Insulin resistance and increased lipolysis in bone marrow derived adipocytes stimulated with agonists of Toll-like receptors

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Abstract

Our objectives were to identify Toll-like receptors (TLRs) in human bone marrow derived adipocytes, to test specific TLR agonists for their ability to induce a proinflammatory response, and to investigate possible metabolic effects after TLR activation, in particular, those associated with insulin resistance and lipolysis. Mesenchymal stem cells were isolated from human bone marrow and differentiated into adipocytes. Total RNA before or after stimulation with agonists specific for TLR was extracted for analysis of expression of TLRs proinflammatory signals and molecules involved in glucose metabolism (IRS-1 and GLUT4). Furthermore, cytokine protein expression was measured from cell lysates. Finally, insulin induced glucose uptake and lipolysis were measured. Human bone marrow-derived adipocytes express TLR1-10. They react to stimulation with specific ligands with expression of inflammatory markers (IL-1beta, IL-6, TNFalpha, IL-8, MCP-1) at the RNA and protein levels. IRS-1 and GLUT4 expression was downregulated after stimulation with the TLR4 and TLR3 specific ligands LPS and poly (I:C), respectively. Insulin-induced glucose uptake was decreased and lipolysis increased. We conclude that adipocytes express TLR 1-10 and react to agonists specific for TLR 1-6. As a consequence proinflammatory cytokine are induced, in particular, IL-6, IL-8, and MCP-1. Since stimulation is followed by decreased insulin-induced glucose uptake and increased lipolysis we conclude that TLRs may be important linking molecules in the generation of insulin resistance in fat tissue.
Insulin Resistance and Increased Lipolysis in Bone Marrow Derived Adipocytes Stimulated with Agonists of Toll-like Receptors

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Key words
- inflammation
- interleukin-6
- poly (I:C)
- LPS
- type 2 diabetes
- obesity
- insulin resistance

Abstract

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Introduction

Type 2 diabetes mellitus (T2DM) is associated with a state of chronic inflammation [1,2]. Upregulation of C-reactive protein and IL-6 as important elements of the acute phase response are predictive markers for the development of T2DM [3-5]. It has been postulated that IL-6 is not necessary and not sufficient for the development of type 2 diabetes [6], but it has also been shown that IL-6 treatment induced insulin resistance and reduced insulin-induced lipogenesis in cultivated murine adipocytes [7]. Insulin resistance also occur in human patients with bacterial sepsis [8] and in healthy humans after administration of bacterial lipopolysaccharide (LPS) [9]. LPS is recognized by Toll-like receptor 4 (TLR4), an important member of a family of receptors that recognize pattern associated molecular patterns and endogenous molecules like heat-shock proteins [10], mRNA [11], and free fatty acids [12]. Activation of TLR4 by LPS results in nuclear translocation of NFκB and subsequent expression of proinflammatory proteins including TNFα, IL-1β, IL-6, INOS as well as chemokines, which contribute to cellular infiltration, tissue damage, and apoptosis. The presence of TLR4 and TLR2 as well as their functionality resulting in the secretion of TNFα was demonstrated in human adipocytes [13]. The presence of TLR2 and reactivity to stimulation with peptidoglycan and subsequent secretion of IL-6 was demonstrated in murine 3T3-L1 adipocytes [14]. The role of IL-6 in type 2 diabetes is controversial, but IL-6 has been shown to decrease Insulin Receptor Substrate-1 (IRS-1) protein expression and insulin-stimulated tyrosine phosphorylation as well as insulin stimulated glucose transport in 3T3-L1 adipocytes [15], which are all considered key events in insulin resistance. It has been estimated that in humans, adipose tissue produces up to one third of the circulating IL-6 [16]. LPS was shown to stimulate lipolysis in primary murine adipocytes [17]. Fatty acids that circulates at
higher concentrations in obese patients have been shown to induce insulin resistance in mice via TLR4 [12]. In the light of this facts it appears important to better characterize the role of TLRs as potential mediators of a proinflammatory response that influence insulin sensitivity and glucose metabolism during infections, general immune stimulation and obesity. The aim of the study was to investigate the expression and the reactivity of Toll-like receptors (TLRs) in human bone marrow derived adipocytes. Furthermore, we wanted to test the ability of TLR agonists to induce a proinflammatory response and if this activation affects adipocyte metabolism, in particular insulin sensitivity and lipolysis.

Materials and Methods

Mesenchymal stem cells
Bone marrow samples were obtained from healthy volunteers (18–63 years, BMI 18–25) during routine orthopedic surgical procedures, in accordance with the local ethical committee and informed consent. Immediately after collection, heparinized bone marrow samples were processed to obtain mesenchymal stem cells. Each 5 ml sample of bone marrow was diluted with 15 ml phosphate-buffered saline (PBS). The 20 ml suspension was then overlaid to 15 ml of Ficoll Histopaque-1077 (Sigma, Deisenhofen, Germany). The solution was centrifuged at 800 g for 25 min at room temperature. The upper phase was aspirated and the interphase transferred to a 15 ml tube. The tube was filled up with PBS and centrifuged at 700 g for 15 min at room temperature. After discarding the supernatant, the pellet was washed with 10 ml PBS. This washing procedure was repeated twice. Finally the cell pellet was resuspended in Dulbecco’s Modified Eagle’s Medium (DMEM, Cambrex, East Rutherford, NJ, USA) containing 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA), and 5 ng human recombinant fibroblast growth factor (FGF, Invitrogen) prewarmed at 37 °C. Cells were seeded in 25 or 75 cm² dishes, with the Reverse Transcription System (Promega, Madison WI, USA) using oligo dT primer and following the manufacturer’s instructions.

Differentiation protocol of adipocytes
After allowing mesenchymal stem cells to reach 100% confluence, adipogenic differentiation was induced by incubation with DMEM containing F12 salts (Cambrex) supplemented with 3% FBS, 50 μg/ml gentamycin (Gibco BRL, Gaithersburg, MD, USA), 100 nM regular insulin (Novo-Nordisk, Küssnacht, Switzerland), 1 μM rosiglitazone (Glaxo-Smith Kline, Worthing, UK), 5 μM transferrin (Calbiochem, San Diego, CA, USA), 1 μM dexamethasone, 0.2 nM 3,5,5-triiodo-3-thyronine, 250 μM 3-isobutyl-1-methylxanthine, 100 μM L-ascorbic acid, 33 μM 2-biotin, and 15 mM β-pantothenic acid (all from Sigma). The medium was changed every 3 days until at least 60% of mesenchymal stem cells underwent adipogenic differentiation, as assessed by formation of lipid droplets.

TLR agonists
TLR agonists were purchased from Invitrogen (LabForce AG Nunningen, Switzerland) and were used at the concentration indicated in Table 1. To assess the effects of TLR agonists on mRNA expression specific for cytokines TLR agonists were added to the cells and incubated for 8 h (unless otherwise indicated). Then supernatants were discarded and the cell monolayer was dissolved in 300 μl RLT solution (Quiagen, Hilden, Germany) and processed for RNA extraction.

RNA extraction and cDNA synthesis
RNA was isolated using the RNeasy Mini RNA isolation kit (Quiagen, Hilden, Germany) following the protocol described by the manufacturer. RNA concentration was measured spectrophotometrically. 1 μg of RNA were then reverse transcribed into cDNA with the Reverse Transcription System (Promega, Madison WI, USA) using oligo dT primer and following the manufacturer’s instructions.

PCR
Conventional PCR was set up in a 20 μl volume using GoTaq Green mastermix (Promega, Catalys AG, Wallisellen, Switzerland). For amplification of TLR a commercially available primer set was used (TLR RT-Primer set, Invitrogen, LabForce AG, Nunningen, Switzerland). The PCR was run on a thermal cycler under following conditions: initial denaturation step: 2 min 95 °C followed from 40 cycles of denaturation 30 s at 95 °C, annealing for 30 s at 55 °C, elongation for 1 min at 72 °C. Finally a 5-min elongation step at 72 °C was performed and 10 μl of the products were assessed on a 2% agarose gel.

Real-time PCR
Real time PCR was performed using a BIO-RAD Cycler iQ and the iQ SYBR green supermix (Bio-Rad, Wallisellen, Switzerland).

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Source/full name</th>
<th>Specific TLR</th>
<th>Working concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pam3CSK4</td>
<td>Tripalmitoylated lipopeptide</td>
<td>1/2</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>HRKL</td>
<td>Heat killed Listeria monocytogenes</td>
<td>2</td>
<td>10⁶ cells/ml</td>
</tr>
<tr>
<td>LTA</td>
<td>LTA from Staphylococus aureus</td>
<td>2</td>
<td>20 μg/ml</td>
</tr>
<tr>
<td>PGN</td>
<td>Peptidoglycan from Staphylococcus aureus</td>
<td>2 (NOD*)</td>
<td>100 μg/ml</td>
</tr>
<tr>
<td>Poly (I:C)</td>
<td>Poly[β(2R,3S,5R,SR')-5-s-4-amino-2-oxopropimidin-1-yl]-3,4-dihydroxyxolan-2-yl)methyl dihydrogen phosphate</td>
<td>3</td>
<td>25 μg/ml</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide from E. coli K12</td>
<td>4</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>Flagellin</td>
<td>Flagellin from Salmonella thphimurium</td>
<td>5</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>FSL1</td>
<td>FSL1 (Pam3CysKPSF)</td>
<td>6/2</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>Imiquimod</td>
<td>Imiquimod (R837)</td>
<td>7</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>ssRNA</td>
<td>Single stranded RNA</td>
<td>8</td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>ODN 2006</td>
<td>Oligonucleotide with unmethylated CpG dinucleotides</td>
<td>9</td>
<td>5 μM</td>
</tr>
</tbody>
</table>

* Others receptors described, i.e., NOD 1, 2 [27]
Samples were tested in duplicates. Reactions were run in a volume of 20 μl using a cDNA amount corresponding to 25 ng of the original RNA. The concentration of oligonucleotide primers in Table 2 was 250 nM. The amplification protocol was as follows: 95 °C 3 min (polymerase activation), 44 cycles of 95 °C 10 s, 62 °C 15 s, 72 °C 30 s. Finally, a melting curve using 0.5 °C steps of 10 s duration starting from 57 °C was determined. Amplification efficiency was determined by using 10-fold serial dilutions of low concentration of specific PCR products. All PCR reactions were tested for amplification efficiency that varied between 90 and 110%.

Threshold cycle values (ct) were normalized using the ΔΔct method using GAPDH (glyceraldehyde 3-phosphate dehydrogenase) as housekeeping gene.

**Validation of GAPDH as housekeeping Gene**

We compared the threshold cycle (ct) values obtained from the standardized amount of RNA used in our stimulation experiments. The ct values for GAPDH obtained from 10 ng of RNA did not significantly differ between different treatment groups (data not shown). Therefore, we used the GAPDH signal for normalization.

**Cytokine assays in adipocyte lysates**

Adipocytes from 3 different donors were grown in 6-well plates and stimulated for 24 h with LPS or poly (I:C) (poly[(2′-5′)-methyl dihydrogen phosphate]). The supernatant was then recognized as a very faint band and is not visible in Fig. 1a. The product obtained for TLR10 was only recognized as a very faint band and is not visible in Fig. 1a. The negative controls without addition of reverse transcriptase did not generate any signal (not shown).

**Silencing of TLR3**

For RNA silencing we used a HSV-amplicon based method described by Saydam et al. [18]. DNA oligonucleotides templates targeting TLR3 were synthesized as 60-mer sense and antisense (19 × 2 nucleotides specific to the targeted TLR3 gene spaced by 9 nucleotides for hairpin and additional 13 nucleotides for restriction enzyme recognition sites).

Sense: 5′-GTCGGATCCATATAGGCGAGCTATACAGGCTACAGTACAGGCTGCTATACGGGGGAGGCCTATACACAGCAGTACGCTCTATACCTTTTTA-3′; Antisense: 5′-AGCTTAAAAAGGAAGTTACGATGTCGAAACGCTAATACGCTCTGCTATACGCAGTACAGGCTGCTATACCTTTTTA-3′.

**Insulin induced glucose uptake**

Glucose uptake experiments were performed as previously described [19]. Briefly, MSC-derived adipocytes were washed 3 times in warm PBS and kept in DMEM/F12 containing 5 mM glucose and 3% FCS. After 24 h, TLR agonists were added. After 2 h of stimulation, fresh medium with TLR agonists was provided. After 2 h, 100 nM insulin was added to half of the wells and incubated for 20 min. d-3H-2-Deoxyglucose (2-[3H(G)], (1 μCi; PerkinElmer, Boston, MA, USA) was added to all wells and incubation was continued for 15 min. Cells were washed 3 times with ice cold PBS and lysed in 0.1% SDS. Radioactivity was measured with a scintillation counter.

**Lipolysis**

Glycerol content in phenol red free supernatants was determined using the UV-method kit from R-Biopharm (Darmstadt, Germany) according to the manufacturer’s protocol.

**Statistical analysis**

Statistical analysis was performed using Prism 5 for Mac OS X, version 5.0. GraphPad Software Inc. La Jolla, CA, USA. The statistical test used is indicated in the results section.

**Results**

**Bone marrow derived adipocytes express TLRs**

Using a set of commercially available oligonucleotide primers specific for TLR1 – 10, we analyzed cDNA, derived from in vitro matured adipocytes. A specific amplification product was obtained for TLR1 – 9 (Table 1). The product obtained for TLR10 was only recognized as a very faint band and is not visible in Fig. 1a. The negative controls without addition of reverse transcriptase did not generate any signal (not shown).

Bone marrow derived adipocytes react to TLR agonists

We tested the functionality of the TLR identified by RT-PCR by stimulating adipocytes with well-characterized commercially available specific agonists (indicated in Table 1). The expression of TNFα and IL-6 was used as readout. Bone marrow-derived adipocytes expressed significantly higher levels of IL-6 and TNFα after stimulation with TLR agonists specific for TLR1-6. In contrast TLR7-9 specific ligands failed to induce proinflammatory cytokines (Fig. 1b).

**Stimulation of TLR 3 and 4 is accompanied by an upregulation of proinflammatory cytokines at protein level**

In the previous experiment we found a strong IL-6 mRNA induction upon stimulation of adipocytes with LPS and poly (I:C). Therefore we focused on these 2 agonist and measured expression of 4 cytokines IL-1β, TNFα, IL-6, and VEGF, and 3 chemokines, IL-8, MCP-1, and MIP-1α at the protein level (Fig. 2a, b). As tested by ANOVA and subsequent Bonferroni multicomparison post-test, a significant increase was observed for all proteins considered except for MIP-1α and VEGF. This indicates that the induction of mRNA expression by activation of TLR3 and TLR4 was followed by the translation of proinflammato-

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Table 1: Oligonucleotide Primers used in RT-PCR

<table>
<thead>
<tr>
<th>Target</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>CAATGCCTCCTCGACACCACCA</td>
<td>GGATGCCTTGAGGCGGCCCT</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>TTGCTGCTAGTGCCTTC</td>
<td>TGCTTCTGAAGGCTAGTG</td>
<td>[30]</td>
</tr>
<tr>
<td>TNFα</td>
<td>CCCCCAGGACTCTCCTCAATC</td>
<td>GGGTTGCTACAATGCGCTACA</td>
<td>[31]</td>
</tr>
<tr>
<td>GLUT4</td>
<td>CGGTACCACCCGCCAGCCCC</td>
<td>CGAGACCAAGGTTGAAGCTG</td>
<td></td>
</tr>
</tbody>
</table>
TLR3 was efficiently downregulated after siRNA treatment
Several potential receptors may mediate the effects of poly (I:C) or double stranded RNA (i.e., the cytoplasmic RNA helicases), RIG-I, and MDA5 [20]. Thus silencing of TLR3 was performed using Herpes simplex virus 1 amplicon vector-mediated siRNA with the purpose to test if the observed effects were due to TLR3 or to other receptors. The expression of TLR3 was reduced to 54% (0.54-fold vs. 1-fold) in absence of stimulation and to 16% upon poly (I:C) stimulation (3-fold vs. 18.6-fold increase). However the 3-fold increase in poly (I:C) stimulated adipocytes was not statistically significant. (ii) Effect of scrambled-siRNA transduction on TLR3 expression: In unstimulated adipocytes TLR3 mRNA was 3.25 times higher. If these cells were treated with poly (I:C) TLR3 was 13.6 times more abundant (Fig. 2c). We conclude that transduction by itself induced a minimal but significant upregulation of TLR3, however, siRNA was strong enough to lead to a substantial reduction of TLR3 mRNA. Furthermore our silencing strategy using herpes simplex virus based amplicons efficiently downregulated TLR3 expression.

Silencing of TLR3 decreases IL-6 expression
(i) Effect of TLR3-siRNA transduction on IL-6 expression: In adipocytes stimulated with poly (I:C) the IL-6 signal obtained after silencing TLR3 was reduced to 41.5%. (51.4-fold vs. 123.6-fold increase) of the signal obtained without silencing with a significant difference between the two (Mann-Whitney test, p < 0.05). (ii) Effect of scrambled-siRNA transduction on TLR3 expression: the IL-6 expression did not significantly differ from that of stimulated not transduced adipocytes (106.4-fold vs. 123.6-fold increase). IL-6 was not significantly increased in unstimulated adipocytes, independently of the siRNA used. We conclude that the IL6 mRNA induced by poly (I:C) treatment was substantially due to TLR3 triggering (Fig. 2b).

IRS-1 mRNA is downregulated after stimulation with poly (I:C) and LPS
IRS-1 is a key molecule in insulin signaling and IRS-1 downregulation at mRNA level has been associated with insulin resistance. Post-translationally IRS-1 activity is regulated via phosphorylation events that can activate or deactivate the signaling cascade. Here we found a decrease of mRNA specific for IRS-1 after 24 h of stimulation with poly (I:C) or LPS (Fig. 3).

GLUT4 mRNA is downregulated after stimulation with poly (I:C) and LPS
GLUT4 mRNA was significantly reduced in adipocytes by 24 h (Fig. 3), but not by an 8-h (not shown) stimulation with poly (I:C) and LPS as tested by the one sample t-test. This indicates that stimulation affects insulin dependent glucose transport.

Influence of poly (I:C) and LPS stimulation on glucose uptake in vitro
We tested insulin-dependent and insulin-independent glucose uptake (uptake of 3H-2-deoxyglucose) in MSC-derived adipocytes from 3 donors. Fig. 4a shows data normalized to basal glucose uptake (spontaneous uptake without addition of insulin) in the absence of TLR stimulation. In control cultures of adipocytes without addition of TLR agonists insulin increased the glucose uptake by 3.2 times. This increase was set to 100% insulin effect. In LPS stimulated adipocytes this effect was reduced to 33.3% and after poly (I:C) stimulation to 33.3%. Coapplication of both agonists reduced insulin mediated glucose uptake to 18.2%.

Influence of poly (I:C) stimulation on lipolysis
To assess the effect of TLR3 activation on lipolysis we compared glycerol content in culture supernatant of adipocytes from 2 donors stimulated for 24 h with increasing concentrations of poly (I:C) to the supernatant of adipocytes stimulated LPS (known to induce lipolysis). We found a significantly increased glycerol concentration comparable to that found in LPS stimulated adipocytes starting from a poly (I:C) concentration of 1 μg/ml. This demonstrated that both, LPS and poly (I:C) induce lipolysis in adipocytes (Fig. 4b).

Discussion and Conclusions
Insulin resistance is the key event and link between T2DM and obesity. Since during endotoxemia insulin sensitivity is also decreased [8,9,21], it appears plausible that this response is mediated by LPS receptors in insulin target organs like muscles and fat tissue [13,22,23]. Here we investigated which TLRs, that recognize molecular patterns derived from pathogens other than Gram negative bacteria are also expressed in in vitro matured human adipocytes and may contribute to the induction of IL-6. Adipose tissue shows a high level of cellular complexity and it has been estimated that mature adipocytes represent only
16% of the population [24]. MSC derived adipocytes display morphological and functional characteristics of mature adipocytes including, catecholamine-regulated lipolysis. They express adiponectin and leptin and proteins involved in lipolysis, including beta2-AR, alpha2A-AR, as well as hormone-sensitive lipase [25]. This cellular model is free of a possible contamination due to infiltrated inflammatory cells that can contribute to the signals obtained from adipocytes derived from adipose tissue. Of note, it has been estimated that the adipose tissue may contribute to one third of the circulating IL-6 [16]. Thus, we screened adipocytes derived from mesenchymal stem cells for expression of known TLRs. We documented expression of TLR1-9 and perhaps 10 (very low signal). We tested the functionality of these receptors by measuring the expression of IL-6 and other cytokines after stimulation with their specific agonists. Ligands specific for TLR1-6 caused an upregulation of IL-6. Despite the expression of TLR7-9 mRNA in adipocytes, we did not find IL-6 induction after stimulation with their specific agonists. One possible explanation for this finding is that despite expression, functional TLR were not translated. Alternatively, it is also possible that translation occurs but the receptor is not accessible to their ligands under in vitro conditions. It is known that both the ligand recognition by TLRs and the functional outcome of ligand binding are governed by the subcellular location of the TLRs and their signaling adaptors [26, 27]. Addition of ligands in vitro may not mimic the situation in vivo where pathogen associated molecular patterns would normally be generated. Thus our experimental conditions were perhaps not suitable for the analysis of this group of TLR. In contrast the stimulation of adipocytes with TLR3 and TLR4 specific ligands induced a clear increase in IL-6 at mRNA level and thus we focused our attention on these 2 receptors. Some effects of LPS on adipocytes have been
been shown that LPS does not induce TNF induced also at the protein level. Under similar conditions it has prepared to IL-6 or IL-8. We found that IL-6 and other proinflammatory cytokines are biologically relevant because proinflammatory activity was assessed by measuring the release of glycerol into culture supernatants of stimulated cultures. Mean relative content ± SEM obtained in unstimulated cultures. Asterisks indicate significance difference (p<0.05). Induction of lipolysis. MSC-derived adipocytes were exposed for 24 h to LPS and increasing concentrations of poly (I:C). Lipolytic activity was assessed by measuring the release of glycerol into culture supernatants. Data shown are from n=6 wells performed on 2 separate occasions.

Fig. 4 a: Effect of stimulation of adipocytes with LPS and poly (I:C) on insulin dependent and independent glucose uptake (Glucose uptake, 3 independent donors. Means ± SEM from 3 independent experiments normalized to untreated controls without addition of insulin (=1). The increase of 3.2 times due to insulin in unstimulated cultures was defined as 100% effect. Numbers indicate insulin effect as percentage of the effect obtained in unstimulated cultures. Asterisks indicate significant difference (p<0.05) when glucose uptake values were compared to insulin treated unstimulated controls. b: Relative glycerol concentration in supernatants of adipocytes as indicator for lipolysis. Mean relative content ± SEM from 2 experiments in triplicate. Asterisks indicate significant difference (p<0.05). The concentration used was in the plateau with respect to IL-6 mRNA induction. Thus this may indicate that downstream pathways are not saturated and the additive effect may have been due to additional activation of the Myd88 independent activation in the TLR3 pathway. All 3 indicators demonstrated insulin resistance after stimulation of adipocytes with TLR3 and TLR4 ligands. LPS has been shown to influence lipolysis. We measured the effect of stimulation with TLR3 and TLR4 ligands on glycerol content as product of lipolysis in cell culture supernatants. We found increased glycerol content in supernatants of stimulated cultures. Thus, if it is known that TLRs induces lipolysis, we have shown for the first time that poly (I:C) as a mimetic of viral infection had a lipolytic effect. Interestingly free fatty acids have been shown to be increased in obese patients and to be able to activate TLR4. This suggests a potential vicious circle, since free fatty acids generated after TLR4 activation may contribute to a stronger activation of TLR4 and increased proinflammatory reaction. Furthermore TLR3 activation during viral infections may generate free fatty acids that can activate TLR4 and reinforce proinflammatory activity and insulin resistance. This suggests that control of TLR activation during infections, obesity, and T2DM or other conditions that may lead to activation of these receptors may be beneficial in control of glucose and lipid homeostasis and maintain insulin sensitivity.

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