Send Orders for Reprints to reprints@benthamscience.net

Understanding and Modulating the Toll Like Receptors (TLRs) and NOD Like Receptors (NLRs) Cross Talk in Type 2 Diabetes

Bhumika Prajapati, Prasant Kumar Jena, Parth Rajput, Kaveri Purandhar and Sriram Seshadri*

Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Abstract: Obesity and Type 2 diabetes are leading health problems which are characterized by low-grade inflammation with an increase in inflammatory cytokines along with the change in the gut microbiota population. Toll like Receptors (TLRs) and NOD like Receptors (NLRs) are very prominent pathogen recognition receptors, which play a significant role in the innate immune system. Both TLRs and NLRs pathways are mediated through different adaptor proteins; commonly found to activate the NF-kB, which induces the expression of proinflammatory cytokines. It has been suggested that TLRs and NLRs have a significant role in the pathogenesis of inflammation mediated insulin resistance, which further develops metabolic complications. TLRs mediated mechanism for insulin resistance involves activation through TLR ligands such as increased free fatty acids and lipid derivatives from adipocytes as well as the skeletal muscles. Moreover, gut microbiota alteration in the type 2 diabetes also plays a key role by increasing the plasma LPS levels, which specifically activates TLR4 and provokes the inflammation mediated insulin resistance. NOD1 and NOD2 are involved in the pathogenesis of diabetes, possibly through the recognition of the gut microbiota. Gut microbiota modulation by antibiotics plays a crucial role in increasing insulin sensitivity, possibly through the TLRs and NLRs mediated signaling responses, which suggest future therapeutic approaches for obesity, insulin resistance and type 2 diabetes. In this review, we focused on the interdependent role of TLRs and NLRs in metabolic diseases and their cross talk for the pathogenesis of inflammatory diseases.

Keywords: Antibiotics, Gut microbiota, Inflammation, NLRs, Obesity, TLRs, Type 2 Diabetes.

INTRODUCTION

Diabetes is the most common chronic endocrine disorder having 382 million people in 2013, which will increase to 592 million by 2035 all over the world [1]. Obesity and Type 2 Diabetes (T2D) are often associated with low-grade systematic inflammation, which is regarded as the activation of the immune systems, increased release of the adipocytes derived bioactive metabolites like free fatty acids (FFA), lipids and proinflammatory cytokines and increased gut derived lipopolysaccharide (LPS), leading to the progression of disease [2, 3]. As early as the 1950s, there was little evidence suggesting a correlation or link between inflammation and insulin-resistant states such as obesity, but the mechanism was unknown. In recent times, researchers have found that IL-1β, TNF-α, Interleukin 6 (IL-6) and C-reactive proteins (CRP) are major acute phase responsive proteins, which are the strongest predictors of the development of obesity which further progress to T2D [4].

TYPE 2 DIABETES, AN INFLAMMATORY DISORDER

Adipose tissue (AT) acts as an origin of inflammation and involves in the secretion of various bioactive molecules such as cytokines, fatty acids and chemokines, which further activates the macrophage infiltration and inflammation. In previous studies of young, obese subjects, TNF-α mRNA expression in AT was found to be 2.5-fold higher as compared to lean subjects and was strongly correlated with the hyperinsulinemia condition of insulin resistance (IR) [5]. Reduction in body weight of obese people had significantly reduced serum TNF-α levels and improved insulin sensitivity [6]. It is suggested that high TNF-α phosphorylates the serine/threonine residues of the insulin molecule and down regulates the insulin receptor signaling (IRS) pathway, which further develop into IR [7]. The increased adipokines and proinflammatory cytokines were observed in a recent case cohort study of middle-aged obese individuals with diabetes, which suggests the major role of inflammation markers in adult onset of diabetes in obese individuals [8].

IL-6 is produced by several cell types including, fibroblasts, endothelial cells, monocytes and macrophages. Adipocytes derived from obese people showed upregulated expression of IL-6 which can further be correlated with the extent of IR [9, 10]. These observations suggest a possible interacting link among IL-6 levels, obesity and inflammation in the development of T2D and demonstrate that IL-6 can be considered as a candidate biomarker for early T2D risk detection [11].

CRP is also a very significant acute phase reactive protein, produced by the liver in response to IL-6 and TNF-α. It is a common marker for inflammation due to its rapidity of induction, cooperative role in innate immune response as well as the ease of measurement [12]. Furthermore, CRP is consistently correlated with certain parameters related to diabetes, such as obesity, lipogenesis and adiponectin [13].

*Address correspondence to this author at the Institute of Science, Nirma University, Sarkhej-Gandhinagar Highway, Chharodi, Ahmedabad-382481, Gujarat, India; Tel: +91 2717 241901-04 ext752; Fax: +91 2717 241916;
E-mails: sriram.seshadri@nirmauni.ac.in and sriramsjpr@gmail.com

1573-3998/14 $58.00+.00

The elevated circulating concentration of CRP was found to be higher in the obese people with IR which reduced upon weight loss along with improvement of insulin sensitivity [14]. Above reports suggest that obesity and inflammation are first steps towards the development and progression of the IR and T2D.

GUT MICROBIOTA AND TYPE 2 DIABETES

Healthy human gut harbors complex organization of the different microorganisms, which plays a key role in the regulation of metabolism and inflammation. Any disturbance or dysregulation in such highly organized microbial assembly by means of stress, diet, antibiotics, genetics and environmental factors, can cause the origin of several gut associated disorders such as T2D. Various metagenomic studies have revealed that there is a change in the gut microbiota composition between healthy and T2D individuals. One recent study has shown that Type 1 Diabetes (T1D) is associated with significant changes in the gut microbiota composition. The significant differences in the number of bacteria such as *Bifidobacterium*, *Lactobacillus*, *Clostridium* and in Firmicutes to Bacteroidetes ratio were observed between the two groups. Moreover, the quantity of bacteria which is essential to regulate the gut integrity was found to be significantly reduced in the children with diabetes as compared to the healthy ones [15].

Recent research clearly shows the association between fecal microbiota composition and the emergence of T2D in Denaturing Gradient Gel Electrophoresis (DGGE). The results of the real-time PCR analysis for *Bacteroides vulgatus*, Bifidobacterial genus, and *Clostridium leptum* subgroup illustrated the major alteration of these three strains at different degrees in diabetic group, and the copy number of Bifidobacterial genus was significantly declined [16]. A metagenome-wide association study (MGWAS) showed that patients with T2D were characterized by moderate microbial dysbiosis with decreased in universal butyrate-producing bacteria, increased opportunistic pathogens and enrichment of the microbial functions such as spot reduction as well as oxidative stress resistance [17]. The real time quantitative PCR (qPCR) investigation showed significantly reduced proportion of the phylum firmicutes and clostridia class in T2D group as compared to control. Moreover, class betaproteobacteria was highly enriched in the diabetic group compared to the non diabetic control [18]. Karlsson *et al.* have studied the gut metagenome composition of T2D and normal communities and found increased number of lactobacillus species while decreased number of clostridium species in T2D group [19]. Such findings could be very beneficial for developing strategies to control the pathogenesis of diabetes by modifying the gut microbiota.

PATTERN RECOGNITION RECEPTORS (PRRS)

The immune system is one of the foremost possessions that nature has bestowed on us to fight against pathogens. Vertebrates are constantly endangered by attack of harmful microorganisms and have evolved immune arms to eliminate the pathogens from the body. The vertebrate immune system majorly consists of two arms: innate and acquired immunity [20]. The innate immune system is germ encoded and non specific host defense system against pathogens and is mediated by phagocytes, macrophages and other immune cells [21, 22]. On the other hand, acquired immunity mainly involves the specific elimination of pathogens in the delayed phase of infection with the generation of immunological memory [20].

Recognition of microbial pathogens is a crucial factor for the initiation of innate immune responses such as inflammation, which is mainly mediated by germline-encoded varieties of extracellular or intracellular pattern recognition receptors (PRRs). PRRs recognize the various molecular structures, which are broadly shared by pathogenic microorganisms such as pathogen-associated molecular patterns (PAMPs) and Danger associated molecular patterns (DAMPs). DAMPs are the molecules released by the stressed cells undergoing necrosis, which act as danger signals to promote the inflammatory response. Upon PAMPs and DAMPs recognition, PRRs commence a series of signaling events that perform first line of host defensive responses, necessary for killing infectious microorganisms [21]. Four different classes of PRRs families have been recognized. These families consist of transmembrane proteins like Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs) and cytoplasmic proteins such as Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) [23, 24]. In spite of their great contribution in defense mechanism, they are known to be involved in the pathogenesis of obesity and T2D. TLRs and NLRs are most extensively studied PRRs known to be involved in inflammation mediated IR. In the present review, an attempt has been made to explain the role of TLRs and NLRs in the context of obesity, IR and T2D.

TOLL LIKE RECEPTORS

At the end of 20th century, Toll was identified as an important receptor for host defense against several bacterial, viral and fungal infections in Drosophila. Later, mammalian homologs of TLR4 were revealed to induce expression of certain genes, which are involved in inflammatory responses against infections [20]. After identification and characterization of TLR4, various other structurally related proteins of TLR4 were identified and named as TLRs. Mammalian and mouse TLRs comprise of a large family consisting of 11 and 13 members respectively. TLRs are divided into two groups depending upon their cellular localization; a group that is expressed extensively on cell surfaces, includes TLR1, TLR2, TLR4, TLR5, TLR6, TLR11 and another group is comprised of TLR3, TLR7, TLR8 and TLR9, which is expressed solely on intracellular vesicles like endoplasmic reticulum, endosomes, lysosomes and endolysosomes. Different types of TLRs sense different types of ligands and attach to the various types of adaptor proteins to start signaling events, which lead to the activation of the inflammatory process (Fig. **1**) [25]. TLRs are mainly characterized by an extracellular leucine-rich repeat (LRR) domain, which is involved in the recognition PAMPs through a cytoplasmic Toll/IL-1 (TIR) domain that activates downstream signaling adaptor protein molecules, including MyD88, IRAKs and TRAF6 (Figs. **1** and **3**) [26].

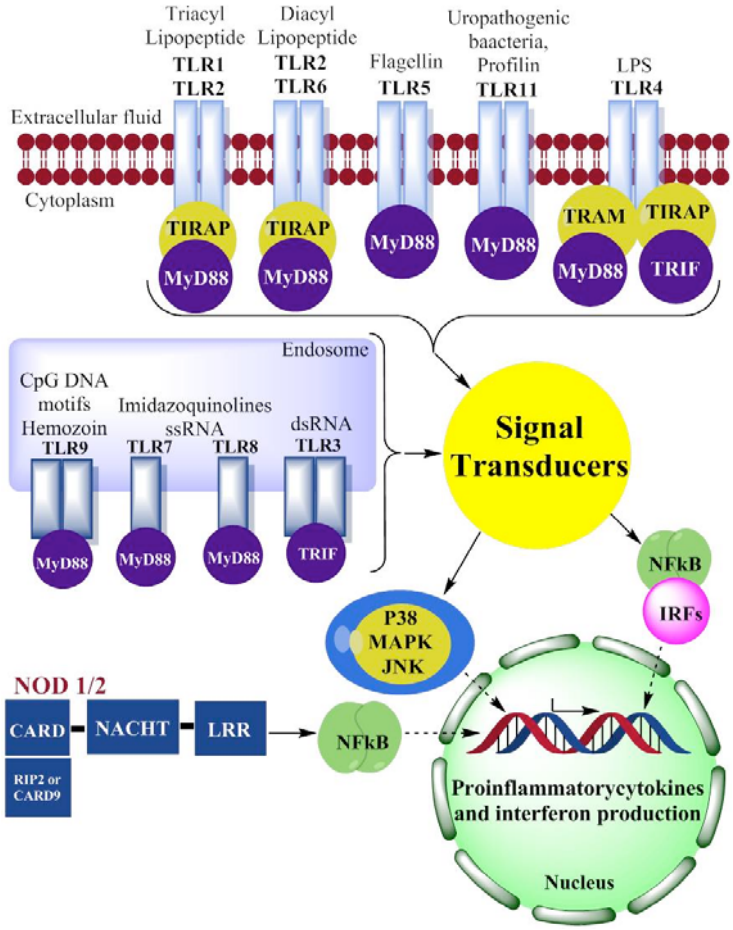

Fig. (1). TLR and NLR signaling pathway: TLR1-11 have been identified in human for recognition of various microbial components and localized on a plasma membrane and endosomes. TLRs sense ligands via their LRR ectodomain which leads to receptor dimerization through adaptor proteins recruitment to the dimeric TIR domain. Signal transduction is processes mainly by the action of signal transducers such as the IRAK and tumor necrosis factor receptor-associated factor (TRAF) family members which leads to the activation of NF-kB and members of the interferon response family (IRF). On other side, activation of NOD1/2 also stimulates receptor oligomerization, followed by their interactions with the adaptor proteins RIP2 or CARD9. Signaling via RIP2 upregulated NF-kB, whereas the use of CARD9 activates Mitogen-activated protein kinase (MAPK), p38 and c-Jun N-terminal associated kinase (JNK) pathways.

TLR2 AND TLR4

TLR2 recognizes a variety of microorganisms derived products like lipoproteins from several pathogens, peptidoglycan moiety from both gram positive and negative microorganisms, Zymosan from some fungi and lipoteichoic acid from certain gram positive microorganisms [27]. It also recognizes LPS of certain non entero bacteria, such *as Leptospira interrogans*, *Porphyromonas gingivalis* and *Helicobacter pylori* species [28].

There are mainly two proposed mechanisms to explain the recognition of the wide spectrum of the microbes derived products by TLR2. It forms hydrophilic dimers with some other structurally associated TLRs like TLR1 and TLR6. Macrophages and monocytes derived from TLR6 knockout (KO) mice have failed to show any production of inflammatory cytokines against Mycoplasma-derived diacyl lipopeptides. However, these cells had shown normal production of inflammatory cytokines in response to tracheal lipopeptides derived from Gram-negative bacteria. Thus, TLR1 and TLR6 are known to be functionally associated with TLR2 for discrimination between diacyl and triacyl lipopeptides [29, 30].

TLR4 is a major receptor found in the recognition of a variety of ligands derived from the gram-negative bacteria. LPS is an important integral component of the outer membrane of gram-negative bacteria which acts as a main ligand for TLR4. It has been shown to be involved in the recognition of a variety of endogenous ligands derived from cellular stress or necrosis, which includes DAMPs like heat shock proteins, mainly HSP60 and HSP70, the extra domain of fibronectins proteins, oligosaccharides moiety of hyaluronic acid, heparan sulfate and fibrinogen at some lesser extent. LPS along with LPS binding proteins initiates a signal pathway through the interaction with membrane-bound receptors like CD14, which are present on monocytes and macrophages [31, 32]. Stimulation of TLRs by various microbial components, *i.e.* PAMPs, triggers expression of several proinflammatory genes that are involved in the immune response [33]. The molecular mechanisms by which TLR ligands induce these gene expressions are now rapidly being elucidated through analyses of TLR-mediated signaling pathways (Fig. **1**). [34-37].

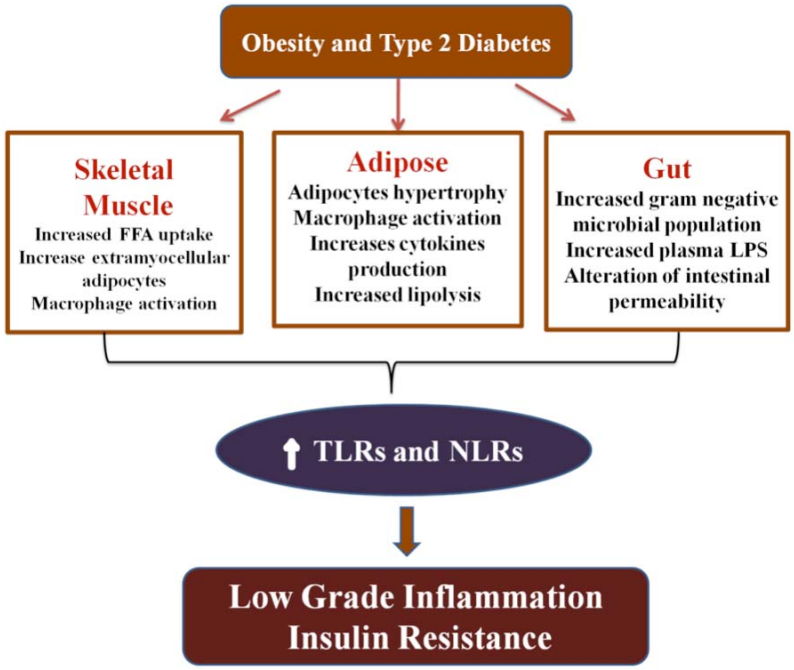

Fig. (2). Obesity mediated development of inflammation and insulin resistance. Obesity induced alteration in the skeletal muscles and adipose tissue increase the plasma free fatty acids. Obesity is also involved with changing in gut microbiota composition towards increase in gram negative microbial population. Both FFA and LPS triggers the upregulation of the TLRs and NLRs mediated inflammatory signaling pathway, which further activates NF-kB and leads to the development of inflammation mediated insulin resistance.

NLRs

The cytosolic NLRs known as nucleotide-binding oligomerization domain (NOD) containing receptors are a specialized group of intracellular proteins, which represent a main component of the host innate immune system. This family of proteins is defined by a tripartite structure mainly consisting of (a) variable N-terminal protein-protein interaction domain, known as caspase recruitment domain (CARD), pyrin domain (PYD), acidic transactivating domain, or baculovirus inhibitor repeat (BIR), (b) a central NOD domain, which mediates oligomerization with own that occurs during activation and (*c*) a C-terminal leucine-rich repeat (LRR) that detects PAMPs [38, 39]. Among all inflammosomes, the present review describes the role of NOD1 and NOD2 in IR and metabolic disorders due to their cross talk with TLRs for the induction of NF-kB mediated inflammation.

NOD1 AND NOD2

NOD1 and NOD2 are the earliest identified and best characterized NLRs, which are classic example of NLR activation of NF-kB and MAPK pathways [40, 41]. NOD1 is also known as CARD4, which recognizes various substructure of the peptidoglycan, namely iE-DAP (γ -D-glutamyl-diaminopimelic acid), which is found to be present in both gram-negative and gram-positive bacteria but specifically, senses the gram negative bacteria. NOD2 is also known as CARD15, which recognizes muramyl dipeptide (MDP), largest active component of peptidoglycan motif, which is present in both gram-negative and gram-positive bacteria. Upon recognition of their respective ligands, both NOD1 and NOD2 self-oligomerize to recruit and activate the adaptor protein RIP2, which is necessary for the activation of both NF-kB and the MAPKs pathways (Fig. **1**) [42-45].

ROLE OF TLRS IN OBESITY MEDIATED INSULIN RESISTANCE

A link between gut microbiota and inflammation in the pathogenesis of certain obesity-related complications is rapidly identified in the recent era. Many dietary factors like FFA, lipids and glucose besides the changes in gut microbiota composition triggers the progression of metabolic disorders through the activation of TLRs and NLRs. Dasu and colleagues have reported that, significant increment in TLR2 and TLR4 along with their ligands and cofactors was found at both mRNA and proteins levels in subjects with T2D [46]. Physiological increase of plasma FFAs causes hepatic IR (i.e increased hepatic glucose production) which results in hepatic accumulation of diacylglycerol (DAG) and activation of proinflammatory cytokines pathway. Furthermore this suggests that obesity associated hepatic inflammation and IR can be due to the elevated levels of plasma FFAs.

The function of the adipose tissue is not only to store and release the fatty acids but also to synthesize and release certain active compounds such as FFA, resistin, TNF-α, IL-6 and others through the activation of macrophages (Fig. **2**) [48, 49]. Among all these compounds, rising plasma FFA levels in the skeletal muscles and AT increase the IR and lowering levels improve insulin sensitivity. The plasma FFA level is usually increased in obesity due to enlargement of the AT mass, which involves intramyocellular accumulation of DAG and long-chain acyl-CoA, which further inhibit the phosphorylation of insulin receptor substrate to down regulate the insulin action [50]. Absence of TLR2 protects against high-fat diet (HFD) induced inflammation and results in greater insulin-stimulated glucose transport in cultured adipocytes [51]. The TLR2 KO mice were protected from the detrimental effects of HFD as compared with TLR2 posi-

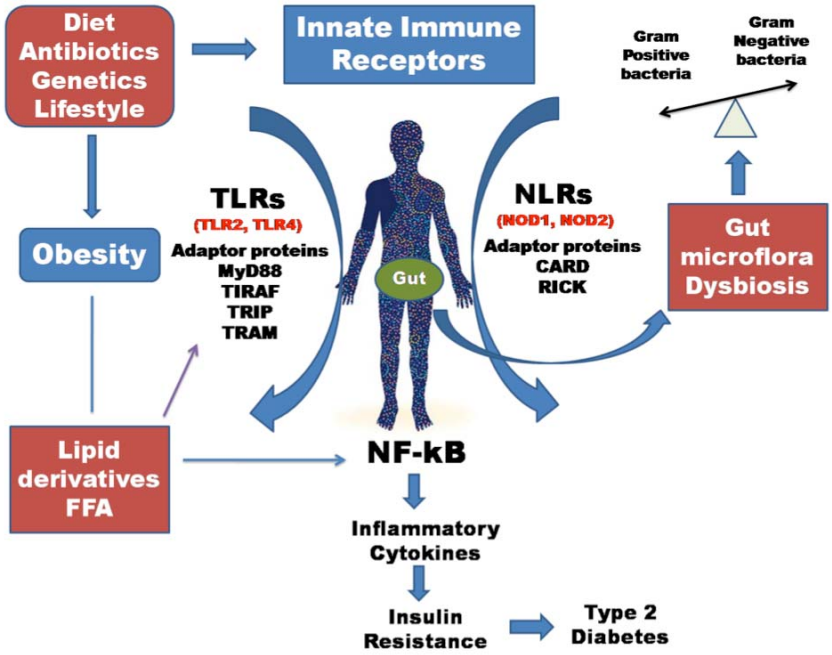

Fig. (3). TLR and NLR cross talk in pathogenesis of Type 2 Diabetes. Innate immune receptors like TLRs (TLR2, TLR4) and NLRs (NOD1, NOD2) play an important role in pathogenesis of the obesity mediated insulin resistance. In obesity, TLRs and NLRs mediated pathway can be activated by increased FFA and LPS levels through the association with their adaptor proteins. Both TLRs and NLRs may mediate activation of central NF-kB pathway which leads to the production of inflammatory cytokines and leads to the insulin resistance.

tive littermate controls with pronounced improvements in glucose tolerance and insulin sensitivity with diminished macrophage infiltration and inflammatory cytokine expression following 20 weeks of HFD feeding. This suggests the molecular link between increased dietary lipid intake and the regulation of glucose homeostasis by regulation of energy utilization and tissue inflammation [52]. Another report suggests that FFAs can activate CD11c (+) myeloid proinflammatory cells through the activation of TLR2/4 and JNK mediated signaling pathways, which promote inflammation and cellular IR [53]. A recent study showed that saturated fatty acids like palmitate treatment in differentiated C2C12 myotubes resulted in a time-dependent inhibition of insulin-activated signal transduction through activation of TLR2 [54]. Short-term inhibition of TLR2 expression using TLR2 oligonucleotides antisense in diet-induced obese mice leads to a downregulated signaling pathway and increased insulin sensitivity [55]. Other studies have reported that TLR2 KO mice exhibit decreased body weight and adiposity along with protection against IR, weight gain and co-morbidities on an HFD than control mice [56].

Murakami and colleagues analyzed the overexpression of TLR2 and TNFα in isolated adipocytes from obese mice using flow cytometry, which was significantly associated with IR [57]. Reyna and colleagues have shown that FFA concentration was directly associated with an increase in TLR4 gene and protein expression, which can be correlated with the IR condition in obese and diabetic subjects [58]. Furthermore, researchers have shown that the TLR4 deficient mouse strain fed on a diet rich in saturated fat protected from systemic inflammation [59]. Increased TLR2 and TLR4 expression was demonstrated on monocytes derived from type 1 diabetes mellitus patients with microvascular complications [60]. Long-chain saturated fatty acids activate TLR4 signaling and promote hypothalamic leptin resistance. Immunopharmacological inhibition of TLR4 protects from monounsaturated fat induced obesity acting as a predominant molecular target for saturated fatty acids in the hypothalamus, which activates the inflammatory response [61]. Previous report suggested that TLR4 deficient mice exhibit greater than their wild-type counterparts, and this effects consequence of reduced inflammatory signaling in AT [62]. When TLR 2 ligand, Zymosan-A (ZymA) was administered at very low doses *in vivo*, which stimulated chronic low-grade systematic inflammation contributing to the formation of new adipocytes. These studies emphasize the important link between obesity and innate immunity, and how dietary fatty acids may aggravate obesity and its related co-morbidities through direct interactions with TLR2 and TLR4 in AT [63]. MyD88 deletion in the central nervous system protected from HFD and palmitate-induced impairment of peripheral glucose metabolism. Thus, we define neuronal MyD88-dependent signaling as a key regulator of diet-induced leptin and IR *in vivo* [64].

One data showed that saturated fatty acids like palmitate stimulates both TLR2 and TLR4 signals in insulin (INS-1) cells, showing the involvement of TLR2 and TLR4 in fatty acid induced IR. Activation of the stress-related JNK pathway through TLR4 stimulation was involved in palmitate-induced INS-1 β cell death, which suggests that activation of an innate immunity signal may be involved in fatty acid induced lipotoxicity in β cells [65]. Elective elevation of palmitate levels among all the other circulating FFA species would be the key to clearly demonstrate the activation of inflammation via the TLR4/myD88 pathway which results in β cell dysfunction [66]. Surface expression of TLR2 and

TLR4 was significantly increased in monocytes in T1D patients and db/db mice at mRNA levels via palmitate and LPS induction, while decreased by pioglitazone treatment. Other researches also demonstrated that expression and activity of the functional receptors of TLR2 and TLR4 were increased in the monocytes of patients with metabolic syndrome, which could be contributed to the increased risk for metabolic disorders [68].

TLR3 and TLR9 contribute to innate immunity and progression of T2D by recognizing the Double-stranded RNA and bacterial unmethylated DNA respectively. One study depicted the critical role of TLR3 signaling and RNase L in the obesity associated IR. They showed that defects in the RNase L activation and TLR3 expression with reduced manganese superoxide dismutase in skeletal muscle cells of the obese people further increases insulin sensitivity [69]. TLR3 KO mice fed with HFD had improved glucose tolerance, reduced hepatic steatosis and down regulated proinflammatory cytokines as compared to Wild Type (WT) mice, indicating that antagonizing TLR3 may be a beneficial approach for the treatment of metabolic diseases [70]. Study in the HFD mediated TLR9 KO mice has suggested that genetic ablation of the TLR9 improved the insulin sensitivity through decreasing the inflammatory responses in AT caused by the activation and accumulation of the macrophages [71].

GUT MICROBIOTA, TLRS AND INSULIN RESISTANCE

In recent times, the gut microbiota composition has also been shown to play a significant role in the development of obesity and IR by affecting fat storage and energy harvesting. Earlier research suggested that T2D might be associated with the dominance of gram-negative bacteria in the gut such as, *Bacteroidetes* and decrease in gram positive helpful bacteria [72, 73]. LPS is a major component of the gram-negative bacteria envelope and a known inducer of inflammation *in vivo*. The proinflammatory component of LPS, endotoxin (or lipid A), is composed of Fatty Acid (FA) and phosphate groups attached to a central Glucosamine dimer. Specific serum binding protein TLR4 transports endotoxin to cells expressing its corresponding receptor through the GPI-linked protein necessary for LPS binding [73]. Diet-induced obese mice exhibit a constant increase in the plasma LPS termed as "metabolic endotoxemia" which further improved by the reduction in *Bacteroides-Prevotella* spp. by antibiotics administration [74]. The gut mucosal permeability is governed by the complex system of the tight junction proteins, including occludin and claudin. In mice, decrease in the population of *bifidobacteria* species in the gut loosens the tight junctions between the cells of the gut lining. The loose junctions or malfunction of the barrier increases the gut permeability and allows LPS from microbes to leak through the gut epithelium which causes a low-grade inflammation and can induce a number of metabolic disorders, including the IR leading to T2D [75]. Creely *et al*. have shown increased TLR2 expression in adipose tissue from T2D patients with strong correlations of plasma endotoxin levels suggesting that gut microbiota related factors are involved in the development of T2D and obesity in humans. The authors had also found that endotoxemia was 2-fold higher in the BMI, sex, and age-matched T2D patient group than in the non diabetic patients [76].

CD14 (LPS co-receptor for TLR4) KO mice were completely resistant to the development of the inflammation induced by both, high-fat feeding or following the chronic low dose LPS administration in the visceral and subcutaneous adipose depots, the liver and the muscle [77]. Gut microbiota contributes to metabolic endotoxemia related to high-fat diet feeding. In the same line of results, recent research has reported that plasma LPS plays an important role in the development of metabolic and vascular abnormalities in diabetic patients [78]. High fat or high carbohydrate food increases the plasma LPS concentration and LPS binding protein which simultaneously increases the expression [79]. Peripheral Blood Mnonuclear cells (PBMCs) isolated from AT of the obese subjects has shown increased TLR2 and TLR4 expression, which further increased pro inflammatory cytokines expression and NF-kB binding [80]. Monitoring gene expression in β-cells exposed for 48 h to the prototypical TLR4 ligand LPS showed a concentration-dependent increase in TLR4 and CD14 transcripts and decreased insulin content and secretion. TLR4 positive mouse insulinoma cells (MIN6) were found to be LPS-responsive with increasing TLR4 and CD14 mRNA levels along with reduced cell viability and insulin content [81]. These results suggest that inflammatory condition of T2D is significantly related to the expression of TLRs like TLR2 and TLR4, but still clear understanding of exact mechanism needs further research.

ROLE OF NOD1 AND NOD2 IN OBESITY MEDIATED INSULIN RESISTANCE

NOD proteins are important innate immune components which are involved in diet-induced inflammation and IR. Acute activation of NOD proteins by mimetics of bacterial peptidoglycan (PGN) causes whole-body inflammatory IR *in vivo* by altering both glucose tolerance and glucose production. NOD1/2 or double KO mice are more insulin tolerant compared with WT control mice after 16 weeks of HFD, evidenced by decreased insulin tolerance. After the HFD, these mice showed lower gonadal adipose and liver masses compared with WT control mice with reduced adipose size. Thus, we can say that mice were protected from the HFD-induced IR, lipid accumulation and inflammation in AT, skeletal muscle and liver [82]. NOD1 activator like bacterial PGN motifs caused acute systemic IR in mice, which further suppressed insulin action in the liver and isolated hepatocytes as well as decreased insulin-mediated glucose uptake in adipocytes [83].

PGN motifs that acts on NOD2 induce muscle cell-autonomous IR suggests that NOD2 alone is capable of acutely inducing IR within muscle cells, possibly through the activation of endogenous inflammatory signals and/or through cytokine production, curbing upstream insulin signals. Therefore, NOD1 ligand-mediated IR, seems to involve cross talk between cells from the various tissues, likely adipose and hepatic, with indirect manifestation in skeletal muscle [83]. Amar and his co-workers have observed the bacterial translocation towards adipose tissue and blood only after one week of high-fat diet mediated inflammation induction. This translocation is prevented in mice lacking the mi-

crobial recognition receptors NOD1 or CD14, suggesting that these receptors have important roles in the development of the low-grade inflammatory state that characterizes IR [84]. Administering PGN-based NOD1 agonists to adipocytes of WT mice leads to the activation of inflammatory cytokines, impairing insulin signaling and decreasing insulin-stimulated glucose uptake, but such effect was found to be absent in NOD1 KO mice [85]. Along with NOD1 and NOD2, the list of innate immune components involved in IR is growing and also includes NOD-like receptors such as NLRP3 [86-88]. It is a daunting but important task to understand how components of the immune system coordinate inflammation resulting in IR.

It has been hypothesized that after stimulation of both TLRs and NLRs by microbial products, the inhibitor of IkB is phosphorylated, leading to degradation and the translocation of NF-kB into the nucleus where it activates the expression of cytokines, which induce the proinflammatory response. TLRs mediate activation of NF-kB possibly through adaptor proteins MyD88, whereas NLRs mediate through interactions with RIP2 (Fig. **1** and **3**) [88].

GUT MICROBIOTA MODULATION, TLRS AND NLRs

Antibiotics have been designed to target the pathogenic microorganism, but they affect some members of commensal microbiota, leaving an imprint on the gut community, long even after their removal [89]. Recent experiments have revealed that treatment with broad-spectrum antibiotics affects the gut microbiota structure with significant decrements in *Bacteroidetes* and a parallel increase in *Firmicutes*. As mentioned above, a high sugar/fat rich diet shifts gut microbiota composition towards increase in gram-negative microbial population, which leads to higher LPS concentration and increases TLRs and NLRs mediated inflammatory responses [90]. Recent research suggests that antibiotic treatment can prevent the progression of T2D at some extent through the modulation of gut microbiota. Oxytetracycline treatment completely restored insulin sensitivity by increasing the insulin binding capacity to the liver membrane [91]. Chronic Oxytetracycline therapy was found to alter the diabetic status of the spontaneously induced diabetic rat (Bio Breed rat) with lowered plasma glucose levels in the fasted as well as in the fed state. These results indicate that the Oxytetracycline treatment was effective in lowering insulin requirements as well as in improving the handling of glucose [92]. Membrez and researchers have modulated the gut microbiota with the combination of broad-spectrum antibiotics like norfloxacin and ampicillin and found that such treatment enhances glucose tolerance in mice through reducing gram-negative microflora population [93]. Gut decontamination with two different combinations of drugs (1) norfloxacin and ampicillin (2) polymyxin and neomycin for two weeks decreased the cecal bacterial DNA content below the level of detection, which significantly improved fasting glycemia and oral glucose tolerance in leptin deficient, diet-induced IR mice [94]. Above mentioned reports suggest that antibiotic treatments may enhance glucose tolerance through the gut microbiota modulation.

A combination of three different antibiotics (Ampicillin, neomycin and metrodinazole) treatment greatly modified the gut microbiota with significant reduction in the levels of *Bacteroidetes* and *Firmicutes*, overall bacteria count and circulating plasma LPS levels. This manipulation also reduced the levels of fasting glucose, insulin, TNF-α and IL-6 along with reduced activity of TLR4, JNK, IKKβ which phosphorylates IRS-1 and subsequently improved glucose tolerance and insulin action in metabolically active tissues. These results suggest that modulation of gut microbiota in obesity through antibiotics can improve insulin signaling and glucose tolerance by reducing circulating LPS levels, and TLR mediated inflammatory signaling [95]. Murphy *et al.* have studied that vancomycin and bacteriocins producing probiotics altered the gut microbiota in the diet induced obese mice. However, only vancomycin treatment had improved metabolic abnormalities via significant reduction in *Firmicutes* and *Bacteroidetes* and increase in *Proteobacteria* [96]. Previously, we showed that gut microbiota modulation with cefdinir microspheres had significantly improved IR, glucose intolerance, triglycerides, and hepatic damages in high-fructose diet fed rats [97].

Until now there has been no report available regarding the participation of NLRs in reduction of diabetic symptoms through the modulation of gut microbiota by antibiotic application. NOD1 and NOD2 may be involved in the inflammatory based process of IR through the recognition of gut microbiota. Reduction of increased gram negative microflora population in diabetic subjects through the application of spectrum specific antibiotics may decrease relative expression of NOD1 as it specifically gets activated by gram negative bacteria. Moreover, it can be hypothesized that relatively decreased expression of TLRs and NLRs through the antibiotics treatment in diabetic individuals, may downregulate the NF-kB signaling. The downregulated activity of NF-kB may reduce the expression of proinflammatory cytokines, which thereby decreases the incidence of IR and metabolic abnormalities. Understanding such a crosstalk of TLRs and NLRs along with the gut microbiota modulation in pathogenesis of IR may provide new insights to develop novel therapeutics for T2D. However, extensive study will be required to understand such a complex pathway and their intercalative role in the progression of metabolic disorders like T2D, obesity and IR.

THE FUTURE MODULATORS OF THE TLRS AND NLRs

TLRs and NLRs successfully fulfill several criteria and indicated them as effective therapeutic targets to treat many inflammatory diseases. Growing research indication through well characterized strategies has also linked immune responses with TLRs and NLRs in the development of metabolic disorders. However, efforts to identify and characterize novel modulators of TLRs and NLRs dependent signaling and inflammation for treatment of metabolic disorders are still not significantly implemented. to treat several complications such as allergy, pain reduction, autoimmunity, inflammation, ischemia, reperfusion, inflammatory bowel disease and rheumatoid arthritis [98]. Several NLRs targeted therapeutics also have been developed to treat rheumatic disease and many others [99, 100]. However, the TLR inhibitory activity of new therapeutic agents in a direction to reduce diabetes induced inflammation is not well documented or

studied. Thus, these approaches need to be applied to target TLR or NLR regulated pathways in diabetes and related metabolic complications.

CONCLUSION

Obesity and T2D are associated with the increased level of adipocytes derived FFA, proinflammatory cytokines and gram negative bacteria derived LPS, which causes inflammation through TLR and NLR signaling pathways. Innate immune receptors such as TLRs and NLRs play a significant role in pathogenesis of obesity mediated insulin resistance through induction of proinflammatory cytokines which further causes downregulation of IRS pathway. Gut microbiota modulation with certain antibiotics has increased insulin sensitivity by decreasing the inflammation through TLR and NF-kB pathway. Role of NOD1/NOD2 can also be studied in relation with gut microbiota and insulin resistance in pathogenesis of T2D. In future, TLRs and NLRs and gut microbiota targeted therapeutics can be developed in direction to treat the metabolic disorders like obesity and T2D. However, it needs extensive research to be understood.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors are thankful to Nirma Education and Research Foundation [NERF], Ahmedabad for providing infrastructure and financial support.

ABBREVIATIONS

T2D	=	Type 2 Diabetes
IR	=	Insulin Resistance
TLR	=	Toll like receptors
NOD	=	Nucleotide Oligomerization domain
FFA	=	Free fatty acid
LPS	=	Lipopolysaccharide
IL-1β	=	Interleukin- 1β
TNF-α	=	Tumor necrosis factor- α
CRP	=	C-reactive protein
VLDL	=	Very low density lipoprotein
PRRs	=	Pattern reorganization receptors
PAMP	=	Pathogen associated molecular patterns
CLR	=	C-type lectin receptors
RLR	=	Retinoic acid inducible gene(RIG) like receptors
LRR	=	c-terminal-Leucine rich repeats
MyD88	=	Myeloid differentiation protein-88
IRAK	=	Interleukin-1 receptor-associated kinase
TRAF6	=	TNF receptor associated factor
IL-6	=	Interleukin-6
AT	=	Adipose tissue
IRS	=	Insulin receptor signaling
IR	=	Insulin resistance
m-RNA	=	Messenger- ribonucleic acid
T1D	=	Type 1 Diabetes
DGGE	=	Denaturing gradient gel electrophoresis
MGWAS	=	Metagenome wide association study
qPCR	=	Quantitative Polymerase cycle reaction
NLR	=	NOD-like receptors
TIR	=	Toll/IL-1 receptor
KO	=	Knockout
CD14	=	Cluster of differentiation-14
HSP60	=	Heat shock protein 60
HSP70	=	Heat shock protein 70
CARD	=	Caspase recruitment domain
PYD	=	Pyrin domain
BIR	=	Baculovirus inhibitor receptor
NF-κB	=	Nuclear factor kappa-light-chain-enhancer of activated B cells
MAPK	=	Mitogen activated protein kinase
iE-DAP	=	γ -D-glutamyl-mdiaminopimelic m diaminopimelic acid
MDP	=	Muramyl dipeptide
RIP2	=	Receptor interacting protein kinase 2
DAG	=	Diacylglycerol
HFD	=	High fat diet
JNK	=	c-Jun N-terminal Kinase
ZymA	=	Zymosan-A
WT	=	Wild type Species
FA	=	Fatty acid
PBMCs	=	Peripheral blood mononuclear cell
MIN6	=	mouse insulinoma cells
PGN	=	Peptidoglycan
AUC	=	Area under curve
NLRP3	=	NOD-like receptor family, pyrin domain containing 3
DNA	=	Deoxy Ribonucleic acid
IKKβ	=	3-Phosphoinositide-dependent Protein Kinase-1-mediated IκB Kinase β

REFERENCES

[1] International Diabetes Federation. Diabetes Atlas 2013; 5th ed. Brussels.

[2] Turnbaugh PJ, Ley RE, Mahowald MA *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444:1027–1031.

[3] Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology 2007; 132: 2169-2180.

[4] Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004; 53: 693-700.

[5] Aldhahi W, Hamdy O. Adipokines, inflammation, and the endothelium in diabetes. Curr Diab Rep 2003; 3: 293-298.

[6] Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes care 2004; 27: 813-823.

[7] Krogh-Madsen R, Plomgaard P, Moller K, Mittendorfer B, Pedersen BK. Influence of TNF-alpha and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans. Am J Physiol Endocrinol Metab 2006; 291: E108-114.

[8] Luft VC, Schmidt MI, Pankow JS *et al.* Chronic inflammation role in the obesity-diabetes association: a case-cohort study. Diabetol Metab Syndr 2013; 5: 31.

[9] Sjoholm A, Nystrom T. Inflammation and the etiology of type 2 diabetes. Diabetes Metab Res Rev 2006; 22: 4-10.

[10] Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 2000; 148: 209-214.

[11] Pearson TA, Mensah GA, Alexander RW, *et al.* Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003; 107: 499-511.

[12] Sattar N, Wannamethee SG, Forouhi NG. Novel biochemical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities? Diabetologia 2008; 51: 926-940.

[13] Pradhan A, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001; 286: 327-334.

[14] Tateya S, Kim F, and Tamori, Y. Recent advances in obesity-induced inflammation and insulin resistance. Front Endocrinol 2013; 4: 93.

[15] Murri M, Leiva I, Gomez-Zumaquero JM *et al.* Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC med 2013; 11: 46.

[16] Wu X, Ma C, Han L *et al.* Molecular characterisation of the faecal microbiota in patients with type II diabetes. Curr Microbiol 2013; 61: 69-78.

[17] Qin J, Li Y, Cai Z, *et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012; 490: 55-60.

[18] Larsen N, Vogensen FK, Jakobsen M. Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. PLoS one 2010; 5(2): e9085.

[19] Karlsson FH, Tremaroli V, Nookaew I *et al.* Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 2013; 498: 99-103.

[20] Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005; 17 (1): 1–14.

[21] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006; 124: 783-801.

[22] Beutler B, Jiang Z, Georgel P *et al.* Genetic analysis of host resistance: Toll-like receptor signaling and immunity at large. Annu Rev Immunol 2006; 24: 353- 389.

[23] Osorio F, Reis e Sousa C. Myeloid C-type lectin receptors in pathogen recognition and host defense. Immunity. 2011; 34: 651- 664.

[24] Dixit E, Kagan JC. Intracellular Pathogen Detection by RIG-I-Like Receptors. Adv Immunol 2013; 117: 99-125.

[25] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010; 11: 373-384.

[26] Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001; 2: 675-680.

[27] Smith M, Mitchell A, Ding S *et al.* Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-κB activation and chemokine expression by epithelial cells. J Biol Chem. 2003; 278: 32552.

[28] Takeuchi O, Kawai T, Muhlradt PF *et al.* Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int Immunol 2001; 13: 933-940.

[29] Alexopoulou L, Thomas V, Schnare M *et al.* Hyporesponsiveness to vaccination with Borrelia burgdorferi OspA in humans and in TLR1- and TLR2-deficient mice. Nat Med 2002; 8: 878-884.

[30] Land WG. The role of post ischemic reperfusion injury and other non antigen-dependent inflammatory pathways in transplantation. Transplantation 2005; 79: 505-514.

[31] Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007; 449: 819-826.

[32] Gao B, Tsan MF. Endotoxin contamination in recombinant human heat shock protein 70 (Hsp70) preparation is responsible for the induction of tumor necrosis factor alpha release by murine macrophages. J Biol Chem 2003; 278: 174-179.

[33] Akira S, Takeda K. Toll-like receptors signaling. Nat Rev Immunol 2004; 4: 499-511.

[34] Kang JY, Nan X, Jin MS, *et al.* Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. Immunity 2009; 31: 873- 884.

[35] Jin MS, Kim SE, Heo JY *et al*. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell 2007; 130: 1071-1082.

[36] Doyle SL, O'Neill LA. Toll-like receptors: from the discovery of NF kappaB to new insights into transcriptional regulations in innate immunity. Biochem Pharmacol 2006; 72: 1102-1113.

[37] Yamamoto M, Sato S, Hemmi H, *et al.* Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 2003; 301: 640-643.

[38] Chen G, Shaw MH, Kim YG, Nunez G. NOD-like receptors: role in innate immunity and inflammatory disease. Annu Rev Pathol 2009; 4: 365-398.

[39] Ting JP, Lovering RC, Alnemri ES, *et al.* The NLR gene family: a standard nomenclature. Immunity 2008; 28: 285-287.

[40] Kobayashi K, Inohara N, Hernandez LD, *et al.* RICK/Rip2/ CARDIAK mediates signaling for receptors of the innate and adaptive immune systems. Nature 2002; 416: 194-199.

[41] Opitz B, Puschel A, Beermann W *et al.* Listeria monocytogenes activated p38 MAPK and induced IL-8 secretion in a nucleotide-binding oligomerization domain 1-dependent manner in endothelial cells. J Immunol 2006; 176: 484- 490.

[42] Hasegawa M, Fujimoto Y, Lucas PC *et al*. A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. EMBO J 2008; 27: 373-383.

[43] Park JH, Kim YG, McDonald C, *et al.* RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. J Immunol 2007; 178: 2380-2386.

[44] Abbott DW, Wilkins A, Asara JM, Cantley LC. The Crohn's disease protein, NOD2, requires RIP2 in order to induce ubiquitinylation of a novel site on NEMO. Curr biol 2004; 14: 2217-2227.

[45] Hayden MS, Ghosh S. Signaling to NF-kappaB. Gen Dev 2004; 18: 2195- 2224.

[46] Dasu MR, Devaraj S, Park S, Jialal I. Increased toll-like receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects. Diabetes care 2010; 33: 861-868.

[47] Boden G, She P, Mozzoli M *et al.* Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. Diabetes 2005; 54: 3458-3465.

[48] Boden G. Obesity and free fatty acids. Endocrinol Metab Clin North Am 2008 37: 635-646, viii-ix.

[49] Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr 2006; 83: 461s-465s.

[50] Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes 2002; 51: 2005-2011.

[51] Davis JE, Braucher DR, Walker-Daniels J, Spurlock ME. Absence of Tlr2 protects against high-fat diet-induced inflammation and results in greater insulin-stimulated glucose transport in cultured adipocytes. J Nutr Biochem 2011; 22: 136-141.

[52] Himes RW, Smith CW. Tlr2 is critical for diet-induced metabolic syndrome in a murine model. FASEB J 2010; 24: 731-739.

[53] Nguyen MT, Favelyukis S, Nguyen AK *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. J Biol Chem 2007; 282: 35279-35292.

[54] Senn JJ. Toll-like receptor-2 is essential for the development of palmitate-induced insulin resistance in myotubes. J Biol Chem 2006; 281: 26865-26875.

[55] Caricilli AM, Nascimento PH, Pauli JR *et al.* Inhibition of toll-like receptor 2 expression improves insulin sensitivity and signaling in muscle and white adipose tissue of mice fed a high-fat diet. J Endocrinol 2008; 199: 399-406.

[56] Song MJ, Kim KH, Yoon JM, Kim JB. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. Biochem Biophys Res Commun 2006; 346: 739-745.

[57] Murakami K, Bujo H, Unoki H, Saito Y. High fat intake induces a population of adipocytes to co-express TLR2 and TNF alpha in mice with insulin resistance. Biochem Biophys Res Commun 2007; 354: 727-734.

[58] Reyna SM, Ghosh S, Tantiwong P *et al.* Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. Diabetes 2008; 57: 2595-2602.

[59] Davis JE, Gabler NK, Walker-Daniels J, Spurloc ME. Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. Obesity (Silver Spring, Md) 2008; 16: 1248-1255.

[60] Devaraj S, Jialal I, Yun M, and Bremer A. Demonstration of increased toll-like receptor 2 and toll-like receptor 4 expression in monocytes of type 1 diabetes mellitus patients with microvascular complications. Metab Clin Exp 2011; 60: 256-259.

[61] Milanski M, Degasperi G, Coope A *et al.* Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci 2009; 29: 359-370.

[62] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116: 3015-3025.

[63] Thomas GP, Hemmrich K, Abberton KM *et al.* Zymosan-induced inflammation stimulates neo-adipogenesis. Int J Obes (Lond) 2008; 32: 239-248.

[64] Kleinridders A, Schenten D, Konner AC *et al.* MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. Cell Metab 2009; 10, 249-259.

[65] Lee SM, Choi SE, Lee JH *et al.* Involvement of the TLR4 (Toll-like receptor4) signaling pathway in palmitate-induced INS-1 beta cell death. Mol Cell Biochem 2011; 354: 207-217.

[66] Eguchi K, Manabe I, Oishi-Tanaka Y *et al.* Saturated fatty acid and TLR signaling link beta cell dysfunction and islet inflammation. Cell Metab 2012; 15: 518-533.

[67] Dasu MR, Park S, Devaraj S, Jialal I. Pioglitazone inhibits Toll-like receptor expression and activity in human monocytes and db/db mice. Endocrinol 2009; 150: 3457-3464.

[68] Jialal I, Huet BA, Kaur H, Chien A, Devaraj S. Increased toll-like receptor activity in patients with metabolic syndrome. Diabetes care 2012; 35: 900-904.

[69] Fabre O, Breuker C, Amouzou C, Salehzada T, Kitzmann M, Mercier J, Bisbal C. Defects in TLR3 expression and RNase L activation lead to decreased MnSOD expression and insulin resistance in muscle cells of obese people. Cell Death Dis 2014; 5: e1136.

[70] Wu X, Ma C, Han L, *et al.* Molecular characterisation of the faecal microbiota in patients with type II diabetes. Curr Microbiol 2010; 61: 69-78.

[71] Nishimoto S, Fakuda D, Shimabukuro M *et al.* Genetic ablation of TLR9 improves insulin resistance through macrophage accumulation in adipose tissue. Eur Heart J 2013 34 (1) doi: 10.1093/eurheartj/eht309.P3267

[72] Backhed F, Ding H, Wang T *et al.* The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004; 101: 15718-15723.

[73] Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. Annu Rev Biochem 2002 71, 635-700.

[74] Cani PD, Bibiloni R, Knauf C *et al.* Gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008; 57: 1470-1481.

[75] Cani PD, Amar J, Iglesias MA *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007; 56: 1761-1772.

[76] Creely SJ, McTernan PG, Kusminski CM *et al.* Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 2007; 292: E740-747.

[77] Brun P, Castagliuolo I, Di Leo *et al.* Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. Am J Physiol-Gastr L 2007; 292: G518-525.

[78] Lassenius MI, Pietilainen KH, Kaartinen K *et al.* Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. Diabetes care 2011; 34:1809-1815.

[79] Ghanim H, Abuaysheh S, Sia CL *et al.* Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. Diabetes care 2009; 32: 2281-2287.

[80] Garay-Malpartida HM, Mourao RF, Mantovani M, Santos IA, Sogayar MC, Goldberg AC. Toll-like receptor 4 (TLR4) expression in human and murine pancreatic beta-cells affects cell viability and insulin homeostasis. BMC Immunol 2011; 12: 18.

[81] Schertzer JD, Tamrakar AK, Magalhaes JG *et al.* NOD1 activators link innate immunity to insulin resistance. Diabetes 2011; 60: 2206-2215.

[82] Schertzer JD, Klip A. Give a NOD to insulin resistance. Am J Physiol Endocrinol Metab 2011; 301: E585-586.

[83] Tamrakar AK, Schertzer JD, Chiu TT *et al.* NOD2 activation induces muscle cell-autonomous innate immune responses and insulin resistance. Endocrinology 2011; 151: 5624-5637.

[84] Amar J, Chabo C, Waget A *et al.* Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol Med 2011; 3: 559-572.

[85] Zhao L, Hu P, Zhou Y, Purohit J, Hwang D. NOD1 activation induces proinflammatory gene expression and insulin resistance in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 2011; 301: E587-598.

[86] Vandanmagsar B, Youm YH, Ravussin A *et al.* The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011; 17: 179-188.

[87] Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2010; 11: 136-140.

[88] Wen L, Ley RE, Volchkov PY *et al.* Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 2008; 455: 1109-1113.

[89] Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PloS one 2010; 5: e9836.

[90] De La Cochetiere MF, Durand T, Lepage P, Bourreille A, Galmiche JP, Dore J. Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. J Clin Microbiol 2005; 43: 5588-5592.

[91] Begin-Heck N, Bourassa M, Heick HM. The effect of oxytetracycline on insulin resistance in obese mice. Biochem J. 1974; 142: 465-475.

[92] Dalpe-Scott M, Heick HM, Begin-Heick N. Oxytetracycline treatment improves the response to insulin in the spontaneously diabetic (BB) rat. Diabetes 1982; 31: 53-59.

[93] Membrez M, Blancher F, Jaquet M *et al.* Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. FASEB J 2008; 22: 2416-2426.

[94] Chou CJ, Membrez M, Blancher F. Gut decontamination with norfloxacin and ampicillin enhances insulin sensitivity in mice. Nestle Nutrition workshop series Paediatric programme 2008; 62: 127-137; discussion 137-140.

[95] Carvalho BM., Guadagnini D, Tsukumo DM *et al.* Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice. Diabetologia 2012; 55: 2823-2834.

[96] Murphy EF, Cotter PD, Hogan A *et al.* Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. Gut 2013; 62: 220-226.

[97] Jena PK, Singh S, Prajapati B, NareshKumar G, Mehta T, Seshadri S. Impact of targeted specific antibiotic delivery for gut microbiota modulation on high-fructose-fed rats. Appl Biochem Biotechnol 2014; 172, 3810-3826.

[98] Basith S, Manavalan B, Lee G, Kim SG, Choi S. Toll-like receptor modulators: a patent review (2006-2010). Expert Opin Ther Pat 2011; 21: 927-944.

[99] O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. Nat Rev Immunol 2011; 11: 163-175.

[100] Sidiropoulos PI, Goulielmos G, Voloudakis GK, Petraki E, Boumpas DT. Inflammasomes and rheumatic diseases: evolving concepts. Ann Rheum Dis 2008; 67: 1382-1389.

Received: February 07, 2014 Revised: May 05, 2014 Accepted: May 14, 2014