Biochemical and Biophysical Research Communications 454 (2014) 95–101

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Naringenin suppresses macrophage infiltration into adipose tissue in an early phase of high-fat diet-induced obesity

Hiroki Yoshida [a,*], Hideaki Watanabe [a], Akiko Ishida [a], Wataru Watanabe [b], Keiko Narumi [c], Toshiyuki Atsumi [d], Chihiro Sugita [a], Masahiko Kurokawa [a,*]

[a] Department of Biochemistry, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan
[b] Department of Microbiology, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan
[c] Department of Clinical Pharmacy, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan
[d] Department of Pharmacognosy, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan

ARTICLE INFO

Article history:
Received 4 October 2014
Available online 18 October 2014

Keywords:
Naringenin
Adipose tissue
Macrophage
MCP-1

ABSTRACT

Obese adipose tissue is characterized by increased macrophage infiltration, which results in chronic inflammation in adipose tissue and leads to obesity-related diseases such as type 2 diabetes mellitus and atherosclerosis. The regulation of macrophage infiltration into adipose tissue is an important strategy for preventing and treating obesity-related diseases. In this study, we report that naringenin, a citrus flavonoid, suppressed macrophage infiltration into adipose tissue induced by short-term (14 days) feeding of a high-fat diet in mice; although naringenin did not show any differences in high-fat diet-induced changes of serum biochemical parameters in this short administration period. Naringenin suppressed monocyte chemoattractant protein-1 (MCP-1) in adipose tissue, and this effect was mediated in part through inhibition of c-Jun NH_2-terminal kinase pathway. Naringenin also inhibited MCP-1 expression in adipocytes, macrophages, and a co-culture of adipocytes and macrophages. Our results suggest a mechanism by which daily consumption of naringenin may exhibit preventive effects on obesity-related diseases.

1. Introduction

Obesity is considered a chronic inflammatory disease, which results from macrophage infiltration into adipose tissue [1–3]. Adipocyte–macrophage interaction augments inflammatory changes in adipose tissue, which contribute to the development of insulin resistance and type 2 diabetes mellitus [3,4]. A variety of chemokines are expressed in adipose tissue, and expression increases as early as a few days after the start of feeding mice a high-fat diet (HFD) [5]. Among these, monocyte chemoattractant protein-1 (MCP-1) plays a pivotal role in obesity-related macrophage infiltration into adipose tissue [6]. Transgenic mice overexpressing MCP-1 in adipose tissue exhibit macrophage accumulation in adipose tissue and insulin resistance, while MCP-1 homozygous knockout mice show the opposite phenotypes [7,8]. The regulation of macrophage infiltration and MCP-1 expression in adipose tissue during the early phase of obesity is an important strategy for preventing obesity-related diseases.

The number of obese individuals and the prevalence of obesity-related diseases are increasing worldwide. Consequently, the annual healthcare costs of obesity-related diseases are increasing, which is a serious social issue. In these circumstances, preventive care has attracted attention, and the development of a novel prophylactic approach is required.

Food-derived biomolecules such as flavonoids have a variety of pharmacological properties and are thought to be useful in the prevention and improvement of various diseases. A cohort study reported that intake of flavonoids reduced the risk of chronic diseases such as cerebrovascular disease, asthma, and type 2 diabetes [9]. However, the molecular mechanisms by which food-derived biomolecules exert beneficial effects remain largely unknown.

Naringenin is a predominant flavanone in grapefruit, and has various pharmacological effects, including antioxidant, anti-inflammatory, and anti-proliferative activities. Studies have shown that naringenin improves obesity-related diseases such as atherosclerosis in disease-model animals [10,11]. In addition, our

Abbreviations: MCP-1, monocyte chemoattractant protein-1; JNK, c-jun N-terminal kinase; IκB-α, inhibitor of κB-α; NF-κB, nuclear factor-κB.

* Corresponding authors. Fax: +81 982 23 5684 (H. Yoshida).
E-mail addresses: h-yoshida@phoenix.ac.jp (H. Yoshida), b2mk@phoenix.ac.jp (M. Kurokawa).

http://dx.doi.org/10.1016/j.bbrc.2014.10.038
0006-291X/

previous studies have shown that naringenin exerts anti-inflammatory effects in adipocytes [12,13], and have also shown that long-term (16 weeks) treatment with naringenin inhibits HFD-induced inflammation in mouse adipose tissue [13]. However, the effects of naringenin on HFD-induced macrophage infiltration and MCP-1 expression in adipose tissue, especially during the early phase of obesity, remain unknown.

In this study, we investigated the effects of naringenin on macrophage infiltration into adipose tissue during 1–14 days of feeding a HFD to mice, and sought to elucidate the mechanisms of these processes.

2. Materials and methods

2.1. Reagents

We purchased the following reagents: dimethyl sulfoxide (DMSO) (Nacalai Tesque, Kyoto, Japan); methyl cellulose (Wako Pure Chemical Industries, Osaka, Japan); naringenin, isobutylmethylxanthine (IBMX), and dexamethasone (DEX) (Sigma, St. Louis, MO); insulin (Cell Science & Technology Institute, Sendai, Japan); phosphate-buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), bovine serum and fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA); SP600125 (BIOMOL, Plymouth Meeting, PA).

2.2. Animals and animal care

Experiments were approved by the animal care committee of Kyushu University of Health and Welfare. Male C57BL/6J mice (age, 7 weeks; weight, 20–22 g) were purchased from the Kyudo Animal Laboratory (Kumamoto, Japan). Mice were housed 5–6/cage and maintained at 24 ± 2 °C on a 12-h light/dark cycle, and were acclimatized for 1 week prior to experimental use. Mice at 8 weeks of age were randomly divided into the following groups: (1) non-treated control mice at 8 weeks of age (day 0); (2) standard diet (STD)-fed mice treated with 0.2 mL vehicle control (0.5 w/v% methyl cellulose solution, p.o. once daily) for 1, 7, or 14 days; (3) HFD-fed mice treated with vehicle control (methyl cellulose, p.o. once daily) for 1, 7, or 14 days; and (4) HFD-fed mice treated with naringenin (100 mg/kg/day, p.o. once daily) for 1, 7, or 14 days. Mice were fed STD (10% of calories from fat, D12450B; Research Diets, New Brunswick, NJ) or HFD (60% of calories from fat, D12492; Research Diets) and water *ad libitum* for each period (1, 7, or 14 days). Mice (n = 5–6) were used for each group for each period. Food intake was measured daily, and body weight was measured before dissection.

2.3. Real-time PCR analysis

The epididymal fat pads were minced and vessels removed. Tissue (100 mg) was homogenized and lysed in TRIzol reagent (Invitrogen) to isolate total RNA. Reverse transcription was performed (ReverTra Ace qPCR RT Master Mix; Toyobo, Osaka, Japan), in accordance with the manufacturer's instructions. PCR amplification was performed using TaqMan Fast Advanced Master Mix (Applied Biosystems, Carlsbad, CA) in a StepOne Plus Real Time PCR System. Primers and TaqMan probes were: Mac-2 (Lgals3), Mm00802901-m1; MCP-1 (Ccl2), Mm00441242-m1; 18S (Rn18s), Mm03928990-g1. The relative quantity of mRNA was determined using the comparative Ct method and was normalized using 18S ribosomal RNA as an endogenous control.

2.4. Immunohistochemical staining

Epididymal fat pads were dissected, immersed in 4% paraformaldehyde, and embedded in paraffin. Sections were incubated in xylene, followed by ethanol, and washed with DH_2O. Sections were rinsed in PBS and incubated in 1% hydrogen peroxide for 30 min. After three washes in PBS for 5 min each, sections were blocked with PBS containing 5% normal goat serum, followed by incubation with a 1:3800 dilution of primary antibody (Anti-Mac-2 [CL8942AP]; Cedarlane, Burlington, Canada) for 60 min. After another three washes in PBS, sections were incubated with a 1:100 dilution of biotinylated secondary antibody (Anti-Rat IgG [BA-9400]; Vector, Burlingame, CA) for 30 min. Sections were washed in PBS before incubating in VECTASTAIN ABC solution (PK-4000; Vector). After further PBS washes, a diaminobenzidine substrate kit (K3466; Dako, Glostrup, Denmark) was used to visualize the reaction product, and nuclear staining was performed using hematoxylin. Sections were dehydrated in a graded series of ethanol solutions, followed by xylene and mounted with coverslips. Mac-2 positive cells/10 mm^2 were counted using an eyepiece fitted with a grid ruler. We excluded perivascular positive cells and localized clumps of positive cells.

2.5. Biochemical analysis of serum

Mice were fasted overnight before intervention. Each parameter was determined using the following kits: glucose, total cholesterol, and triglyceride, Cholestech LDX (Alere Medical, Tokyo, Japan); free fatty acid, NEFA-V2 (Alfresa, Osaka, Japan); insulin, AKRIN-031 (Shibayagi, Gunma, Japan).

2.6. Western blot analysis

Epididymal fat pads (100 mg) were homogenized and lysed in PRO-PREP Protein Extraction Solution (iNtRON Biotechnology, Kyungki-Do, Korea). Cell culture media (1.8 mL) were concentrated using centrifugal filter devices (Amicon Ultra 3K Device; Millipore, Darmstadt, Germany), following the manufacturer's instructions. Tissue extracts and concentrated culture media were separated on 10% SDS–PAGE gels and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). Membranes were blocked with TBS solution containing 0.1% Tween 20 (TBS-T) and 2% blocking agent (GE Healthcare, Buckinghamshire, UK). After five washes (2, 2, 15, 5, and 5 min) in TBS-T, membranes were incubated with a 1:2000 dilution of primary antibody. After another five washes in TBS-T, membranes were incubated with a 1:10,000 dilution of the corresponding secondary antibody. Chemiluminescence reagent ECL Advance (GE Healthcare) was used to visualize blots. Anti-MCP-1 (#2029), anti-β-actin (#4967), anti-phospho-JNK (#4668), and anti-IκB-α (#9242) were obtained from Cell Signaling Technology (Beverly, MA). Protein bands were quantified by signal intensity analysis with ImageQuant TL software (GE Healthcare).

2.7. Cell culture and treatments

3T3-L1 cells (Health Science Research Resources Bank, Osaka, Japan) were maintained in DMEM supplemented with 10% bovine serum. After pre-adipocytes reached confluence in 12-well plates, they were induced to differentiate into mature adipocytes by replacing medium with 10% FBS-supplemented DMEM containing 0.5 mM IBMX, 0.25 μM DEX, and 5 μg/mL insulin for 2 days. Medium was then replaced with 10% FBS-supplemented DMEM containing 5 μg/mL insulin, and this was changed every 2–3 days for the next 6–7 days. RAW 264 cells (RIKEN BioResource Center, Tsukuba, Japan) were cultured in DMEM supplemented with 10% FBS. Adipocytes and macrophages were co-cultured in a contact system,

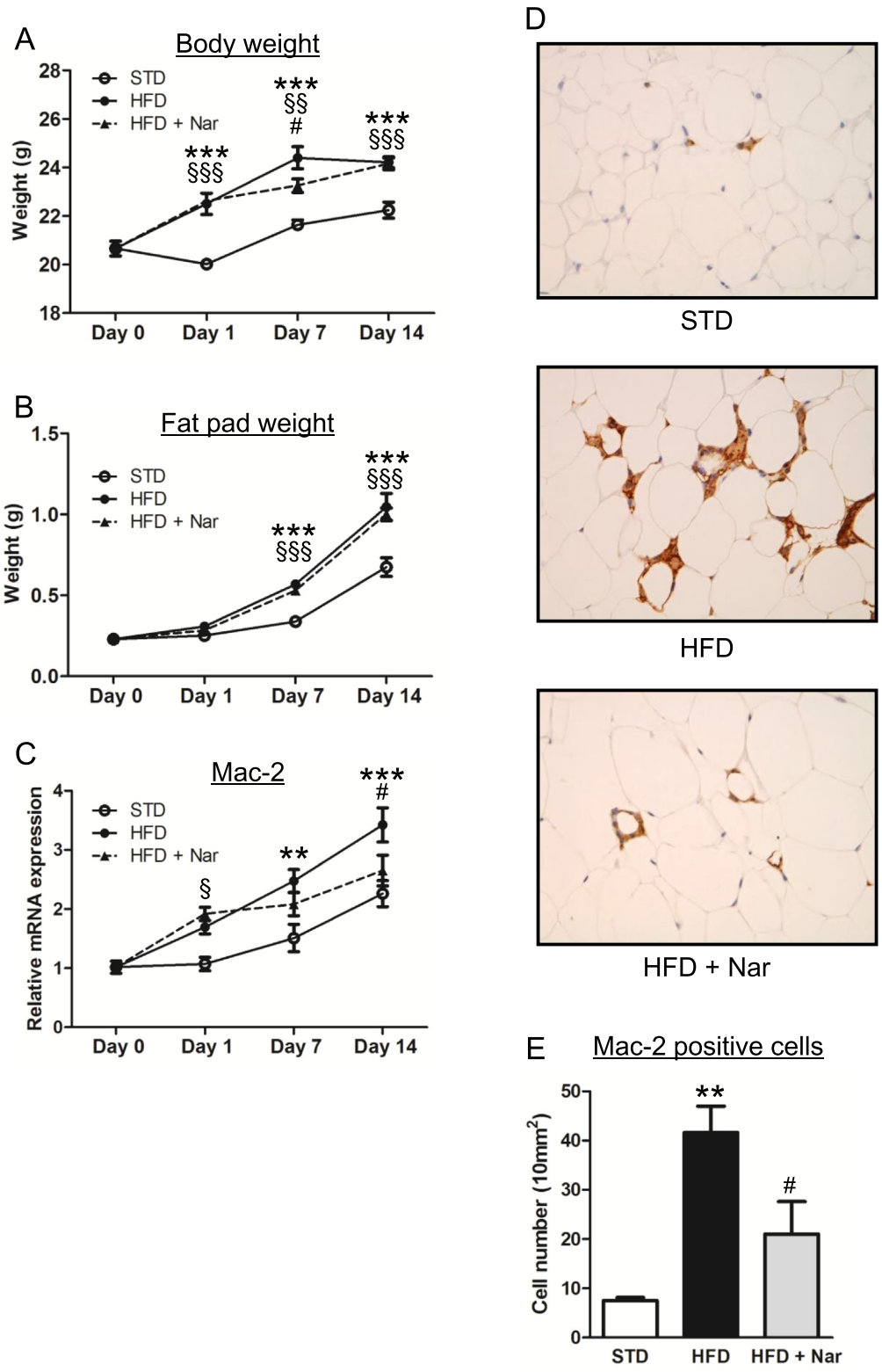

Fig. 1. Effects of naringenin on macrophage infiltration into adipose tissue in HFD-fed mice. C57BL/6J mice (n = 5–6) were used for each group for each administration period. (A) Body weight. (B) Epididymal fat pad weight. (C) Real-time PCR analysis of Mac-2 expression in adipose tissue. (D) Immunohistochemical analysis of Mac-2 positive cells in adipose tissue from mice administered for 14 days. (E) Quantified data of immunohistochemical analysis. $^{**}P < 0.01$ and $^{***}P < 0.001$ for STD *vs.* HFD. $^{§}P < 0.05$, $^{§§}P < 0.01$ and $^{§§§}P < 0.001$ for STD *vs.* HFD + Nar. $^{\#}P < 0.05$ for HFD *vs.* HFD + Nar. STD, standard diet; HFD, high-fat diet; Nar, naringenin.

as described previously [4]. Briefly, RAW 264 cells (1×10^5 cells/mL) were plated onto dishes with differentiated 3T3-L1 adipocytes. For each experiment, we treated cells with naringenin or SP600125 (SP), a JNK inhibitor. We used 0.5% DMSO as vehicle control.

2.8. ELISA

MCP-1 levels in cell culture media were measured using a specific ELISA kit (88-7391, eBioscience, Santa Clara, CA) according to

the manufacturer's instructions. The lower limit of detection of the kit was 15 pg/mL for MCP-1. The intra- and inter-assay coefficients of variation for ELISA results were less than 10%.

2.9. Statistical analysis

Data are presented as mean ± SE, and analysis was performed using GraphPad Prism 5. Differences between groups were assessed by an unpaired *t*-test, one-way ANOVA, or two-way ANOVA. Multiple comparisons were made using a *post hoc* Bonferroni's test. Differences between groups were considered to be significant at $P < 0.05$.

3. Results

3.1. Naringenin suppresses HFD-induced macrophage infiltration into mouse adipose tissue in the early pathological process of obesity-related disease

To determine if naringenin affects macrophage infiltration into adipose tissue in the early phase of obesity, we administered naringenin to HFD-fed mice for 1, 7, or 14 days. Although naringenin did not affect HFD-induced body weight gains and epididymal fat pad weight gains, it did suppress gene expression of Mac-2, a marker of macrophages [14], in epididymal fat pads on day 14 (Fig. 1A–C). We also confirmed the effects of naringenin by immunohistochemical staining. As shown in Fig. 1D and E, administration of naringenin for 14 days reduced HFD-induced Mac-2 positive cells in adipose tissue. During the 14-day administration period, HFD significantly increased serum glucose, total cholesterol, and triglyceride levels compared with the STD, but not free fatty acid and insulin. Naringenin did not affect HFD-induced changes of serum biochemical parameters (Table 1).

3.2. Naringenin suppresses MCP-1 expression by inhibiting JNK pathway in adipose tissue of HFD-fed mice

To elucidate the mechanism by which naringenin suppresses HFD-induced macrophage infiltration into adipose tissue, we examined the effect of naringenin on MCP-1 expression. As shown in Fig. 2A, administration of naringenin for 14 days inhibited HFD-induced MCP-1 gene expression in adipose tissue. We also measured the effects of naringenin on MCP-1 expression at the protein level. Naringenin inhibited the expression of protein on day 14 (Fig. 2B and C). Previous studies have shown that c-Jun NH_2-terminal kinase (JNK) and nuclear factor-kappaB (NF-κB) pathways are involved in the regulation of MCP-1 expression in adipocytes [6,15]. Thus, we determined whether naringenin influences the activation of JNK and NF-κB pathways by assessing JNK phosphorylation and inhibitor of kappaB-α (IκB-α) degradation. Administration of naringenin inhibited HFD-induced JNK phosphorylation in adipose tissue. However, neither HFD feeding nor naringenin administration affected the expression of IκB-α (Fig. 2B and C).

Table 1
Biochemical parameters of C57BL/6J mice administered for 14 days.

	STD	HFD	HFD + Nar
Glucose (mg/dL)	93.8 ± 3.46	135.8 ± 7.81***	127.2 ± 3.65***
T-CHO (mg/dL)	118.6 ± 4.03	143.3 ± 2.86***	145.5 ± 3.38***
TG (mg/dL)	130.5 ± 5.97	170.7 ± 11.0**	172.0 ± 5.91**
NEFA (μEq/L)	1595 ± 123.8	1388 ± 67.5	1406 ± 109.9
Insulin (pg/mL)	336.2 ± 130.0	348.8 ± 66.65	427.7 ± 111.1

Data are mean ± SE ($n = 5–6$).
** $P < 0.01$ and *** $P < 0.001$ *vs.* STD.
STD, standard diet; HFD, high-fat diet; Nar, naringenin; T-CHO, total cholesterol; TG, triglyceride, NEFA, free fatty acid.

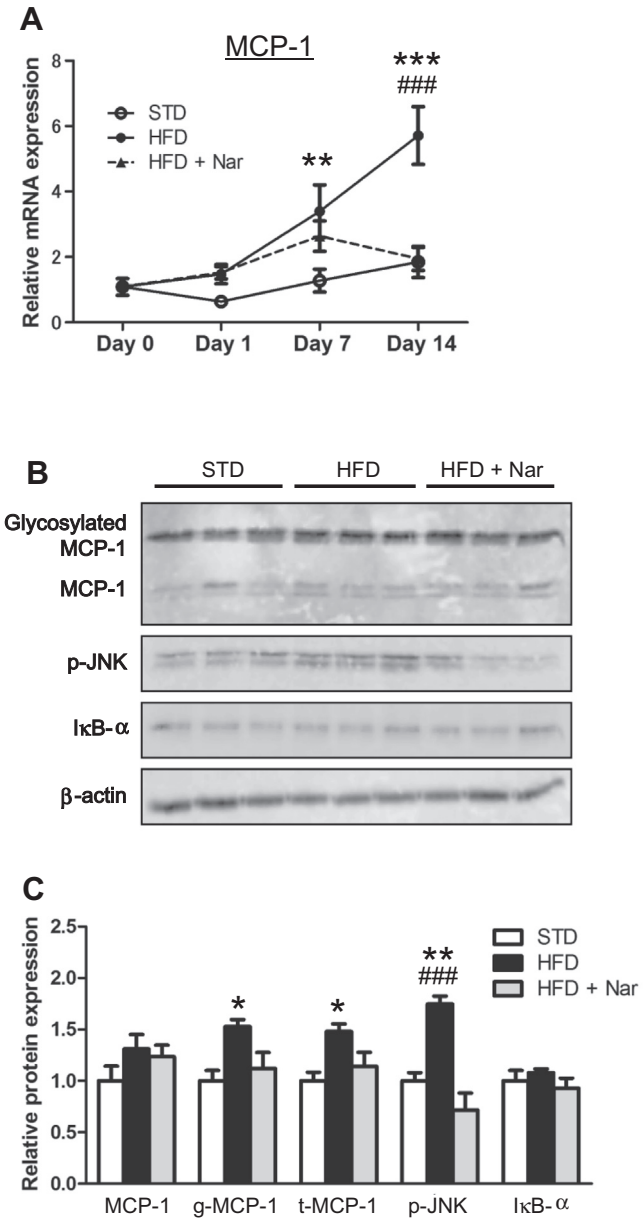

Fig. 2. Effects of naringenin on MCP-1 expression in adipose tissue of HFD-fed mice. (A) Real-time PCR analysis of MCP-1 expression in adipose tissue. C57BL/6J mice ($n = 5–6$) were used for each group for each administration period. (B) Western blot analysis of MCP-1 in adipose tissue from mice administered for 14 days ($n = 3$ mice in each group). (C) Quantified data of Western blot analysis. Signals for each protein was normalized to β-actin and quantified. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ for STD *vs.* HFD. ###$P < 0.001$ for HFD *vs.* HFD + Nar. g-MCP-1, glycosylated MCP-1; t-MCP-1, total MCP-1 (MCP-1 + g-MCP-1).

3.3. Naringenin suppresses MCP-1 production in vitro

Adipose tissue comprises adipocytes and stromal vascular cells. Therefore, to elucidate the source of MCP-1 in adipose tissue and also to determine whether naringenin affects MCP-1 production at the source, we first used 3T3-L1 adipocytes. Naringenin inhibited MCP-1 mRNA expression and secretion in a dose-dependent manner (Fig. 3A and B). We next used RAW 264 cells, because monocytes/macrophages are also known to be the major source of MCP-1 [16]. As shown in Fig. 3C and D, naringenin suppressed MCP-1 production; however, MCP-1 secretion from RAW 264 cells

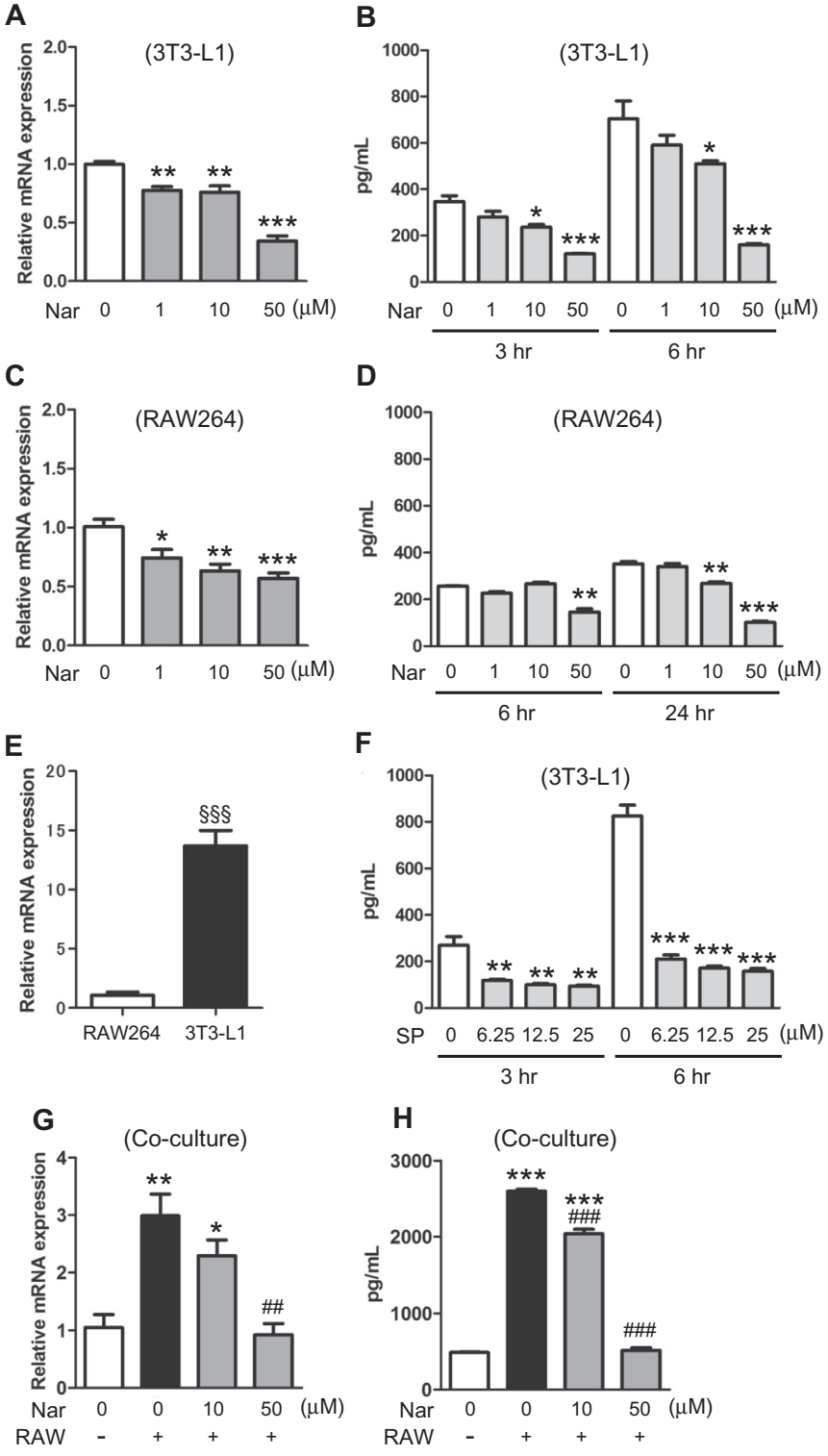

Fig. 3. Effects of naringenin on MCP-1 expression *in vitro*. (A) Real-time PCR analysis of MCP-1 expression in 3T3-L1 adipocytes treated with naringenin for 3 h. (B) ELISA of MCP-1 in culture media of 3T3-L1 adipocytes treated with naringenin for 3 or 6 h. (C) Real-time PCR analysis of MCP-1 expression in RAW 264 cells treated with naringenin for 3 h. (D) ELISA of MCP-1 in culture media of RAW 264 cells treated with naringenin for 6 or 24 h. (E) Real-time PCR analysis of MCP-1 expression in RAW 264 cells and 3T3-L1 adipocytes. (F) ELISA of MCP-1 in culture media of 3T3-L1 adipocytes treated with SP600125 for 3 or 6 h. (G) Real-time PCR analysis of MCP-1 expression in co-culture of 3T3-L1 adipocytes and RAW 264 cells. 3T3-L1 adipocytes were pre-treated with naringenin for 30 min and then co-cultured with RAW 264 cells for 3 h. (H) ELISA of MCP-1 in culture media of co-culture of 3T3-L1 adipocytes and RAW 264 cells for 3 h. Data are mean ± SE ($n = 3$) of two independent experiments (A–H). (I) Western blot analysis of MCP-1 levels in culture media from 3T3-L1 adipocytes and RAW 264 cells co-cultured for 24 h. (J) Quantification of western blot data. The signal for each protein was normalized to non-specific bands (NS) and quantified. Data are presented as means ± SE of three independent experiments (J). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* vehicle control of each treatment period. $^{§§§}P < 0.001$ *vs.* RAW 264 cells. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs.* vehicle-treated co-cultures. SP, SP600125; RAW, RAW 264 cells; NS, non-specific bands.

was lower than that from 3T3-L1 adipocytes. In addition, we compared mRNA expression levels of MCP-1 between RAW 264 cells and 3T3-L1 adipocytes, and the latter showed the higher expression level (Fig. 3E). These results suggest that adipocytes are the

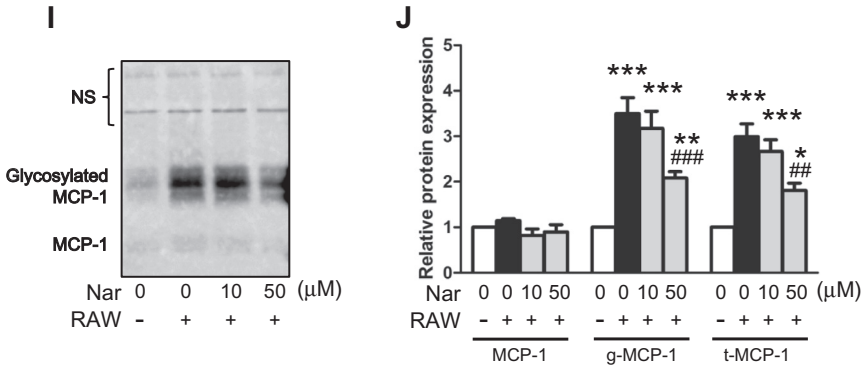

Fig. 3 (*continued*)

major source of MCP-1 in adipose tissue, and naringenin decreases MCP-1 production in adipocytes, which contributes largely to suppress macrophage infiltration into adipose tissue.

Because our *in vivo* data showed that naringenin inhibited the activation of JNK pathway in mouse adipose tissue (Fig. 2B and C), we examined whether the JNK pathway is involved in the regulation of MCP-1 secretion from adipocytes using SP600125, an inhibitor of JNK pathway. As shown in Fig. 3F, JNK inhibitor suppressed MCP-1 secretion from 3T3-L1 adipocytes.

As adipocyte–macrophage interaction induces the abundant production of inflammatory adipokines including MCP-1 in adipose tissue, we next determined the effects of naringenin on adipocyte–macrophage interaction using a co-culture system of 3T3-L1 adipocytes and RAW 264 cells. As shown in Fig. 3G–J, naringenin suppressed co-culture-induced MCP-1 mRNA expression and secretion. These results suggest that naringenin inhibits the exacerbation of MCP-1 production stimulated by interaction between adipocytes and infiltrated macrophages.

4. Discussion

Macrophage infiltration into adipose tissue is a key pathological process, which induces chronic inflammation in adipose tissue and leads to obesity-related diseases such as type 2 diabetes and atherosclerosis. The regulation of macrophage infiltration into adipose tissue is an important strategy for preventing and treating these diseases. In the current study, we report, for the first time, that the citrus flavonoid naringenin suppressed HFD-induced macrophage infiltration into adipose tissue via inhibition of MCP-1 production in the early phase of obesity. This result reveals a mechanism by which daily consumption of naringenin may exhibit preventive effects on obesity-related diseases.

Naringenin did not affect HFD-induced body weight gains or epididymal fat pad weight gains on day 14, although it significantly reduced HFD-induced changes in body weight on day 7 temporarily (Fig. 1A and B). In our previous study, we observed that administration of naringenin for 16 weeks protected HFD-fed mice from adipose tissue inflammation and hyperglycemia without significant changes in body weight and epididymal fat pad weight [13]. These results suggest that naringenin exerts beneficial effects by regulating the functions of adipose tissue or adipocytes, although it does not reduce body weight or the amount of fat.

Studies have reported that various flavonoids exert beneficial effects against obesity-related diseases, accompanied by the reduction of macrophage infiltration into adipose tissue [17–19]. In these studies, the effects of flavonoids were determined after long-term (more than 12 weeks) treatment and it is unclear if the flavonoids affected macrophage infiltration or MCP-1 expression in the early phase of obesity. In the current study, we observed that naringenin did not block the initiation of macrophage infiltration into adipose tissue on day 1, but naringenin suppressed HFD-induced accumulation of macrophages by degrees during the 14-day administration period (Fig. 1C–E). Naringenin also caused suppression of MCP-1 expression in adipose tissue (Fig. 2A), although it did not show any effect on HFD-induced changes of serum biochemical parameters on day 14 (Table 1). These findings suggest that our experimental model makes it possible to conduct screening of useful biomolecules in the short term by measuring expression changes of Mac-2 and MCP-1 before pathophysiological changes appear.

It has been reported that the bioavailability of naringenin is low, and the mean peak plasma concentration was 4.6–10.2 μM after a single oral administration of 135 mg of naringenin in six healthy volunteers [20]. In our preliminary experiment, we measured the serum concentration after a single oral administration of 100 mg/kg of naringenin in C57BL/6J mice, and the mean peak concentration was 4.9–10.0 μM (data not shown). Our *in vitro* data showed that 10 μM of naringenin significantly suppressed MCP-1 production in adipocytes (Fig. 3A and B). These results suggest that the physiological concentration of naringenin exerts suppressive activity on MCP-1 expression in adipocytes.

MCP-1 is highly glycosylated in human and murine cells, and this post-translational modification affects the biological activity of MCP-1. The glycosylated form of MCP-1 shows lower chemotactic activity for monocytes and lymphocytes [21,22], but exhibits enhanced functional stability compared with unglycosylated MCP-1 [23]. Our results indicate that naringenin can significantly suppress the production of glycosylated MCP-1 in adipose tissue from HFD-fed mice and in adipocyte and macrophage co-cultures. By contrast, naringenin did not induce significant changes in the production of unglycosylated MCP-1 (Figs. 2B,C and 3I,J). However, the expression of unglycosylated MCP-1 is much lower than that of the glycosylated MCP-1; a large fraction of the MCP-1 pool appears to be glycosylated. Additionally, naringenin significantly suppressed the mRNA expression levels of *MCP-1* (Figs. 2A and 3G). These results suggest that naringenin reduces MCP-1 expression at the transcriptional level, which reflects the reduction of glycosylated MCP-1 protein. However, we cannot exclude the possibility that naringenin affects post-translational modifications of the MCP-1 protein. Further studies using recombinant MCP-1 will be needed to distinguish these possibilities.

Obese adipose tissue is characterized by the recruitment of not only macrophages but also other leukocytes. T lymphocytes are known to accumulate in HFD-induced obese adipose tissue, and $CD8^+$ T cells contribute to macrophage recruitment and adipose tissue inflammation [5,24]. A recent study reported that the fraction and function of regulatory B cells, which restrain immune

responses, are reduced in obese adipose tissue [25]. Further studies using these cells are necessary to clarify the effects of naringenin on leukocyte recruitment and function in adipose tissue.

The results of this study suggest that naringenin suppresses macrophage infiltration into HFD-induced obese adipose. Our findings provide evidence for the usefulness of naringenin in obesity-related metabolic disorders.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

We are grateful to Ms. Yukiko Shimoda, Mr. Takeshi Horiguchi, Mr. Mamoru Sato, and Ms. Narika Hamamura (Kyushu University of Health and Welfare) for their kind support and helpful suggestions. This work was supported in part by a Grant-in-Aid for Scientific Research (Grant No. 24790182 to H. Yoshida) from the Japan Society for the Promotion of Science.

References

[1] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante Jr., Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Invest. 112 (2003) 1796–1808.
[2] H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia, H. Chen, Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest. 112 (2003) 1821–1830.
[3] T. Suganami, Y. Ogawa, Adipose tissue macrophages: their role in adipose tissue remodeling, J. Leukoc. Biol. 88 (2010) 33–39.
[4] T. Suganami, J. Nishida, Y. Ogawa, A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 2062–2068.
[5] B.K. Surmi, A.H. Hasty, The role of chemokines in recruitment of immune cells to the artery wall and adipose tissue, Vascul. Pharmacol. 52 (2010) 27–36.
[6] P. Jiao, Q. Chen, S. Shah, J. Du, B. Tao, I. Tzameli, W. Yan, H. Xu, Obesity-related upregulation of monocyte chemotactic factors in adipocytes: involvement of nuclear factor-kappaB and c-Jun NH_2-terminal kinase pathways, Diabetes 58 (2009) 104–115.
[7] H. Kanda, S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, S. Kitazawa, H. Miyachi, S. Maeda, K. Egashira, M. Kasuga, MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity, J. Clin. Invest. 116 (2006) 1494–1505.
[8] N. Kamei, K. Tobe, R. Suzuki, M. Ohsugi, T. Watanabe, N. Kubota, N. Ohtsuka-Kowatari, K. Kumagai, K. Sakamoto, M. Kobayashi, T. Yamauchi, K. Ueki, Y. Oishi, S. Nishimura, I. Manabe, H. Hashimoto, Y. Ohnishi, H. Ogata, K. Tokuyama, M. Tsunoda, T. Ide, K. Murakami, R. Nagai, T. Kadowaki, Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance, J. Biol. Chem. 281 (2006) 26602–26614.
[9] P. Knekt, J. Kumpulainen, R. Jarvinen, H. Rissanen, M. Heliovaara, A. Reunanen, T. Hakulinen, A. Aromaa, Flavonoid intake and risk of chronic diseases, Am. J. Clin. Nutr. 76 (2002) 560–568.
[10] E.E. Mulvihill, J.M. Assini, B.G. Sutherland, A.S. DiMattia, M. Khami, J.B. Koppes, C.G. Sawyez, S.C. Whitman, M.W. Huff, Naringenin decreases progression of atherosclerosis by improving dyslipidemia in high-fat-fed low-density lipoprotein receptor-null mice, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 742–748.
[11] J.M. Assini, E.E. Mulvihill, B.G. Sutherland, D.E. Telford, C.G. Sawyez, S.L. Felder, S. Chhoker, J.Y. Edwards, R. Gros, M.W. Huff, Naringenin prevents cholesterol-induced systemic inflammation, metabolic dysregulation, and atherosclerosis in Ldlr(−)/(−) mice, J. Lipid Res. 54 (2013) 711–724.
[12] H. Yoshida, N. Takamura, T. Shuto, K. Ogata, J. Tokunaga, K. Kawai, H. Kai, The citrus flavonoids hesperetin and naringenin block the lipolytic actions of TNF-alpha in mouse adipocytes, Biochem. Biophys. Res. Commun. 394 (2010) 728–732.
[13] H. Yoshida, W. Watanabe, H. Oomagari, E. Tsuruta, M. Shida, M. Kurokawa, Citrus flavonoid naringenin inhibits TLR2 expression in adipocytes, J. Nutr. Biochem. 24 (2013) 1276–1284.
[14] M.K. Ho, T.A. Springer, Mac-2, a novel 32,000 Mr mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies, J. Immunol. 128 (1982) 1221–1228.
[15] K. Takahashi, S. Yamaguchi, T. Shimoyama, H. Seki, K. Miyokawa, H. Katsuta, T. Tanaka, K. Yoshimoto, H. Ohno, S. Nagamatsu, H. Ishida, JNK- and IkappaB-dependent pathways regulate MCP-1 but not adiponectin release from artificially hypertrophied 3T3-L1 adipocytes preloaded with palmitate in vitro, Am. J. Physiol. Endocrinol. Metab. 294 (2008) E898–E909.
[16] S.L. Deshmane, S. Kremlev, S. Amini, B.E. Sawaya, Monocyte chemoattractant protein-1 (MCP-1): an overview, J. Interferon Cytokine Res. 29 (2009) 313–326.
[17] J. Dong, X. Zhang, L. Zhang, H.X. Bian, N. Xu, B. Bao, J. Liu, Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPKalpha1/SIRT1, J. Lipid Res. 55 (2014) 363–374.
[18] T. Murase, K. Misawa, Y. Minegishi, M. Aoki, H. Ominami, Y. Suzuki, Y. Shibuya, T. Hase, Coffee polyphenols suppress diet-induced body fat accumulation by downregulating SREBP-1c and related molecules in C57BL/6J mice, Am. J. Physiol. Endocrinol. Metab. 300 (2011) E122–E133.
[19] X. Terra, V. Pallares, A. Ardevol, C. Blade, J. Fernandez-Larrea, G. Pujadas, J. Salvado, L. Arola, M. Blay, Modulatory effect of grape-seed procyanidins on local and systemic inflammation in diet-induced obesity rats, J. Nutr. Biochem. 22 (2011) 380–387.
[20] F.I. Kanaze, M.I. Bounartzi, M. Georgarakis, I. Niopas, Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects, Eur. J. Clin. Nutr. 61 (2007) 472–477.
[21] Z.G. Liu, A. Haelens, A. Wuyts, S. Struyf, X.W. Pang, P. Proost, W.F. Chen, J. van Damme, Isolation of a lymphocyte chemotactic factor produced by the murine thymic epithelial cell line MTEC1: identification as a 30 kDa glycosylated form of MCP-1, Eur. Cytokine Netw. 7 (1996) 381–388.
[22] P. Proost, S. Struyf, M. Couvreur, J.P. Lenaerts, R. Conings, P. Menten, P. Verhaert, A. Wuyts, J. Van Damme, Posttranslational modifications affect the activity of the human monocyte chemotactic proteins MCP-1 and MCP-2: identification of MCP-2(6–76) as a natural chemokine inhibitor, J. Immunol. 160 (1998) 4034–4041.
[23] P. Ruggiero, S. Flati, V. Di Cioccio, G. Maurizi, G. Macchia, A. Facchin, R. Anacardio, A. Maras, M. Lucarelli, D. Boraschi, Glycosylation enhances functional stability of the chemotactic cytokine CCL2, Eur. Cytokine Netw. 14 (2003) 91–96.
[24] S. Nishimura, I. Manabe, M. Nagasaki, K. Eto, H. Yamashita, M. Ohsugi, M. Otsu, K. Hara, K. Ueki, S. Sugiura, K. Yoshimura, T. Kadowaki, R. Nagai, CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity, Nat. Med. 15 (2009) 914–920.
[25] S. Nishimura, I. Manabe, S. Takaki, M. Nagasaki, M. Otsu, H. Yamashita, J. Sugita, K. Yoshimura, K. Eto, I. Komuro, T. Kadowaki, R. Nagai, Adipose natural regulatory B cells negatively control adipose tissue inflammation, Cell Metab. (2013).