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# Absence of Tlr2 protects against high-fat diet-induced inflammation and results in greater insulin-stimulated glucose transport in cultured adipocytes☆

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**Abstract**

We have previously shown that toll-like receptor-4 (Tlr4) is involved in obesity-induced inflammation in adipose tissue (AT). However, less is known about the role of Tlr2 in this process. To determine the involvement of this receptor in obesity-induced inflammation, we utilized male $Tlr2^{-/-}$ mice that were backcrossed onto a mouse model of diet-induced obesity (DIO). Mice were fed either low-fat control (LFD) or high-fat diet (HFD) *ad libitum* for 16 weeks. Despite negligible differences in body weight or energy intake, $Tlr2^{-/-}$ mice were protected from HFD-induced adiposity as was evident by reduced epididymal fat pad weight and carcass lipid content. Corresponding with these effects was a blunted accumulation of F4/80-positive macrophages in AT of $Tlr2^{-/-}$ mice. Furthermore, transcript abundance of proinflammatory mediators, including monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-α (TNFα) and nitric oxide synthase-2 (NOS2) in AT of $Tlr2^{-/-}$ mice, was lower or less responsive to DIO. There were no significant differences in serum markers of insulin sensitivity (data not shown). However, adipocytes derived from stromal vascular cells (SVCs) isolated from AT of $Tlr2^{-/-}$ mice had considerably greater basal and insulin-stimulated glucose uptake as compared with those obtained from $Tlr2^{+/+}$ mice. Furthermore, the absence of $Tlr2^{-/-}$ precluded the induction of insulin resistance by zymosan A (ZymA) but not by palmitate. These data indicate that Tlr2 may be directly involved in HFD-induced inflammation and may also regulate basal and insulin-stimulated glucose uptake in adipocytes.


*Keywords:* Obesity; Inflammation; Fatty acids; Toll-like receptor; Adipose tissue

## 1. Introduction

The incidence of diabetes is considerably higher in obese individuals *vs*. those with normal body mass index. Obesity is now generally recognized as a disease marked by chronic inflammation. Furthermore, obesity-linked inflammation, characterized by increased expression of proinflammatory cytokines and infiltration of adipose tissue (AT) with macrophages, is causally linked with insulin resistance and frank diabetes [1–3]. The strong association between obesity, inflammation and insulin resistance suggests that AT plays a prominent role in the onset and progression of these comorbidities of obesity.

Several distinct inflammatory pathways, including nuclear factor kappa B (NFκB) and jun N-terminal kinase (JNK) [1,2], have been associated with macrophage infiltration in AT and stimulation of proinflammatory cytokines and chemokines, such as tumor necrosis factor-α (TNFα) and monocyte chemotactic protein-1 (MCP-1). The underlying mechanism contributing to the proinflammatory response in AT is not fully elucidated, but may involve signaling through specific toll-like receptors (Tlrs). Shi et al. [4] reported attenuation of lipid-induced NFκB activation and insulin resistance in AT of Tlr4 null mice. Additional studies [5–8] have confirmed that Tlr4 deficiency improved insulin sensitivity and lowered inflammation in diet-induced obesity (DIO). We recently reported that the Tlr4-deficient 10ScN mouse strain, which has a 74-kb deletion from chromosome 4 that precludes expression of Tlr4, is specifically protected from systemic inflammation in response to a diet high in saturated fat [9]. However, because Tlr4 deficiency did not completely attenuate AT-specific inflammation, we sought to determine whether alternative receptors may also contribute to DIO-induced inflammation in AT.

One potential candidate is the closely related Tlr2. Expression of this innate immune receptor is increased in AT of obese and diabetic individuals [10,11], and is also shown to be activated by saturated fatty acids (SFAs) [12]. Thus, the objective of this study was to determine whether the absence of Tlr2 would attenuate AT-specific inflammation in DIO.

## 2. Methods and materials

### 2.1. Animals and animal care

Male B6.129-Tlr2tm1Kir/J (Cat No. 004650; $Tlr2^{-/-}$) and C57BL/6J (Cat No. 000664) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Heterozygotes containing the Tlr2 mutation were intercrossed and then backcrossed to

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C57BL/6J for nine generations. The strain was then maintained by breeding homozygotes for the Tlr2 mutation ($Tlr2^{-/-}$). C57BL/6J mice with intact Tlr2 ($Tlr2^{+/+}$) were bred from separate litters and used as controls for $Tlr2^{-/-}$ mice. Animals were housed individually in a room with an automatically controlled 12-h light/dark cycle. Mice were acclimated to cages and provided unlimited access to food and water.

Animals from each genotype were randomly assigned to low-fat (LFD) or high-fat diet (HFD) ($n$=15/genotype/diet) for 16 weeks as previously described [9]. All diets were semipurified, powdered diets based on American Institute of Nutrition recommendations [13] (Table 1). The LFD contained 12% of total calories from lipid (soybean oil), whereas the HFD contained 60% of total calories from lipid (mixture of lard and purified palmitate). Food intake was measured daily and used to calculate total energy intake. Mice were fasted overnight (6–8 h) and euthanized by $CO_2$ asphyxiation for all blood and tissue collections. All experimental protocols for animal care and use were approved by the Institutional Animal Care and Use Committee at Iowa State University, Ames, IA, USA.

### 2.2. Determination of body composition

Frozen mouse carcasses (excluding epididymal fat pad) were weighed and autoclaved for 2.5 h at 230°C and thereafter blended for 3 min in water. Carcass homogenate was freeze dried for 48 h. The resulting product was then reblended under liquid nitrogen and subsequently used to determine percentage lean and fat mass as previously described [14,15].

### 2.3. Analysis of metabolic parameters

Blood glucose, total cholesterol and triacylglycerides were measured using the CardioChek PA system (Polymer Technology Systems, Inc., Indianapolis, IN, USA). Serum insulin (Millipore, Billerica, MA, USA), C-reactive protein (CRP) (Immunology Consultants Laboratory, Inc., Newberg, OR, USA) and MCP-1 (R&D Systems, Minneapolis, MN, USA) were measured using commercial assay kits. Nonesterified fatty acids (NEFA) were measured with the NEFA-C kit (Wako, Inc., Richmond, VA, USA). Serum endotoxin determinations were performed using a kit based upon a Limulus amoebocyte extract (LAL kit; Hycult Biotechnology, The Netherlands). Samples were diluted 1:40 and internal control of recovery calculation was included in the assessment.

### 2.4. Quantitative real-time PCR

Total RNA was extracted and cDNA synthesized as described previously [9]. The abundance of each gene product was calculated by regressing against the standard curve generated in the same reaction with their respective plasmid. All genes of interest were normalized to beta actin and expressed as fold change. Primer sequences for genes of interest are provided in Table 2.

### 2.5. Immunohistochemistry

Frozen AT was fixed and stained for expression of F4/80 with monoclonal antibody (Serotec) as previously described [9].

### 2.6. Stromal vascular cell isolation and culture

Epididymal fat pads from $Tlr2^{-/-}$ and $Tlr2^{+/+}$ mice were added to sterile digestion cocktail (1× Krebs Ringer buffer and 200 U/ml collagenase) for 1 h in 37°C shaking water bath. The stromal vascular cells (SVCs) were recovered from beneath the adipocyte layer and centrifuged at 800×$g$ for 4 min. The resulting pellet was resuspended in red cell lysis buffer (pH 7.2; 155 mM $HH_4Cl$, 5.7 mM $K_2HPO_4$ and 0.1 mM EDTA) for 6 min and then diluted with 1× PBS and centrifuged for 800×$g$ for 15 min. Following a second wash with PBS, the pellet was resuspended in low-glucose DMEM containing 10% FBS and 5% streptomycin/penicillin mixture and the SVCs were transferred to six-well plates.

At 70–80% confluence, adipocyte differentiation cocktail (growth media containing 1.5 mM IBMX, 1.0 μM dexamethasone, 1.7 μM insulin and 0.4 mM biotin) was added to cells for 48 h and subsequently replaced with growth media containing insulin. At 14 days postdifferentiation, approximately 40% of SVCs exhibited adipocyte phenotype (*e.g.*, lipid accumulation). Cells were then treated with PBS, 0.5 mM sodium palmitate or 20 μg/ml ZymA from *Saccharomyces cerevisiae* for 48 h and used thereafter for analysis of glucose uptake as previously described [16].

### 2.7. Statistical analyses

Data were tested for normality and analyzed using the mixed-model analysis with Bonferroni adjustment. Diet and genotype were considered fixed effects, and block was implemented as a random effect. Interaction means±S.E. are presented in figures, and when the interaction of main effects (diet and genotype) was $P$<.15, then *post hoc* comparisons were made using the least significant means (LSMEANS) separation (pdiff) procedure. Differences among LSMEANS were considered significant at $P$<.05 and trends are noted when $P$<.10. Significant main effect means±S.E. are presented in figure legends.

Table 1
Diet composition

| Ingredient | Low-fat control[a] (g/kg) | High-fat palmitate[a] (g/kg) |
|---|---|---|
| Casein[b] | 140 | 196 |
| Sucrose[b] | 100 | 100 |
| Corn starch[b] | 455.692 | 88.192 |
| Maltodextrin[b] | 155 | 155 |
| Soybean oil[b] | 50 | |
| Lard[b] | | 235 |
| Palmitate[c] | | 125 |
| Cholesterol[b] | | 1.5 |
| Cellulose[b] | 50 | 50 |
| Vitamin mix[b] | 10 | 10 |
| Mineral mix[b] | 35 | 35 |
| Choline bitartrate[b] | 2.5 | 2.5 |
| L-Cysteine[b] | 1.8 | 1.8 |
| THBQ[b] | 0.008 | 0.008 |
| Total (g) | 1000 | 1000 |

[a] Adapted from AIN-93M diet.
[b] Harlan Teklad, Madison, WI, USA.
[c] Nu-Check Prep, Inc., Elysian, MN, USA.

Table 2
Primer sequences for quantitative real-time polymerase chain reaction

| Target | Forward primer | Reverse primer |
|---|---|---|
| Beta actin | TGAGAGGGAAATCGTGCGTGACAT | ACCGCTCGTTGCCAATAGTGATGA |
| Tlr2 | TCCCTTGACATCAGCAGGAACACT | GCAGCCGAGGCAAGAACAAAGAAA |
| Tlr4 | CCGCTCTGGCATCATCTTCATTGT | TCCTCCCATTCCAGGTAGGTGTTT |
| MCP-1 | CCAAGAAGGAATGGGTCCAGACAT | ACAGAAGTGCTTGAGGTGGTTGTG |
| TNF-α | CCAACGGCATGGATCTCAAAGACA | AGATAGCAAATCGGCTGACGGTGT |
| NOS2 | TCTTTGACGCTCGGAACTGTAGCA | CGACCTGATGTTGCCATTGTTGGT |
| F4/80$^+$ | TGCCACAACACTCTCGGAAGCTAT | TCCTGGAGCACTCATCCACATCTT |

## 3. Results

### 3.1. Absence of Tlr2 reduced adiposity in HFD-fed mice

Absence of Tlr2 was associated with a 20% reduction in epididymal fat pad weight (Fig. 1A, genotype effect, $P$=.0042), as well as a trend increase in carcass lean mass compared to $Tlr2^{+/+}$ mice (Fig. 1B, genotype, $P$=.099). Moreover, carcass lipid content was 25% greater in HFD-fed *vs.* LFD-fed $Tlr2^{+/+}$ mice (Fig. 1C, Diet*Genotype, $P$=.046), whereas $Tlr2^{-/-}$ mice fed HFD did not exhibit a similar increase in carcass lipid content compared to LFD-fed mice (Diet*Genotype, $P$=.12). We observed no significant difference in body weight (Fig. 1D, genotype, $P$=.35) or total energy intake (data not shown) between genotypes indicating that the observed changes in body composition of $Tlr2^{-/-}$ mice must be in part mediated by alterations in metabolism.

### 3.2. F4/80-positive macrophage accumulation is reduced in AT of $Tlr2^{-/-}$ mice

Despite significantly lower fat pad weights, $Tlr2^{-/-}$ mice exhibited a trend increase in adipocyte size compared to $Tlr2^{+/+}$ mice (Fig. 2A–B, genotype, $P$=.058). To determine whether the increase in adipocyte hypertrophy influenced the presence of macrophages in AT of $Tlr2^{-/-}$ mice, we used immunohistochemical staining to detect the macrophage-specific marker, F4/80. Here we demonstrated that the number of F4/80-positive cells was significantly reduced in AT of $Tlr2^{-/-}$ mice, regardless of diet (Fig. 2B–C, genotype, $P$=.0053). There was also a >20% increase in F4/80-positive cells in AT of $Tlr2^{+/+}$ mice fed HFD *vs.* LFD (Diet*Genotype, $P$=.0011), whereas $Tlr2^{-/-}$ mice actually exhibited a slight reduction in F4/80-positive cells in response to HFD compared to LFD (Diet*Genotype, $P$=.092). These findings were confirmed through measurement of

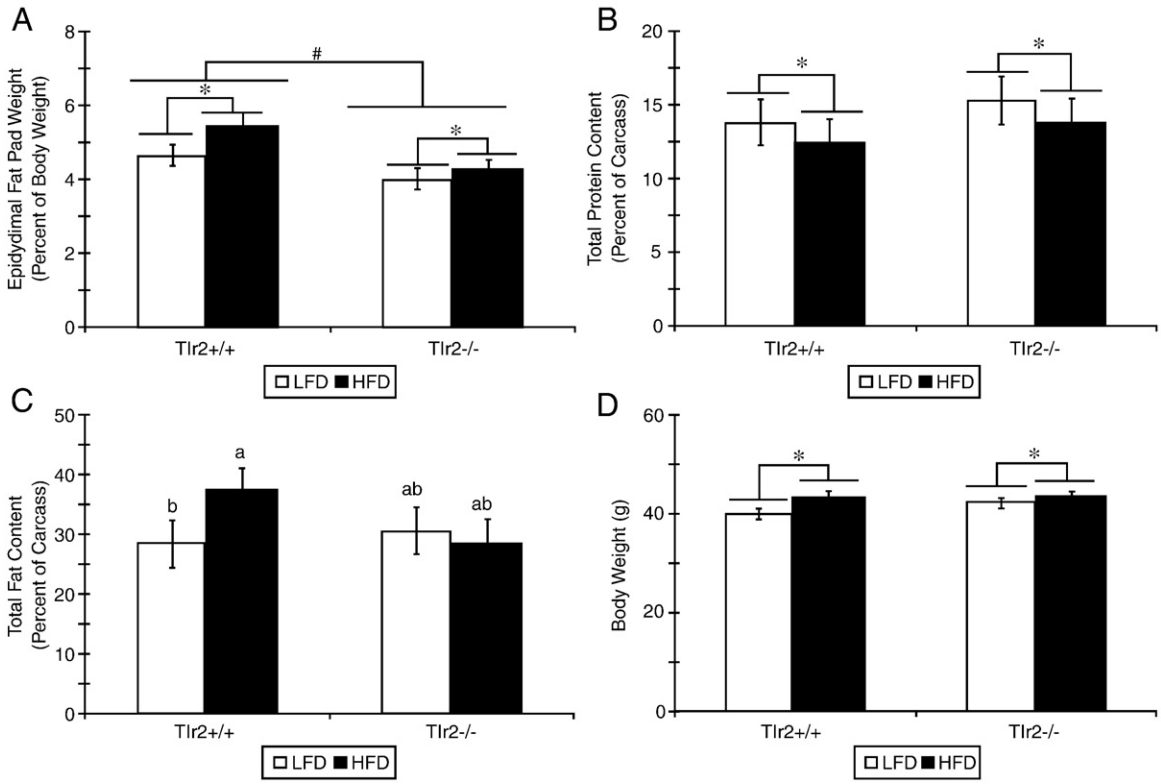


Fig. 1. Tlr2$^{-/-}$ mice exhibit reduced adiposity compared to Tlr2$^{+/+}$ mice. Tlr2$^{-/-}$ mice after 16 weeks on LFD (white bars) or HFD (black bars) diet. All graphed values represent LS interaction means±S.E. Significant main effects ($P$<.05) are denoted by '*' (diet) and '#' (genotype). Letters represent significant differences among means as determined by the Bonferroni correction when interaction term was $P$<.15. (A) Epididymal fat pad weight expressed as percent of total body weight [$n$=15, diet effect (LFD *vs.* HFD) $P$=.048, 4.33 *vs.* 4.83 ±0.18; genotype effect (Tlr2$^{+/+}$ *vs.* Tlr2$^{-/-}$) $P$=.0042, 5.04 *vs.* 4.12±0.20]. (B) Total carcass protein content (excluding epididymal fat pad) expressed as percent of carcass weight [$n$=5, diet effect (LFD *vs.* HFD) $P$=.011, 14.6±1.33 *vs.* 13.2±1.41; genotype effect (Tlr2$^{+/+}$ *vs.* Tlr2$^{-/-}$) $P$=.099, 13.2±1.56 *vs.* 14.6±1.24]. (C) Total carcass fat content (excluding epididymal fat pad) expressed as percent of carcass weight [$n$=5, Diet*Genotype interaction, $P$=.12]. (D) Body weight [$n$=15, diet effect (LFD *vs.* HFD) $P$=.026, 41.2 *vs.* 43.5±0.70].

F4/80 transcript abundance (Fig. 2D, Genotype, $P$=.0085). Moreover, there was an approximately sixfold increase in F4/80 transcript abundance in AT of Tlr2$^{+/+}$ mice fed HFD relative to LFD (Diet*Genotype, $P$=.0002), but not in Tlr2$^{-/-}$ mice fed the same diet (Diet*Genotype, $P$=.31). Thus, absence of Tlr2 significantly lowered the accumulation of F4/80-positive macrophages in AT independently of adipocyte size.

### 3.3. Systemic and AT-specific inflammation was reduced in Tlr2$^{-/-}$ mice

There were no significant differences in serum CRP, NEFA or endotoxin concentrations regardless of genotype or diet (data not shown). However, Tlr2$^{-/-}$ mice were specifically protected against the HFD-induced serum MCP-1 (Fig. 3A, Diet*Genotype, $P$=.029). These data closely correspond with the observed reduction in F4/80-positive macrophages in AT of Tlr2$^{-/-}$ mice. Accordingly, we then measured the inflammatory status of AT to determine whether absence of Tlr2 protected mice from HFD-induced inflammation. The expression of Tlr2 could not be detected in Tlr2$^{-/-}$ mice (Fig. 3B, genotype, $P$<.0001), but HFD had an approximately threefold induction of Tlr2 transcript abundance *vs.* LFD-fed Tlr2$^{+/+}$ mice (diet, $P$=.028). Corresponding with this increased expression of Tlr2 was a nearly threefold induction of MCP-1 (Fig. 3C) and TNFα (Fig. 3D) transcript abundance in AT of HFD-fed, compared to LFD-fed, Tlr2$^{+/+}$ mice (Diet*Genotype, $P$=.014 and $P$=.0003, respectively). There was also an overall reduction in MCP-1 and TNFα expression in Tlr2$^{-/-}$ mice irrespective of diet (genotype, $P$=.0089 and $P$=.0017, respectively). Transcript abundance of the oxidative stress inducer, NOS2, was also reduced in AT of Tlr2$^{-/-}$ mice compared to Tlr2$^{+/+}$ mice (Fig. 3E, genotype, $P$<.0001). More specifically, NOS2 transcript abundance was almost 10-fold lower in LFD-fed Tlr2$^{-/-}$ mice relative to Tlr2$^{+/+}$ mice fed either LFD or HFD (Diet*Genotype, $P$<.0001). These data indicated that absence of Tlr2 protected mice from HFD-induced inflammation in AT, which may be due to a reduction in macrophage accumulation.

### 3.4. Systemic markers of insulin sensitivity were not improved in Tlr2$^{-/-}$ mice, but isolated SVCs exhibited an improvement in insulin sensitivity

There were no observed differences in blood glucose, serum insulin or glucose-to-insulin ratio in Tlr2$^{-/-}$ and Tlr2$^{+/+}$ mice (data not shown). However, primary SVCs isolated from epididymal fat pads of Tlr2$^{-/-}$ mice had greater glucose uptake compared to Tlr2$^{+/+}$ mice (Fig. 4, genotype, $P$<.0001). This included both basal and insulin-stimulated uptake (Diet*Genotype, $P$<.0001). Furthermore, the reduction in insulin-stimulated glucose uptake caused by zymosan A in SVCs from Tlr2$^{+/+}$ mice was completely attenuated in SVCs isolated from Tlr2$^{-/-}$ mice (Diet*Genotype, $P$=.23). Conversely, absence of Tlr2 did not attenuate palmitate-induced insulin resistance (Diet*Genotype, $P$<.0001). Thus, despite no impact on in vivo markers of insulin sensitivity, absence of Tlr2 improved basal and insulin-stimulated glucose uptake, as well as Tlr2 agonist-induced insulin resistance in SVCs, which may be related to the reduction in inflammatory status of AT.

## 4. Discussion

Tlr signaling pathways in AT have been strongly implicated as major contributors to the molecular mechanisms involved in obesity-linked inflammation, especially in relation to diets high in SFAs

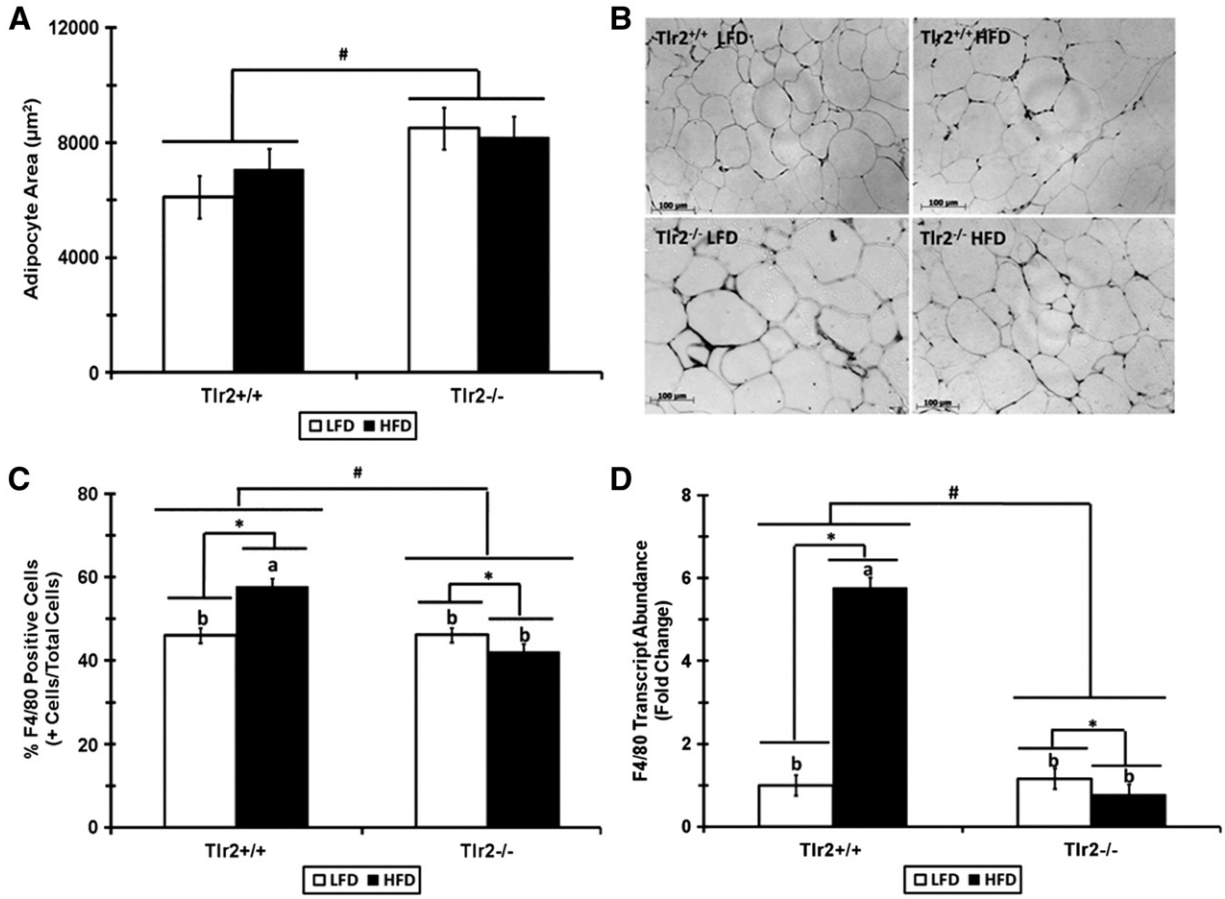


Fig. 2. F4/80-positive macrophage accumulation is reduced in AT of $Tlr2^{-/-}$ mice. All measures were obtained from $Tlr2^{-/-}$ and $Tlr2^{+/+}$ mice after 16 weeks on LFD (white bars) or HFD (black bars) diet. All graphed values represent LS interaction means±S.E. Significant main effects ($P<.05$) are denoted by '*' (diet) and '#' (genotype). Letters represent significant differences among means as determined by the Bonferroni correction when interaction term was $P<.15$. (A) Adipocyte size expressed as square micrometer [$n=5$, genotype effect ($Tlr2^{+/+}$ *vs.* $Tlr2^{-/-}$) $P=.058$, 6583 *vs.* 8344±520]. (B) Representative images from AT of $Tlr2^{+/+}$ and $Tlr2^{-/-}$ mice fed either LFD or HFD. (C) Percent of F4/80-positive cells (positive cells/total cells) [$n=5$, diet effect (LFD *vs.* HFD) $P=.05$, 46.1 *vs.* 49.9±1.19; genotype effect ($Tlr2^{+/+}$ *vs.* $Tlr2^{-/-}$) $P=.0053$, 51.9 *vs.* 44.1±1.45; Diet*Genotype interaction, $P=.0008$]. (D) F4/80 transcript abundance in AT as fold change [$n=5$, diet effect (LFD *vs.* HFD) $P=.017$, 1.0 *vs.* 0.51±0.22; genotype effect ($Tlr2^{+/+}$ *vs.* $Tlr2^{-/-}$) $P=.0085$, 1.0 *vs.* 0.40±0.24; Diet*Genotype interaction, $P=.001$].

[4,6,7,17]. Herein, we report the novel finding that $Tlr2^{-/-}$ mice are protected from HFD-induced adiposity without significant changes in body weight, energy intake or adipocyte size. Mice lacking a functional Tlr2 had smaller epididymal fat pad weights, greater carcass lean mass and lower carcass lipid content, albeit with no discernable difference in energy intake or overall body weight. In contrast with our previous findings in mice lacking Tlr4 [9], the absence of Tlr2 was not associated with a decrease in adipocyte size and, in fact, there was a trend increase in adipocyte size as compared to $Tlr2^{+/+}$ mice. This is perhaps reflective of an overall change in metabolic rate or energy expenditure such that $Tlr2^{-/-}$ mice had lower adiposity and greater protein content. Furthermore, despite the reduction in fat pad weight adipocyte size tended to be larger in AT of $Tlr2^{-/-}$ mice indicating a potential reduction in adipocyte cell number. Although not yet confirmed in adipogenic progenitors, Shishido et al. [18] have determined that $Tlr2^{-/-}$ mice had reduced neointimal hyperplasia in response to vascular injury, which may suggest a role of Tlr2 in cell proliferation.

The fatty acid composition of dietary fat influences not only the degree of obesity, but also its metabolic comorbidities, including inflammation and insulin resistance [19]. In this study, we determined that $Tlr2^{-/-}$ mice were protected from HFD-induced inflammation in AT. The ability of a highly saturated fat diet to induce inflammation through Tlr4 is well documented in vitro [5,20]. However, only a limited number of studies [12,21] have implicated Tlr2 as a potential target for SFA. Senn [21] reported that Tlr2 siRNA reversed palmitate-induced activation of IKKβ/NFκB in C2C12 myotubes. Our data suggests that Tlr2 may also mediate some inflammatory responses to FA and therefore contribute to localized and systemic inflammation and insulin resistance.

Obesity-induced inflammation in AT is evident by macrophage infiltration and proinflammatory gene expression, which contributes to the development of insulin resistance [22,23]. We have previously reported that Tlr4-deficient 10ScN mice exhibit lower macrophage accumulation in AT [9]. In the present study, we determined that the macrophage-specific marker, F4/80, was markedly reduced in $Tlr2^{-/-}$ mice. This reduction was more apparent in mice fed the HFD and also corresponded with lower proinflammatory gene expression (*e.g.*, TNFα and MCP-1). Additionally, transcript abundance of the oxidative stress marker, NOS2, was reduced overall in $Tlr2^{-/-}$ mice, but the absence of Tlr2 failed to protect against HFD-induced NOS2 transcript abundance. This effect is likely reflective of SFA signaling through Tlr4. In fact, we recently found that mice lacking Tlr4 are protected against HFD-induced NOS2 expression (unpublished data). Collectively, these findings support a major role for Tlrs in obesity-induced oxidative stress, although Tlr4 seems to have a more prominent role in SFA-induced oxidative stress than Tlr2. This confirms our previous in vitro work that demonstrated Tlr2 and Tlr4 agonist-mediated oxidative stress in adipocytes [16].

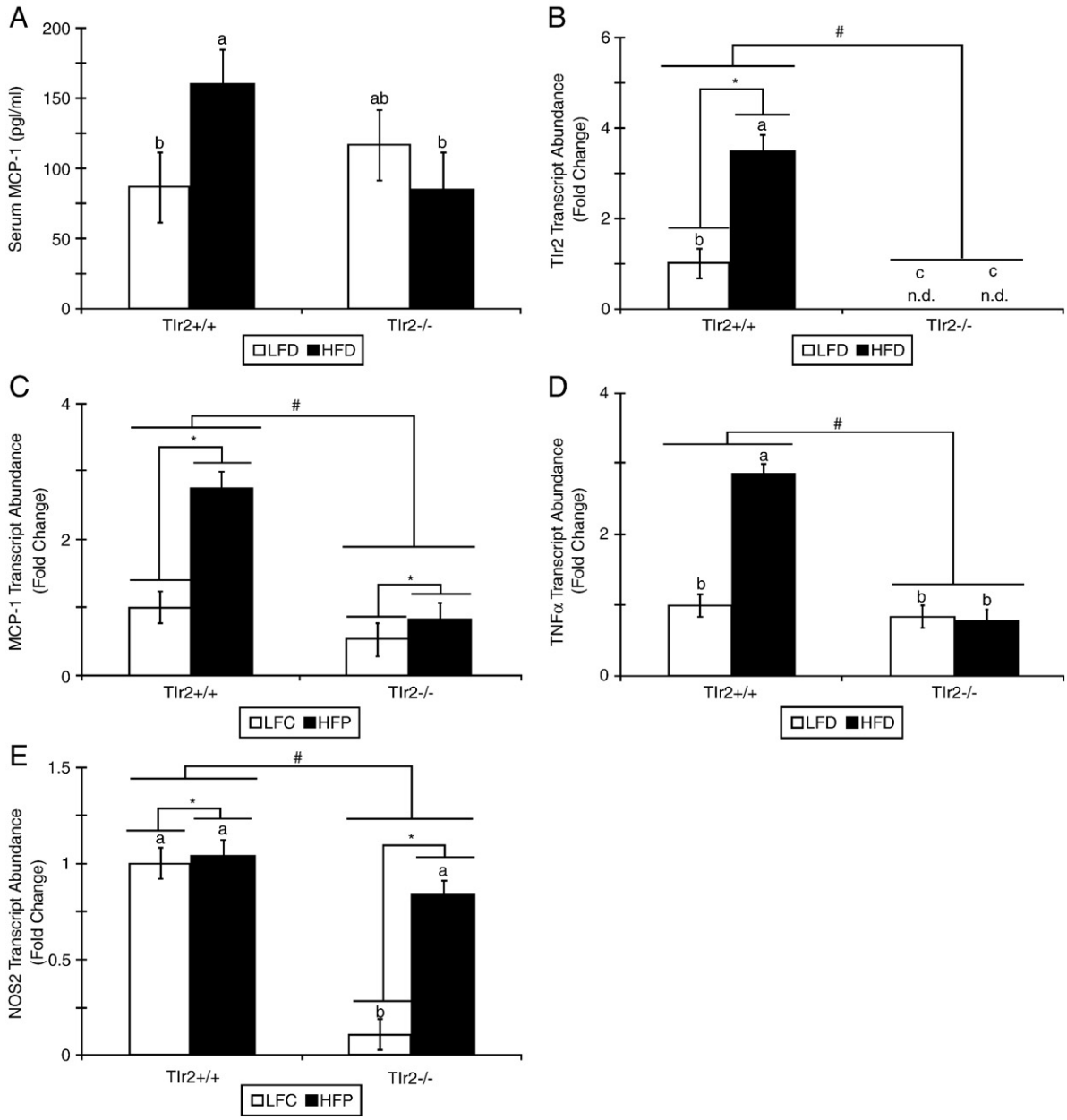


Fig. 3. Systemic and AT-specific inflammation was reduced in $Tlr2^{-/-}$ mice. All measures were obtained from WT and $Tlr2^{-/-}$ mice after 16 weeks on low-fat control (white bars) or high-fat palmitate (black bars) diet. All graphed values represent LS interaction means±S.E. Significant main effects ($P<.05$) are denoted by '*' (diet) and '#' (genotype). Letters represent significant differences among means as determined by the Bonferroni correction when the interaction term was $P<.15$. (A) Serum MCP-1 ($n=5$, Diet*Genotype interaction, $P=.037$). (B) Tlr2 transcript abundance in AT expressed as fold change [$n=5$, diet effect (LFD *vs.* HFD) $P=.022$, 1.0 *vs.* 6.34±0.23; genotype effect ($Tlr2^{+/+}$ *vs.* $Tlr2^{-/-}$) $P<.0001$, 1.0 *vs.* 9.73E10$^{-7}$± 0.23; Diet*Genotype interaction $P=.15$]. (C) MCP-1 transcript abundance in AT expressed as fold change [$n=5$, diet effect (LFD *vs.* HFD) $P=.012$, 1.0 *vs.* 2.04±0.21; genotype effect ($Tlr2^{+/+}$ *vs.* $Tlr2^{-/-}$) $P=.0089$, 1.0 *vs.* 0.40±0.23]. (D) TNFα transcript abundance in AT expressed as fold change [$n=5$, diet effect (LFD *vs.* HFD) $P=.0078$, 1.0 *vs.* 1.66±0.14; genotype effect ($Tlr2^{+/+}$ *vs.* $Tlr2^{-/-}$) $P=.0017$, 1.0 *vs.* 0.48±0.15; Diet*Genotype interaction $P=.0053$]. (E) NOS2 transcript abundance in AT expressed as fold change [$n=5$, diet effect (LFD *vs.* HFD) $P<.0001$, 1.0 *vs.* 2.88±0.07; genotype effect ($Tlr2^{+/+}$ *vs.* $Tlr2^{-/-}$) $P<.0001$, 1.0 *vs.* 0.29±0.08; Diet*Genotype interaction $P=.0053$].

Caricilli et al. [24] reported that neutralization of Tlr2 with an antisense oligonucleotide attenuated insulin resistance in mice fed a HFD. Furthermore, disruption of Tlr2 specifically improved insulin sensitivity in AT and muscle of DIO mice. Our data did not substantiate that the absence of Tlr2 improved serum markers of insulin sensitivity in vivo. Consequently, we chose to determine whether insulin sensitivity is altered in adipocytes derived from SVCs obtained from $Tlr2^{-/-}$ *vs.* $Tlr2^{+/+}$ mice fed the LFD, and whether the absence of Tlr2 altered the effects of fatty acid (*e.g.*, palmitate) and the classical Tlr2 ligand, ZymA, on insulin-stimulated glucose uptake. Basal glucose uptake was greater in mice lacking $Tlr2^{-/-}$, and the response to insulin was greater. Furthermore, the absence of $Tlr2^{-/-}$ abrogated the induction of insulin resistance by ZymA (the classical Tlr2 ligand) but did not protect against palmitate-induced insulin resistance. This is likely attributable to the activation of Tlr4 signaling by palmitate and perhaps to the activation of intracellular nucleotide oligomerization domain (NOD) receptors. NOD1 is constitutively expressed in murine and human preadipocytes, and NOD2 expression is increased by LPS or TNFα in murine preadipocytes [25]. Also, laurate has been identified as a ligand for NODs in HCT116 cells (which do not express measurable Tlr2 or Tlr4) and causes a dose-dependent increase in IL8 [26]. We are currently exploring the potential role of NODs in fatty acid-induced inflammation and insulin resistance in adipocytes.

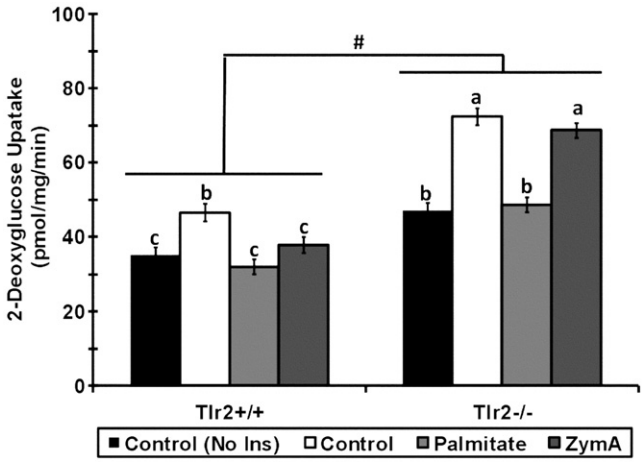


Fig. 4. Systemic markers of insulin sensitivity were not improved in Tlr2$^{-/-}$ mice, but isolated SVCs exhibited an improvement in insulin sensitivity. Fourteen-day post-differentiated SVCs were treated with 0.5 mM sodium palmitate, 10 μg/ml LPS or 20 μg/ml ZymA for 48 h. A non-insulin-stimulated control was included for reference. All graphed values represent LS interaction means±S.E. Significant main effects ($P$<.0001) are denoted by '#' (genotype). Letters represent significant differences among means as determined by the Bonferroni correction when the interaction term was significant ($P$<.15). 2-Deoxyglucose uptake in SVCs expressed as percentage of insulin-stimulated glucose uptake ($n$=8, Treatment*Genotype interaction $P$=.0007).

In summary, our study provides substantial evidence for the involvement of Tlr2 in obesity-induced inflammation and insulin resistance. More specifically, absence of this receptor protected against increases in some markers of systemic and AT-specific inflammation, especially in mice fed a HFD. We therefore suggest that Tlr2 also represents a potential dietary and pharmaceutical target for reducing inflammation and insulin resistance associated with DIO.

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