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# Potential Mechanisms for Marbling Content Differences in M. *Longissimus Dorsi* From Wagyu And Brahman Cattle

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## Objectives

Intramuscular fat (marbling) affects consumer perceptions of meat quality. Recent studies indicated that intramuscular adipocytes are derived from fibroadipogenic progenitors (FAPs), a type of bipotent mesenchymal progenitor cells in extracellular matrix of muscle fiber. Platelet-derived growth factor receptor α (PDGFRa) is a marker in identifying FAPs. The amount of PDGFRa positive cells refers to the abundance of FAPs, a key factor determining the intramuscular adipogenic efficiency of the animal. In addition, it has been shown that both satellite cells and skeletal muscle fibers regulate the activity of FAPs. In this study, we aimed to identify some underlying mechanisms for the differences in intramuscular fat accumulation between Wagyu, a high marbling breed, and Brahman, a low marbling breed, through a comparison study.

## Materials and Methods

Five cattle of each breed approaching mature body composition (Wagyu born January to May 2017; Brahman two 2-yr-old and three 3-yr-old, born April 2017 and 2015) were selected for this study. Biopsy samples of M. *Longissimus* muscle (LM) were taken after local anesthesia between the 12th and 13th ribs. Fresh samples were frozen in isopentane chilled by liquid nitrogen immediately after trimming fat and connective tissue. Other samples were fixed for 4 h in ice-cold 4% paraformaldehyde, soaked overnight in 30% sucrose solution, and then processed in Optimal Cutting Temperature embedding medium. Frozen samples were stored at –80°C before cryosectioning (5 mm) at –25°C for immunochemical staining. Unfixed muscle tissue sections were only used for muscle fiber determinations by rinsing with 1X Tris Buffer Saline (TBS) 3 times ,5 min each, before blocking in 10% goat serum and 1% bovine serum albumin, followed by overnight incubation with primary antibodies at 4°C. Subsequent secondary antibody staining at room temperature for 1 h. TBS/T (0.3% Triton) was used for membrane permeabilization to identify satellite cells, FAPs, and basement membrane. Mounting media containing 4,6-diamidino-2-phenylindole (DAPI) was used for nucleus staining. Primary antibodies against PDGFRα, paired box 7 (PAX7), laminin, and different isoforms of skeletal muscle myosins were used to identify FAPs, satellite cells, basement membrane, and different types of muscle fiber, respectively. All the sections were visualized under a Nikon inflorescence microscope, and images were analyzed by Image J software to identify different muscle fiber types and positive signals of PDGFRα and PAX7. Minimum diameter of each muscle fiber type was the average of 30 randomly selected fibers per section. Data were analyzed with R-studio using 't.test ()' function with the critical value being equal to 0.05.

## Results

Wagyu muscle demonstrated a greater ($P$ = 0.01) number of FAPs compared with Brahman muscle. A trend toward a higher ($P$ = 0.06) abundance of satellite cells in Wagyu muscle than in Brahman muscles was also identified. No differences were found in muscle fiber diameter or muscle fiber type composition between these two breeds of cattle.

## Conclusion

The greater number of FAP cells observed in the LM of Wagyu cattle than in Brahman cattle suggests that the higher marbling content of Wagyu meat is at least partially attributed to the more abundant adipogenic progenitor cells, which increases the capacity and efficiency of intramuscular adipogenesis in Wagyu cattle.