

Project Summary

Regulation of Marbling Development in Beef Cattle by Specific Fatty Acids

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**Study Completed
May 2010**



Funded by The Beef Checkoff

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Background

Marbling, or intramuscular (i.m.) fat, is one of the primary factors used to determine quality grades of beef carcasses. The positive relationship between marbling and overall palatability has long been recognized. Despite the importance of i.m. fat deposition in determining product quality, the goal of maintaining or increasing i.m. fat while reducing subcutaneous (s.c.), intermuscular (seam) and internal fat depots, which reduce carcass yield and value, has proven to be challenging. A fundamental understanding of the biological mechanisms controlling the accumulation of fat in distinct adipose tissue depots of cattle is lacking, and this hampers our ability to develop effective strategies to increase marbling or decrease unwanted carcass fat.

Mature adipocytes result from the differentiation of preadipocytes, which are sometimes referred to as stromal-vascular (SV) cells. Preadipocyte differentiation is a transformation from a fibroblast-like cell to a lipid-filled cell, and this transformation coincides with the expression of transcription factors, genes, enzymes and other proteins that allow adipocytes to effectively store and release lipid. Because i.m. fat accumulation is dependent upon an increase in the number of adipocytes, differentiation of preadipocytes is an important regulatory step in the deposition of marbling.

The specific goals of this research project were to utilize cell culture models to investigate the effect of specific fatty acids on differentiation of: i. SV cells and mature adipocytes within i.m. and s.c. adipose tissue explants (Smith); ii. muscle-derived preadipocytes (Johnson); and iii. i.m. and s.c. preadipocytes (Doumit). Specific fatty acids have the potential to regulate the differentiation of preadipocytes, and evidence exists that regulation of adipose tissue deposition may differ among anatomical depots. The goal of this project was to determine if specific fatty acids, or combinations of fatty acids, could selectively stimulate the differentiation of preadipocytes from i.m. fat, or selectively inhibit the differentiation of s.c. preadipocytes.

Methodology

Researchers used adipose tissues at different stages of growth, preadipocytes isolated from market cattle and muscle satellite cells to test the effects of fatty acids on i.m. and s.c. preadipocytes in culture.

In pure cultures of clonally derived preadipocytes, arachidonic acid stimulated a higher proportion of i.m. than s.c. preadipocytes to accumulate lipid (Figure 1). This finding warrants further investigation to determine more precisely the mode of action, since arachidonic acid is a precursor for many signaling molecules. In general, s.c. preadipocytes have a greater propensity than i.m. preadipocytes to form large lipid droplets in response to fatty acid treatments, while i.m. preadipocytes typically accumulate many small lipid droplets.

Findings

Researchers compared the effects of oleic acid addition on primary cultures of muscle-derived cells and i.m. and s.c. preadipocytes. These data showed that these three bovine primary cells are differentially affected by oleic acid (Figure 2). The mRNA levels of the adipogenic transcription factors, C/EBP β and PPAR γ , increased in response to oleic acid in i.m. adipocytes but not to the

same extent in s.c. adipocytes and muscle-derived cells. Western blot analysis revealed that oleic acid increased PPAR γ protein in both i.m. and s.c. adipocytes. These data demonstrated that oleic acid may have autocrine or paracrine effects in further stimulating marbling development, but is less important in regulating the development of s.c. adipose development. Therefore, we concluded that oleic acid is a critical factor in enhancing in marbling adipose tissue.

Oleic acid also increased phosphorylated AMP-activated protein kinase (pAMPK) and GPR43 in muscle satellite cells that had been treated to *trans*-differentiate into adipocytes. It has been documented that these GPRs transduce extracellular signals across the plasma membrane, activating cellular responses through a variety of second messenger cascades such as AMPK α . AMPK complex, which regulates cellular energy balance, is an important enzyme regulating lipid metabolism in adipose tissue.

Treatment of i.m. and s.c. adipose tissue explants with 40 μ M α -linolenic, oleic, stearic, *trans*-vaccenic or conjugated linoleic acid (*trans*-10,*cis*-12) increased adipocyte volume, but the effects were dependent on age of cattle and adipose tissue depot (Figures 3 & 4). Remarkably, media α -linolenic and *trans*-vaccenic acid increased adipocyte volume linearly between 12 and 16 months of age.

There was a peak in PPAR γ gene expression in both i.m. and s.c. adipose tissue (Figure 3), indicating that between 12 and 14 months of age, a population of preadipocytes was actively differentiating to become lipid-filled cells. This was depressed strongly by all media fatty acids in the explant culture system, but only at 14 months of age. At 12 months of age, *trans*-vaccenic acid stimulated PPAR γ gene expression in i.m. adipose tissue, and stearic acid stimulated PPAR γ gene expression in s.c. adipose tissue. Both fatty acids are absorbed at relatively high concentrations in young cattle during the transition from a forage-based diet to a grain-based diet.

Unlike PPAR γ , peak SCD gene expression was not observed until the last sampling period, 16 months of age (Figure 4). This is consistent with a general increase in monounsaturated fatty acids in i.m. and s.c. adipose tissues over time on feed. As in the preadipocyte and myoblast system (Figure 2), oleic acid (and fatty acids in general) depressed SCD gene expression in i.m. adipose tissue. This effect was more variable in s.c. adipose tissue, which had an overall higher expression than i.m. adipose tissue.

Implications

Reduced beef quality has been identified as a major issue in the U.S. beef industry and is believed to reduce the overall demand for beef. By increasing marbling, important palatability factors such as flavor and juiciness are enhanced, which improves consumer satisfaction and thereby overall beef demand. Researchers demonstrated the ability to differentially regulate the development of marbling and subcutaneous fat depots, which provides sustainability to the cattle feeding sector. These findings demonstrate clearly that marbling and subcutaneous fat depots are regulated by separate and distinct factors. Specific fatty acids in plasma or within the fat depots can promote marbling fat development while at the same time can cause muscle precursor cells to develop into marbling fat cells. The long-term goal of this work is to develop intervention strategies, like specific implants or feed additives, that can be administered at the optimal time during the feeding period to “turn on” marbling fat cells while having no effect on subcutaneous fat cells.

In theory, this could help achieve higher, or as high, marbling scores earlier in the finishing period and be able to lower overall cost of gains, thereby increasing beef quality while at the same time improving profitability in the beef industry.

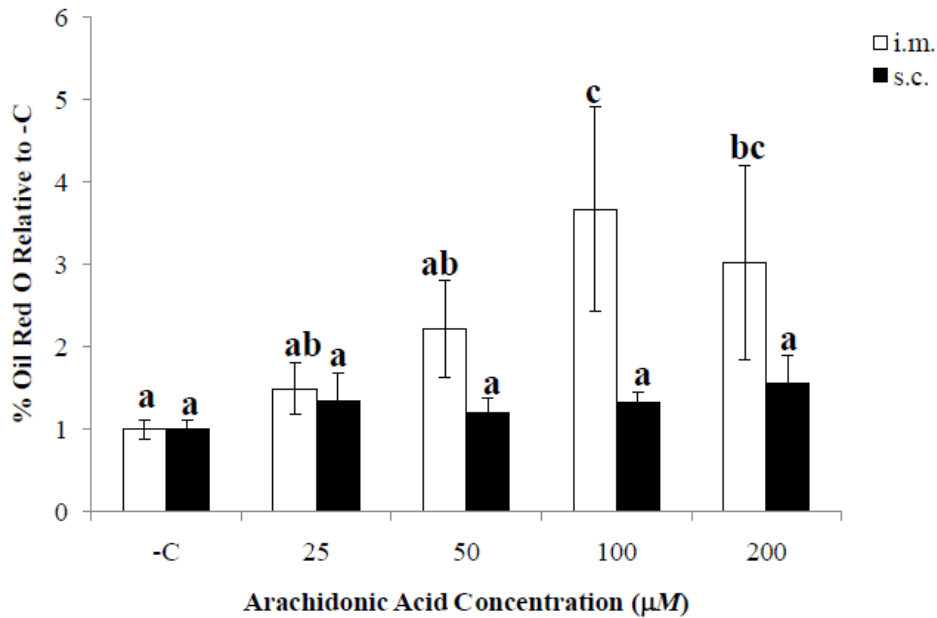
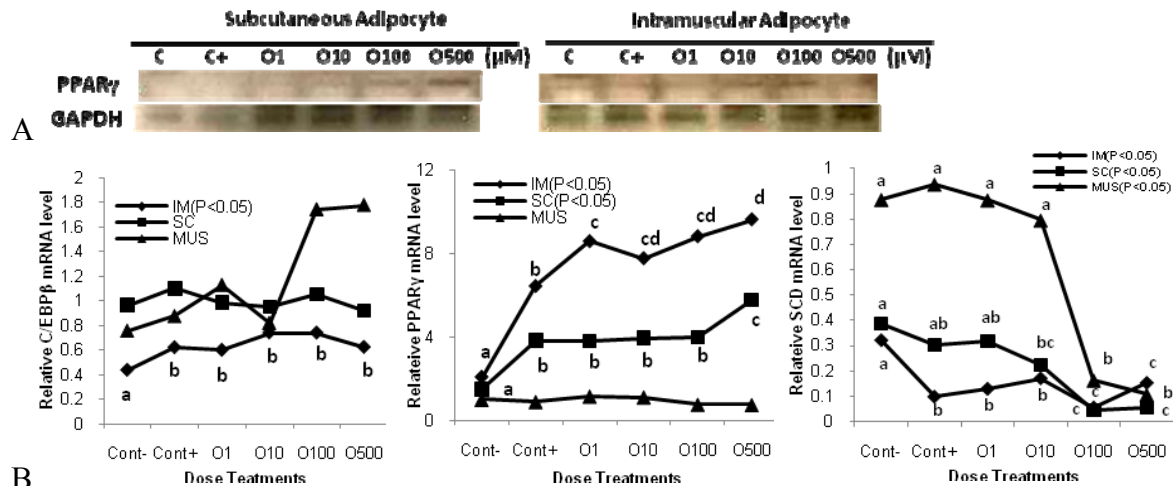


Figure 1. Effect of arachidonic acid concentration on percentage of differentiated bovine preadipocytes. Clonally-derived bovine preadipocytes isolated from intramuscular (i.m.), and subcutaneous (s.c.) adipose tissue were grown to confluence and subsequently exposed to the indicated arachidonic acid concentrations in differentiation medium for 10 days. The percentage of differentiated preadipocyte cells was determined by oil red O staining of lipid, and quantified by microscopy. Bars represent means \pm SEM. Treatment ($P < 0.05$) and depot ($P < 0.01$). ^{a-} Means without a common superscript differ ($P < 0.05$).



B

Figure 2. Relative mRNA levels of adipogenic transcription factors (C/EBP and PPAR) and enzymatic protein SCD in total mRNA and protein isolated from cultured s.c. and i.m. adipose tissue. Bars are means \pm SE relative to control. ^{abcd} Bars differ from control ($P < 0.05$). Values are the means of 3 replicates. IM and SC preadipocyte treated basal media (5% FBS/DMEM; Cont-) with insulin (10 μM) and ciglitzone (10 μM) (Cont+) or insulin, ciglitzone with oleic acid (1 μM ; O1, 10 μM ; O10, 100 μM ; O100, and 500 μM ; O500).

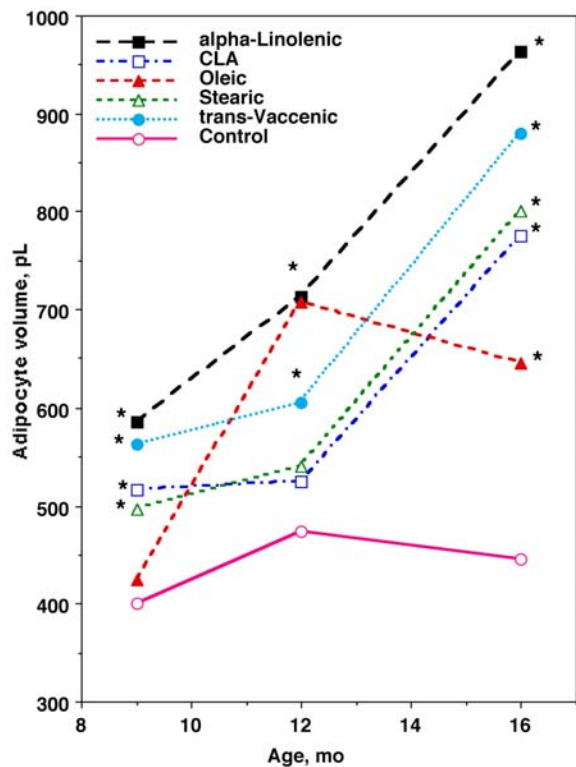


Figure 3. Intramuscular adipocyte volume over time and treatment. *Significantly different from control ($P < 0.05$).

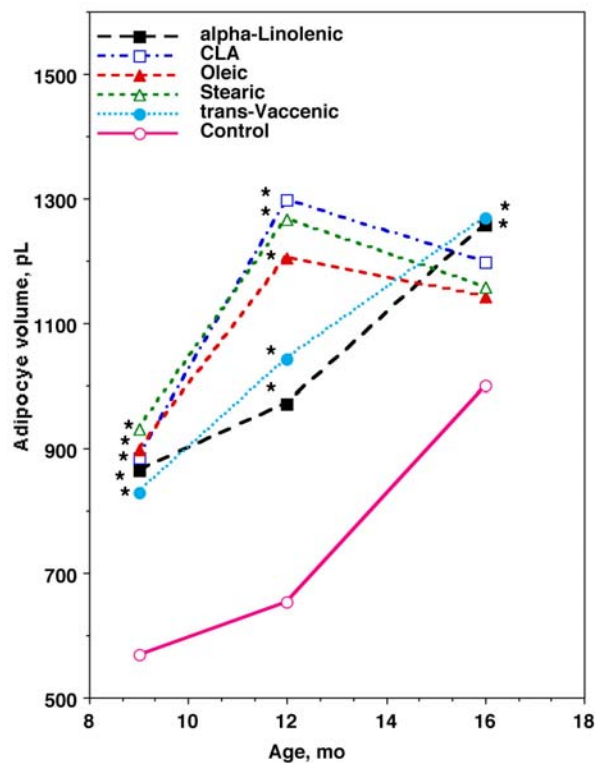


Figure 4. Subcutaneous adipocyte volume over time and treatment. *Significantly different from control ($P < 0.05$).

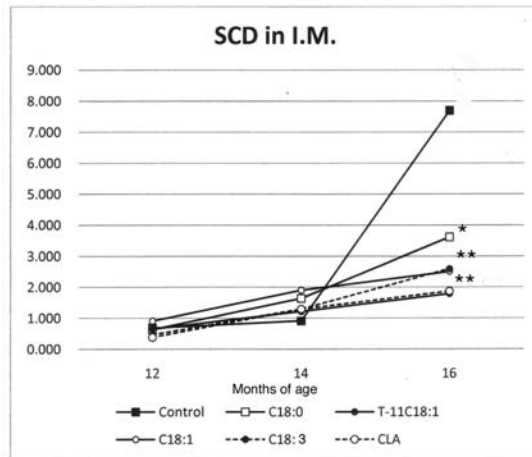
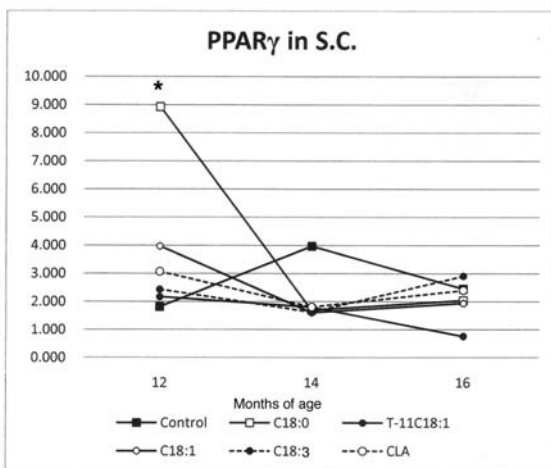
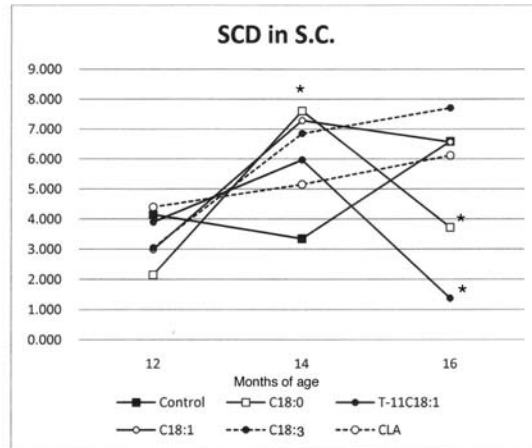
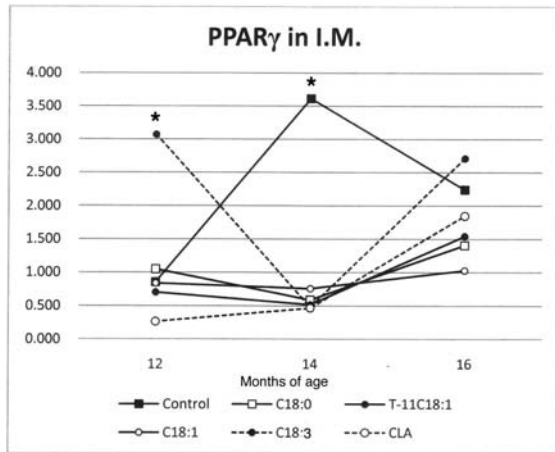


Figure 3. PPAR γ gene expression in i.m. (top) and s.c. (bottom) adipose tissues incubated with and without media fatty acids. *Significantly different from control ($P < 0.05$).

Figure 4. SCD gene expression in s.c. (top) and i.m. (bottom) adipose tissues incubated with and without media fatty acids. *Significantly different from control ($P < 0.05$).

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