Journal of Food and Nutrition Research, 2023, Vol. 11, No. 3, 269-276
Available online at http://pubs.sciepub.com/jfnr/11/3/12
Published by Science and Education Publishing
DOI:10.12691/jfnr-11-3-12

Sulforaphane (SFN) Exerts Anti-inflammatory Effects on LPS-stimulated RAW 264.7 and Mouse Bone Marrow Driven Macrophages by Modulating p65 Phosphorylation and Histone Acetylation

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Received February 07, 2023; Revised March 14, 2023; Accepted March 27, 2023

Abstract Obesity-induced chronic inflammation contributes to the development of insulin resistance and type 2 diabetes and largely pertains to macrophages, primarily derived from bone marrow, (BMDMs) in insulin-sensitive tissues. Sulforaphane (SFN) is a major bioactive of cruciferous vegetables with a potent anti-inflammatory property. However, potential molecular modes of action remain unclear. The phosphorylation of nuclear factor-kappa B (NF-κB) and subsequent histone acetylation are critical signaling pathways for the transcription of pro-inflammatory genes in macrophages in obesity. Therefore, we tested our hypothesis that SFN mitigates the expression of pro-inflammatory genes in macrophages by suppressing p65 phosphorylation and histone acetylation using RAW 264.7 cell line and primary mouse BMDMs. In RAW 264.7 macrophages, SFN significantly inhibited the lipopolysaccharide (LPS)-induced pro-inflammatory genes expression, including *iNos*, *Cox-2*, *Tnfα*, *Mcp-1* and *Il-6*, and suppressed CD11c immunofluorescence. Moreover, SFN significantly inhibited p65 phosphorylation and acetylation levels of histone H4, while it increased the histone deacetylase 3 (HDAC3) expression. Similarly, SFN inhibited p65 phosphorylation and histone H4 acetylation with HDAC3 expression increased in the BMDMs. These results revealed that SFN exerts anti-inflammatory effects via modulation of p65 phosphorylation and histone acetylation in macrophages. Our work suggests that supplementation of SFN or SFN-containing vegetables may serve as an anti-inflammatory diet component for mitigating obesity-related inflammation and related metabolic disorders.

Keywords: *Sulforaphane, NF-κB, RAW264.7 macrophage, bone marrow-derived macrophage, inflammation*

Cite This Article: Seok-Yeong Yu, Zhenhua Liu, Soonkyu Chung, and Young-Cheul Kim, "Sulforaphane (SFN) Exerts Anti-inflammatory Effects on LPS-stimulated RAW 264.7 and Mouse Bone Marrow Driven Macrophages by Modulating p65 Phosphorylation and Histone Acetylation." *Journal of Food and Nutrition Research*, vol. 11, no. 3 (2023): 269-276. doi: 10.12691/jfnr-11-3-12.

1. Introduction

Obesity-induced sustained inflammation is a significant risk factor for the development of metabolic disorders such as insulin resistance, type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD) [1,2]. Substantial evidence indicates that chronic inflammation in obesity is mediated by macrophage accumulation in adipose tissues, derived mainly from bone marrow, and their subsequent inflammatory responses [3,4]. Conversely, the selective depletion of macrophages leads to a resistance against diet-induced obesity and inflammation [5]. Even though several anti-inflammatory drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) are commercially available, safety concerns have been raised due to side effects, including increased mortality in patients with metabolic disorders in clinical trials [6,7,8]. Alternatively, consumption of certain fruits and vegetables has been shown to attenuate obesity-induced inflammation and decrease the risk of obesity-induced chronic diseases [9]. Therefore, identifying such anti-inflammatory food sources and dietary bioactive component(s) would be important for the development of dietary approaches to attenuate obesity-induced chronic inflammation.

Nuclear factor-kappa B (NF-κB) is composed of five proteins such as p65 (Rel-A), Rel-B, c-Rel, p52, and p50, and is a key transcriptional factor that regulates the expression of pro-inflammatory genes such as inducible nitric oxidase (iNOS), tumor necrosis factor alpha (TNFα), monocyte chemoattractant protein-1 (MCP-1) as well as distinct surface markers for inflammatory macrophages including CD11c [10,11]. Among the NF-κB family, p65

subunit, which forms a heterodimer with p50, has been recognized as a major target molecule for inducing pro-inflammatory gene expression in obesity and related metabolic diseases [10,12]. In a quiescent state, the promoter regions of pro-inflammatory genes are restrained by histone deacetylation, which is primarily mediated by histone deacetylase 3 (HDAC3) [13]. Upon activation, p65 subunit is phosphorylated, and histone acetylation occurs on the promoter regions of pro-inflammatory genes, which in turn allows p-p65 to bind its responsive element [13,14]. Therefore, suppressing p65 phosphorylation and/or preventing histone acetylation would serve as molecular targets to mitigate inflammation.

Sulforaphane (SFN) is a metabolite of glucoraphanin which is abundantly found in cruciferous vegetables such as broccoli and brussels sprouts. SFN is well-known as an inducer of NF-E2-related factor (NRF2) signaling by increasing Nrf2 mRNA expression and expression of Nrf2-targeted antioxidant genes, including heme oxygenase-1 (HO-1) [15]. Observational studies indicate that a high consumption of cruciferous vegetables is linked with decreased circulating levels of pro-inflammatory markers and reduced risks for T2DM incidence in humans [16,17]. In line with this, the supplementation of glucoraphanin, a precursor of SFN, significantly improved glucose tolerance, mitigated the expression of pro-inflammatory genes, and reduced the number of CD11c-positive inflammatory macrophage in the liver and adipose tissue (AT) in diet-induced obese mice [18]. Furthermore, Nrf2 knockout (KO) in peritoneal macrophages mitigated the inhibitory effects SFN on the expression of pro-inflammatory genes, suggesting Nrf2 as a potential target molecule which SFN acts through to exert its anti-inflammatory roles in macrophages [18,19]. However, The inhibitory effects of SFN on the production of pro-inflammatory mediators including nitrate, IL-1β, and PGE2 were still significantly potent in Nrf2 KO peritoneal macrophages and increased as its concentration increases from 5 to 20 μM [20]. Based on these studies, it is likely that SFN may trigger another anti-inflammatory mechanisms via Nrf2-independent manner. In this study, we investigated whether SFN inhibits p65 phosphorylation and/or histone acetylation using two models of macrophage, RAW 264.7 macrophage cell line and primary mouse BMDMs, by treating with physiologically achievable concentrations of SFN (≤ 20 μM) in vivo [18,19,20]. We hypothesized that SFN would inhibit inflammatory response in RAW 264.7 macrophage and primary BMDMs by mitigating p65 phosphorylation and histone acetylation.

2. Materials & Methods

2.1. Materials

L-Sulforaphane (SFN) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lipopolysaccharide from E. Coli O111:B4 (LPS) and 2-mercaptoethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (high glucose, DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, penicillin/streptomycin (P/S), fetal bovine serum (FBS), macrophage colony-stimulating factor were purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. Cell Culture

RAW 264.7 macrophages (ATCC® TIB-71TM) were propagated in DMEM containing 10% FBS and 1% P/S. Cells were seeded at the density of 1 x 10^5 cells/mL in 6-well plates and incubated overnight. Next day, the cells were subject to SFN-pretreatment for 1 hour followed by 12-hour treatment of SFN in the presence of LPS (100 ng/mL). For the experiments with bone marrow-derived macrophages (BMDMs), femurs and tibias were harvested from FVB mice at 4-5 weeks old, and the bone marrow cells were collected and cultured in RPMI 1640 medium containing 10% FBS, 0.05 μM 2-mercaptoethanol and 1% P/S overnight. Next day, the floating bone marrow cells were collected, seeded in 6-well plates at the density of 0.2 x 10^6 cells/mL, and differentiated into BMDM in RPMI 1640 medium containing 10% FBS, 1% P/S, 0.05 μM 2-mercaptoethanol and M-CSF at 10 ng/mL for 5 days. Then, the cells were pre-treated with SFN for 1-hour and co-treated with LPS for 12 hours. For Western blot analysis, RAW264.7 macrophages were co-treated with SFN and LPS for 1 hours, and BMDMs were co-treated for 2.5 hours to address potential molecular mechanisms for the transcription of pro-inflammatory genes.

2.3. Nitric Oxide (NO-) Production

After 12-hr treatment with SFN in the presence or absence of LPS, the conditioned medium from RAW 264.7 macrophages were collected, mixed with Griess reagent (Sigma-Aldrich) at the ratio of 1 to 1 and incubated for 15 mins. The absorbance was measured at 540 nm.

2.4. Nitroblue Tetrazolium (NBT) Assay

O_2^- production was measured as previously described [22]. After SFN treatment for 12 hours in the presence or absence of LPS, RAW 264.7 macrophages were exposed to 0.2% NBT in PBS for 60 min. Formazan was dissolved with dimethyl sulfoxide (DMSO), and the absorbance was measured at 570 nm.

2.5. Quantitative Real-time-PCR (qRT-PCR)

Total RNAs were isolated using TRIzol™ reagent (Thermo Fisher Scientific) and cDNAs were obtained using RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific) by following the manufacturer's protocol. qRT-PCR was performed using ViiA™ 7 RT-PCR system (Life technologies, Grand Island, NY). The target genes were normalized by 18S gene expression, and the relative expression was calculated using the $2^{-(\Delta\Delta nd)}$. Primers used were mouse-specific and listed in Table 1.

Table 1. The list of primer sequences used

Gene name	Forward (5'-3')	Reverse (5'-3')
Nrf2	GACATCCTTTGGAGGCAAGA	GCCTTCTCCTGTTCCTTCTG
Ho-1	CGCCTTCCTGCTCAACATT	TGTGAGGGACTCTGGTCTTT
iNos	TACGCCTTCAACACCAAGG	CTTGTCACCACCAGCAGTAG
Cox2	CTGGACCCATTCCTTCTTGG	GATACACCTCTCCACCAATGAC
Tnfα	AAGCATGATCCGCGACGTG	ATAGGCACCGCCTGGAGTTC
Mcp-1	CCGGAGCCATCAATCAAGAA	CTGGACCCATTCCTTCTTGG
Il-6	TGCCTTCTTGGGACTGATGC	TTCTGCAAGTGCATCATCGTT
18s	CGCTTCCTTACCTGGTTGAT	GAGCGACCAAAGGAACCATA

Nrf2, Nuclear factor erythroid 2-related factor 2; Ho-1, Heme oxygenase-1; iNos, inducible nitric oxide synthase; Cox2, cyclooxygenase 2; Tnfα, tumor necrosis factor alpha; Mcp-1, monocyte chemoattractant protein-1; Il-6, interleukin 6.

2.6. Immunofluorescence (IF) Staining

Briefly, RAW264.7 macrophages were seeded and propagated in 4-well chamber slides and used for SFN treatment. Then, the cells were fixed with 4.5% paraformaldehyde for 20 min and permeabilized with 0.5% Triton X-100 for 10 min at RT. For IF staining, the cells were blocked in 2% BSA-containing PBS supplemented with 0.1% tween 20 (PBST) for 1 hour at RT, incubated with primary antibody (1:100) in PBST overnight at 4°C, followed by the incubation with secondary antibody (1:200) overnight at 4°C. Imaging for the immune-labeled cells was performed using a Nikon Eclipse 50i Fluorescence microscope (Nikon, Japan), and the images were processed using the Adobe Photoshop (Adobe System, San Jose, CA).

2.7. Western Blotting

Cell lysates were prepared using RIPA buffer (Thermo Fisher Scientific) supplemented with a protease and phosphatase inhibitors and deacetylase inhibitor cocktail (Thermo Fisher Scientific). 20 µg of lysates was separated on 10% SDS polyacrylamide gel, transferred to PVDF membranes, and then incubated with primary antibody overnight at 4°C, followed by secondary antibody incubation for 1 to 1.5 hour at room temperature. All of primary antibodies targeting p65, p-p65 Ser536, acetylated histone H3 (Lys9/Lys14) and acetylated histone H4 (Lys8) [acyl-H3 and acyl-H4 in short] were purchased from Cell Signaling Technology (Beverly, MA, USA). Primary antibodies to HDAC3 and β-actin were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Then, the membrane was incubated in Clairty^{TM} Western ECL Blotting substrate (Bio-Rad, Hercules, CA) for 5 min and exposed to X-ray film. The band intensities were quantified using ImageJ software [National Institutes of Health (NIH), Bethesda, MD, US].

2.8. Statistics

The data were presented as the means ± S.D of three independent experiments (n=3). To evaluate statistically significance, unpaired t-test was used, and the differences were considered statistically significant at $P < 0.05$. All data were prepared and analyzed using SigmaPlot version 11.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. SFN Exerts Antioxidant Effects on RAW 264.7 Macrophages

SFN exerts antioxidant effects by increasing Nrf2 expression and its related down-stream target genes such as *heme oxygenase-1 (HO-1)* [19]. We first investigated the antioxidant effects of SFN on RAW264.7 macrophages to validate that SFN works at the molecular level as previously reported (Figure 1). We chose the concentration range within 20 µM, which showed no cytotoxic effects of SFN in MTT assay (Data not shown) and also is physiologically achievable in mice fed brussels sprout extracts [20]. SFN significantly increased the expression levels of *Nrf2* and *Ho-1* genes in RAW264.7 macrophages compared to control cells while LPS showed no effects on the expression of *Nrf2* and *Ho-1* genes (Figure 1 A). The mRNA expression levels of Ho-1 were statistically greater in the cells treated with 20 µM than with 10 µM. Next, we measured the effect of SFN on the production of ROS ($O2^-$) and RNS (NO^-) in RAW 264.7 macrophages (Figure 1 B). LPS significantly increased the production of $O2^-$ and NO^- in RAW 264.7 macrophages compared to unstimulated cells, whereas SFN significantly suppressed LPS-induced $O2^-$ and NO^- levels in a dose-dependent manner. Thus, these data indicate that SFN exerts antioxidant activities in RAW264.7 macrophages via Nrf2-dependent mechanisms, as previously reported [19].

3.2. SFN Suppresses LPS-induced Expression of CD11c and Inflammatory Genes in RAW 264.7 Macrophages

SFN was shown to decrease the expression of pro-inflammatory genes and accumulation of inflammatory CD11c-positive macrophages in tissues in diet-induced obese mice [18]. Thus, we evaluated the anti-inflammatory effects of SFN in RAW264.7 macrophages stimulated with LPS by analyzing the expression of pro-inflammatory genes and CD11c immunofluorescence staining (Figure 2). LPS-stimulated RAW264.7 macrophages exhibited greater CD11c fluorescent intensity than unstimulated controls (Figure 2 A). CD11c fluorescent intensity, however, was notably diminished in the cells in the presence of SFN at 10 and

20 µM. Moreover, SFN significantly decreased LPS-induced upregulation of inflammatory genes such as *iNos, Cox-2, Tnfα, Mcp-1 and Il-6* in RAW 264.7 macrophages compared to LPS-stimulated controls (Figure 2 B & Figure 2C). The inhibitory effects of SFN on LPS-induced inflammation were also dose-dependent.

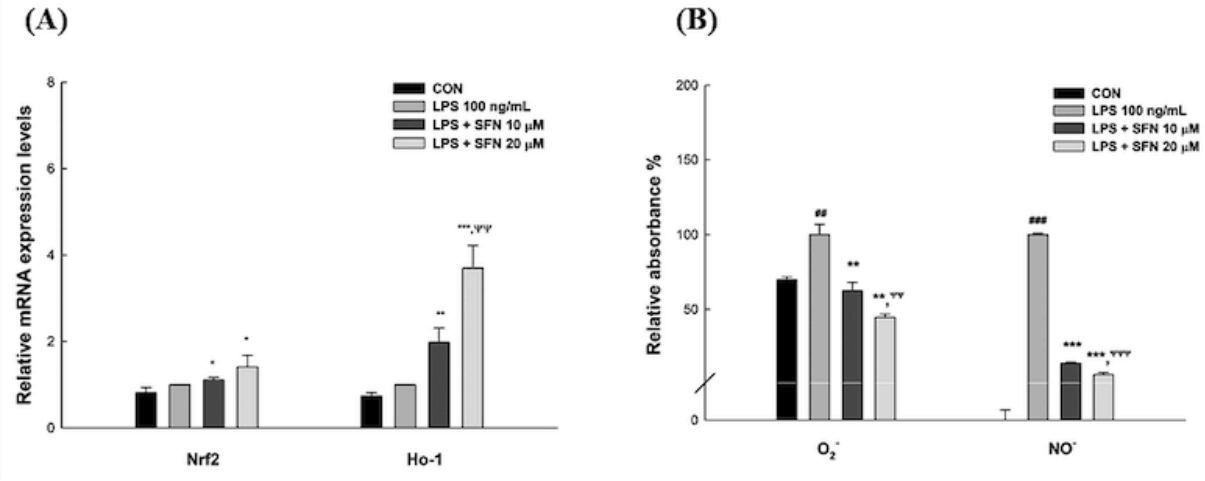

Figure 1. The effects of SFN on the expression of Nrf2 and Ho-1 genes and production of superoxide anion ($O2^-$) and nitric oxide (NO^-) in LPS-stimulated RAW 264.7 macrophages. Cells were preincubated with SFN for 1 hour and co-treated with LPS for 12 hours. (A) Gene expression by RT-PCR and (B) $O2^-$ and NO^- production. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 as compared to control (unstimulated cells), * P<0.05, ** P<0.01, *** P<0.001 as compared to only LPS-stimulated cell. $^{\Psi}$ P<0.05, $^{\Psi\Psi}$ P<0.01, $^{\Psi\Psi\Psi}$ P<0.001 as compared to SFN at 10 µM. n = 3, bars represent mean ± S.D

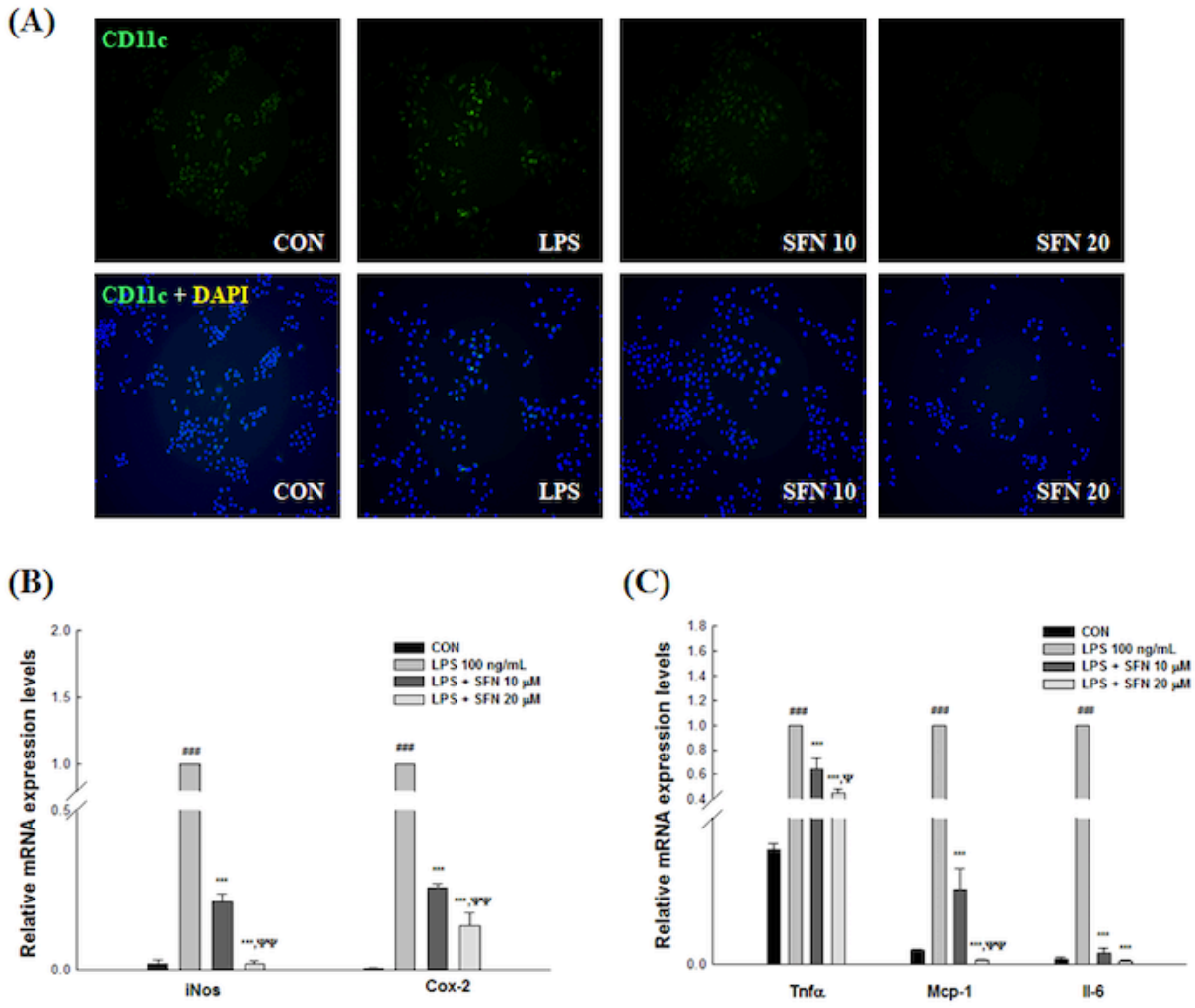

Figure 2. The effects of SFN on CD11c fluorescence intensity and expression of pro-inflammatory genes in LPS-stimulated RAW 264.7 macrophages. Cells were preincubated with SFN for 1 hour and co-treated with LPS for 12 hours. (A) Representative picture of CD11c immunofluorescence staining. (B & C) Gene expression by RT-PCR. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 as compared to control (unstimulated cells), * P<0.05, ** P<0.01, *** P<0.001 as compared to only LPS-stimulated cell. $^{\Psi}$ P<0.05, $^{\Psi\Psi}$ P<0.01, $^{\Psi\Psi\Psi}$ P<0.001 as compared to SFN at 10 µM. n = 3, bars represent mean ± S.D

3.3. SFN Suppresses the Phosphorylation of p65 and Acetylation Levels of Histone H4 and Increases HDAC3 Expression in RAW 264.7 Macrophages

It was reported that the anti-inflammatory effects of SFN in macrophages occur independently of Nrf2-mediated antioxidant effects [19]. To gain more insight into potential mechanisms by which SFN inhibits LPS-induced inflammation in RAW264.7 macrophages, we investigated the effects of SFN on p65 phosphorylation and histone deacetylation (Figure 3). Consistent with mRNA expression data, we found that SFN significantly decreased LPS-stimulated p65 phosphorylation and increased the expression levels of HDAC3 (20 μM) (Figure 3 A & B). In addition, SFN markedly suppressed the histone acetylation levels, especially histone H4, in RAW 264.7 macrophages, showing the inverse correlation between HDAC3 expression and histone H4 acetylation levels. It was also observed that LPS stimulation increased HDAC3 expression in RAW 264.7 macrophages. However, no changes on the acetylation levels of histone H3 and H4 by LPS were observed. Taken together, these findings indicate that SFN may exert anti-inflammatory effects on macrophages by inhibiting p65 phosphorylation and histone acetylation.

3.4. SFN Upregulates the Expression of Antioxidant Genes and Suppresses Anti-inflammatory Genes in Mouse BMDMs Stimulated LPS

The recruitment of bone marrow-derived macrophages and subsequent inflammatory activation are identified as key mechanisms for obesity-induced chronic inflammation in tissues [4]. To test the in vivo relevance of anti-inflammatory roles of SFN in macrophages, we fractionated primary murine bone marrow cells, differentiated into macrophages, and stimulated with LPS in the presence and absence of SFN at 10 and 20 μM (Figure 4). Unlike the findings from RAW 264.7 macrophages, LPS treatment significantly reduced Nrt2 gene expression compared to unstimulated cells (Figure 4 A). However, consistent with the data obtained from RAW 264.7 cells, 20 μM of SFN treatment significantly increased the *Nrf2 and Ho-1* mRNA expression in BMDMs compared to BMDMs treated only with LPS. Moreover, SFN treatment at 20 μM concentration significantly decreased LPS-induced expression of inflammatory genes including *iNos, Cox-2, Tnfα, Mcp-1 and Il-6*, whereas 10 μM SFN inhibited *iNos, Mcp-1 and Il-6* only in BMDMs (Figure 4 B).

3.5. SFN Suppresses p65 Phosphorylation and Increases HDAC3 Expression in BMDMs

Potential mechanisms by which SFN suppresses LPS-induced upregulation of pro-inflammatory genes were investigated in BMDMs. Consistent with the findings in RAW264.7 macrophages (Figure 3), SFN treatment at 20 μM concentration remarkably reduced the phosphorylation of p65 subunit in BMDMs (Figure 5 A). Moreover, neither LPS nor SFN altered the protein expression levels of p65 per se. In addition, we observed that SFN at 20 μM significantly increased the expression levels of HDAC3 with a concomitant decrease in the acetylation levels of histone H4, but not H3, in BMDMs stimulated with LPS (Figure 5 B). Collectively, SFN was able to suppress the p65 phosphorylation and histone H4 acetylation in both RAW 264.7 and primary BMDM macrophages, resulting in downregulation of the transactivation of proinflammatory genes.

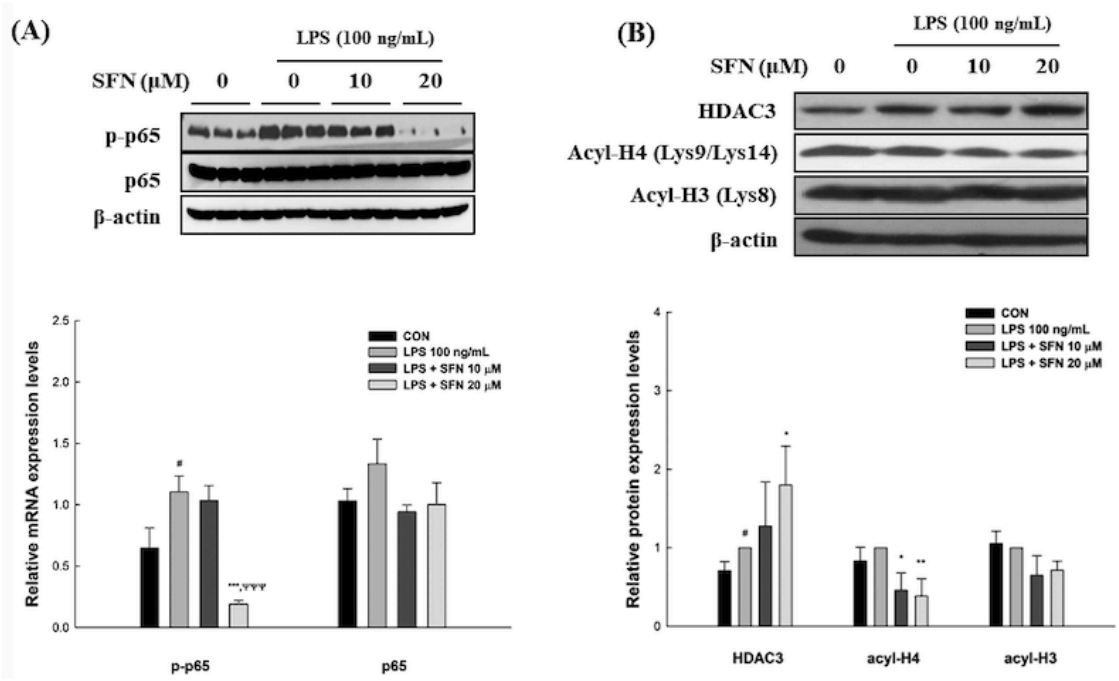

Figure 3. The effects of SFN on p65 phosphorylation, histone acetylation and HDAC3 expression in LPS-stimulated RAW 264.7 macrophages. (A) p65 and p-p65 protein expression. (B) Representative picture of HDAC3, acyl-H4 and acyl-H3. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 as compared to control (unstimulated cells), * P<0.05, ** P<0.01, *** P<0.001 as compared to only LPS-stimulated cell. $^{\Psi}$ P<0.05, $^{\Psi\Psi}$ P<0.01, $^{\Psi\Psi\Psi}$ P<0.001 as compared to SFN at 10 μM. n = 3, bars represent mean ± S.D

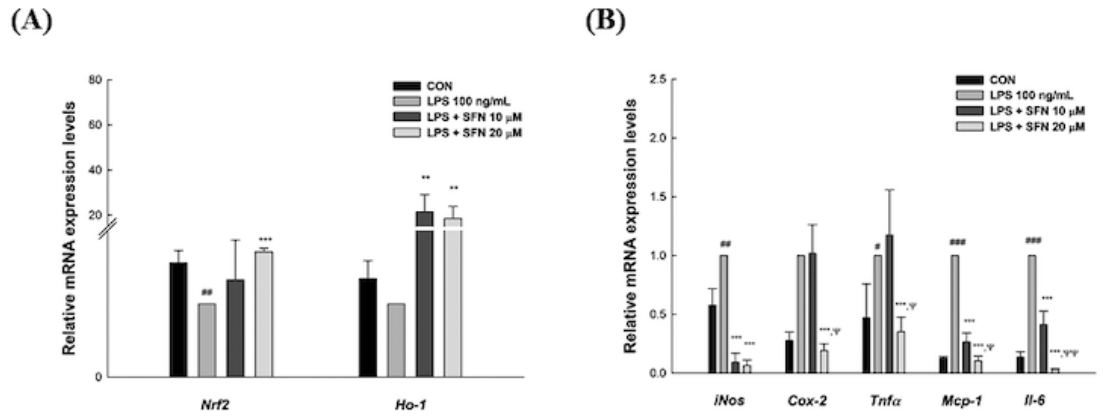

Figure 4. The effects of SFN on the expression of anti-oxidant or pro-inflammatory genes in LPS-stimulated mouse BMDMs. Cells were preincubated with SFN for 1 hour and co-treated with LPS for 12 hours. (A & B) Gene expression by RT-PCR. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 as compared to control (unstimulated cells), * P<0.05, ** P<0.01, *** P<0.001 as compared to only LPS-stimulated cell. $^{\Psi}$ P<0.05, $^{\Psi\Psi}$ P<0.01, $^{\Psi\Psi\Psi}$ P<0.001 as compared to SFN at 10 μM. n = 3, bars represent mean ± S.D

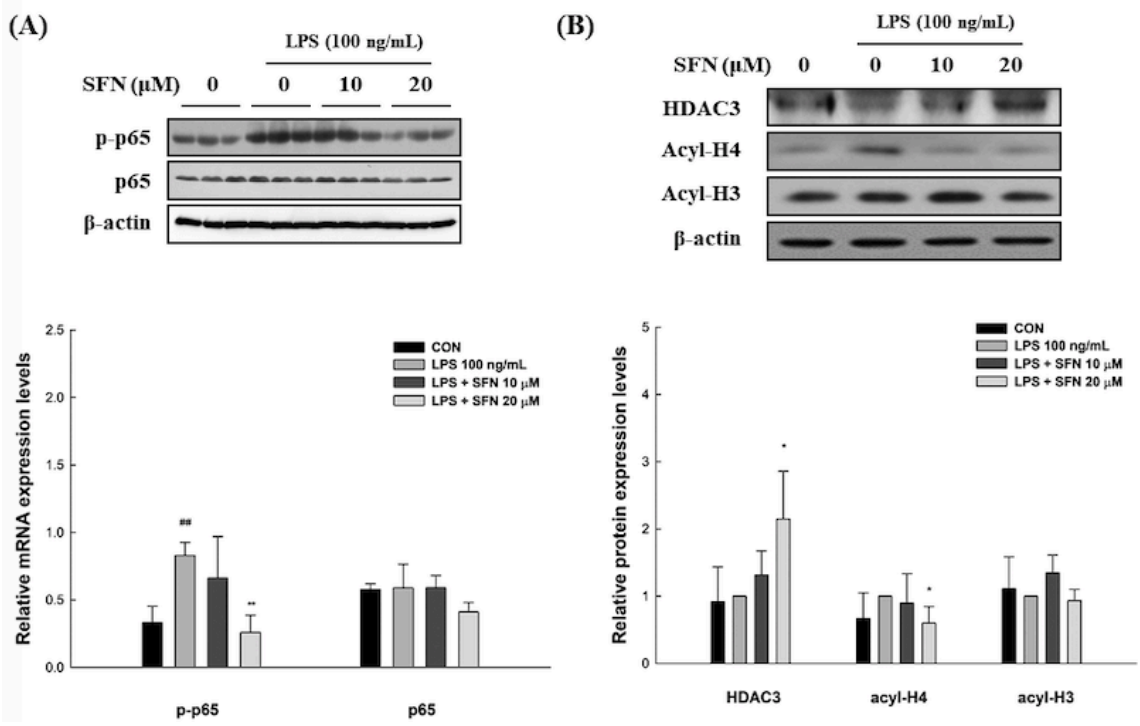

Figure 5. The effects of SFN on p65 phosphorylation, histone acetylation and HDAC3 expression in LPS-stimulated BMDMs. (A) p65 and p-p65 protein expression. (B) Representative picture of HDAC3, acyl-H4 and acyl-H3. $^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 as compared to control (unstimulated cells), * P<0.05, ** P<0.01, *** P<0.001 as compared to only LPS-stimulated cell. $^{\Psi}$ P<0.05, $^{\Psi\Psi}$ P<0.01, $^{\Psi\Psi\Psi}$ P<0.001 as compared to SFN at 10 μM. n = 3, bars represent mean ± S.D

4. Discussion

The accumulation and subsequent inflammatory activation of tissue macrophages, which are mainly originated from bone marrow, mediates sustained local and systemic inflammation in obesity [3,4]. Substantial evidence recognizes obesity-induced chronic inflammation as a significant risk factor for morbidity and mortality of metabolic complications such as insulin resistance, T2DM and CVD [1,2]. Epidemiological studies have also shown that the consumption of cruciferous vegetables such as, brussels sprouts and broccoli, is inversely correlated with blood pro-inflammatory markers and T2DM incidence [16,17]. In line with these observations in humans, SFN, a major bioactive compound of cruciferous vegetables, has been demonstrated to exert anti-inflammatory effects by reducing the expression of pro-inflammatory genes in in vitro macrophages as well as accumulation of inflammatory CD11c-positive macrophages in tissues from diet-induced obese mice [18,19,21]. However, potential mechanisms for the anti-inflammatory effects of SFN in macrophages remain unclear. In the present study, we found that SFN at the physiological concentrations (10 and 20 μM) significantly inhibited LPS-induced expression of pro-inflammatory genes and CD11c fluorescence intensity in RAW264.7 macrophages [19,20,21]. Consistently, the inhibitory effects of SFN were found in primary mouse BMDMs which represent tissue macrophages in obesity [3,4]. Moreover, we suggest the inhibition of p65 phosphorylation and histone acetylation in both cell types as potential mechanisms by which SFN exerts its anti-inflammatory effects indicating our tested hypothesis is accepted.

The disproportionate increase in phosphorylated p65 protein and subsequent inflammatory responses has been proposed as a key mechanism for obesity-mediated macrophage accumulation and expression of pro-inflammatory genes in AT [10,12]. The inhibition of p65 phosphorylation by synthetic NF-κB inhibitor was shown to exert a significant loss of the expression of pro-inflammatory genes and macrophage accumulation in ex vivo cultured AT from diet-induced obese mice [10]. In the present study, we found that SFN at 10 and 20 μM, which falls within a range of physiologically achievable concentrations in mice [20], suppressed LPS-induced inflammation activation in RAW264.7 macrophages as observed by reduced fluorescence intensity of CD11c surface marker and expression of pro-inflammatory genes such as *iNos*, *Cox-2*, *Tnfα, Mcp-1 and Il-6*. The inhibitory effects of SFN on LPS-induced upregulation of pro-inflammatory genes were consistently observed in mouse primary BMDMs, which are the major source of obesity-induced tissue macrophages and inflammation [4]. In addition, we found that SFN inhibited p65 phosphorylation in both RAW 264.7 and mouse BMDMs. Our data are consistent with the previous observations in RAW264.7 and peritoneal macrophages that SFN inhibited the expression of pro-inflammatory genes such as *Tnfα and Il-6* at 10 and 20 μM concentrations [19,21]. Also, it was demonstrated that the supplementation of SFN precursor molecule, glucoraphanin, significantly reduced expression of pro-inflammatory genes and suppressed p65 phosphorylation in tissues from diet-induced obese mice [18]. Therefore, our findings suggest the suppression of p65 phosphorylation by SFN as a potential mechanism for the anti-inflammatory effects of SFN in macrophages.

HDAC3 is shown to be a key mediator that restrains the expression of pro-inflammatory genes during quiescent state in human and mouse macrophages [13,23]. Histone acetylation on the promoter regions of pro-inflammatory genes is thus required for p65-mediated transactivation of pro-inflammatory genes in macrophages [13]. It was reported that the treatment of trichostatin A (TSA), an inhibitor of HDAC, enhanced LPS-induced inflammation in mouse BMDMs [24]. To the best of our knowledge, we made the first observation that SFN increases the expression levels of HDAC3 with concomitant reduction of acetylation levels of histone H4 in both RAW 264.7 macrophages and primary BMDMs. In contrast to our findings, SFN has been shown to suppress HDAC activity and expression in in vitro cancer cells, serving as a key molecular mechanism for inducing cancer cell apoptosis [25]. However, Chen et. al. recently reported that SFN reduced the acetylation levels of histone H3 and H4 in HaCaT keratocytes and HCT116 colon cancer cells, while TSA enhanced histone H3 and H4 acetylation levels, suggesting the epigenetic regulatory effect of SFN through inhibition of histone acetylation [17]. Our data clearly indicate that the anti-inflammatory effects of SFN are mediated, at least in part, by epigenetic regulation of histone deacetylation to suppress the transactivation of pro-inflammatory genes, which is mediated by p-p65 in macrophages. We also observed differential regulations of histone acetylation by SFN on histone H3 and H4, showing a significant reduction only in histone H4 acetylation in RAW 264.7 macrophages and mouse primary BMDMs. Our results are supported by the observation that the histone H4, but not H3, is rapidly acetylated to loosen the DNA for transcription in response to inflammatory stimuli [26].

In summary, we have demonstrated that SFN, is a major bioactive compound of cruciferous vegetables, exerts potent anti-inflammatory effects in RAW264.7 and mouse primary BMDMs against LPS stimulation. Our results suggest that the inhibition of p65 phosphorylation and/or HDAC3 protein expression would be potential mechanisms by which SFN suppresses the transactivation of inflammatory responses in macrophages. Taken these together, our observations support that SFN-containing vegetables may serve as an anti-inflammatory diet to curb current epidemics of obesity and its associated inflammatory diseases

Acknowledgements

This study was supported by the NIFA/USDA, the Massachusetts Agricultural Experiment Station and the Nutrition department of the University of Massachusetts Amherst, under project number MAS00587 to Y-C. Kim. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA.

Conflict of Interest

The authors declare that there is no conflicting interest in this manuscript.

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