Hesperetin suppresses LPS/high glucose-induced inflammatory responses via TLR/MyD88/NF-κB signaling pathways in THP-1 cells

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ABSTRACT

BACKGROUND/OBJECTIVES: Unregulated inflammatory responses caused by hyperglycemia may induce diabetes complications. Hesperetin, a bioflavonoid, is a glycoside in citrus fruits and is known to have antioxidant and anticarcinogenic properties. However, the effect of inflammation on the diabetic environment has not been reported to date. In this study, we investigated the effect of hesperetin on proinflammatory cytokine secretion and its underlying mechanistic regulation in THP-1 macrophages with co-treatment LPS and hyperglycemic conditions.

MATERIALS/METHODS: THP-1 cells differentiated by PMA (1 μM) were cultured for 48 h in the presence or absence of hesperetin under normoglycemic (5.5 mM/L glucose) or hyperglycemic (25 mM/L glucose) conditions and then treated with LPS (100 ng/mL) for 6 h before harvesting. Inflammation-related proteins and mRNA levels were evaluated by enzyme-linked immunosorbent assay, western blot, and quantitative polymerase chain reaction analyses.

RESULTS: Hesperetin (0–100 μM, 48 h) treatment did not affect cell viability. The tumor necrosis factor-α and interleukin-6 levels increased in cells co-treated with LPS under hyperglycemic conditions compared to normoglycemic conditions, and these increases were decreased by hesperetin treatment. The TLR2/4 and MyD88 activity levels increased in cells co-treated with LPS under hyperglycemic conditions compared to normoglycemic conditions; however, hesperetin treatment inhibited the TLR2/4 and MyD88 activity increases. In addition, nuclear factor-κB (NF-κB) and Acetyl-NF-κB levels increased in response to treatment with LPS under hyperglycemic conditions compared to normoglycemic conditions, but those levels were decreased when treated with hesperetin. SIRT3 and SIRT6 expressions were increased by hesperetin treatment.

CONCLUSIONS: Our results suggest that hesperetin may be a potential agent for suppressing inflammation in diabetes.

Keywords: Diabetes mellitus; hesperetin; inflammation; macrophages; NF-kappa B

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by abnormal insulin secretion or insulin resistance and associated hyperglycemia, in which blood glucose levels are elevated \[1,2\]. The global diabetes prevalence in 2019 was estimated to be 9.3% \[3\]. Over the past decade, studies on the cellular and molecular mechanisms of diabetes and its complications have revealed a close association between nutrient excess and derangements in mediators of immunity and inflammation \[4\]. High glucose levels have been shown to induce inflammatory cytokine secretion in both clinical and experimental settings \[5,6\].

Nuclear factor-κB (NF-κB) is a transcription factor involved in the expression of several genes, such as those regulating inflammatory and immune responses \[7,8\]. NF-κB has also been involved in the association between chronic diabetes complications, cardiovascular disease, and microvascular complications \[9\]. Among the toll-like receptors (TLRs), TLR2/4 has an important role in inflammation and diabetes \[10\]. In several inflammatory diseases, expression of TLRs and the downstream signal adapter, myeloid differentiation factor 88 (MyD88), are also increased, leading to NF-κB activation and induction of genes encoding inflammatory mediators such as cytokines and cyclooxygenase-2 (COX-2) \[11\].

An accumulation of evidence suggests that a chronic elevation of lipopolysaccharide (LPS) could have a role in the pathogenesis of insulin resistance in vivo. An elevated LPS concentration contributes to low-grade systemic inflammation, a central feature of obesity, insulin resistance, and type 2 diabetes \[12\]. TLRs can recognize various components of bacterial cell walls such as LPS, peptidoglycans, and lipopeptides. TLR4 binding to LPS activates NF-κB and leads to the production of inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) \[13\]. Liang et al. \[12\] reported that metabolic endotoxemia could be involved in the pathogenesis of insulin resistance in obese and type 2 diabetes subjects and that targeting TLR4 might be beneficial in these individuals.

IL-6, a proinflammatory cytokine secreted during inflammation by monocytes and macrophages, activates lymphocytes to increase antibody production, and its expression is elevated in inflammatory lesions. TNF-α also has an important role in triggering inflammatory responses by activating macrophages and increasing the production of other proinflammatory cytokines \[14\].

Sirtuins (SIRTs) are members of the class III group of histone deacetylases (HDACs). Interest in these proteins is increasing because studies have shown that SIRTs are linked to age-related diseases, such as cancer, diabetes, and neurological diseases \[15-18\]. Among the SIRTs, SIRT3 is reported to act as an NAD+-dependent deacetylase, and SIRT6 acts as ADP-ribosyl-transferase \[19\]. SIRT3 and SIRT6 are important in glucose and lipid metabolism \[20\]. SIRT3 is associated with the generation of reactive oxygen species (ROS), and the predominant source of ROS is mitochondria. Excessive ROS levels may contribute to the development of type 2 diabetes \[21\]. A recent study has shown that SIRT6 could regulate the expression of NF-κB \[22\].

Hesperetin is an aglycone form of hesperidin, which is present in peels of several citrus fruits, and research on the health effects of hesperetin is progressing actively \[23\]. Hesperetin has been studied for its potential hypolipidemic \[24\] and anti-cancer properties \[25\]. Moreover, hesperetin exerts effects on diabetes-induced retinal oxidative stress, neuroinflammation,
and apoptosis in rats [26]. However, the protective effects and mechanism of hesperetin action on the diabetic environment have not been fully described.

Thus, in this study, we investigated the effects and mechanism of action of hesperetin on diabetic inflammation in THP-1 cells. By using experimental conditions that mimic diabetic complications, we investigated whether hesperetin treatment can modulate inflammation through the TLR/MyD88/NF-κB signaling pathways and SIRT expression.

**MATERIALS AND METHODS**

**Reagents**

Hesperetin, LPS, 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT), and PMA were obtained from Sigma Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was obtained from Biosesang (Seongnam, Korea). Quantitative polymerase chain reaction (qPCR) primers were procured from Bioneer (Daejeon, Korea). The BCA protein assay kit was procured from Thermo Fisher Scientific (Waltham, MA, USA).

**Cell culture and sample treatment**

The human monocyte cell line THP1 was obtained from Korean Cell Line Bank (Seoul, Korea). THP1 cells were cultured in RPMI 1640 medium (Welgene, Daegu, Korea) supplemented at 37°C in 5% CO₂. For monocyte to macrophage differentiation, THP1 cells (4 × 10⁶ cells/mL) were seeded in a cell culture dish in RPMI 1640 medium with 1 μM PMA for 48 h. Differentiated THP1 cells were treated with hesperetin for 48 h in the absence or presence of LPS for 6 h under normoglycemic (NG, 5.5 mM/L glucose) or hyperglycemic (HG, 25 mM/L glucose) conditions. Next, medium was collected for measurement of cytokine release. Cells were washed in PBS and then harvested.

**Cell viability assay**

Cytotoxicity effects of hesperetin on cultured THP1 cells were measured by MTT assay. THP1 cells were seeded at 1 × 10⁵ cells/well in a 24-well plate and treated with hesperetin for 48 h. The cells were then treated with LPS (100 ng/mL) for 6 h under NG or HG conditions. Then, MTT solution (100 μL; 1 mg/mL) was added, and the cells incubated for a further 3 h. After incubation, the supernatant was removed, and the precipitated formazan was solubilized in 150 μL of 100% DMSO. Absorbance was measured at 570 nm with an EZRead 400 Microplate reader (Biochrom, Cambridge, UK).

**Western blot**

Protein (20 μg) was mixed with buffer (100 mM Tris at pH 7.5, 2% sodium dodecyl sulfate, 2% glycerol, 1% 2-mercaptoethanol, and 0.01% bromophenol blue), incubated at 100°C for 5 min, and loaded on 10% polyacrylamide gels. Electrophoresis was performed using the Mini Protein Tetra Cell (Bio-Rad, CA, USA). Proteins were transferred to nitrocellulose membranes (Carlsbad, CA, USA), and the membranes blocked using blocking buffer (5% non-fat dried milk) for 2 h. The membranes were incubated with appropriate primary antibodies, then washed and incubated with the appropriate secondary antibody. Next, the membranes were visualized by applying the Western Blotting Luminol Reagent (Santa Cruz Biotecnology, Dallas, TX, USA) and performing autoradiography on a ChemiDoc XRS+ System (Bio-Rad). Equal loading of protein was normalized with β-actin and TATA-binding protein (TBP). The NFκB, COX-2, β-actin, and TBP were purchased from Santa Cruz Biotechnology. Acetyl-NFκB
Ac-NF-κB, IL-6, SIRT3, and SIRT6 were purchased from Cell Signaling Technology (Beverly, MA, USA). Signal quantification was performed using ImageJ software.

**qPCR**

Total RNA was isolated with TRIzol reagent (Ambion, Life Technologies, Carlsbad, CA, USA). The RNA concentration was measured using an absorbance ratio of 260 and 280 nm and a NanoDrop 2000 (Thermo Fisher Scientific). The cDNA was synthesized from total RNA using an Omniscript RT kit (QIAGEN, Hilden, Germany). The qPCR was performed with the CFX96 Touch Real-Time PCR system (Bio-Rad) and iQ SYBR Green Supermix (Bio-Rad) and the following primers (5′ to 3′, Forward [F], Reverse [R]); human TLR2 (F): GGGTCATCATCAGCCTCTCC, (R): GTGGATGTAATCGTTGTCACTGGA, human TLR4 (F): CAGAGTTGCTTTCAATGGCATC, (R): GGAGGTCCAAGAACTAATGTCAGA, human MyD88 (F): TGCTGGAGCTGGGACCCACGATTGAGAGG, (R): TCAGACACACACACTCTCAACTGATAG, human NFκB (F): GACAAGGTGCCAAGAGCTGACAT, (R): TCATACTGTTAACAAGGCCT, human SIRT1 (F): CGGAAACCAATACCTCCACCT, (R): CACCCCATCGCTTCAGTGAAG, human SIRT3 (F): ACATCGATGGCTGAGGAGATG, (R): CATGAGCTTCAACAGGCTTGA, human SIRT6 (F): GTGCCGCTCGGCTCCACAG, (R): GGAGGTCCGCGCTGCTCCTG, human β-actin (F): CACCCCGTGCTGCTGAC, (R): CCAGAGGCGTACAGGGATAG. Data were analyzed using the 2^−ΔΔCT method and normalized using β-actin as a housekeeping gene.

**Immunofluorescence staining**

The cells were seeded at 1 × 10^5 cells/well in a 24-well plate and subsequently treated with hesperetin with LPS (100 ng/mL) treated prior to immunofluorescence staining. The cell medium was removed, and the cells washed twice with PBS. The PBS was removed, and the cells were fixed with 4% paraformaldehyde (PFA) for 30 min. Next, the 4% PFA was removed and the cells washed in PBS. Cells were incubated with NF-κB p65 and Ac-NF-κB p65 antibodies. For detection of nucleic acid, 4′,6-diamidino-2-phenylindole (DAPI) solution was incubated at 37°C. The mounting solution was dropped on a slide glass, and signal quantification was evaluated using a microscope (Leica Microsystems, Wetzlar, Germany).

**Enzyme-linked immunosorbent assay (ELISA)**

The IL-6 and TNF-α ELISA kits were purchased from Abcam (Cambridge, MA, USA) and used to evaluate the effect of hesperetin on cytokine levels in LPS-activated, PMA-differentiated THP-1 macrophages under NG or HG conditions. The cells were seeded at 1 × 10^5 cells/well in 24-well plates and treated with hesperetin for 48 h followed by LPS (100 ng/mL) for 6 h before harvesting. Cytokine concentrations in the supernatants of THP-1 macrophage cell cultures were determined by using the ELISA kits according to the manufacturer’s instructions.

**Statistical analysis**

Results are reported as means ± SD. Each experiment was performed at least three times. Statistical significance was assessed by applying one-way analysis of variance (ANOVA; SPSS version 25.0 software) followed by Dunnett’s post hoc test. Statistical significance is expressed as *P < 0.05 and **P < 0.01 compared with normoglycemic (NG) results or ’P < 0.05 and ’’P < 0.01 compared with the LPS + hyperglycemic + hesperetin 0 μM (H0) results.
RESULTS

Cytotoxic range of hesperetin in LPS-treated THP1 macrophages under high glucose conditions
The concentration of LPS and/or glucose required to create an environment that mimics diabetic conditions at the cellular level was determined. Inflammation was confirmed by measuring the COX-2 expression level. Fig. 1A shows the results of an experiment to determine LPS concentration in the absence of glucose treatment. The COX-2 expression level increased in cells with LPS 100 ng/mL and LPS 500 ng/mL compared to that in untreated LPS. However, treatment with LPS 500 ng/mL resulted in a slight decrease in COX-2 expression compared to that with LPS 100 ng/mL. Thus, we used a concentration of LPS 100 ng/mL in this experiment. Fig. 1B shows the results of an experiment to determine the COX-2 expressions at various concentration of glucoses with a 100 ng/mL LPS concentration. MTT assay was performed to study the effect of hesperetin on cell viability after 48 h incubation (Fig. 1C). THP1 cells (1 × 10^5 cells/mL) were cultured in the presence of LPS (100 ng/mL) under NG or HG conditions. Cells were exposed to different concentrations of hesperetin (0–100 μM) for 48 h, and the results showed that hesperetin treatment and LPS stimulation did not affect cell viability in THP1 macrophages.

Hesperetin suppresses release of proinflammatory cytokines in LPS-treated THP1 macrophages under hyperglycemic conditions
To examine whether hesperetin could inhibit high glucose and LPS-induced inflammatory cytokine expressions in differentiated THP1 cells, the cells were treated with 10–50 μM hesperetin for 48 h, then stimulated using LPS 100 ng/mL for 6 h. Cell culture media were collected for measurement of cytokine release using ELISA (Fig. 2A and B) and western blotting (Fig. 2C). In the HG and LPS + HG conditions, TNF-α and IL-6 expressions were significantly increased compared to that under NG conditions and significantly decreased when the cells were treated with hesperetin.
Hesperetin regulates TLR-mediated signaling pathway-related TLR2, TLR4, and MyD88 gene expressions in LPS-stimulated THP1 macrophages under hyperglycemic conditions

qPCR was performed to confirm the mRNA expressions of TLR2/4 and MyD88 in THP1 macrophages exposed to high glucose and LPS-induced inflammatory conditions that mimic complex diabetic diseases. The inhibitory effect of hesperetin on the activation of TLR2/4 and MyD88 was also investigated. The mRNA level expressions of TLR2/4 and MyD88 increased under HG and LPS + HG conditions compared to that under NG conditions. However, the expressions of these molecules were significantly decreased following hesperetin treatment (Fig. 3). Modulating TLRs may be a useful strategy in preventing diabetic complications, given the crucial role of inflammation in microvascular and associated complications [27]. Our results suggested that hesperetin regulates inflammatory responses within the TLR2, TLR4, and MyD88 pathways.

Hesperetin reduces NFκB protein and mRNA expressions in LPS-treated THP1 macrophage under hyperglycemic conditions

Western blotting, qPCR, and immunofluorescence assay were performed to examine the expression of NFκB in THP1 cells stimulated with LPS and cultured under hyperglycemic conditions. The potential inhibitory effect of hesperetin on the activation of NFκB was
also investigated. NFκB protein expression in the nucleus increased in the HG and LPS + HG conditions and decreased when cells were treated with hesperetin (Fig. 4A, B, and C). Consistent with protein expression results, the mRNA level of NFκB increased in HG and LPS + HG conditions compared to that under the NG condition but decreased significantly in response to treatment with hesperetin (Fig. 4D). Using immunofluorescence, the expression of NFκB and the active form of Ac-NF-κB were shown to be increased under HG and LPS + HG conditions and decreased in cells treated with 50 μM hesperetin (Fig. 4E and F). These results indicated that hesperetin treatment could suppress NFκB expression by blocking NFκB translocation to the nucleus.
Hesperetin increases SIRT3 and SIRT6 expressions in LPS-treated THP1 macrophages under hyperglycemic conditions

We analyzed the effect of hesperetin treatment on the expression levels of SIRT3 and SIRT6 in THP-1 macrophages cultured under diabetes-mimicking conditions by performing western

Fig. 4. Effect of hesperetin on NF-κB protein and mRNA expressions in LPS-treated THP-1 macrophages under hyperglycemic conditions. NF-κB levels were evaluated by western blot analysis of cell lysates (A). The percentage of NF-κB (whole)/β-actin (B) and the percentage of NF-κB (nuclear)/TBP (C) were revealed by image J. Cells (1 × 10^6 cells/mL) were treated with hesperetin for 48 h, and NF-κB mRNA levels were evaluated by qPCR (D). Immunofluorescence images show downregulation of NF-κB (E) and Ac-NF-κB (F) in LPS-treated THP-1 macrophages under hyperglycemic conditions following hesperetin treatment. Differentiated THP-1 cells were cultured in the presence of LPS (100 ng/mL) under normoglycemic or hyperglycemic conditions in the absence or presence of hesperetin (50 μM) for 48 h (400 × Magnification). Signal quantification was performed using ImageJ software. The data are representative of at least three independent experiments. Data were analyzed using the 2−ΔΔCT method.

NF-κB, nuclear factor-κB; TBP, TATA-binding protein; NG, normoglycemic 5.5 mM/L glucose; HG, hyperglycemic 25 mM/L glucose; LPS, lipopolysaccharide; H0, LPS + hyperglycemic + hesperetin 0 μM; DAPI, 4′,6-diamidino-2-phenylindole.

**P < 0.01 compared with NG; ***P < 0.01 compared with H0.
blot and qPCR. As shown in Fig. 5A, western blotting showed that SIRT3 protein expression increased under the NG condition but decreased under the HG and LPS + HG conditions. Conversely, SIRT3 protein expression increased following treatment with hesperetin. Consistent with this, SIRT3 and SIRT6 mRNA levels increased significantly with hesperetin 25 and 50 μM treatment (Fig. 5B); similarly, SIRT6 mRNA levels increased (Fig. 5C), but there was no significance to the changes.

**DISCUSSION**

High blood glucose levels in type 2 diabetic patients are reported to increase inflammatory cytokines and ROS expressions via the polyol pathway, nicotinamide adenine dinucleotide phosphate oxidase, advanced glycation end products pathway, and the mitochondrial electron transport system [28, 29]. Increased Inflammatory cytokine and ROS levels contribute to malignant circulation, which contributes to an increased incidence of complications and reduced insulin secretion [30-32].

Several natural components present in common foods can inhibit the expression of inflammatory cytokines. For instance, curcumin can regulate high glucose-induced
proinflammatory cytokine production in monocytes [33]. The present study investigated hesperetin, a flavanone glycoside in citrus fruits. The study aimed to determine whether hesperetin could be a therapeutic agent against inflammation, particularly inflammatory responses that contribute to diabetes and diabetic complications. Increased secretion of inflammatory cytokines is known to reduce insulin sensitivity and induce diabetes, and abnormal expression levels are accompanied by systemic low-level inflammatory conditions, which are ultimately responsible for the onset of diabetic complications [34,35].

Several studies have suggested that inflammatory markers, such as TNF-α and IL-6, are associated with diabetes and glucose disorders [36,37]. According to Xie et al. [38] blueberries can reduce proinflammatory cytokine TNF-α and IL-6 production in mouse macrophages. Also, Guha et al. [39] reported that a high glucose level can upregulate the expression of TNF-α in THP-1 cells. In support of those studies, herein, we show that the expression of TNF-α and IL-6 is significantly decreased by hesperetin treatment compared to that under hyperglycemic and LPS + hyperglycemic conditions. Thus, hesperetin is considered to have anti-inflammatory properties effective against diabetes.

Increased TLR4 and NF-κB levels have been reported in patients with inflammatory chronic kidney disease, non-alcoholic fatty liver disease, and diabetes and can lead to increases in other proinflammatory cytokines [40,41]. In addition, an increase in TLR2 in an LPS-induced inflammatory environment leads to enhanced NF-κB activation and COX-2 expression [42]. According to Ghanim et al. [43] in normal-weight men and women, when taken with a high-fat/high-carbohydrate meal, orange juice can prevent meal-induced oxidative and inflammatory stresses, including increased endotoxin levels and TLR expression. Previous studies have also reported that phytochemicals such as resveratrol [41] and curcumin [27] are effective against diabetes by inhibiting TLR signaling. Our results have shown that hesperetin treatment can significantly reduce TLR2, TLR4, and MyD88 mRNA levels in LPS-treated THP-1 macrophages under hyperglycemic conditions, suggesting that hesperetin inhibits TLR activation in LPS-induced inflammation.

The expression of inflammatory cytokines is necessarily accompanied by NF-κB activity. When NF-κB is activated by LPS and a high glucose level, it migrates from the cytoplasm to the nucleus and induces gene expression [44]. This results in the expression of proinflammatory cytokines that lead to diabetic complications. According to Yang et al. [45] epigallocatechin gallate inhibits inflammatory agents such as LPS and proinflammatory cytokines such as TNF-α. Another study showed that resveratrol inhibits the expressions of COX-2 and inducible nitric oxide synthase by inhibiting NF-κB activation induced by proinflammatory stimulants such as LPS and H2O2 [46,47]. Our data showed that NF-κB expression and mRNA levels increased in THP-1 macrophages treated with LPS and/or maintained under hyperglycemic conditions and decreased following treatment with hesperetin. Furthermore, as a result of immunofluorescence staining, the expression of Ac-NF-κB was reduced by treatment with hesperetin. Thus, hesperetin inhibits the activation of NF-κB, which is mediated by several proinflammatory stimuli in diabetic complications.

The activity of SIRTs in metabolic syndrome is presumed to be related to aging, nutrition, and type 2 diabetes [48]. SIRT3 and SIRT6 are associated with dysregulated metabolism and other complications observed in diabetes. [48]. Elliott et al. [49] reported a decrease in fasting and postprandial blood glucose levels and decreased postprandial insulin levels in type 2 diabetes patients treated with resveratrol. Our data showed that SIRT3/6 protein and
mRNA expressions in THP-1 macrophages maintained in a diabetes-mimicking environment increased following treatment with hesperetin. Therefore, hesperetin may produce effective therapeutic outcomes in diabetes by regulating SIRT3/6 expression.

In the present study, we observed that treatment with hesperetin modulates inflammatory cytokine release, NF-κB acetylation, and SIRT3/6 expression via the TLR/MyD88/NF-κB signaling pathways. Thus, we propose that hesperetin may be a potential therapeutic agent for preventing diabetes and diabetic complications.

REFERENCES

1. Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. Lancet 2000;355:773-8.
2. Urakami T, Kubota S, Nitadori Y, Harada K, Owada M, Kitagawa T. Annual incidence and clinical characteristics of type 2 diabetes in children as detected by urine glucose screening in the Tokyo metropolitan area. Diabetes Care 2005;28:1876-81.
3. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, Colagiuri S, Guariguata L, Motala AA, Ogurtsova K, Shaw JE, Bright D, Williams RJ. IDF Diabetes Atlas Committee. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract 2019;157:107843.
4. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest 2011;121:2111-7.
5. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: a clinical perspective. Endocr Rev 2001;22:36-52.
6. Shanmugam N, Kim YS, Lanting L, Natarajan R. Regulation of cyclooxygenase-2 expression in monocytes by ligation of the receptor for advanced glycation end products. J Biol Chem 2003;278:34834-44.
7. de Winther MP, Kanters E, Kraal G, Hofer MH. Nuclear factor kappaB signaling in atherogenesis. Arterioscler Thromb Vasc Biol 2005;25:904-14.
8. Arkan MC, Heveren AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 2005;11:191-8.
9. Yang SM, Kim SY, Lee KY, Kim YS, Nam MS, Park IB. Inflammatory markers are associated with microvascular complications in type 2 diabetes. J Korean Diabetes Assoc 2007;31:472-9.
10. Dasu MR, Ramirez S, Isseroff RR. Toll-like receptors and diabetes: a therapeutic perspective. Clin Sci (Lond) 2012;122:203-14.
11. Rhee SH, Hwang D. Murine TOLL-like receptor 4 confers lipopolysaccharide responsiveness as determined by activation of NF kappa B and expression of the inducible cyclooxygenase. J Biol Chem 2000;275:34035-40.
12. Liang H, Hussey SE, Sanchez-Avila A, Tantrivong P, Musi N. Effect of lipopolysaccharide on inflammation and insulin action in human muscle. PLoS One 2013;8:e63983.
13. Park BS, Lee JO. Recognition of lipopolysaccharide pattern by TLR4 complexes. Exp Mol Med 2013;45:e66-66.
14. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. Int J Mol Sci 2019;20:20.
15. Haigis MC, Sinclair DA. Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol
2010;5:253-95. PubMed | Crossref

16. Bosch-Presegué L, Vaquero A. The dual role of sirtuins in cancer. Genes Cancer 2011;2:648-62. PubMed | Crossref

17. Ma Y, Chen H, He X, Nie H, Hong Y, Sheng C, Wang Q, Xia W, Ying W. NAD+ metabolism and NAD(+)-
dependent enzymes: promising therapeutic targets for neurological diseases. Curr Drug Targets
2012;13:222-9. PubMed | Crossref

18. Roth M, Chen WY. Sorting out functions of sirtuins in cancer. Oncogene 2014;33:1609-20. PubMed | Crossref

19. Frye RA. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins
(sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. Biochem Biophys Res
Commun 1999;260:273-9. PubMed | Crossref

20. Michishita E, Park JY, Burneskis JM, Barrett JC, Horikawa I. Evolutionarily conserved and nonconserved
cellular localizations and functions of human SIRT proteins. Mol Biol Cell 2005;16:4623-35. PubMed | Crossref

21. Wang CH, Wei YH. Roles of mitochondrial sirtuins in mitochondrial function, redox homeostasis, insulin
resistance and type 2 diabetes. Int J Mol Sci 2020;21:5266. PubMed | Crossref

22. Kawahara TL, Michishita E, Adler AS, Damian M, Berber E, Lin M, McCord RA, Ongaiigu KC, Boxer
LD, Chang HY, Chua KF. SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene
expression and organismal life span. Cell 2009;136:62-74. PubMed | Crossref

23. Parhiz H, Roohbakhsh A, Soltani F, Rezaee R, Iranshahi M. Antioxidant and anti-inflammatory properties
of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and
experimental models. Phytother Res 2015;29:323-31. PubMed | Crossref

24. Aranganathan S, Nalini N. Efficacy of the potential chemopreventive agent, hesperetin (citrus flavanone),
on 1,2-dimethylhydrazine induced colon carcinogenesis. Food Chem Toxicol 2009;47:2594-600. PubMed | Crossref

25. Morin B, Nichols LA, Zalasky KM, Davis JW, Manthey JA, Holland LJ. The citrus flavonoids hesperetin and
nobiletin differentially regulate low density lipoprotein receptor gene transcription in HepG2 liver cells. J
Nutr 2008;138:1274-81. PubMed | Crossref

26. Kumar B, Gupta SK, Srinivasan BP, Nag TC, Srivastava S, Saxena R, Jha KA. Hesperetin rescues retinal
oxidative stress, neuroinflammation and apoptosis in diabetic rats. Microvasc Res 2013;87:65-74. PubMed | Crossref

27. Jialal I, Kaur H. The role of toll-like receptors in diabetes-induced inflammation: implications for vascular
complications. Curr Diab Rep 2012;12:172-9. PubMed | Crossref

28. Amaral S, Oliveira PJ, Ramalho-Santos J. Diabetes and the impairment of reproductive function: possible
role of mitochondria and reactive oxygen species. Curr Diabetes Rev 2008;4:46-54. PubMed | Crossref

29. Nishikawa T, Araki E. Investigation of a novel mechanism of diabetic complications: impacts of
mitochondrial reactive oxygen species. Rinsho Byori 2008;56:712-9. PubMed | Crossref

30. Jain SK, Kannan K, Lim G, Matthews-Greer J, McVie R, Bocchini JA Jr. Elevated blood interleukin-6
levels in hyperketonemic type 1 diabetic patients and secretion by acetocetate-treated cultured U937
monocytes. Diabetes Care 2003;26:2139-43. PubMed | Crossref

31. Jain SK, Kannan K, Lim G, McVie R, Bocchini JA Jr. Hyperketonemia increases tumor necrosis factor-
alpha secretion in cultured U937 monocytes and Type 1 diabetic patients and is apparently mediated by
oxidative stress and cAMP deficiency. Diabetes 2002;51:2287-93. PubMed | Crossref

32. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and
interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab
2001;280:E745-51. PubMed | Crossref
33. Yun JM, Jialal I, Devaraj S. Epigenetic regulation of high glucose-induced proinflammatory cytokine production in monocytes by curcumin. J Nutr Biochem 2011;22:450-8.

34. Matulewicz N, Karczewska-Kupczewska M. Insulin resistance and chronic inflammation. Postepy Hig Med Dosw 2016;70:1245-58.

35. Wu H, Ballantyne CM. Metabolic inflammation and insulin resistance in obesity. Circ Res 2020;126:1549-64.

36. Hossain M, Faruque MO, Kabir G, Hassan N, Silkdar D, Nahar Q, Ali L. Association of serum TNF-α and IL-6 with insulin secretion and insulin resistance in IFG and IGT subjects in a Bangladeshi population. Int J Diabetes Mellit 2010;2:165-8.

37. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999;353:1649-52.

38. Xie C, Kang J, Ferguson ME, Nagarajan S, Badger TM, Wu X. Blueberries reduce pro-inflammatory cytokine TNF-α and IL-6 production in mouse macrophages by inhibiting NF-κB activation and the MAPK pathway. Mol Nutr Food Res 2011;55:1587-91.

39. Guha M, Bai W, Nadler JL, Natarajan R. Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells via hyperglycemia-induced oxidant stress-dependent and -independent pathways. J Biol Chem 2000;275:17728-39.

40. Choi RY, Ham JR, Lee MK. Esculetin prevents non-alcoholic fatty liver in diabetic mice fed high-fat diet. Chem Biol Interact 2016;260:13-21.

41. Lee H, Yang SJ. In vitro and in vivo effects of piceatannol and resveratrol on glucose control and TLR4-NF-κB pathway. J Korean Soc Food Sci Nutr 2017;46:267-72.

42. Youn HS, Lee JY, Fitzgerald KA, Young HA, Akira S, Hwang DH. Specific inhibition of MyD88-independent signaling pathways of TLR3 and TLR4 by resveratrol: molecular targets are TBK1 and RIP1 in TRIF complex. J Immunol 2005;175:3339-46.

43. Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaiesheh S, Mohaney P, Dandona P. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression. Am J Clin Nutr 2010;91:940-90.

44. Hua KF, Wang SH, Dong WC, Lin CY, Ho CL, Wu TH. High glucose increases nitric oxide generation in lipopolysaccharide-activated macrophages by enhancing activity of protein kinase C-α/δ and NF-κB. Inflamm Res 2012;61:1107-46.

45. Yang F, de Villiers WJ, McClain CJ, Varilek GW. Green tea polyphenols block endotoxin-induced tumor necrosis factor-production and lethality in a murine model. J Nutr 1998;128:2334-40.

46. Tsai SH, Lin-Shiau SY, Lin JK. Suppression of nitric oxide synthase and the down-regulation of the activation of NFkappaB in macrophages by resveratrol. Br J Pharmacol 1999;126:673-80.