JARQ 60 (4), 383-387 (2026) https://www.jircas.go.jp

Fat Depot-Specific Expression of Immune Cell Marker Genes in Fattening Wagyu Cattle

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Abstract

In this study, we investigated the fat depot-specific expression of immune cell marker genes of regulatory T-cells (*FOXP3*), T lymphocytes (*CD3E*), B lymphocytes (*CD79A*), and macrophages (*CD68*) in fattening Wagyu cattle at three anatomical sites (subcutaneous, intramuscular, and visceral adipose tissue). The expression of the *FOXP3* gene in subcutaneous adipose tissue was significantly higher than in intramuscular and visceral adipose tissue. In contrast, the expression of *CD3E*, *CD79A*, and *CD68* genes in visceral adipose tissue was significantly higher than in subcutaneous and intramuscular adipose tissue. These results indicate that the anatomical location of bovine adipose tissue influences immune cell populations in a fat depot-specific manner.

Discipline: Animal Science
Additional key words: adipose tissue, intramuscular, subcutaneous, visceral

Introduction

Obesity-induced adipose tissue inflammation is strongly associated with immune cell populations. T lymphocytes (T-cells), B lymphocytes (B-cells), and macrophages (MACs) promote obesity-induced adipose tissue inflammation in humans and rodents (McLaughlin et al. 2017). In contrast, regulatory T-cells (Tregs) suppress the inflammation of adipose tissue in humans and rodents (Liu et al. 2021).

The severity of obesity-induced adipose tissue inflammation varies by anatomical site. Studies in humans and rodents have shown that depot-specific differences in adipose tissue inflammation are affected by immune cell populations. In obesity, the inflammatory state of visceral adipose tissue is higher than that of subcutaneous adipose tissue (Bruun et al. 2004). MAC and T-cell populations in obese visceral adipose tissue are larger than those in subcutaneous adipose tissue (Cancello et al. 2006, Duffaut et al. 2009). Diet-induced obesity leads to infiltration of B-cells into visceral adipose tissue (Winer et al. 2011). In contrast, the Treg population in visceral adipose tissue from obese individuals is smaller than that in subcutaneous adipose tissue (Feuerer et al. 2009). However, depot-specific differences in immune cell populations within the adipose tissue of ruminants have not yet been determined. Moreover, the immune cell populations of ectopic (intramuscular) adipose tissue, (e.g., in humans and rodents,) remain unclear.

We investigated the fat depot-specific expression of immune cell marker genes for Tregs (*FOXP3*), T-cells (*CD3E*), B-cells (*CD79A*), and MACs (*CD68*) in fattening Wagyu cattle at three different anatomical sites (subcutaneous, intramuscular, and visceral adipose tissue).

Materials and methods

1. Animals

Fattening Wagyu (Japanese black cattle) steers (n = 7) were used in this study. All animals received humane care as outlined in the Guide for the Care and Use of Experimental Animals (National Agriculture and Food Research Organization, Approval No. 30 – 7). They were fed concentrate (78% total digestible nutrients and 14% crude protein on a dry matter basis) and orchard grass hay (56% total digestible nutrients and 8% crude protein

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Received 17 September 2025; accepted 16 December 2025; Advanced Epub 30 July 2026.
https://doi.org/10.6090/jarq.25S14

on a dry matter basis) *ad libitum* from 10 months of age until slaughtered at 30 months of age. At slaughter, subcutaneous adipose tissue and intramuscular adipose tissue within the longissimus muscle were collected between the third and fourth lumbar vertebrae. Visceral adipose tissue was collected from pericolonic fat. Adipose tissue samples for determining gene expression were collected in RNAlater reagent (Ambion, Carlsbad, CA, USA) and stored at –80°C until analysis as described by Yamada et al. (2018). Adipose tissue samples for determining the adipocyte size were fixed with osmium tetroxide as described by Yamada et al. (2020b).

2. Adipose tissue analysis

The expression of immune cell marker genes was analyzed by real-time PCR as described in Yamada et al. (2018). In brief, total RNA was extracted from adipose tissue using the RiboPure Kit (Ambion, Carlsbad, CA, USA) according to the manufacturer's protocol. The first-strand cDNA was reverse-transcribed using the ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan). Real-time PCR was performed with a Mini Opticon (Bio-Rad, Munich, Germany) using THUNDERBIRD SYBR qPCR Mix (Toyobo). The reaction conditions were as follows: initial denaturation at 95°C for 60 s, followed by 40 cycles at 95°C for 15 s, 55°C for 15 s, and 70°C for 30 s. The primer sequences were *FOXP3*, 5′- AGC AAA TGG CGT CTG CAA G -3′ (forward) and 5′- TTG CTC CAG AGA TTG CAC CAC -3′ (reverse); *CD3E*, 5′- GCT ACA TGA CCG AAG GCA ACA A -3′ (forward) and 5′- GAG TGA CAC AGA TGT CAA CCA CGA -3′ (reverse); *CD79A*, 5′- GTG CCT GGA ACA CTG CTG CT -3′ (forward) and 5′- TGC CCA CGT CCT GGT AGG TA -3′ (reverse); *CD68*, 5′- CAG AAG GCA GAG GGT ACA GG -3′ (forward) and 5′- ACA CAG CCA ACC TCA TGA CA -3′ (reverse); and *RPLP0*, 5′-CAA CCC TGA AGT GCT TGA CAT -3′ (forward) and 5′-AGG CAG ATG GAT CAG CCA -3′ (reverse). Ribosomal protein large P0 (*RPLP0*) is a stable housekeeping gene commonly used as a reference for normalizing target gene expressions in adipose tissue (Ohsaki et al. 2007). Expression levels of immune cell marker genes were normalized to *RPLP0* as an internal control. Adipocyte size (diameter) was measured using WinROOF software (Mitani Corporation, Fukui, Japan) as described in Yamada et al. (2020b).

3. Statistical analysis

Results were presented as means ± SE. Statistical significance was determined by analysis of variance (ANOVA), followed by Tukey's post hoc test. Values of $P < 0.05$ were considered significant.

Results

Figure 1 shows the fat depot-specific expression of immune cell marker genes in subcutaneous (S), intramuscular (I), and visceral (V) adipose tissues. Expression of the *FOXP3* gene in subcutaneous adipose tissue was significantly higher than in intramuscular and visceral adipose tissue (Fig. 1A). In contrast, the expression of *CD3E* (Fig. 1B), *CD79A* (Fig. 1C), and *CD68* (Fig. 1D) genes in visceral adipose tissue was significantly higher than in subcutaneous and intramuscular adipose tissue.

Figure 2 shows osmium tetroxide- fixed adipocytes from subcutaneous (S), intramuscular (I), and visceral (V) adipose tissue. Larger adipocytes were more abundant in visceral adipose tissue (Fig. 2C) than in subcutaneous (Fig. 2A) and intramuscular adipose tissue (Fig. 2B). The mean size of visceral adipocytes was significantly larger than that of subcutaneous and intramuscular adipocytes (Fig. 2D).

Discussion

This study showed that expression of the *FOXP3* gene in subcutaneous adipose tissue was significantly higher than that in intramuscular and visceral adipose tissue, suggesting that the populations of Tregs in the subcutaneous adipose tissue of fattening Wagyu cattle may be larger than those in visceral and intramuscular adipose tissue. We also showed that the expression of *CD3E*, *CD79A*, and *CD68* genes in visceral adipose tissue was significantly higher than in subcutaneous and intramuscular adipose tissue. These results suggest that the populations of T-cells, B-cells, and MACs in the visceral adipose tissue of fattening Wagyu cattle may be larger than those in subcutaneous and intramuscular adipose tissue.

In humans, visceral adipose tissue exhibits a higher inflammatory state than subcutaneous adipose tissue (Bruun et al. 2004). Our previous study showed that visceral adipose tissue exhibits a higher inflammatory state than subcutaneous adipose tissue in fattening Wagyu cattle (Yamada et al. 2018). These results suggest that the elevated prevalence of pro-inflammatory immune cells (B-cells, T-cells, and MACs) in the visceral adipose tissue of fattening Wagyu cattle contributes to its heightened inflammatory profile compared with subcutaneous adipose tissue. The present results also suggest that the higher prevalence of anti-inflammatory immune cells (Tregs) contributes to the lower inflammatory state observed in subcutaneous adipose tissue as compared to visceral adipose tissue in fattening

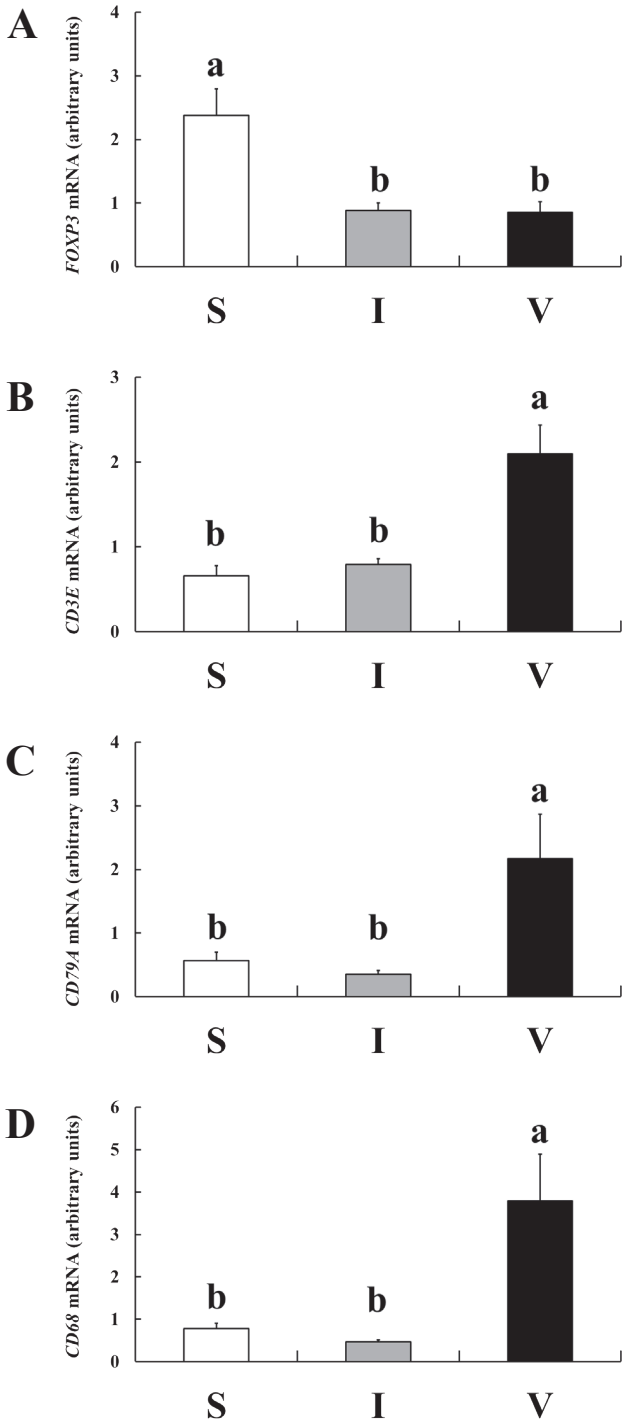


Fig. 1. Fat depot-specific expression of immune cell marker genes ((A) *FOXP3*, (B) *CD3E*, (C) *CD79A*, and (D) *CD68*) in subcutaneous (S), intramuscular (I), and visceral (V) adipose tissue of fattening Wagyu cattle (n = 7)

RPLP0 was used as an internal control. Results are presented as means ± SE. a, b: $P < 0.05$

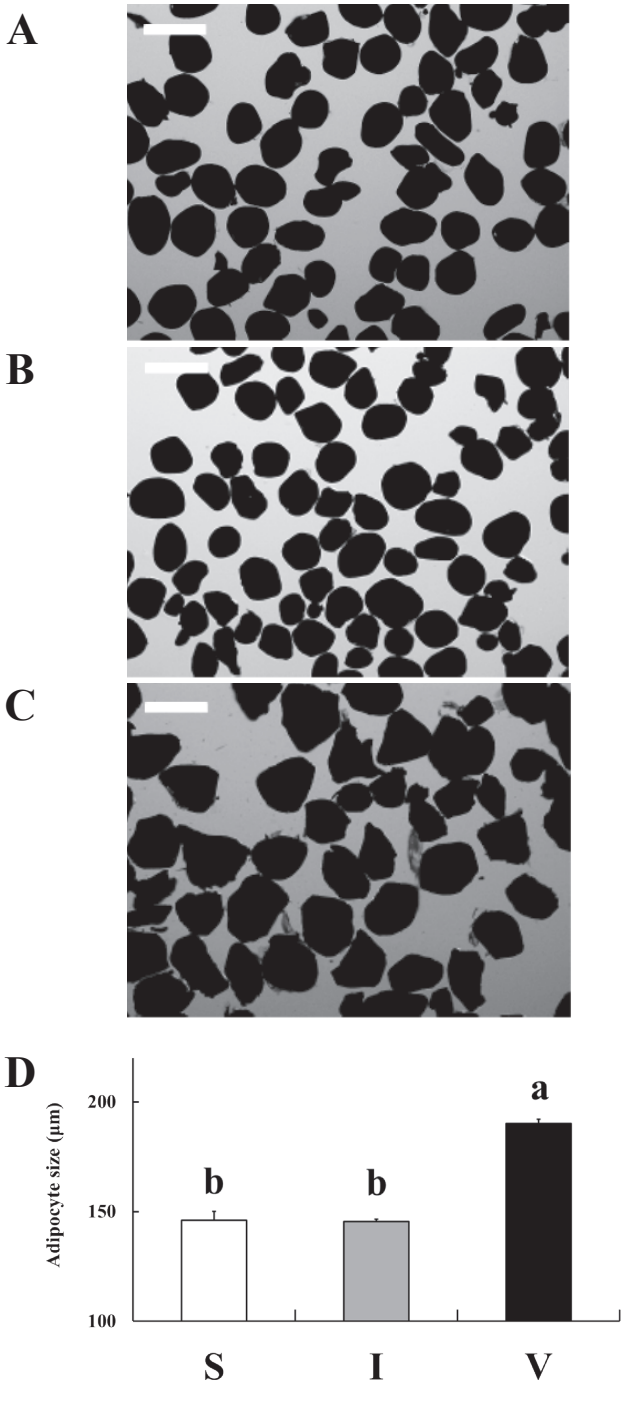


Fig. 2. Fat depot-specific differences in adipocyte size in fattening Wagyu cattle (n = 7)

Osmium tetroxide-fixed adipocytes from (A) subcutaneous, (B) intramuscular, and (C) visceral adipose tissue (magnification: × 40). The white scale bar represents 300 μm. (D) Mean adipocyte size of subcutaneous (S), intramuscular (I), and visceral (V) adipose tissue. Results are presented as means ± SE. a, b: $P < 0.05$

Wagyu cattle.

Hypertrophic adipocytes were exposed to hypoxic stress, which stimulates an inflammatory response in adipose tissue (Hosogai et al. 2007). Studies in humans and rodents have indicated that hypertrophic adipocytes stimulate pro-inflammatory immune cell infiltration into adipose tissue and promote adipose tissue inflammation (McLaughlin et al. 2017). The current study showed that the mean size of visceral adipocytes was significantly larger than that of subcutaneous and intramuscular adipocytes, suggesting that the increased population of pro-inflammatory immune cells (B-cells, T-cells, and MACs) in the visceral adipose tissue of fattening Wagyu cattle is associated with larger adipocyte size in the visceral depot than in subcutaneous and intramuscular adipose tissue.

This study showed that the population of anti-inflammatory Tregs in subcutaneous adipose tissue was larger than in intramuscular adipose tissue. We also found no difference in adipocyte size between subcutaneous and intramuscular depots. These results suggest that the fat depot-specific differences of Treg populations in fattening Wagyu cattle between subcutaneous and intramuscular depots are not affected by adipocyte size. In our previous study, we found that intramuscular adipose tissue from Wagyu cattle was more highly susceptible to cellular senescence than that from Holstein cattle (Yamada et al. 2020a). In contrast, subcutaneous adipose tissue from Wagyu cattle showed low levels of cellular senescence, with no differences observed between the Wagyu and Holstein breeds (Yamada et al. 2020a). These results indicate that susceptibility to cellular senescence is lower in subcutaneous adipose tissue than in intramuscular adipose tissue. In obesity, the inflammatory state of adipose tissue promotes cellular senescence and impairs adipose tissue function (Minamino et al. 2009, Pérez et al. 2016). In contrast, clearance of senescent cells prevents the development of tissue dysfunction (Baker et al. 2011). In addition, accumulation of Tregs promotes tissue repair by suppressing the inflammatory state (Nayer et al. 2024). Therefore, the greater abundance of Tregs in the subcutaneous adipose tissue of Wagyu cattle may contribute to the suppression of adipose tissue senescence, suggesting that differences in the senescence-associated state of adipose tissue in Wagyu cattle between subcutaneous and intramuscular regions could be influenced by depot-specific distributions of anti-inflammatory Tregs.

In human and murine immune systems, immune cell lineages are composed of distinct subsets defined by the differential expression of surface antigens, lineage-specifying transcription factors, and characteristic cytokine and chemokine secretion profiles (Fang et al. 2018). Guzman et al. (2014) reported that γδ T cells are a major regulatory T cell population in the bovine immune system. Further studies are needed to clarify fat depot-specific differences in the composition of immune cell subsets in cattle.

In conclusion, this study found that the anatomical location of bovine adipose tissue influences immune cell populations in a fat depot-specific manner. Our findings suggest that depot-specific differences in bovine adipose tissue inflammation are influenced by immune cell populations.

Acknowledgements

This work was supported in part by a Grant-in-Aid for JSPS KAKENHI (18K05958).

References

Baker, D. J. et al. (2011) Clearance of $p16^{Ink4a}$-positive senescent cells delays ageing-associated disorders. *Nature*, **479**, 232-236.

Bruun, J. M. et al. (2004) Higher production of IL-8 in visceral vs. subcutaneous adipose tissue. Implication of nonadipose cells in adipose tissue. *Am. J. Physiol*., **286**, E8-E13.

Cancello, R. et al. (2006) Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. *Diabetes*, **55**, 1554-1561.

Duffaut, C. et al. (2009) Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. *Arterioscler. Thromb. Vasc. Biol*., **29**, 1608-1614.

Fang, P. et al. (2018) Immune cell subset differentiation and tissue inflammation. *J. Hematol. Oncol*., **11**, 97.

Feuerer, M. et al. (2009) Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med*., **15**, 930-939.

Guzman, E. et al. (2014) Bovine γδ T cells are a major regulatory T cell subset. *J. Immunol*., **193**, 208-222.

Hosogai, N. et al. (2007) Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes*, **56**, 901-911.

Liu, L. et al. (2021) The role and research progress of the balance and interaction between regulatory T cells and other immune cells in obesity with insulin resistance. *Adipocyte*, **10**, 66-79.

McLaughlin, T. et al. (2017) Role of innate and adaptive immunity in obesity-associated metabolic disease. *J. Clin. Invest*., **127**, 5-13.

Minamino, T. et al. (2009) A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat. Med*., **15**, 1082-1087.

Nayer, B. et al. (2024) Local administration of regulatory T cells promotes tissue healing. *Nat. Commun*., **15**, 7863.

Ohsaki, H. et al. (2007) Stearoyl-CoA desaturase mRNA

expression during bovine adipocyte differentiation in primary culture derived from Japanese Black and Holstein cattle. *Comp. Biochem. Physiol. Part A*, ***148***, 629-634.

Pérez, L. M. et al. (2016) 'Adipaging': ageing and obesity share biological hallmarks related to a dysfunctional adipose tissue. *J. Physiol*., **594**, 3187-3207.

Winer, D. A. et al. (2011) B lymphocytes promote insulin resistance through modulation of T lymphocytes and production of pathogenic IgG antibody. *Nat. Med*., **17**, 610-617.

Yamada, T. et al. (2018) Fat depot-specific differences of macrophage infiltration and cellular senescence in obese bovine adipose tissues. *J. Vet. Med. Sci*., **80**, 1495-1503.

Yamada, T. et al. (2020a) Breed differences in macrophage infiltration and senescence state in adipose tissues of Wagyu and Holsteins. *Anim. Sci. J*., **91**, e13443.

Yamada, T. et al. (2020b) Fat depot-specific effects of body fat distribution and adipocyte size on intramuscular fat accumulation in Wagyu cattle. *Anim. Sci. J*., **91**, e13449.