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RESEARCH ARTICLE

High-fat diet stimulates IL-1 type I receptor-mediated inflammatory signaling in the skeletal muscle of mice

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Recently, substantial attention has been focused on the association between obesity and chronic inflammation. The aim of the present study was to investigate whether high-fat diet (HFD)-induced obesity induces the activation of the IL-1 type I receptor (IL-1RI)-mediated inflammatory signaling cascade in the skeletal muscle of mice. Male C57BL/6J mice were fed either an HFD or a normal diet (ND) for 12 wk. Compared with the results in mice receiving the ND, the HFD increased the expression of IL-1RI and downstream signaling proteins, such as myeloid differentiation primary response gene 88 (MyD88), IL-1R-associated kinase 4 and phospho-transforming growth factor-activated kinase 1 in the skeletal muscle. Additionally, activities of both inhibitor of κB kinase β and inhibitor of κB degradation were significantly elevated in the skeletal muscle of mice fed with an HFD compared with mice receiving an ND. In contrast, the levels of other downstream transcription factors, such as activator protein-1 and INF regulatory factor 5, were not affected by the HFD. These results suggest that the IL-1RI-MyD88-nuclear factor κB signaling pathway might be involved in the induction of the inflammatory response in the skeletal muscle of mice fed with an HFD.

Keywords:
IL-1 type I receptor / Inhibitor of κB kinase / Metaflammation / MyD88 / Nuclear factor κB

1 Introduction

The consumption of a high-fat diet (HFD) by both rodents and humans results in an increase of free fatty acids (FFAs) or an influx of saturated fatty acids from the diet. This may induce adipogenesis and associated metabolic diseases [1, 2]. The general consensus is that HFD-induced obesity is characterized by a low-grade and chronic activation of the inflammatory response, a phenomenon recently termed metabolically triggered inflammation (metaflammation) [3]. The physiological mechanisms linking obesity to metaflammation include the production of various adipocytokines (*e.g.* adiponectin, resistin or leptin) and the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-6 and IL-1 by the expanded adipose tissues [4–7]. Furthermore, elevated FFAs in the serum and visceral fat tissue in obese humans, as well as in animal models of obesity, have been shown to induce inflammatory signaling and insulin resistance in white adipose tissues [1, 2]. Recently, Shi *et al.* [8] suggested that toll-like receptor 4 (TLR4) may be one mechanism by which fatty acids induce inflammation and insulin resistance.

Although many studies have demonstrated that both the circulating levels of TNF-α and IL-6 and their expression levels in adipose tissue are elevated in subjects who are

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Abbreviations: AP-1, activator protein-1; **FFAs,** free fatty acids; **GST,** glutathione-*S*-transferase; **HFD,** high-fat diet; **HRP,** horseradish peroxidase; **IκB,** inhibitor of κB; **IKK,** inhibitor of κB kinase; **IL-1RI,** IL-1 type I receptor; **IRAK,** IL-1R-associated kinase; **IRF,** INF regulatory factor; **JNK,** c-Jun kinase; **MyD88,** myeloid differentiation primary response gene 88; **ND,** normal diet; **NFκB,** nuclear factor κB; **TAK1,** transforming growth factor-activated kinase 1; **TAB1,** TAK1-binding protein 1; **TIR,** Toll/IL1R; **TLR4,** Toll-like receptor 4; **Tollip,** Toll interacting protein; **TNF-α,** tumor necrosis factor-α; **TRAF6,** TNF-receptor-associated factor 6

obese or have type 2 diabetes [9, 10], few studies have implicated IL-1, a strong proinflammatory cytokine, in these diseases. IL-1 consists of two isoforms, IL-1α and IL-1β, both of which induce similar biological functions through the IL-1 type I receptor (IL-1RI) [11]. Circulating levels of IL-1β have been correlated with the BMI of obese subjects [7], and the expression of IL-1β is increased in the visceral adipose tissue of obese subjects [6]. The cytoplasmic domain of the IL-1 receptor (IL-1R) is similar to TLRs and can activate similar intracellular proinflammatory signaling pathways. Signaling through the IL-1R or TLRs activates several transcription factors, including members of the nuclear factor κB (NFκB) family, activator protein-1 (AP-1) family and INF regulatory factor (IRF) family. The activation of these transcription factors then, in turn, increases the expression of multiple proinflammatory genes that function to shape the immune response [12, 13]. Once IL-1 binds to IL-1RI, the adaptor protein MyD88 is recruited to the Toll/IL-1R (TIR) domain of IL-1RI. The interaction of IL-1RI with MyD88 leads to the autophosphorylation of IL-1R-associated kinase (IRAK), a serine/threonine kinase, and ultimately results in either NFκB activation or activation of AP-1 *via* signaling through the mitogen-activated protein kinase pathway [13].

As skeletal muscle is one of the major sites of obesity-induced inflammation and the associated insulin resistance [3], it is plausible that the inflammatory signals resulting from signaling through TLRs or IL-1R may be produced in the skeletal muscle of rodents fed an HFD. However, to date, the relationship between obesity and the molecules involved in IL-1R-mediated inflammation in peripheral tissues, including skeletal muscle, has not been examined. Furthermore, although a mechanistic role for the inhibitor of κB (IκB) kinase β (IKK)-IκB-NFκB pathway in lipid-induced insulin resistance has been demonstrated in human muscle and L6 rat myotube cells [14–16], the upstream receptors inducing the inflammatory signals through this pathway in the skeletal muscle have not been fully identified. Therefore, the present study aims to determine whether IL-1RI-mediated proinflammatory signaling is regulated in the skeletal muscle of mice with HFD-induced obesity.

2 Materials and methods

2.1 Regents

Antibodies against IL-1RI, MyD88, transforming growth factor-activated kinase 1 (TAK1)-binding protein 2 (TAB2), β-actin, IκBα, lamin B, c-Jun, phosphorylated c-Jun (Ser63), c-Fos, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, HRP-conjugated donkey anti-goat IgG and HRP-conjugated goat anti-rabbit IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against TAB1 and toll interacting protein (Tollip), glutathione-*S*-transferase (GST)-tagged c-Jun and protein agarose beads were purchased from Upstate Biotechnology (Lake Placid, NY, USA). Anti-IRAK4, TAK1, phospho-TAK1 (Thr184/187) and IKKβ were purchased from Cell Signaling Technology (Beverly, MA, USA). The anti-rabbit and anti-mouse HRP-conjugated secondary antibodies were obtained from Zymed Laboratories (San Francisco, CA, USA). [γ-^{32}P]ATP was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). ECL chemiluminescent detection reagent was purchased from Amersham (Arlington Heights, IL, USA).

2.2 Animals and experimental diets

Twenty 5-wk-old male C57BL/6J mice (Orient, Gyeonggi-do, Korea) were housed in a temperature- ($21 \pm 2.0^{\circ}C$) and humidity-controlled ($50 \pm 5\%$) room with a 12-h light/12-h dark cycle and were fed a commercial diet (Purina, St. Louis, MO, USA) for 1 wk. Mice were randomly divided into normal diet (ND, $n = 10$) and HFD groups ($n = 10$). The HFD contained 200 g fat/kg (170 g lard plus 30 g corn oil) and 1% cholesterol by weight. The HFD was formulated to provide 40% of the total energy generated by the diet from fat by replacing carbohydrates with lard and corn oil, but it contained the same amount of vitamins and minerals *per* kilojoule as the ND did. At the end of the 12-wk feeding period, the mice were anesthetized with diethyl ether following overnight fasting. Blood was drawn from the abdominal aorta into a vacuum tube and the gastrocnemius muscles were removed, weighed and frozen in liquid nitrogen. This study adhered to the *Guide for the Care and Use of Laboratory Animals* developed by the Institute of Laboratory Animal Resources of the National Research Council and was approved by the Institutional Animal Care and Use Committee of Yonsei University in Seoul, Korea.

2.3 Biochemical analyses

The serum glucose concentration was measured using an Express Plus Chemistry Analyzer (Chiron Diagnostics, Emeryville, CA, USA). Serum insulin and leptin levels were measured by radioimmunoassay (Linco Research, St. Louis, MO, USA) and total serum cholesterol, triglyceride and FFA levels were determined with commercial kits (Bio-Clinical System, Gyeonggi-do, Korea). Hepatic lipids were extracted through the procedure developed by Folch *et al.* [17] using chloroform–methanol mixture (2:1 v/v). The dried lipid residues were dissolved in 2 mL ethanol for cholesterol and triglyceride measurements. The hepatic cholesterol, triglyceride and FFA concentrations were analyzed using the same enzymatic kits that were used for serum analyses.

2.4 RNA isolation and semiquantitative RT-PCR analysis

The total RNA was isolated individually from the skeletal muscle of each mouse using Trizol (Invitrogen, Carlsbad,

CA, USA) and then pooled for RT-PCR analysis ($n = 10$). Four micrograms of the total RNA were reverse-transcribed using the Superscript II kit (Invitrogen) according to the manufacturer's recommendations. The PCR was programmed as follows: initial denature at 94°C for 10 min followed by 95°C for 30 s; 55°C for 30 s; 72°C for 45 s, cycled 30 times and 72°C for 10 min. The sequences of the designed primers were as follows: IL-1RI – sense: 5′-GTGCTACTGGGGCTCATTTGT-3′ and antisense: 5′-GGAGTAAGAGGACACTTGCGAAT-3′; IL-1RII – sense: 5′-GTGTGGTGGTTGGCTAACAG-3′ and antisense: 5′-TCCCTTGTGACTGGATCAAA-3′; TLR1 – sense: 5′-TCTCTGAAGGCTTTGTCGATACA-3′ and antisense: 5′-GACAGAGCCTGTAAGCATATTCG-3′; TLR2 – sense: 5′-TCTAAAGTCGATCCGCGACAT-3′ and antisense: 5′-TACCCAGCTCGCTCACTACGT-3′; TLR4 – sense: 5′-ACCTCTGCCTTCACTACAGA-3′ and antisense: 5′-AGGGACTTCTCAACCTTCTC-3′; TLR6 – sense: 5′-AACAGGATACGGAGCCTTGA-3′ and antisense: 5′-CCAGGAAAGTCAGCTTCGTC-3′; IRF3 – sense: 5′-ACATCTCCAACAGCCAGCCTAT-3′ and antisense: 5′-AGTCCATGTCCTCCACCAAGTC-3′; IRF5 – sense: 5′-AATACCCCACCACCTTTTGA-3′ and antisense: 5′-TTGAGATCCGGGTTTGAGAT-3′; glyceraldehyde-3-phosphate dehydrogenase – sense: 5′-AGAACATCATCCCTGCATCC-3′ and antisense: 5′-TCCACCACCCTGTTGCTGTA-3′. The glyceraldehyde-3-phosphate dehydrogenase gene was used as an internal control for semi-quantitative RT-PCR.

2.5 Western blotting

The skeletal muscles of each mouse were pooled and homogenized at 4°C in extraction buffer (100 mM Tris-HCl pH 7.4, 5 mM EDTA, 50 mM NaCl, 50 mM sodium pyrophosphate, 50 mM NaF, 100 mM orthovanadate, 1% Triton X-100, 1 mM PMSF, 2 μg/mL aprotinin, 1 μg/mL pepstatin A and 1 μg/mL leupeptin). The tissue homogenates were centrifuged (13 000 × *g*, 20 min, 4°C) and the resulting supernatants (whole-tissue extracts) were used for the Western blot analyses. Nuclear extracts from skeletal muscles of each mouse were prepared as described previously [18]. The protein concentrations of the whole-tissue extracts and nuclear extracts were determined by Bradford assay (Bio-Rad, Hercules, CA, USA). The protein samples (100–200 μg/lane) were separated in an 8% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Amersham Pharmacia Biotech). Membranes were then blocked for 2 h in 5% bovine serum albumin in Tris-buffered saline/Tween buffer (20 mM Tris-base, pH 7.5, 150 mM NaCl and 0.2% Tween-20). Membranes were then probed with a 1:1000 dilution of the primary antibody. After incubation with the corresponding secondary antibody, signals were detected using the chemiluminescent detection system (Amersham, Uppsala, Sweden) and were quantified using the Quantity One analysis software (Bio-Rad).

2.6 Kinase assay

Proteins were isolated from the skeletal muscles of mice as described above. Pre-cleared extracts were immunoprecipitated at 4°C overnight using rabbit polyclonal antibodies directed against either N-terminus of c-Jun kinase (JNK) or IKKβ. The immunoprecipitates were suspended in 50 μL of a reaction mixture containing 1 × kinase buffer (Cell Signaling Technology), 1 μg GST-c-Jun or GST-IκBα as a substrate and 10 μCi [γ-^{32}P]ATP and incubated at 30°C for 45 min. The reaction was terminated by adding 2.5 × SDS sample buffer, then boiled at 99°C for 5 min and centrifuged at 14 000 × *g* for 2 min. The supernatant was subjected to SDS-PAGE and the gel was stained with Coomassie brilliant blue G250, destained and exposed to X-ray film.

2.7 Statistical analyses

The data for body weight gain and serum biochemistries are presented as mean ± SEM of ten mice. The RT-PCR data shown is a representative experiment from a total of three independent experiments using the RNA samples pooled from ten mice *per* group. Western blot and kinase activity data are presented as mean ± SEM of the triplicate analyses of the protein samples pooled from ten mice *per* group. Statistical significance was defined as $p < 0.05$ and was determined by Student's *t*-test using the SPSS software.

3 Results

The mice fed an HFD for 12 wk had a significantly greater body weight (109% greater, $p < 0.001$) and higher serum levels of total cholesterol ($p < 0.001$), FFA ($p < 0.001$), glucose

Table 1. Body weight gain, serum, and hepatic biochemistries

	ND	HFD
Body weight gain (g/12 wk)	7.21 ± 0.21	15.05 ± 1.15**
Serum		
Total cholesterol (mmol/L)	2.80 ± 0.13	5.01 ± 0.20**
Triglyceride (mmol/L)	0.49 ± 0.01	0.57 ± 0.03
FFA (μEq/L)	469.0 ± 18.25	708.1 ± 21.09**
Glucose (mmol/L)	9.37 ± 0.36	11.90 ± 0.76*
Insulin (pmol/L)	36.60 ± 6.21	68.25 ± 11.80*
Leptin (pmol/L)	7.20 ± 0.73	22.12 ± 0.96**
Liver		
Weight (mg/100 g body weight)	3.35 ± 0.08	6.19 ± 0.25**
Cholesterol (μmol/g liver)	10.80 ± 0.64	23.89 ± 1.07**
Triglyceride (μmol/g liver)	28.60 ± 2.76	43.52 ± 2.13**
FFA (μEq/g liver)	2.19 ± 0.22	7.90 ± 0.39**

Each value represents the mean ± SEM, $n = 10$. *$p < 0.05$, **$p < 0.001$.

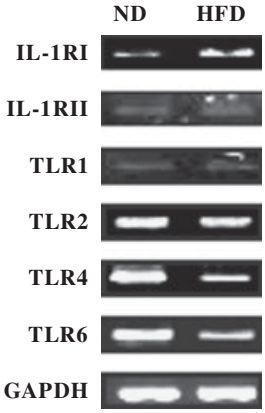

Figure 1. An HFD upregulates IL-1RI but downregulates TLR gene expression in the skeletal muscle of mice. The total RNA was isolated from the skeletal muscle of each mouse ($n = 10$) and pooled for RT-PCR analysis of IL-1RI, IL-1RII, TLR1, TLR2, TLR4 and TLR6 mRNA levels.

($p < 0.05$), insulin ($p < 0.05$) and leptin ($p < 0.001$) than the mice fed an ND (Table 1). Feeding mice the HFD led to significant increases in the relative liver weight (an 85% increase, $p < 0.001$) and in the hepatic levels of cholesterol (121% higher, $p < 0.001$), triglycerides (52% greater, $p < 0.001$) and FFA (261% greater, $p < 0.001$) compared with those for the ND mice (Table 1).

The HFD-induced changes in the expression of IL-1R and TLRs in the skeletal muscle were evaluated through semi-quantitative RT-PCR analyses. Compared with an ND, an HFD resulted in the upregulation of IL-1RI, but not IL-1RII, in the skeletal muscle of mice. Mice with HFD-induced obesity exhibited reduced mRNA levels of TLR2, TLR4 and TLR6 in their skeletal muscles compared with those in the ND mice (Fig. 1). However, the expression level of TLR1 in skeletal muscle was not altered by the HFD.

To determine whether the HFD altered the protein levels of the signaling molecules involved in the IL-1RI-mediated inflammatory signaling pathway, Western blot analyses were performed using a whole-tissue extract prepared from

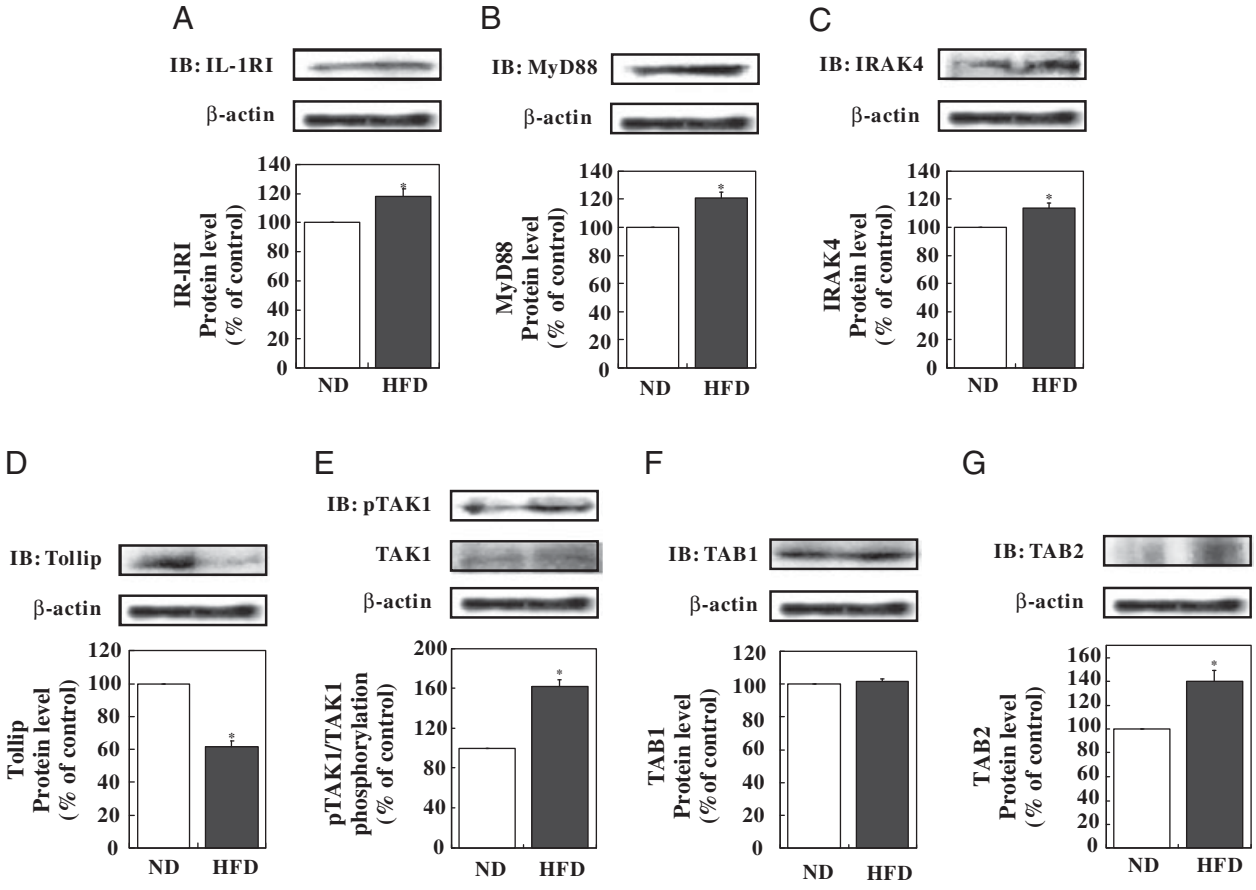

Figure 2. An HFD enhances the expression of adaptor proteins and signaling molecules related to the MyD88-dependent innate inflammatory signaling pathway in the skeletal muscle of mice. Proteins from whole-tissue extract (100 μg/lane) were probed using the following antibodies: (A) anti-IL-1RI; (B) anti-MyD88; (C) anti-IRAK4; (D) anti-Tollip; (E) anti-phosphorylated TAK1 (Thr184/187) and TAK1; (F) anti-TAB1; (G) anti-TAB2. The immunoblots are representative experiments of three independent experiments. Each bar represents the mean ± SEM, $^*p < 0.05$.

the skeletal muscle of mice fed either an ND or an HFD. These results showed that an HFD led to an 18, 21 and 14% increase in the IL-1RI, MyD88 and IRAK4 protein levels, respectively. The HFD also resulted in a 38% reduction in the expression of Tollip ($p<0.05$, Figs. 2A–D). Additionally, the HFD resulted in a 62% increase in the phosphorylation of TAK1 at Thr184/187 ($p<0.05$, Fig. 2E). Mice fed an HFD had an elevated level of TAB2 protein (a 40% increase, $p<0.05$) in the skeletal muscle, while the TAB1 protein level remained unchanged (Figs. 2F and G). We observed that catalytic activity of IKKβ (an 8.2-fold increase, $p<0.05$) and degradation of IκB were significantly increased (a 55% reduction, $p<0.05$) in the skeletal muscle of mice fed HFD than in the ND mice (Figs. 3A and B).

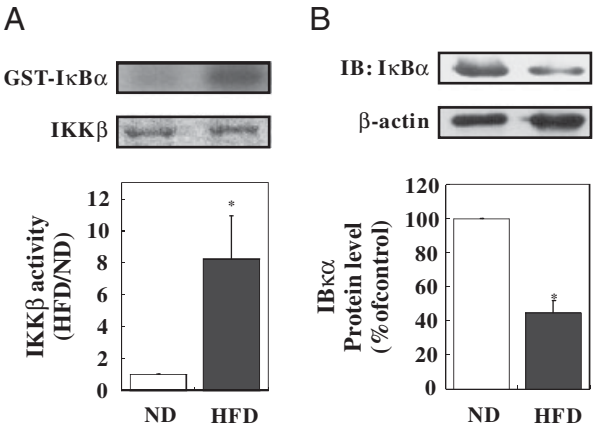

Figure 3. An HFD increases IKK activity and IκBα degradation in the skeletal muscle of mice. (A) IKKβ activity was determined by the radioactive kinase assay using GST-IκBα as a substrate. (B) Whole-tissue lysates (200 μg/lane) obtained from skeletal muscles of mice fed an ND or an HFD were probed using anti-IκBα. The blots are representative experiments of three independent experiments. Each bar represents the mean ± SEM, $^{*}p<0.05$.

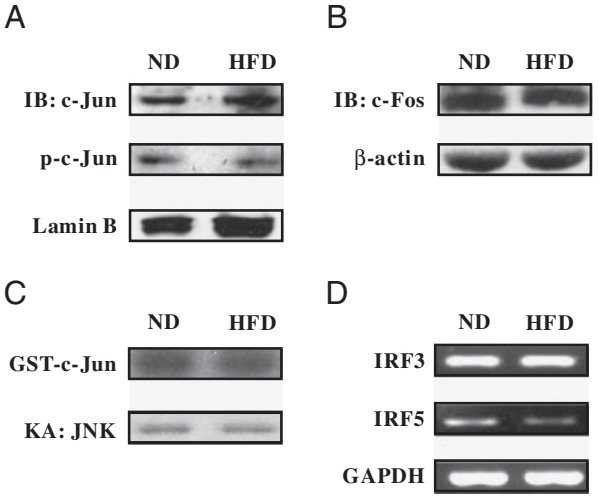

Figure 4. An HFD does not affect the activity and expression of AP-1 functional subunits, c-Jun and c-Fos, and IRF3. (A) Proteins from nuclear extracts were probed using anti-c-Jun, anti-phospho-c-Jun (Ser63) and anti-lamin B. (B) Proteins from whole-tissue extract (100 μg/lane) were probed using anti-c-Fos and anti β-actin. (C) JNK activity was determined by the radioactive kinase assay using GST-c-Jun as a substrate. (D) RT-PCR analysis of IRF3 and IRF5 genes.

The HFD-induced changes in the expression and activation of transcription factors such as AP-1, IRF5 and the IRF3, other than those leading to the activation of NFκB, were determined. The immunoblot results of nuclear extracts indicated that the HFD barely affected the level of the phosphorylated, as well as the total, form of c-Jun protein in the skeletal muscle of mice (Fig. 4A). Moreover, the level of c-Fos protein, another principal component of the AP-1 transcription factor, was unaffected by the HFD (Fig. 4B). Similarly, the kinase assay results indicate that the phosphorylation of c-Jun was not affected in the skeletal muscle of mice fed an HFD (Fig. 4C). Semi-quantitative RT-PCR analysis demonstrated that the HFD did not affect the expression of IRF3 and slightly decreased the IRF5 mRNA level in the skeletal muscle (Fig. 4D).

4 Discussion

The HFD used in the current study successfully induced the accumulation of visceral adipose tissue, along with other obesity-related complications including dyslipidemia, fatty liver, hyperinsulinemia and hyperleptinemia in mice (Table 1). DNA microarray analyses of over 40 000 genes have been performed on the skeletal muscle of mice used in the current study. The gene expression profiles indicate that the expression of IL-1RI (2.20 ± 0.04-fold) and TAB2 (1.89 ± 0.23-fold) were upregulated, whereas the expression of Tollip (0.5 ± 0.07-fold) and TIR-domain-containing adaptor protein (0.48 ± 0.05-fold) genes were downregulated by the HFD (data not shown). These microarray results are confirmed by the RT-PCR analyses demonstrating a higher IL-1RI expression in the skeletal muscle of mice fed an HFD than mice fed an ND (Fig. 1). Moreover, the HFD-induced up- and downregulation of TAB2 and Tollip, respectively, observed in the microarray experiments are supported by the results of the immunoblots (Fig. 2).

The TLRs are type I integral membrane glycoproteins and members of a larger superfamily of proteins that includes the IL-1R [19]. TLRs and IL-1Rs have a conserved region of ~200 amino acids in their cytoplasmic tail that is known as the TIR domain. TLRs play an important role in the innate immune system by activating inflammatory pathways in response to microbial agents. TLR4 functions as the receptor for both LPS found in cell walls of Gram-negative bacteria and saturated FFAs in inflammatory cells [20] and in conventional insulin-target tissues such as fat [8, 21] and muscle [22, 23]. TLR2, a subclass of TLRs which dimerizes with TLR1 or TLR6, is activated by the specific fatty acid composition, such as triacyl or diacylipoprotein [24–26].

Recent reports have demonstrated the increased expression of TLR2 and TLR4 in adipose tissue from obese rodent models (*ob/ob*, *db/db* and HFD-induced obese mice) and obese subjects compared with their normal-weight counterparts [8, 27]. While most studies investigating the role of TLR-driven NFκB signaling in metabolic diseases have been conducted in adipocytes or fat tissue derived from obese animal models, few studies have evaluated TLR signaling in the skeletal muscle. In the present study, the RT-PCR results indicate that the expression levels of TLR2, TLR4 and TLR6 were downregulated in the skeletal muscle of mice with HFD-induced obesity compared with normal-weight mice fed an ND (Fig. 1). Interestingly, these same genes were upregulated by the HFD in the visceral adipose tissue of these mice (data not shown). These results contradict recent observations in humans in which TLR4-mediated NFκB signaling was activated in muscle biopsies obtained from obese and type 2 diabetes subjects but not from lean subjects [28]. This suggests that the roles that TLR2, TLR4 and TLR6 play as modulators of the cross-talk between the inflammatory and metabolic pathways in diet-induced obesity appear to be tissue and/or species specific.

The RT-PCR and immunoblot results demonstrated that the HFD upregulated the expression of IL-1RI at both mRNA and protein levels, whereas it did not affect the expression of IL-1RII in the skeletal muscle of mice (Figs. 1 and 2). While IL-1RI mediates IL-1 signaling, IL-1RII acts as a decoy receptor, inhibiting IL-1 signaling [11]. After IL-1R stimulation, MyD88 is recruited to the cytoplasmic TIR domain where it facilitates the association of IRAK4 with the receptor complex. The binding of MyD88 to IRAK4 facilitates IRAK4-mediated phosphorylation of IRAK1. Activated IRAK1 phosphorylates Tollip, which subsequently leaves the receptor complex and enables TNF-receptor-associated factor 6 (TRAF6) to bind to this complex. The IRAK1-TRAF6 complex then disengages from the receptor and interacts with another preformed complex consisting of TAK1, TAB1 and either TAB2 or TAB3 at the plasma membrane. This interaction induces the phosphorylation of TAB2/TAB3 and TAK1 and then, together with TRAF6 and TAB1, the complex translocates to the cytoplasm. The subsequent activation of TAK1 in the cytoplasm leads to the activation of IKKs, degradation of IκB and the release of NFκB [13, 19]. We found that prolonged consumption of an HFD led to changes in the protein levels of various signaling molecules involved in IL-1RI-mediated NFκB activation, such as MyD88, IRAK4, Tollip, TAB2 and phosphorylated TAK1, in the skeletal muscle of mice (Fig. 2). Additionally, the IKK kinase assay revealed that consumption of the HFD significantly increased the phosphorylation of IκBα and induced the degradation of IκBα in the mouse skeletal muscle (Fig. 3). These results suggest that the HFD induced the IL-1RI-mediated NFκB activation in the skeletal muscle of mice.

The Western blot analyses of nuclear extracts, along with the JNK assays, demonstrated that c-Jun and c-Fos are not involved in the HFD-induced proinflammatory signaling pathway in the skeletal muscle of mice (Figs. 4A–C). After recognizing specific ligands, TLRs utilize either MyD88-dependent or MyD88-independent signaling pathways. The MyD88-independent pathway, used in TLR3 and TLR4 signaling, leads to the activation of IRF3 followed by the induction of IFN-inducible genes [19, 24]. Recently, an integral role of IRF5 in the induction of genes activated by TLRs has been suggested [29]. The observations that, at the mRNA level, the HFD did not affect the expression of IRF3 and slightly decreased the expression of IRF5 in the skeletal muscle of mice (Fig. 4D) suggest that the inflammation induced by an HFD is not mediated through TLRs. In conclusion, these results provide the first line of evidence that the long-term consumption of an HFD activates the IL-1RI-mediated proinflammatory signaling pathway in the skeletal muscle of mice and this signal is transduced *via* MyD88-IKK-NFκB signaling cascades. The HFD-induced activation of IL-1RI-mediated proinflammatory signaling pathway might be associated with insulin resistance manifested in the skeletal muscle of animals with diet-induced obesity.

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The authors have declared no conflict of interest.

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