnosis has recently been harmonized globally (7). Insulin resistance and low-grade inflammation are common manifestations and could play a role in the pathogenesis of obesity and MetS and its sequelae (4–8). There appears to be a strong correlation between insulin resistance and low-grade inflammation.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received August 6, 2013. Accepted October 25, 2013.
First Published Online November 1, 2013

Abbreviations: BMI, body mass index; CCR, C-C chemokine receptor; CRP, C-reactive protein; CVD, cardiovascular disease; DAMP, damage-associated molecular pattern; DIO, diet-induced obesity; FFA, free fatty acid; HFD, high-fat diet; HMGB1, high-mobility group box 1; HSP, heat shock protein; IKK, IκB kinase; IRAK, IL-1 receptor associated kinase; JNK, Jun N-terminal kinase; KO, knockout; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; MetS, metabolic syndrome; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; Ox-LDL, oxidized LDL; PAI-1, plasminogen activator inhibitor-1; PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood mononuclear cell; PM, peripheral monocyte; SAT, sc adipose tissue; SFA, saturated fatty acid; T2DM, type 2 diabetes mellitus; TLR, Toll-like receptor; TRIF, Toll/IL-1 receptor domain containing adaptor protein-inducing interferon-β; VAT, visceral adipose tissue.

Dysregulation of adipose tissue biology plays a potential role in the initiation of inflammatory events in obesity and MetS causing release of biomediators, such as monocyte chemotactic protein-1 (MCP-1), that induce macrophage infiltration in adipose tissue. Activated macrophages both resident and immigrant in the adipose tissue further trigger an inflammatory cascade causing release of various proinflammatory cytokines such as TNF-α, IL-6, etc, via nuclear factor-κB (NF-κB) activation and signaling (6). The major complications associated with increased obesity and MetS include type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), fatty liver disease, obstructive sleep apnea, and certain obesity-related cancers (6, 7). Recently, studies have provided insight into the expression and activation of innate immune receptors such as Toll-like receptors (TLRs) in various cells. TLRs appear to contribute to the chronic inflammatory state of obesity and MetS. Because both obesity and MetS presage diabetes and CVD, understanding the status of TLRs in these common disorders will clearly provide insights into their sequelae and management. Hence, the main focus of this review will be on the potential role of TLRs in human obesity and MetS.

Toll-like Receptors

TLRs are transmembrane pattern recognition receptors that play a potential role in pathogen recognition and innate immune response by activating various inflammatory signaling pathways as depicted in Figure 1 (9). There are more than 10 members known in the mammalian TLR family (11 in humans and 13 in mice). Most TLRs are on the cell surface, except for TLR3, -7, -8, and -9, which reside mainly in the endosomes. TLRs initiate an immune response after recognition of specific ligands such as pathogen-associated molecular patterns (PAMPs) derived from pathogens as well as tissue damage and inflamma-

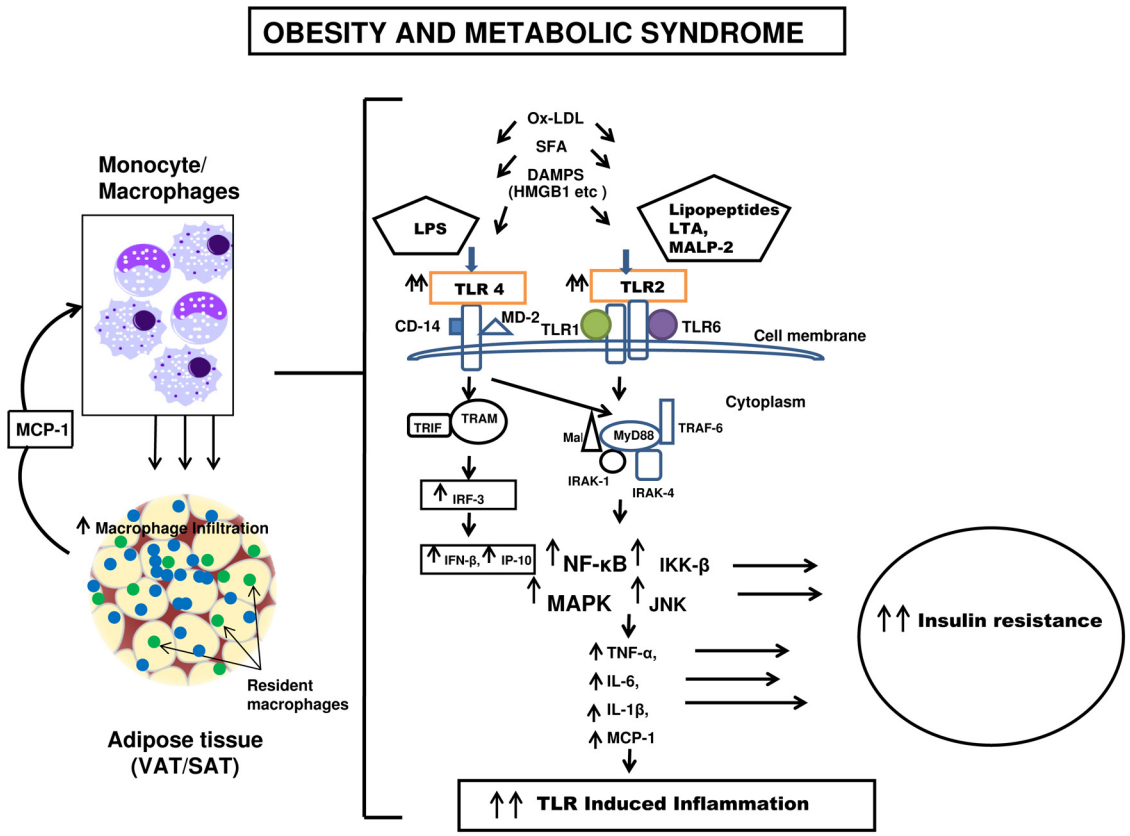

Figure 1. Schema depicting role of TLR2 and TLR4 in obesity- and MetS-induced inflammation and insulin resistance. TLR2 and TLR4 are increased in adipose tissue of obese and MetS subjects, depicted by both resident (green) and immigrant macrophages (blue) and circulating monocytes/macrophages. They activate downstream signaling proteins via MyD88 and non-MyD88 pathways leading to activation of IKK-β-NF-κB, JNK resulting in increased inflammation. Potential agonists for TLRs include LPS, lipopeptides, DAMPs, SFA, and Ox-LDL. In addition, adipose tissue undergoes expansion, releasing FFAs, cytokines, and chemokines such as MCP-1, etc, which further plays a role in recruitment of monocytes/macrophages that release IL-6, TNF-α, and IL-1β. Biomediators of the TLR pathway, eg, JNK, IKK-β, TNF, IL-1, and IL-6, induce insulin resistance by impairing insulin receptor substrate-1 (IRS-1) and AKT phosphorylation, glucose transporter type 4 translocation, etc (81). ↑ refers to increasing levels. MALP, macrophage-activating lipopeptide (TLR2/6 agonist); IP-10, interferon-γ inducible protein 10; LTA, lipoteichoic acid; TRAM, TRIF- related adaptor molecule; IRF-3, interferon regulatory factor 3; TRAF-6, TNF-associated factor 6.

tion-induced nonmicrobe danger signals such as damage-associated molecular patterns (DAMPs) (9, 10). Various endogenous ligands, such as saturated fatty acids (SFAs), modified low-density lipoproteins (LDLs), heat shock proteins (HSPs), high-mobility group box 1 (HMGB1), extracellular matrix degradation products, and advanced glycation end-products, act as DAMPs and are recognized by TLRs, especially TLR2 or TLR4, triggering a proinflammatory response as shown in Figure 1 (9, 10).

MyD88 and Non-MyD88 Pathways in TLR Signaling

The recognition of ligands by TLRs triggers activation of various adaptor proteins of the myeloid differentiation factor 88 (MyD88)-dependent pathway or non-MyD88 downstream signaling pathways (TLR4 and TLR3) causing subsequent inflammatory responses (Figure 1). Of various TLRs, TLR2 and TLR4 signaling cascades are the best studied as they relate to metabolism and CVD. TLR2 forms a heterodimer with either TLR1 or TLR6 upon recognition of PAMPs such as bacterial lipopeptides or mycobacterial lipopeptides, respectively. This leads to activation of cytoplasmic adaptor proteins such as MyD88, which in turn causes phosphorylation and activation of IL-1 receptor associated kinase (IRAK). IRAK-1 along with TNF-associated factor 6 subsequently leads to activation of IκB kinase (IKK) complex MAPKs, causing release of various biomediators such as IL-6, TNF-α, MCP-1, and IL-1β via activation of NF-κB as shown in Figure 1 (5, 9, 10). TLR5, which is localized on the cell surface, also recognizes PAMPs present on the outer membrane of Gram-negative bacteria. Bacterial flagellin, a component of flagella, stimulates TLR5 expressed on the basolateral surface of intestinal epithelium only if it crosses intestinal epithelium, unlike TLR2 and TLR4 which recognize PAMPs on the apical surface. Activation of TLR5 further mediates its inflammatory response via MyD88 signaling similar to TLR2 and TLR4 (9).

TLR4 responds to ligands such as lipopolysaccharide (LPS), also referred to as endotoxin, and initiates its response by forming a complex with myeloid differentiation factor 2 (MD-2) leading to activation of both MyD88-dependent and non-MyD88-dependent signaling cascades. The non-MyD88 pathway recruits and activates adaptor proteins such as Toll/IL-1 receptor domain containing adaptor protein-inducing interferon-β (TRIF) and TRIF-related adaptor molecule, followed by subsequent activation of interferon regulatory factor 3 and releasing type 1 interferons and interferon-γ inducible protein 10 (9, 10). NF-κB serves as an important key transcriptional factor initiating an inflammatory cascade (Figure 1) (9). Thus, TLR-mediated activation of NF-κB is a pivotal regulator controlling the expression of various inflammatory cytokines, such as IL-6, TNF-α, MCP-1, IL-1β, etc (9), as depicted in Figure 1. Various studies have provided evidence of increased expression of TLRs, especially TLR2 and TLR4, in atherosclerotic plaques and animal models of atherosclerosis, obesity, and nonalcoholic steatohepatitis (4, 5, 10, 11). Knockout (KO) of either TLR2 or TLR4 results in decreased atherosclerosis in animal models of atherosclerosis (11). TLR4KO or MyD88KO mice fed a high-fat diet (HFD) showed significant reduction of aortic plaque areas, plaque lipid content, macrophage infiltration, and circulating levels of proinflammatory cytokines in atherosclerosis-prone apolipoprotein E-deficient mice (5, 11). Thus, both TLR2 and TLR4 can contribute to the increased risk of CVD in both obesity and MetS.

The major focus of this review is the evolving role of TLRs in inflammation, insulin resistance, and adiposity because these are harbingers of T2DM, the major sequelae of both obesity and MetS.

Markers of Inflammation in Obesity and MetS

Chronic low-grade inflammation and insulin resistance are key characteristics contributing to obesity and MetS. Concerning obesity, there appears to be controversy and conflict with respect to the subtypes, eg, metabolically healthy obese, especially criteria used to define subtypes, and future risk for diabetes and CVD (12–15). By most definitions, metabolically healthy obese appears to be typified by a low inflammatory burden (16) and, in the largest study, appeared to account for 32% of obese persons (17). Be that as it may, most studies define obesity as a proinflammatory state (18). On the other hand, MetS can occur in both obese and nonobese persons, and it connotes a high cardiometabolic risk burden in both.

As we have reviewed previously (6), MetS is a proinflammatory state characterized by increased levels of the prototypic marker of inflammation, C-reactive protein (CRP), as well as proinflammatory cytokines and chemokines and plasminogen activator inhibitor-1 (PAI-1). Adipose tissue plays a critical role in obesity-induced inflammation and acts as a source of increased cytokines, probably as a result of increased macrophage activity into the adipose tissue (19). The circulating biomarkers of inflammation that are dysregulated in MetS and obesity are summarized in Table 1.

Table 1. Biomarkers of Inflammation That Are Dysregulated in Obesity and MetS

↑ High-sensitivity CRP, serum amyloid A, fibrinogen
↓ Adiponectin, omentin
↑ Leptin, PAI-1, chemerin
Cytokines/chemokines: ↑ IL-1, IL-6, IL-8, MCP-1, TNF; and ↓ IL-10
↑ Monocytic TLR2 and TLR4

↑ represents increased expression, and ↓ represents decreased expression.

TLRs in Obesity and MetS

TLR activation in obesity

In vitro studies

Obesity is associated with elevated levels of circulating free fatty acids (FFAs), which can activate various proinflammatory pathways promoting insulin resistance (20). SFAs enhance TLR signaling and activity of NF-κB in macrophages (21). Youssef-Elabd et al (22) demonstrated that exposure of human abdominal sc adipose tissue (SAT) explants and adipocytes (n = 6) to SFAs (2 mM) stimulates TLR4 signaling, MyD88 expression, and up-regulated NF-κB activity with significantly increased secretion of IL-6 and TNF-α. In addition to FFA, TLR2 agonists (Pam3CSK4) or TLR4 agonists (LPS) activate TLR2/4 expression on macrophages, adipocytes, and adipose tissue, resulting in increased release of various inflammatory biomediators (5, 23–25). These studies clearly document TLRs in adipose tissue and suggest a role of TLR-mediated dysregulation in obesity-induced inflammation.

Animal studies

Studies in animal models have provided evidence for a role for TLR activity in the development of insulin resistance and obesity. Murakami et al (26) found increased expression of TLR2 in visceral adipose tissue (VAT) in contrast to SAT of mice fed a HFD compared to controls. The expression of TLR2 also induced expression of TNF-α in murine fat tissues. Caricilli et al (27) demonstrated that suppression of TLR2 using TLR2 antisense oligonucleotide improves insulin sensitivity and signaling with decreased expression of IKK-β and MAPK8 in muscle and white adipose tissue of diet-induced obesity (DIO) mice models. Ehses et al (28) also reported that TLR2KO mice on a HFD-induced obesity background had less tissue inflammation, increased insulin sensitivity, insulin secretion, and improved glucose tolerance, suggesting that targeting TLR2 could be a strategy to ameliorate the insulin resistance and inflammation induced by DIO.

Studies have also reported that increased circulating levels of FFAs contribute to visceral obesity and promote fat accumulation in skeletal muscle and liver with concomitant insulin resistance (20). Shi et al (23) reported that FFAs result in increased TLR4 signaling in adipose tissue, liver, and macrophages in mice. Song et al (29) demonstrated enhanced TLR4 mRNA expression in adipose tissue of obese db/db mice, suggesting the potential link of TLR4 signaling in obesity and inflammation. Tsukumo et al (30) further observed reduced adiposity, enhanced insulin signaling in adipose tissue, muscle, and liver, and inhibition of IKK-β in preventing DIO in C3H/HeJ mice (that have a loss of function point mutation of TLR4), compared to control animals fed a HFD. Other groups have also shown that inactivation of TLR4 resulted in improved insulin resistance and tissue inflammation in DIO mice models (5, 11). Loss of function mutation in TLR4 gene was also found to decrease macrophage infiltration and promote polarization of macrophages to the M2 reparative phenotype (31).

More recently, Kim et al (32) provided evidence of increased expression of TLR1–9 and TLR11–13 in murine adipose tissue in response to obesity induced by HFD or leptin deficiency (ob/ob mice), causing activation of MyD88 and non-MyD88 signaling cascades as well as downstream up-regulation of NF-κB activity and subsequent release of chemokines and cytokines. Obesity-induced overexpression of TLR genes and the associated inflammatory signaling cascade were more pronounced in VAT than SAT and were greater in DIO mice than ob/ob mice. However, a weakness of this study was that all readouts were mRNA and not the proteins.

Thus, collectively the above studies in animal models indicate that TLR2 and TLR4 expression is increased in obesity in most studies. Furthermore, global deficiency of TLR2 and TLR4 in DIO animal models ameliorates inflammation, insulin sensitivity, and weight gain (in few studies) pointing to a pathogenic role of TLRs in obesity and insulin resistance via induction of inflammation (5, 23, 27, 28, 30).

Human studies

Similar to animal studies, human studies (Table 2) also provide evidence of enhanced TLR2/4 activation in adipose tissue and peripheral monocytes (PMs) of obese subjects. Creely et al (24) found increased TLR2 expression and activity in abdominal SAT of obese (n = 5) compared to lean control subjects (n = 5). In this small study, they showed increased plasma endotoxin (LPS) levels, the classical ligand for TLR4; however, TLR4 was not increased in their obese patients. They did not report on TLR-mediated signaling in obese subjects vs lean controls (Table 2). Reyna et al (33) demonstrated increased expression of TLR4 with enhanced NF-κB activity and subsequently increased release of IL-6 in muscle biopsies of obese subjects

Table 2. TLR Expression and Activity in Human Obesity and Nascent MetS

TLR Expression	Tissue	Diagnosis	Downstream Targets	Cytokine/ Chemokines	First Author, Year (Ref.)
↑ TLR2 (mRNA, protein), no effect on TLR4 (protein)	Abdominal SAT	Obese (n = 5)	NA	NA	Creely, 2007 (24)
↑ TLR4 (mRNA and protein)	Muscle biopsies	Obese (n = 8)	↑ NF-κB	↑ IL-6	Reyna, 2008 (33)
↑ TLR1, ↑ TLR2 (mRNA) in OFT	OFT, SAT	Obese subjects (n = 12)	NA	NA	Poulain-Godefroy, 2010 (34)
↑ TLR4 (mRNA) in PMs, no significant difference in SAT	PMs, SAT	Obese (n = 8)	NA	PMs: ↑ IL-8, ↑ CCR1, ↑ CCR2, ↑ CCR3	Mraz, 2011 (35)
↑ TLR4 (mRNA) in VAT	VAT, SAT	Obese (n = 32)	NA	NA	Catalán, 2012 (36)
No effect on TLR2, TLR4 (protein)	TLR4 agonists (LPS) and TLR2 agonist (PAM) stimulated whole blood monocytes	Obese subjects with established coronary (n = 78) or carotid artery (n = 104) disease	NA	↑ TNF-α	Scholtes, 2011 (37)
↑ TLR2, ↑ TLR4 (mRNA) in PBMCs and SAT of obese and overweight	PBMCs, SAT	Obese (n = 8), overweight (n = 7)	↑ MyD88, ↑ IRAK-1	↑ TNF-α, ↑ IL-6	Ahmad, 2012 (38)
↑ TLR2, ↑ TLR4 (mRNA, protein)	Monocytes	Nascent MetS (n = 49)	↑ NF-κB	↑ CRP, ↑ IL-6, ↑ IL-1β, ↑ IL-8, ↑ sTNFR1	Jialal, 2012 (45)
↑ TLR2, ↑ TLR4 (mRNA)	Monocytes	MetS (n = 9)	NA	↑ TNF-α, ↑ IL-6	Hardy, 2013 (48)

Abbreviations: NA, not available; OFT, omental fat tissue; PAM, tripalmitoylcysteinylseryl-(lysyl)4; sTNFR1, soluble TNF receptor-1. ↑ represents increased expression compared to matched controls.

(n = 8) compared to lean subjects (n = 7). Also, the circulating levels of FFAs were significantly higher in obese compared to lean subjects (Table 2). Poulain-Godefroy et al (34) reported significantly enhanced mRNA expression of TLR1, TLR2, TLR4, and TLR6 in omental fat tissue compared to sc fat tissue. Importantly, they demonstrated increased mRNA for TLR1 and TLR2 in omental tissue of obese normoglycemic subjects (n = 12) compared to lean controls (n = 7). However, they did not compare TLRs in SAT. They found correlations of TLR expression within and between each depot, hence providing evidence of TLR-induced inflammation occurring globally in adipose tissue in obesity (Table 2). Mraz et al (35) demonstrated significantly increased TLR4 mRNA along with increased mRNA expression of IL-8, C-C chemokine receptor (CCR) 1, CCR2, and CCR3 in PMs of obese nondiabetic subjects (n = 8) compared to healthy controls (n = 15). However, no significant differences were observed in the SAT of these obese subjects. The authors provided no explanation for these discrepant data. In this study, they did not report on receptor status or signaling but reported only mRNA. However, the SAT of obese subjects showed increased mRNA expression of IL-18 and numerous chemokines.

Vitseva et al (25) also reported significantly increased TLR4 mRNA and protein expression colocalized with adiponectin in adipocytes from human sc abdominal fat from obese subjects (n = 16) with increased NF-κB activity and release of IL-6 and TNF-α. However, a limitation of this study was the failure to compare TLR4 expression between obese and nonobese control subjects. Catalán et al (36) recently reported significantly increased TLR4 mRNA expression in VAT, but not SAT, in normoglycemic obese subjects (n = 32) compared to lean controls (n = 13) (Table 2).

Scholtes et al (37) also found significantly increased TLR2- and TLR4-induced signaling and inflammation in obese patients with atherosclerosis. Whole blood monocytes in obese patients with established coronary (n = 78) or carotid artery disease (n = 104) were exposed to TLR4 ligand (LPS) and TLR2 ligand (Pam3CSK4). There was a significant increase in TLR2/4-induced secretion of TNF-α in obese groups compared to nonobese patients. However, surprisingly, there was no increase in TLR2 or TLR4 expression assayed by flow cytometry. These patients had CVD and were on multiple therapies that could modulate TLR, explaining the failure to see an increase in TLR expression with agonists, or their methodology using whole blood could explain the failure to see up-regulated TLRs due to interferents in whole blood, unlike the studies in monocytes and adipose tissue (Table 2).

Ahmad et al (38) also found significantly increased expression of TLR2, TLR4, and MyD88 in both peripheral blood mononuclear cells (PBMCs) and SAT of obese (n = 8) and overweight (n = 7) subjects compared to lean (n = 6) controls. There was significant correlation of expression of TLR2, TLR4, MyD88, and IRAK-1 with body mass index (BMI). mRNA expression of IL-6 and TNF-α was also reported to be significantly elevated in obese compared to lean controls and was significantly associated with TLR expression. Thus, most of the above studies support an association of increased mRNA/protein levels and various biomediators in adipose tissue and PMs of obese patients (Table 2).

Interestingly, in a recent study, Hulsmans et al (39) identified three microRNAs (miR-181a, -181b, and -181d), as possible negative regulators of TLR/NF-κB signaling, and these were significantly decreased in monocytes of obese persons.

TLR activation in MetS

Animal studies

Shi et al (23) demonstrated increased TLR4 expression and activity in diet-induced MetS in a mouse model, where TLR4KO mice were protected from HFD-induced insulin resistance with lesser tissue inflammation. Himes and Smith (40) suggested a potential role for TLR2 in the diet-induced MetS in a mouse model showing that TLR2-deficient mice had decreased adiposity, insulin resistance, hepatic steatosis, and diminished macrophage infiltration and inflammatory cytokine expression in adipose tissue compared to wild-type mice. Vijay-Kumar et al (41) found that TLR5 genetically deficient mice (TLR5KO) displayed an altered microbiota species composition and exhibited features of MetS such as hyperlipidemia, hypertension, insulin resistance, and increased adiposity. There was also increased release of proinflammatory cytokines such as IL-1β and interferon-γ in TLR5KO, compared to wild-type adipose tissue, causing speculation that changes in gut microbiota contribute to the MetS. Transferring gut microbiota from TLR5KO to wild-type germ-free mice resulted in the development of several features of MetS. These data indicate that alterations of host microbiota by HFD, etc, contribute to inducing innate immune receptors in the gut and could predispose to metabolic complications. This exciting finding needs to be tempered by the report of Kim et al (32) in which they showed increased TLR5 expression in both DIO and genetic leptin-deficient animal models. Hence, TLR5 needs to be studied in human gut microbiota, adipose tissue, and monocytes to appreciate its relevance to the pathobiology of MetS.

Human studies

There are few human studies investigating TLR expression and activity in MetS. Bremer et al (42) previously reported significantly increased concentrations of circulating markers of inflammation in nascent MetS patients without the confounding of diabetes or CVD, compared to controls. Most of these abnormalities persisted after correction for adiposity, suggesting that MetS per se is a proinflammatory state. SAT also secreted increased levels of IL-6, IL-1, IL-8, MCP-1, leptin, retinol-binding protein-4, PAI-1, CRP, and serum amyloid A along with increased macrophages and crown-like structures in MetS than controls. Thus, dysregulation of SAT in MetS could potentially increase the risk for diabetes and CVD. In a subsequent report, Jialal and colleagues (43) showed that both plasma- and SAT-secreted chemerin was increased, whereas both plasma- and SAT-secreted omentin 1 was decreased. Once again, these perturbations continued to be significant using BMI and waist circumference as covariants emphasizing that MetS is a proinflammatory state. The increase in plasma resistin was not significant after adjustment for adiposity. Also, it needs to be pointed out that 20% of the MetS patients in these studies were not obese (44), further underscoring that the proinflammatory state of MetS is not a simple function of adiposity but is due to dysregulation of adipose tissue and monocytes created by the unique metabolic milieu due to the cardiometabolic cluster of risk factors.

Jialal et al (45) demonstrated significantly increased mRNA and surface expression of TLR2 and TLR4 on monocytes of these nascent MetS subjects compared to controls, even after adjustment for waist circumference. Priming of monocytes with LPS resulted in significantly increased IL-6, IL-1β, and IL-8 as well as increased NF-κB activity in MetS compared to controls. In this study, increased plasma levels of FFAs and endotoxin levels correlated significantly with TLR4 expression but not TLR2 (Table 2). Recently, Jialal et al (46) reported a significant decrease in the global antioxidant defense nuclear factor erythroid 2-related factor and increased levels of various oxidative stress biomarkers, nicotinamide adenine dinucleotide phosphate hydrogenase oxidase activity, nitrotyrosine, and oxidized LDL (Ox-LDL) in MetS compared to controls. Because it has been previously shown that Ox-LDL triggers TLR4 (47), Jialal et al further correlated TLR4 expression on monocytes with Ox-LDL, showing a significant correlation ($r = 0.31$; $P < .05$).

In a preliminary report, Hardy et al (48) appear to have confirmed these findings of increased TLR2 and TLR4 expression and activity by studying nine adolescents with MetS compared to BMI-matched controls. They showed an increase in mRNA expression for TLR2 and TLR4, TNF and IL-6, and circulating TNF and IL-6. However, they did not report on downstream signaling. Thus, both of these studies (45, 48) show that TLR2 and TLR4 are increased in MetS and could be a hub for the proinflammatory state.

Thus, the above reports collectively support the notion of increased TLR activity, especially TLR2 and TLR4, and their associated signaling cascades playing a pivotal role in accentuating the inflammatory state and insulin resistance and thus accelerating the progression of human obesity, MetS, and its sequelae. However, the above reports do not provide clear insights into the triggers of increased TLR activity, and this needs to be elucidated in future studies. Further understanding of the pathophysiology of innate immune response induced primarily by TLRs has the potential to ameliorate the chronic inflammatory burden in obesity by selective therapeutic targeting.

Potential Activators of the TLRs in Obesity and MetS

The role of TLR2/4 agonists and the exact mechanisms for increased TLR2/4- mediated inflammatory response in obesity and MetS is largely a hiatus and urgently needs scientific inquiry. Studies have demonstrated the role of LPS serving as a ligand to induce TLR4 signaling in macrophages, liver, and adipocytes (23). Also, increased levels of FFAs are associated with increased macrophage infiltration in white adipose tissue, which induces proinflammatory signaling cascade possibly leading to the development of insulin resistance in obese humans and animal models of obesity (5). Jialal et al (45) also reported elevated levels of TLR2/4 expression with increased fatty acids and endotoxin levels in MetS compared to controls. Hence, both obesity and MetS are characterized by increasing levels of various exogenous/endogenous activators including endotoxins (LPS), fatty acids, Ox-LDL, HMGB1, and HSP-60, which are thought to mediate increased TLR activation. The Witztum group (49) has shown that Ox-LDL promotes cytokine production via TLR4. Also, HMGB1 binding to TLR4 and mediating signaling is thought to define its cytokine-like and cytokine-inducing activities (50). HSP-60 and -70 bind to TLR2 and TLR4 and activate cytokine production (51). However, there is no clear evidence that HMGB1 and HSPs mediate increased inflammation in obesity or MetS, and this needs to be studied further. To date, the classical ligand for TLR4, endotoxin, has been shown to be increased in both obesity and MetS. This is an intriguing finding, and it raises the issue of increased gut permeability in obesity and MetS due to perturbations in the gut microbiota resulting in the increase in circulating endotoxin levels (52–54). It appears that obesity is associated with an alteration in gut microbiota composition, causing a perturbation in tight junction proteins that results in an increase in gut permeability, which induces a metabolic endotoxemia explaining the increase in plasma LPS in obesity and MetS. In addition, circulating zonulin, a marker and modulator of intestinal permeability, has been shown to be increased with obesity and insulin resistance (55). Recent data from Serino et al (56) indicate that insulin resistance in humans could be linked to the gut microbiome. Also, Leber et al (57) have demonstrated increased gut permeability in MetS patients compared with healthy controls.

Furthermore, levels of SFA are increased in both obesity and MetS and can trigger an increase in TLR activity. Finally, Ox-LDL also triggers TLR expression and is increased in both obesity and MetS (58). However, other potential triggers such as HMGB1, CD14, HSP-60, etc, need investigation to determine their role in triggering TLR response in obesity and MetS.

TLRs as Candidates of Therapeutic Modulation

Thus, there is increased TLR activation in obesity and MetS. Both obesity and MetS presage the development of diabetes, CVD, and other complications. Increasing evidence from various human studies has demonstrated increased TLR expression and activity, especially TLR2 and TLR4, contributing to increased inflammation in both type 1 diabetes and T2DM (10). Thus, modulation of TLR-mediated inflammation at the early stages of devel-

Table 3. Strategies That Modulate TLRs In Vivo

Therapy	Cell/Targets	TLR Expression	TLR Signaling/Cytokines	First Author, Year (Ref.)
Exercise	Human monocytes	↓ TLR2, ↓ TLR4	↓ IL-6, ↓ TNF-α, ↓ IL-1β	Lancaster, 2005 (60); McFarlin, 2006 (63)
Diet-induced weight loss	Human PBMCs	↓ TLR2, ↓ TLR4	↓ CCL5, ↓ TNFRSF1A	de Mello, 2008 (65)
Phytochemicals		↓ TLR2, ↓ TLR4	↓ NF-κB, ↓ cJUN, ↓ TNF-α	Zhao, 2011 (66); Hirai, 2010 (67); Yeop, 2010 (68); Yoshida, 2013 (69)
Statin therapy	Human monocytes	↓ TLR4	↓ IRAK phosphorylation, ↓ IL-6, ↓ IL-12, ↓ TNF-α	Methe, 2005 (72); Stoll, 2006 (73)
		↓ TLR4	↓ TNF-α, ↓ MCP-1	Niessner, 2006 (74)
Incretin-based therapies such as sitagliptin	Human mononuclear cells	↓ TLR2, ↓ TLR4	↓ TNF-α, ↓ IKK-β, ↓ IL-6, ↓ cJUN, ↓ NF-κB	Chaudhuri, 2012 (79); Makdissi, 2012 (80)

Abbreviations: CCL, chemokine (C-C motif) ligand; cJUN, c-Jun-N-terminal kinases; TNFRSF1A, TNF receptor superfamily, member 1A.
↓ represents decreased expression.

opment of obesity to MetS may have a major impact in forestalling diabetes and CVD (59).

Exercise can modulate inflammation. Previous studies have demonstrated that strenuous exercise significantly decreased surface expression of TLR2 and TLR4 on human monocytes in vivo (60–63). Lambert et al (64) also reported decreased TLR4 expression after exercise in obese elderly patients. de Mello et al (65) reported that improved insulin sensitivity after diet-induced weight loss was associated with down-regulation of TLR2 and TLR4 expression in PBMCs from human subjects with abnormal glucose tolerance and MetS. However, the exact mechanisms by which exercise or diet-induced weight loss modulate TLR2/4 expression in obesity and MetS require further investigation.

Studies have demonstrated reduction of TLR-induced inflammation using phytochemicals and omega-3 fatty acids (66–68). Yoshida et al (69) reported that the citrus flavonoid naringenin reduced TLR2 expression, inhibiting TNF-α and NF-κB activation in adipocytes in vitro as well as in adipose tissue of HFD-fed mice, hence suggesting its role as an anti-inflammatory agent in obesity-related disorders. Ghanim et al (70) have shown that orange juice can decrease HFD-induced inflammation and TLR2 and TLR4 mRNA and protein expression in human mononuclear cells. We have previously shown decreased vitamin D levels in type 1 diabetes along with a significant negative association between vitamin D levels and high sensitivity-CRP, TLR4 expression, and NF-κB activity (71).

Pharmacological agents also modulate TLR2 and TLR4 expression. Statin therapy significantly reduces monocytic TLR2 and TLR4 expression (72–74). Peroxisome proliferator-activated receptor-γ agonists decrease TLR expression and activity in monocytes, microglia, and astrocytes (75–77). Angiotensin receptor blockers such as candesartan reduce TLR2 and TLR4 activation and NF-κB p65 activity in human monocytes in vitro (78). The Dandona group (79, 80) has examined the effect of incretin-based therapies, including both exenatide and sitagliptin therapy. They found significantly decreased mRNA expression of TLR2, TLR4, TNF-α, and IL-1β in mononuclear cells as well as significantly reduced circulating levels of MCP-1, IL-6, and serum amyloid A after 12 weeks of exenatide in obese patients with T2DM (79). Similarly, 12 weeks of sitagliptin therapy significantly decreased mRNA expression of TNF-α, TLR2, TLR4, c-Jun-N terminal kinase-1 (c-JNK-1), IKK-β, and CCR2 in mononuclear cells of T2DM patients. There was also reduced protein expression of c-JNK-1, IKK-β, and TLR4 and in plasma concentrations of CRP, IL-6, and FFAs after sitagliptin therapy, hence suggesting anti-inflammatory effects of incretin- based therapy possibly by modulating TLRs (80).

Thus, whereas many pharmacological and nutraceutical agents can modulate TLRs (Table 3), there are, to date, no approved therapeutic agents targeting TLR2/TLR4 that have been shown to reduce obesity, MetS, and diabetes-associated inflammation. Hence, there is an urgent need to identify both nutritional and pharmacological modulators of TLR activity in obesity and MetS in both monocytes and adipose tissue depots to determine whether such strategies can reduce this epidemic of MetS and diabetes.

Conclusion

Collectively, several lines of evidence point to the role of TLRs, especially TLR2 and TLR4, in accentuating inflammation and insulin resistance in both obesity and MetS. Obvious agonists that are increased in both obesity and MetS that trigger TLR activity include SFA, endotoxins, Ox-LDL, and DAMPs. TLR activation promoting inflammatory signaling cascade and insulin resistance could contribute to the pathogenesis of obesity and MetS (81) (Figure 1). Future studies in humans based on targeting TLRs could provide therapeutic approaches in forestalling the progression of obesity to MetS and its complications.

Acknowledgments

The authors thank Gerred Smith, University of California Davis Medical Center, for assisting with manuscript preparation.

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This work was supported by an American Diabetes Association Clinical Research Grant.

Author Contributions: H.K. researched data, contributed to discussion, wrote, reviewed, and edited the manuscript. S.D. researched data, contributed to discussion, reviewed, and edited the manuscript. I.J. researched data, contributed to discussion, and reviewed and edited the drafting and critical revision of the manuscript for important intellectual content.

I.J. is the guarantor of this work and, as such, had full access to all the articles discussed in this review and takes responsibility for the integrity and the accuracy of the review based on the input of both of his coauthors.

Disclosure Summary: There are no potential conflicts of interest related to this article, and the authors have nothing to disclose.

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