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ORIGINAL ARTICLE

REDUCED IL-1β PRODUCTION IN DIET-INDUCED OBESE MICE IMPAIRS HOST DEFENSE AGAINST SKIN *STAPHYLOCOCCUS AUREUS* INFECTION

Yuichi Sakamoto, Kouji Narita and Akio Nakane

Abstract Obesity leads to a state of chronic inflammation associated with increased levels of proinflammatory cytokines including interleukin-1β (IL-1β) that plays a critical role in host defense against *Staphylococcus aureus* infection. In this study, we investigated host defense against skin *S. aureus* infection in high-fat diet (HFD)-induced non-diabetic obesity by focusing on IL-1β production from subcutaneous adipose tissue derived-macrophages (SATDMs). Bacterial numbers in skin lesions of HFD-fed mice were higher than those of normal-fat diet (ND)-fed control mice on day 3 after subcutaneous *S. aureus* infection. IL-1β production from SATDMs of HFD-fed mice was less than that of ND-fed mice after stimulation with formalin-killed *S. aureus* and ATP. In addition, NLRP3 mRNA expression and protein level of activated caspase-1 in SATDMs of HFD-fed mice were lower than those of ND-fed mice after stimulation. Conversely, IL-1β production, NLRP3 mRNA expression and protein level of activated caspase-1 from visceral adipose tissue-derived macrophages of HFD-fed mice were higher than those of ND-fed mice. These results suggest that reduced IL-1β production in SATDMs of HFD-fed obese mice might be involved in impairment of host defense against skin *S. aureus* infection.

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Key words: Obesity; skin *Staphylococcus aureus* infection; interleukin-1β; inflammasome.

原　著

食餌性肥満マウスにおけるIL-1β産生減少にともなう皮膚黄色ブドウ球菌感染防御機能の低下

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抄録　肥満体における脂肪組織では interleukin-1β(IL-1β)を含む炎症性サイトカインが関与した慢性炎症状態にある．一方，IL-1β は黄色ブドウ球菌感染に対する宿主防御に重要な役割を果たす．本研究では高脂肪食を摂取した非糖尿病性肥満マウス(HFD)を用いて黄色ブドウ球菌皮下感染に対する宿主感染防御機構を IL-1β に焦点を当て検討した．黄色ブドウ球菌皮下感染後の HFD 皮膚生菌数は普通食摂取マウス(ND)と比べ増加した．ホルマリン処理黄色ブドウ球菌と ATP 刺激による HFD 皮下脂肪由来マクロファージの IL-1β 産生，NLRP3 mRNA 発現およびカスパーゼ - 1の活性化は ND 皮下脂肪由来マクロファージと比べ低下していた．これらの結果から，肥満による皮下脂肪由来マクロファージの IL-1β 産生の低下は，黄色ブドウ球菌皮膚感染防御能の低下に関与することが示唆された．

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Introduction

Obesity is a serious health problem in the world and is characterized by excess body fat stored in subcutaneous and visceral adipose tissue. Metabolic syndrome including obesity, insulin resistance, elevated fasting plasma glucose and hyperlipidemia is defined as a cardio-cerebrovascular risk factor[1, 2]. Obesity induces inflammatory state associated with the activation of adipose tissue macrophages and a variety of chronic interleukin-1β (IL-1β)-driven metabolic diseases including atherosclerosis and type 2 diabetes[2, 3]. Obese individuals are more likely than individuals with normal weight to suffer from various types of infections including postoperative infections and other nosocomial infections and to develop serious complications of common infections such as *Staphylococcus aureus* bacteremia[4-6]. Previous studies showed that numbers of mature monocytes were reduced and responses of peripheral lymphocytes to mitogen stimulation were impaired in obese patients[7, 8]. Strandberg et al. reported that chronic high-fat diet-induced obesity disturbed innate immune functions, and impaired the ability to clear sepsis caused by *S. aureus*[9].

S. aureus is a Gram-positive bacterium leading to skin, respiratory, bone, joint, and endovascular infections[10]. Complicated skin *S. aureus* infection sometimes spreads into deeper tissues and causes systemic infection. IL-1β plays a critical role in host defense against *S. aureus* activating neutrophil recruitment[11, 12]. Impaired IL-1 receptor or Toll-like receptor signaling causes recurrent and severe cutaneous *S. aureus* infection[13-15]. IL-1β secretion is induced by caspase-1 activation via several types of inflammasome which include Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing 1 (NLRP1), NLRP3, CARD domain containing 4 (NLRC4) and absent in melanoma 2 (AIM2) inflammasomes[16].

S. aureus reportedly induces IL-1β production via activation of NLRP3 inflammasome[17]. Obesity also induces the assembly of NLRP3 inflammasome which is involved in increased caspase-1 activation and matured IL-1β production in adipose tissue macrophages[18]. However, the role of IL-1β produced by subcutaneous adipose tissue macrophages in host defense against skin *S. aureus* infection in chronic inflammatory state induced by obesity remains unclear.

In this study, we investigated host defense against skin *S. aureus* infection in diet-induced non-diabetic obesity by focusing on IL-1β production from subcutaneous adipose tissue derived-macrophages.

Materials and Methods

Mice and Dietary intervention

BALB/c female mice were purchased from Clea Japan, Tokyo, Japan. Four-week-age female mice were started to be fed either a normal-fat diet (ND, 5.6% of calories from fat; Oriental Yeast Co., ltd. Tokyo, Japan) or a high-fat diet (HFD, 62.2% of calories from fat; Oriental Yeast; HFD-60) for 13 weeks until 17 weeks of age. Mice were maintained under specific-pathogen-free conditions at the Institute for Animal Experimentation, Hirosaki University Graduate School of Medicine. Food and water were given *ad libitum*. All animal experiments in this study were performed in accordance with the Guidelines for Animal Experimentation of Hirosaki University.

Fasting blood glucose analysis

ND-fed and HFD-fed mice were fasted (water *ad libitum*) for 18-21 h before fasting blood glucose (FBG) analysis. Ten micro litter of blood from cut tail was analyzed for FBG by the capillary action of the test tip with Medisafe Mini (Terumo Corp., Tokyo, Japan).

Bacterial strains and culture condition

S. aureus 834 strain, a clinical sepsis isolate, was used in this study[19]. The bacteria were grown in tryptic soy broth (BD Diagnosis Systems, Sparks, MD) for 15 h, harvested by centrifugation, and washed with sterile phosphate-buffered saline (PBS). The washed bacteria were diluted with PBS to appropriate cell concentrations as determined spectrophotometrically at 550 nm.

Subcutaneous *S. aureus* inoculation

ND-fed and HFD-fed mice were shaved on the back and inoculated subcutaneously with 5×10^5 or 1×10^8 colony-forming units (CFU) per mouse of *S. aureus* 834 using a 27-gauge needle. Skin specimens were aseptically removed with 8 mm punch biopsy (KAI Medical Inc., Honolulu, HI, USA) and minced with scissors on day 3 after bacterial inoculation. Spleens and kidneys were aseptically removed on days 3, 6, and 10 after subcutaneous inoculation with 1×10^8 CFU of *S. aureus*. Bacterial counts in skin, spleen, and kidneys were enumerated by preparing organ homogenates in PBS and plating 10-fold serial dilutions on mannitol salt agar (Eiken Chemical Co., Ltd., Tokyo, Japan) or tryptic soy agar (BD Diagnosis Systems). Colonies were counted after 24 h of incubation at 37°C.

Cell culture

Subcutaneous adipose tissue and visceral adipose tissue of ND-fed and HFD-fed mice were aseptically removed and incubated in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 0.2% collagenase (Sigma-Aldrich, St Louis, MO, USA) for 40 min. Subcutaneous adipose tissue-derived macrophages (SATDMs), visceral adipose tissue-derived macrophages (VATDMs) and spleen-derived macrophages (SPMs) were obtained by squeezing and filtering through stainless steel mesh (size, 100) in RPMI 1640 medium. Erythrocytes were lysed with 0.83% NH_4Cl. After being washed three times with RPMI 1640 medium, the macrophages were incubated in RPMI 1640 medium supplemented with 10% fetal calf serum (JRH Biosciences, Lenexa, KS, USA), 1% L-glutamine (Wako Pure Chemical Industries, Osaka, Japan), 100 U of penicillin G per ml, 100 μg/ml of streptomycin and 50 ng/ml recombinant mouse macrophage colony-stimulating factor (Wako) at 37°C for 4 to 7 days. The macrophages were suspended at a concentration of 1×10^6 cells/ml and transferred into a 24-well culture plate.

Stimulation with formalin-killed *S. aureus* and ATP

To determine IL-1β production in cell culture supernatant, SATDMs, VATDMs and SPMs were stimulated with 1×10^8 CFU/ml of formalin-killed *S. aureus* (FKSA) for 6 h, followed by stimulation with 1 mM ATP for 12 h. For real-time PCR experiments, SATDMs, VATDMs and SPMs were stimulated with 1×10^8 CFU/ml of FKSA for 1 h, followed stimulation with 1 mM ATP for 10 min. For Western blotting experiments, SATDMs, VATDMs and SPMs were stimulated with 1×10^8 CFU/ml of FKSA for 6 h, followed by stimulation with 1 mM ATP for 1 h. The culture supernatants and the cells were collected after stimulation. The samples were stored at -80°C until further analyses.

Real-time quantitative reverse transcription-PCR (RT-PCR)

Total mRNAs from cultured cells were isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First strand cDNAs were synthesized by reverse transcription of 1 μg total mRNA using random primers (Takara, Shiga, Japan) and Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Gene expression levels were determined by real-time PCR analysis using the SYBR green Supermix (Bio-

Rad Laboratories, Hercules, CA, USA). The PCR condition of all primers is as follows: denaturation at 94°C for 30 sec; annealing at 55°C for 30 sec; extension at 72°C for 120 sec. The following oligonucleotides were used: for NLRP3, 5'-CGAGACCTCTGGGAAAAAGCT-3' and 5'-GCATACCATAGAGGAATGT GATGTACA-3'; for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-TGAAGGTCGGTGTGAACGGATTTGG-3' and 5'-ACGACATACTCAGCACCAGCATCAC-3'. Dissociation curves were used to detect primer-dimer conformation and nonspecific amplification. The threshold cycle (*CT*) of each target product was determined and set in relation to the amplification plot of GAPDH. The detection threshold was set to the log linear range of the amplification curve and kept constant (0.05) for all data analysis. The difference between the *CT* values (ΔCT) of two genes was used to calculate the relative expression; i.e., relative expression $2^{-(CT \text{ of target gene} - CT \text{ of GADPH})} = 2^{-\Delta CT}$.

Cytokine assay

The amounts of IL-1β in the culture supernatants of SATDMs, VATDMs and SPMs were determined by double-sandwich enzyme-linked immunosorbent assay (ELISA) using a mouse IL-1β Cytoset kit (Biosource USA, Camarillo, CA, USA) according to manufacturer's instructions.

SDS-PAGE and Western blotting

Macrophages were lysed with lysis buffer (2% TritonX-100 in PBS) supplemented with a complete protease inhibitor mixture (Roche, Mannheim, Germany), and mixed with SDS sample buffer. Cell culture supernatants were concentrated by precipitation with 10% trichloroacetic acid and resuspended in SDS sample buffer. Samples were subjected to SDS-PAGE on 12% gel. The proteins were transferred onto an Immobilon-P transfer membrane (Millipore, Bedford, MA, USA) using a semidry blotter. The membrane was blocked for 1 h at room temperature in PBS, 5% (w/v) nonfat dried milk. The membrane was incubated in rabbit anti-caspase 1 polyclonal antibody (Millipore) for 2 h at room temperature in PBS. After being washed with PBS, the membrane was incubated with horseradish peroxidase (HRP)-labeled anti-rabbit IgG for 2 h at room temperature. The membrane was then washed and the signals were detected using SuperSignal West Dura Extended Duration Substrate (Thermo Scientific/Pierce, Rockford, IL, USA).

Histological analysis

Skin specimens were obtained at 0, 24 h and 3 days after subcutaneous infection with *S. aureus*, fixed with 10% phosphate-buffered formalin and embedded in paraffin. One or two-micrometer-thick sections were prepared. The sections were mounted on glass slides. The paraffin sections were deparaffinized with xylene and rehydrated using graded concentrations of ethanol. The sections were stained with hematoxylin and eosin.

Immunohistochemical analysis

The tissue sections were quenched with peroxidase blocking solution (0.3% H_2O_2) for 15 min and incubated with proteinase K working solution for 30 min at room temperature. After processing with blocking solution (2% bovine serum albumin in PBS) for 1 h, the sections were incubated with rat anti-mouse F4/80 (1:100, AbD Serotec, Kidlington, UK) for 30 min at room temperature in a humid chamber. After washing, the sections were incubated with HRP-conjugated goat anti-rat IgG2b (1:250, AbD Serotec) for 30 min at room temperature. The signals were visualized using diaminobenzidine substrate. The sections were counterstained with hematoxylin for 1 min, rinsed with distilled water and dehydrated by sequential immersion

in gradient ethanol and xylene. The coverslips were then mounted onto slides by using mounting medium.

Statistical Analysis

Data for body weight, FBG, bacterial numbers in the skin, cytokines production and mRNA expression are expressed as the medians ± interquartile range or dot plots. Statistical significant differences were calculated using a Mann-Whitney *U*-test. The cut-off for statistical significance was set at a P value of 0.05 or below.

Results

HFD-induced obesity and fasting blood glucose

To prepare diet-induced obese mice, four-week-age female mice were maintained with free access to a HFD until 17 weeks of age. In 9-17 weeks of age, body weights of HFD-fed mice significantly increased compared with those of ND-fed mice (Figure 1A). To define non-diabetic mice, FBG levels were analyzed. The FBG levels in both groups were below 126 mg/dl in 13, 15 and 17 weeks of age. From these results, HFD-fed obese mice were non-diabetic (Figure 1B).

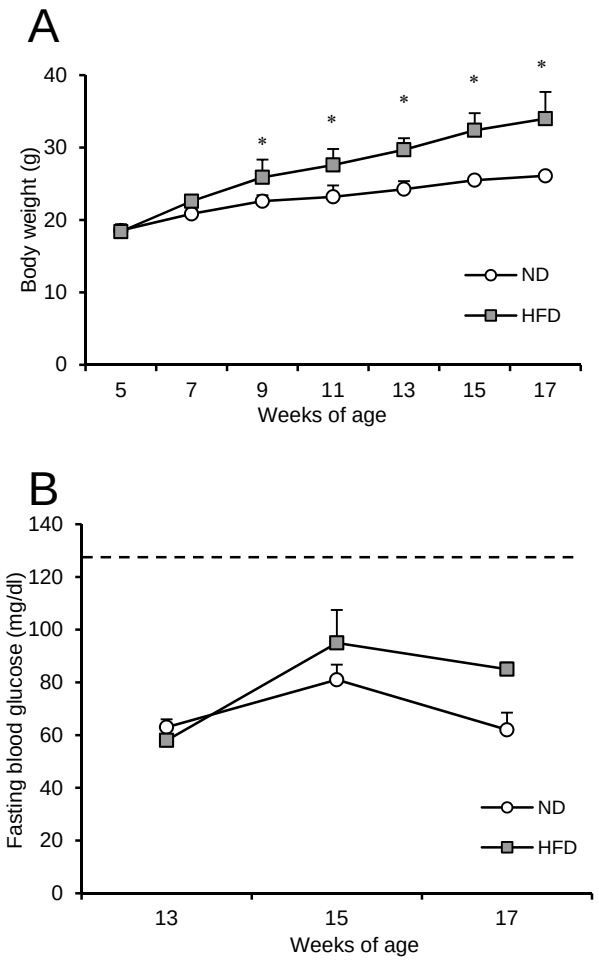

Figure 1 Comparison of body weight gain and fasting blood glucose in ND-fed and HFD-fed mice.
(A) Body weights of ND-fed and HFD-fed BALB/c female mice were measured until 17 weeks of age. Data are represented as the medians ± interquartile range for a group of 11 to 14 mice. An asterisk represents a statistically significant difference from ND-fed mice ($P < 0.05$). (B) ND-fed and HFD-fed mice were fasted for 18 to 21 h before FBG analysis, and ten micro litter of blood from cut tail was analyzed. Dotted line represents 126 mg/dl. Data are represented as the medians ± interquartile range for a group of 4 to 12 mice.

Bacterial numbers after subcutaneous *S. aureus* inoculation

We next evaluated whether HFD-induced obesity affects innate immunity to skin *S. aureus* infection. HFD-fed and ND-fed mice were inoculated with two different doses of *S. aureus* subcutaneously. Three days after subcutaneous inoculation with 5×10^5 CFU of *S. aureus*, bacterial numbers in skin lesions of HFD-fed mice were increased compared with those of ND-fed mice. Whereas bacterial numbers in skin lesions of HFD-fed and ND-fed mice were comparable after subcutaneous inoculation with 1×10^8 CFU of *S. aureus* (Figure 2A). It is known that conditions such as cellulitis can cause sepsis. Hence we evaluated bacterial numbers in the organs after subcutaneous inoculation with *S. aureus*. Bacterial counts increased in the spleens and kidneys of ND-fed mice than those of HFD-fed mice on day 6 after subcutaneous inoculation with 1×10^8 CFU of *S. aureus* (Figure 2B), while the bacteria were not detected in the organs of both ND-fed and HFD-fed mice after

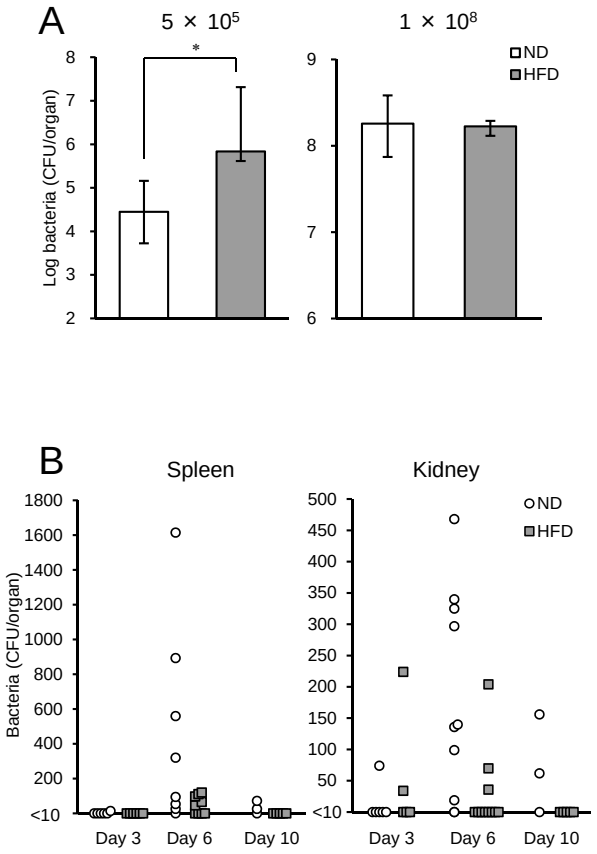

Figure 2 Bacterial numbers in the skin lesions and organs of ND-fed and HFD-fed mice.
(A) Skin specimens of ND-fed and HFD-fed mice were taken as described in the materials and methods, and bacterial numbers were determined on day 3 after inoculation with 5×10^5 or 1×10^8 CFU of *S. aureus*. Data are represented as the medians ± interquartile range for a group of 5 to 9 mice. An asterisk represents a statistically significant difference ($P < 0.05$). (B) Bacterial numbers in the spleens and kidneys of ND-fed and HFD-fed mice were determined on days 3, 6 and 10 after subcutaneous inoculation with 1×10^8 CFU of *S. aureus*. Data are represented as dot plots. White circles represent ND-fed mice. Gray squares represent HFD-fed mice.

inoculation with 5×10^5 CFU of *S. aureus* (data not shown).

Histology of skin lesions after *S. aureus* inoculation

Skin histology of non-infected mice was shown in Figure 3A. Subcutaneous adipose tissue layer became apparently hypertrophic and the size of adipocytes enlarged in HFD-fed mice. Macrophages in subcutaneous adipose tissue were detected as F4/80 positive cells as shown in Figure 3B, and the macrophage density was lower in subcutaneous adipose tissues of HFD-fed mice compared with that of ND-fed mice. Skin histology of ND-fed and HFD-fed mice after subcutaneous inoculation with *S. aureus* was shown in Figure 3C-3F. Although inflammatory cell infiltration was shown in subcutaneous lesions of both ND-fed and HFD-fed groups, the cell infiltration was more prominent in ND-fed mice than HFD-fed mice on days 1 and 3 after subcutaneous infection with 5×10^5 CFU of *S. aureus* (Figure 3C and 3E). After subcutaneous infection with 1×10^8 CFU of *S. aureus*, higher inflammatory cells infiltration was shown in subcutaneous layer as well as muscle layer of ND-fed mice, whereas the lesions of HFD-fed mice were almost restricted in subcutaneous layer (Figure 3D and 3F).

IL-1β production in adipose tissue-derived macrophages after stimulation with FKSA and ATP

To evaluate IL-1β production from SATDMs, VATDMs and SPMs of ND-fed and HFD-fed mice, these cells were stimulated with FKSA as one of the pathogen-associated molecular patterns and followed by stimulation with extracellular ATP as one of the damage-associated molecular patterns. IL-1β production from SATDMs of HFD-fed mice was reduced compared with that from ND-fed mice after stimulation with FKSA and ATP (Figure 4A). In contrast, the stimulation enhanced IL-1β production from VATDMs of HFD-fed mice compared with that from ND-fed mice (Figure 4B). Whereas amounts of IL-1β in cell culture supernatants of SPMs of HFD-fed mice after stimulation with FKSA and ATP were similar to those of ND-fed mice (Figure 4C).

NLRP3 mRNA expression in adipose tissue derived-macrophages after stimulation with FKSA and ATP

We assessed the involvement of NLRP3

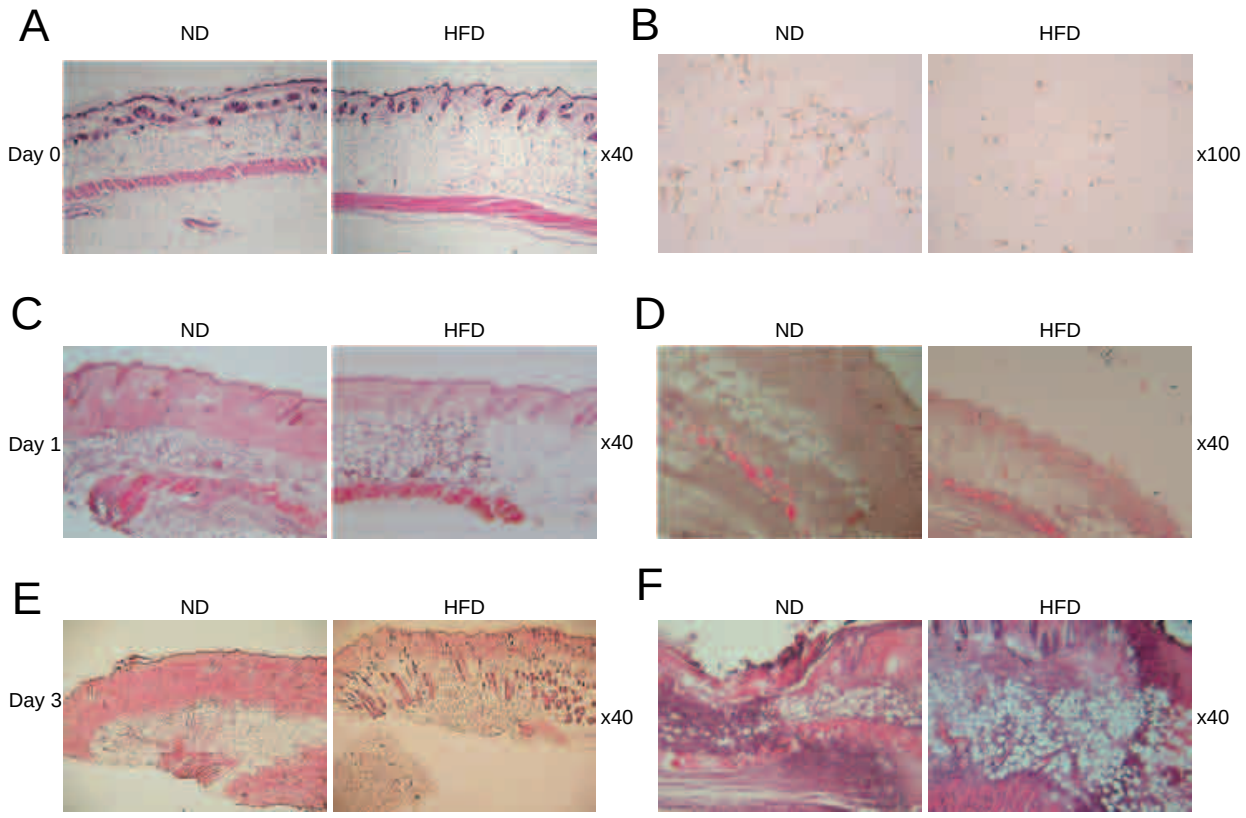

Figure 3 Histology of skin lesions of ND-fed and HFD-fed mice after inoculation with *S. aureus*.
(A) Skin specimens from back of non-infected mice were taken and the tissue samples were stained with hematoxylin and eosin. (B) Subcutaneous adipose tissues were immunostained with anti-F4/80 antibody, macrophages were detected as F4/80 positive cells. (C-F) Skin specimens from *S. aureus*-infected mice were taken and the tissues samples were stained with hematoxylin and eosin. The skin specimens were collected on day 1 (C and D) or on day 3 (E and F) after subcutaneous inoculation with 5×10^5 CFU (C and E) or 1×10^8 CFU (D and F) of *S. aureus*.

inflammasome in IL-1β production by stimulation FKSA and ATP. NLRP3 mRNA expression in SATDMs from ND-fed but not HFD-fed mice tends to increase after stimulation, although significant difference was not detected between before and after the stimulation (Figure 5A). In VATDMs and SPMs from HFD-fed mice but not ND-fed mice, NLRP3 mRNA expression was significantly increased after stimulation with FKSA and ATP (Figure 5B and 5C). In addition to NLRP3 mRNA expression, NLRP1a and NLRC4 mRNA expression was analyzed. Neither mRNA expression in SATDMs, VATDMs and SPMs from both ND-fed and HFD-fed mice increased after stimulation with FKSA and ATP (data not shown).

Caspase-1 activation in adipose tissue-derived macrophages

To investigate caspase-1 activation in SATDMs, VATDMs and SPMs from ND-fed and HFD-fed mice, Western blot analysis was performed using the culture supernatants and the lysates of these cells. The protein levels of p20 subunit of activated caspase-1 in SATDMs from HFD-fed mice decreased compared with those from ND-fed mice after stimulation. In contrast, the levels of p20 subunit in SPMs and VATDMs from HFD-fed mice were higher than those from ND-fed mice (Figure 6).

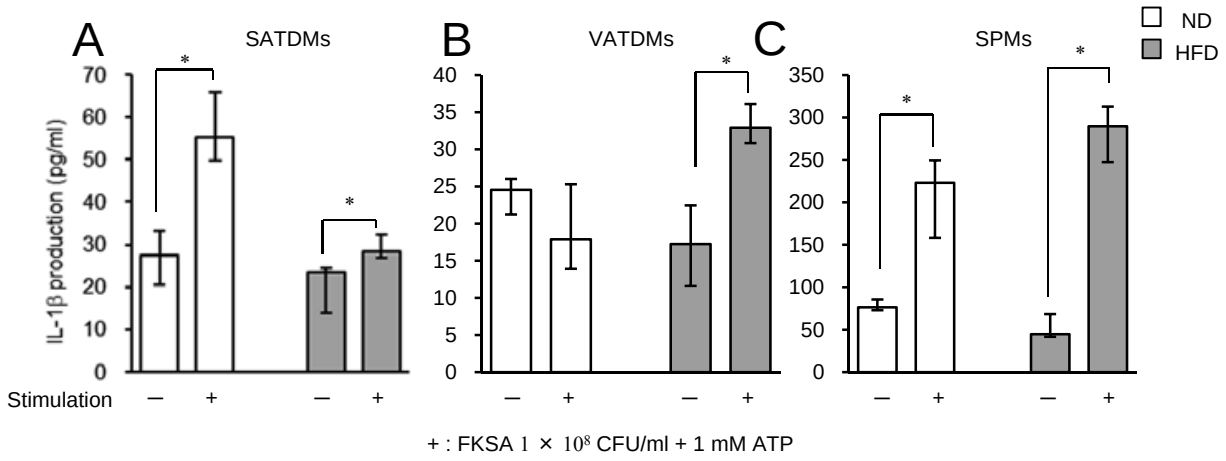

Figure 4 IL-1β production from SATDMs, VATDMs and SPMs.
SATDMs (A), VATDMs (B) and SPMs (C) were stimulated with 1 × 10^8 CFU of FKSA for 6 h, followed by stimulation with 1 mM ATP for 12 h. IL-1β production in cell culture supernatants was determined by ELISA. Data are represented as the medians ± interquartile range for a group of 4 to 6 mice. An asterisk represents a statistically significant difference from the ND-fed mice ($P < 0.05$).

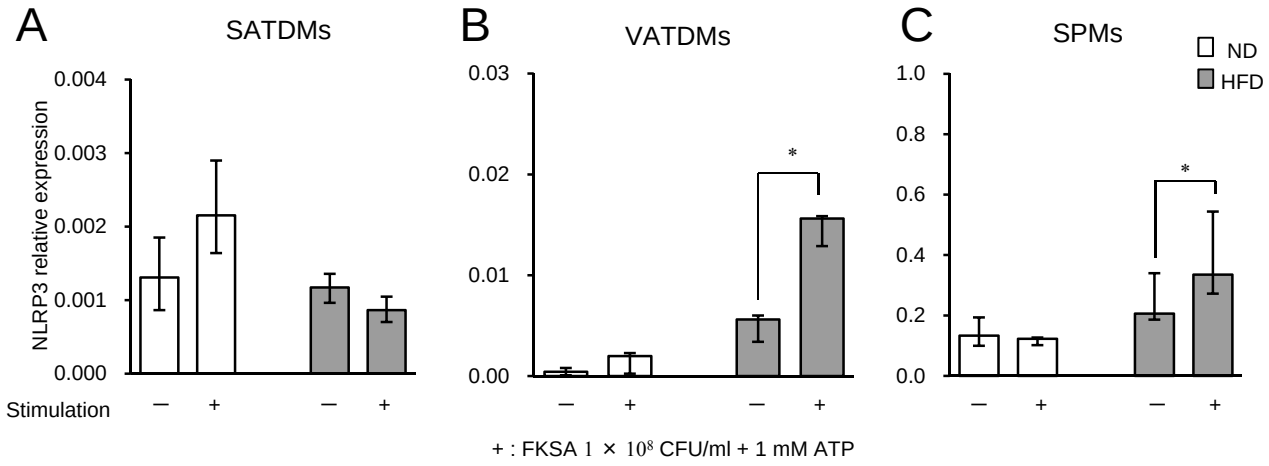

Figure 5 NLRP3 mRNA expression of SATDMs, VATDMs and SPMs.
SATDMs (A), VATDMs (B) and SPMs (C) were stimulated with 1 × 10^8 CFU of FKSA for 1 h, followed by stimulation with 1 mM ATP for 10 min. NLRP3 mRNA expression was determined by real-time quantitative PCR. Data are represented as the medians ± interquartile range for a group of 4 to 6 mice. An asterisk represents a statistically significant difference from the ND-fed mice ($P < 0.05$).

Discussion

It is has been known that IL-1β produced by macrophages plays a critical role in host defense against *S. aureus* infection[11, 12]. On the other hand, IL-1β produced from visceral adipose tissue macrophages also plays a role in obesity-induced chronic inflammatory state[2, 3]. The effects of non-diabetic obesity on pathology of skin *S. aureus* infection, IL-1β production in SATDMs in the skin lesions, and IL-1β-dependent host defense against skin *S. aureus* infection are still unclear. In this study, we investigated effects of obesity on innate immunity to skin *S. aureus* infection and the IL-1β production from SATDMs in HFD-

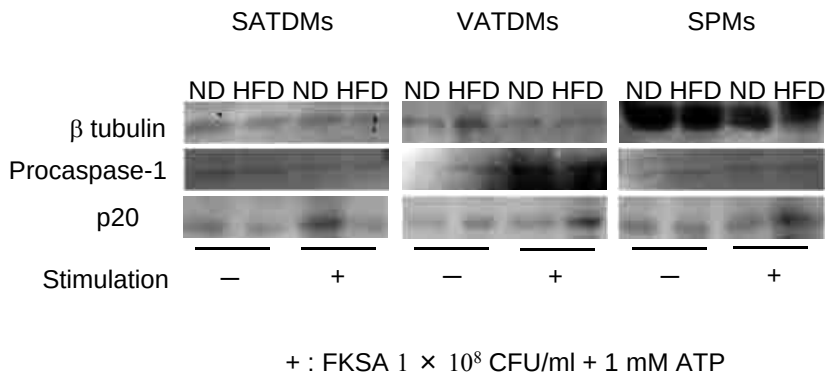

Figure 6 Caspase-1 activation in SATDMs, VATDMs and SPMs.
SATDMs (A), VATDMs (B) and SPMs (C) were stimulated with 1×10^8 CFU of FKSA for 6 h, followed by stimulation with 1 mM ATP for 1 h. Protein levels of procaspase-1 and β-tubulin in cell lysates and activated caspase-1 in cell culture supernatants were determined by Western blot analysis. p20: a subunit of activated caspase-1.

fed non-diabetic obese mice.

Bacterial numbers in skin lesion of ND-fed mice were reduced compared with those of HFD-fed mice on day 3 after subcutaneous inoculation with 5×10^5 CFU of *S. aureus* (Figure 2A). For clearance of *S. aureus* in skin lesion, neutrophil abscess formations associated with IL-1β are required[12]. Mölne et al. reported that the neutrophil-depleted mice intradermally inoculated with *S. aureus* developed crusted ulcerations and exhibited increased levels of IL-6 and specific antibodies to staphylococcal cell wall components[20]. In this study, inflammatory cell infiltration was impaired in subcutaneous adipose tissue of HFD-fed mice compared with that of ND-fed mice after subcutaneous infection with 5×10^5 CFU of *S. aureus* (Figure 3C and 3E). These results suggest that impaired host defense against skin *S. aureus* infection in non-diabetic obese mice is associated with reduced infiltration of inflammatory cells into the infection sites. Although bacterial clearance in skin lesions was impaired in HFD-fed mice after inoculation with 5×10^5 CFU of *S. aureus*, bacterial numbers in skin lesions of HFD-fed and ND-fed mice were comparable on day 3 after inoculation with 1×10^8 CFU of *S. aureus* (Figure 2A). The dose of 1×10^8 CFU of *S. aureus* might be too large to clear the pathogen in innate immunity because remarkable destruction of skin tissue such as abscess formation expanded to muscle layer and ulcer formation was shown in ND-fed mice on day 3 after inoculation, although inflammatory cell infiltration which is required for clearance of *S. aureus* was prominent in the skin lesions of ND-fed mice on day 1 after infection (Figure 3D and 3F). On the other hand, bacterial numbers in the spleens and kidneys of ND-fed mice increased compared with those of HFD-fed mice after subcutaneous inoculation with 1×10^8 CFU of *S. aureus* (Figure 2B). After subcutaneous infection with 1×10^8 CFU of *S. aureus*, inflammatory cells infiltrated into subcutaneous layer as well as muscle layer of ND-fed mice, whereas the lesions of HFD-fed mice were almost restricted in subcutaneous adipose tissue (Figure 3D and 3F). These results suggest that thick subcutaneous adipose tissue layer of HFD-fed mice could suppress the spread of bacteria after infection with high dose of *S. aureus*, although bacterial clearance in skin lesions was impaired in HFD-fed mice after infection with low dose of *S. aureus*.

IL-1β is required for host defense against *S. aureus* infection to recruit neutrophils[11, 12]. Several

types of inflammasome play an important role in the regulation of caspase-1 activation which is required for conversion of pro-IL-1β to mature IL-1β[21, 22]. Caspase-1 activation via inflammasome was reported to be involved in the protection against the bacterial infections, such as *Salmonella Typhimurium, Legionella pneumophilia* and *Pseudomonas aeruginosa*[23-25]. In this study, NLRP3 mRNA expression slightly increased in SATDMs of ND-fed mice, while the expression was significantly enhanced in VATDMs and SPMs of HFD-fed mice after stimulation with FKSA and ATP (Figure 5). It should be noted that NLRP1a and NLRC4 mRNA expression did not increase in SATDMs, VATDMs and SPMs by the stimulation (data not shown). Increased IL-1β production in SATDMs of ND-fed mice, VATDMs and SPMs of HFD-fed mice by stimulation with FKSA and ATP was coincident with higher NLRP3 mRNA expression in these macrophages by stimulation (Figures 4 and 5). Caspase-1 activation and NLRP3 mRNA expression increased in SATDMs of ND-fed mice and VATDMs and SPMs of HFD-fed mice after the stimulation (Figures 5 and 6). In contrast, SATDMs of HFD-fed mice increased neither NLRP3 mRNA expression nor caspase-1 activation after stimulation with FKSA and ATP (Figures 5 and 6). These results suggest that NLRP3 mRNA expression and caspase-1 activation of SATDMs of HFD-fed mice in skin *S. aureus* infection was attenuated compared with that of SATDMs of ND-fed mice.

It has been reported that activated caspase-1 production and NLRP3 inflammasome activity increased in visceral adipose tissue in diet-induced obese animal models[18, 26]. Coincidence with the previous studies, VATDMs and SPMs showed strong caspase-1 activation and NLRP3 mRNA expression after stimulation with FKSA and ATP. However, SATDMs of HFD-fed obese mice did not show such activation and expression in this study. Macrophages in adipose tissue are divided into two different profiles. M1 macrophages produce inflammatory cytokines, whereas M2 macrophages produce anti-inflammatory cytokines[27, 28]. In this study, although we did not verify the difference of macrophage types in adipose tissues, different responses between SATDMs of HFD-fed and ND-fed mice in caspase-1 activation and NLRP3 mRNA expression might be due to different type of macrophages.

Our data showed that host defense of HFD-fed non-diabetic obese mice against skin *S. aureus* infection was impaired. IL-1β production, caspase-1 activation and NLRP3 mRNA expression of SATDMs of HFD-fed mice were attenuated compared with those of ND-fed mice after stimulation with FKSA and ATP. The present study suggests that reduction of IL-1β production in subcutaneous adipose tissues-macrophages which is associated with caspase-1 activation induced by NLRP3 inflammasome might be involved in impaired host defense against skin *S. aureus* infection in diet-induced non-diabetic obesity.

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