**Project Title:** Characterization of Intramuscular Adipogenesis in Cattle

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## Background

Every year the U.S. beef cattle industry produces over 2 billion kg of excess fat. One of the reasons for this excess fat in feedlot steers might be increased time on feed in an effort to improve the palatability and acceptability of meat for the U.S. consumer. This and other related production inefficiencies involving inferior muscling and excessive fat deposition cost the beef industry an estimated 7.4 billion dollars annually. On the other hand, marbling or the deposition of intramuscular (i.m.) adipose tissue is considered to be highly desirable in the cattle industry, as marbling is believed to positively influence the palatability and tenderness of beef. In fact, under the current USDA beef grading system, carcass value is primarily determined by the abundance of marbling on a cross-section of *Longissimus dorsi* muscle. Although heritability for tenderness, and thus marbling, has varied from moderate/high to low in various studies in beef cattle, studies in laboratory animals suggest that the deposition and differentiation of adipose tissue is under significant genetic control. Although a significant amount of work has been done in laboratory animals, little is understood about the genetic regulation of fat deposition in cattle. Lack of such knowledge is a critical hindrance in our efforts at minimizing the economic loss due to unnecessary subcutaneous and peritoneal fat deposition and maximizing the highly desired intramuscular fat deposition.

## Methodology

*Evaluation of Adipocyte Differential Gene Expression through PCR Subtraction Hybridization*

For this experiment, 48 fall-weaned crossbred steers (231 + 25 kg) were harvested at the Food and Agricultural Products Research & Technology Center. During a 143-d grazing period, treatments were high (HGW; 1.28 kg/d) or low (LGW; 0.48 kg/d) daily BW gain on wheat pasture or dormant native range (NR; 0.21 kg/d). Before finishing, four steers per treatment were harvested and adipose tissue samples collected. Grazing treatments resulted in 0.69, 0.08, and 0.00 cm of 12th-rib fat, 2.25, 1.30, and 0.00 % kidney, pelvic, and heart fat (KPH) and 275, 75, and 0 marbling score (100 = practically devoid, 200 = trace, and 300 = slight) for HGW, LGW, and NR, respectively. All remaining steers were individually fed a high-grain diet for 86, 111, and 162 d for HGW, LGW and NR, respectively, to a common 1.27 cm of backfat. At final harvest, six steers per treatment were selected for harvest for adipose tissue collection. At harvest, subcutaneous and intramuscular adipose tissue samples were obtained after exsanguination by cutting through the hide at the 2nd to 6th lumbar vertebrae region of the loin. RNA was extracted from adipose tissue collect at slaughter and subjected to PCR subtraction hybridization to detect genes that are turned on or off during adipogenesis in beef cattle.



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## Findings

*Evaluation of Adipocyte Differential Gene Expression through PCR Subtraction Hybridization*

We have completed (Ross et al., 2005) a research study utilizing a sensitive molecular technique called PCR subtractive hybridization that identified genes involved with differentiation of adipocytes in subcutaneous and intramuscular adipose deposits of finishing steers. Differential screening confirmed that 12 templates were expressed greater in NR steers and 35 templates were expressed greater in HGW steers. A total of 47 templates were subjected to dideoxy chain termination sequencing. Identity and homology information of specific genes is presented in Table 1. Three differentially expressed templates, specifically osteonectin, ferritin heavy chain (HC) and decorin, were evaluated using real-time PCR. Decorin is suspected of playing an important role in tissue morphogenesis and participates in cell proliferation and differentiation. Osteonectin is a component of the extracellular matrix involved with cell-matrix interactions. Osteonectin is up-regulated in obesity and may affect key functions of the adipose tissue. Ferritin HC is an intracellular protein involved with differentiation of 3T3 cells into adipocytes and has been reported to increase as pre-adipocytes differentiate into mature adipocytes.

Real-time PCR of decorin, osteonectin, and ferritin HC expression in adipose tissue from beef steers indicated that gene expression was influenced by previous nutrition and adipose tissue depot. Differences in gene expression between intramuscular and subcutaneous adipose tissue were observed in adipose tissue from steers harvested before and after finishing. A significant adipose depot x nutritional background interaction for osteonectin ($P = 0.004$) and ferritin HC ($P = 0.03$) gene expression in adipose from HGW and NR steers harvested prior to finishing (Figure 1). Subcutaneous fat from HGW steers had 13-fold greater gene expression compared NR steers. However, osteonectin gene expression in intramuscular adipose was only 2.4-fold greater in HGW than NR steers. Osteonectin gene expression was not different ($P = 0.42$) between adipose depots in NR steers. Ferritin HC gene expression in intramuscular adipose did not differ ($P = 0.25$) between HGW and NR steers. However, ferritin HC gene expression was four-fold greater in HGW subcutaneous and 2.4-fold greater in HGW intramuscular adipose than from subcutaneous adipose from NR steers (Figure 1). Gene expression of decorin in adipose tissue prior to finishing was 2.5-fold greater ($P = 0.25$, Figure 2) in subcutaneous compared with intramuscular adipose tissue. High-gain wheat steers had 2.1-fold greater ($P = 0.03$) decorin gene expression than NR steers prior to finishing.

One assumption was that energy restricted steers (NR) would have more immature adipocytes than non-restricted steers (HGW), therefore depot and harvest date gene expressions were evaluated. A significant depot x harvest interaction was observed for both osteonectin ($P = 0.006$) and ferritin HC ($P = 0.02$) gene expression (Figure 3). Osteonectin gene expression was lowest in subcutaneous, but not different ($P = 0.45$) from intramuscular adipose tissue. However, osteonectin gene expression in increased 14.7-fold ($P < 0.001$) during finishing in subcutaneous adipose tissue. Likewise, osteonectin gene expression in intramuscular adipose increased 3.1-fold ($P < 0.05$) during finishing. Ferritin HC gene expression profile (Figure 3) was similar to osteonectin before finishing. The lowest ferritin HC gene expression was in subcutaneous adipose prior to finishing, but it was not different ($P = 0.23$) from intramuscular adipose. After finishing, ferritin HC gene expression increased nearly seven-fold ($P < 0.001$) compared to before finishing. Intramuscular adipose ferritin HC gene expression increased 2.9-fold ($P = 0.03$) compared to subcutaneous adipose gene expression prior to finishing, however intramuscular ferritin HC gene expression did not increase ($P = 0.13$) during finishing compared to pre-finishing levels of expression. Results indicated nutritional management of steers prior to the feedlot stage affects adipose tissue depots differently.



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Our data suggests that nutritional background alters gene expression in adipose depots, and that depots are influenced differently.

### *Characterization of Bovine Adipocyte Differentiation in Primary Culture and Gene Expression as a Result of Substrate Stimulation*

A limited amount of published data has indicated that there are clear metabolic differences between the sites of bovine adipose deposition. Our hypothesis is that differences exist in the factors that stimulate adipocyte differentiation in the various adipose depots. We believe that the stimulants utilized to investigate gene expression during terminal adipocyte differentiation of 3T3 cells (standard cell line used in adipocyte differentiation) may not necessarily induce the same response in all bovine adipocytes depending upon the location of preadipocytes collected. We have developed a primary bovine adipocyte cell culture system to delineate differences between adipose depots.

We cultured adipocytes from the intramuscular, KPH and subcutaneous adipose depots. Adipogenesis involves a specific series of events to initiate and maintain the differentiation pathway. Studies utilizing 3T3 cells have characterized early events in the differentiation of preadipocytes into adipocytes, including the expression profiles of two families of transcription factors induced in this early phase, the CAAT/enhancer binding proteins (C/EBPs) and the peroxisome proliferator-activated receptors (PPARs). These transcription factors are responsive to various adipogenic inducers including insulin, dexamethasone, long-chain fatty acids, and retinoids. We have previously investigated the change in adipocyte gene expression of PPARγ during marbling of steers (Childs et al., 2002). However, evaluation of PPARγ expression revealed no significant difference across slaughter groups. This suggested that differentiation of the intramuscular adipocytes had occurred prior to day 86 of the feedlot phase.

Cultured intramuscular, subcutaneous and KPH cells have been collected at confluence (0 hr), 6, 12, 24, 48, and 72 hrs after the initiation of differentiation. We developed a PCR-based assay to determine mRNA expression of PPARγ, stearoyl-CoA desaturase 1, lipoprotein lipase, acyl-CoA synthetase, and fatty acid synthase in intramuscular, subcutaneous, KPH, and 3T3-L1 samples. Our objective was to determine if cultured bovine stromal-vascular cells isolated from bovine adipose tissue from different depots are an adequate model for differentiation compared with the 3T3-L1 cell line by examining known genes expressed in the known 3T3-L1 model using real-time PCR methodology.

To date our cell culture data of bovine adipocytes from intramuscular, subcutaneous, and KPH indicates that the primary cell culture of bovine adipocytes differentiated to adipocytes has been successful, although different depending upon the source of the adipocytes. Evidence supporting this conclusion includes increased PPARγ gene expression in subcutaneous adipose cells (Figure 4). Additionally, the gene expression of PPARγ resulted in the subsequent gene expression of stearoyl-CoA desaturase 1 (Figure 5) a gene marker for differentiation, and lipoprotein lipase (Figure 6). Further evidence is found in the gene expression of acyl-CoA synthetase (Figure 8) which is a gene involved in lipid oxidation. Acyl-CoA synthethase gene expression decreased (Time effect, $P < 0.001$) during differentiation.

Our preliminary data also supports the hypothesis that bovine adipocytes from different depots have different gene expression when stimulated to differentiate to mature adipocytes in cell culture. Gene expression of PPARγ exhibited depot ($P < 0.001$) and a depot x time of differentiation interaction ($P < 0.001$, Figure 4). Subcutaneous PPARγ gene expression was nearly four-fold greater at 48 h than 0 h of differentiation, and six-fold greater than intramuscular and KPH PPARγ gene expression at 48 h. Gene expression of PPARγ at 48 h is



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of importance because the 3T3 model cell line exhibits increasing levels of PPARγ at this time. Stearoyl-CoA desturase 1 gene expression also exhibited a depot x time interaction ($P = 0.04$). Stearoyl-CoA desaturase 1 gene expression in subcutaneous adipocytes increased nearly seven-fold from 0 to 72 h (Figure 5). Meanwhile, gene expression of stearoyl-CoA desaturase 1 in intramuscular and KPH adipocytes increased no more than 0.8 and 1.7-fold from 6 to 72-h. Lipoprotein lipase gene expression in cultured bovine adipocytes was similar to that of PPARγ and stearoyl-CoA desaturase 1 by exhibiting a depot x time interaction ($P < 0.001$, Figure 6). Gene expression in subcutaneous adipocytes increased over seven-fold from 0 h to 24 and 48 h, whereas lipoprotein lipase gene expression in intramuscular and KPH adipocytes decreased or remained the same from 0 h through 72 h of differentiation. Gene expression of fatty acid synthase exhibited a depot x time interaction ($P < 0.001$, Figure 7). Expression of fatty acid synthase decreased in all depots from 0 to 72 h. The greatest decrease in fold expression occurred in intramuscular adipose, whereas the subcutaneous had the least change in fold expression of fatty acid synthase. An obvious explanation for these findings is not readily apparent. However, the small magnitude of change in gene expression may indicate adequate expression of fatty acid synthase is already occurring in cultured bovine adipocytes at differentiation. Similar to the other genes examined the gene expression of acyl-CoA synthetase exhibited a depot x time interaction ($P < 0.001$, Figure 8). Gene expression of acyl-CoA synthetase decreased in all depots during differentiation with the greatest decrease in expression occurring in intramuscular adipocytes. After initiation of differentiation the gene expression of acyl-CoA synthetase changed very little during the 72 h period in adipocytes from the three depots.

Results from RT-PCR of 3T3 cultured cells will be forthcoming with completion of all RT-PCR assays. We believe that the data from the adipocyte cell culture system demonstrate that differences exist for activation of adipocyte terminal differentiation from different depots. Additionally, if adipocytes from different depots have different gene expression profiles under the standard conditions of cell culture system then the adipocytes likely have different stimulatory or regulatory inputs to evolve into mature lipid-filled adipocytes. These data represent one more step in understanding the physiology and potential differences between adipose depots. This increased understanding will lay the groundwork for additional work that could result in manipulation of adipose depots in the whole animal at a future date.

*Development and Application of a Bovine Adipose Microarray*

We have compared global gene expression over time of cellular differentiation and the source of the stromal-vascular cells with the microarray that has been developed by Dr. DeSilva. Utilization of the bovine adipocyte cDNA microarray provides an excellent opportunity to analyze which genes are activated during adipogenesis and determine if additional gene expression differences between adipose depots exist.

We have constructed a cDNA library consisting of clones derived from bovine adipose tissue. 20,000 clones have been picked and individually arrayed. We have completely sequence analyzed ~3000 clones. Initial results prove that bovine adipose tissue is of much higher complexity than previously thought. We have selected 1051 unique clones from this library as the template for the cDNA microarray. Using this microarray we have compared gene expression in primary adipocyte cell lines. Around 100 transcripts out of the 1089 printed on the array, showed fluctuations in expression during the process of differentiation. Expression changes were minimal during the first 12 hours of differentiation. Remarkable changes were observed after 24 hours of differentiation and that pattern was continued up to 120 hours.

Genes showing 1.8-fold up or down regulation during more than one time point were selected and clustered in to seven groups. These genes showed consistent up-regulation



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during the entire process of differentiation. A subset of genes that are up- or down-regulated are depicted in Tables 2 and 3.

### *Characterize potential roles of candidate molecules in processes of growth and differentiation in adipose and muscle*

*Activation of Adipocyte Differentiation with Thiazolidinediones and Linoleic Acid*

Several chemical compounds including thiazolidinediones and linoleic acid mediate transcriptional activation of PPARγ. In addition, expression of PPARγ and activation by these ligands leads to the characteristic differentiation, lipid accumulation in adipocytes, changes in cell morphology, and greater expression of genes characteristic of adipocyte phenotype. Feeding trials have also indicated that zinc can mediate increases in marbling in finishing cattle. We are currently determining if these activators increase the expression of PPARγ in cultured stromal-vascular cells harvested from subcutaneous and intramuscular adipocytes. If expression of PPARγ is increased, these agents might serve as useful research tools and/or metabolic modifiers for altering adipogenesis in ruminant tissues. The 3T3 cell line is the standard model used for adipocyte differentiation research. Establishment of this model in our laboratory will allow for comparison of results in bovine adipocytes to results previously published for the 3T3 cells. If successful in culture, we hope to utilize the ligands to determine if adipogenesis can be stimulated in cattle.

## Implications

The U.S. beef cattle industry produces over 2 billion kg of excess fat, which costs the industry an estimated 7.4 billion dollars annually. In contrast, marbling (intramuscular fat) is considered to be highly desirable due to its association with palatability and tenderness of beef. Heritability for marbling has varied from moderate/high to low in various studies in beef cattle; however, studies in laboratory animals suggest that the deposition and differentiation of adipose tissue is under significant genetic control. Although a significant amount of work has been done in laboratory animals, little is understood about the genetic regulation of fat deposition in cattle. The objectives of this research were to generate candidate genes via whole-genome scanning in adipose tissue during animal growth and fat cell maturation in cattle, and to characterize potential roles of candidate molecules in processes of adipose tissue growth and differentiation. With resources from this grant, we have published a research study utilizing a sensitive molecular technique called PCR subtractive hybridization that identified genes involved with differentiation of adipocytes in subcutaneous and intramuscular adipose deposits of finishing steers. Results indicated nutritional management (grazing wheat pasture vs. low-quality forage) of steers prior to the feedlot stage affects adipose tissue depots (subcutaneous vs. intramuscular fat) differently. We subsequently hypothesized that differences exist in the factors that stimulate adipocyte differentiation in the various adipose depots. We believe that the stimulants utilized to investigate gene expression during terminal adipocyte differentiation of 3T3 cells (standard cell line used in adipocyte differentiation research) may not necessarily induce the same response in all bovine adipocytes depending upon the location of preadipocytes collected. Therefore, a primary bovine adipocyte cell culture system has been developed to delineate differences between adipose depots. Data from the adipocyte cell culture system demonstrate that differences exist for activation of adipocyte terminal differentiation from different depots. These data represent one more step in understanding the physiology and potential differences between adipose tissue depots. This increased understanding will lay the groundwork for additional research that could result in manipulation of adipose depots in the whole animal at a future date. The overall goal is to decrease excessive subcutaneous and abdominal fat while maintaining or increasing marbling in beef cattle.



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Table 1. Identity, size, and homology of bovine cDNA clones from high gain wheat (HGW) vs native range (NR) subtraction.

| Expressed Pattern | Identity | Base Pairs to BLAST | GenBank Accession # | Homology |
|---|---|---|---|---|
| HGW | Histone H3.3A | 345 | X51897<br>NM_002107<br>BG013857 | Rabbit 94.4% (319/338)<br>Human 93.2% (316/339)<br>Human 93.2% (316/339) |
| | *Elongation factor 1 alpha* mRNA | 527 | AJ238405<br><br>AF013213<br>*AB060107* | Bovine 99.0% (502/507)<br><br>*Bovine 99.0% (502/507)*<br>*Bovine 99.0% (502/507)* |
| | *Ribosomal protein S6* | *765* | *BC02760*<br>NM_001010<br>BC013296 | *Human 91.9% (192/209)*<br>Human 91.9% (192/209)<br>Human 91.9% (192/209) |
| | *Satellite 1 downstream DNA* | *790* | *AB010556*<br>K00133<br>J00037 | *Bovine 89.2% (486/545)*<br>Bovine 90.9% (413/454)<br>Bovine 92.6% (654/706) |
| | Cathepsin K* | *316* | *NM_000396*<br>BC016058 | Human 91.2% (83/91)<br>Human 91.2% (83/91) |
| | Cathepsin O | *316* | *X82153*<br>U13665 | Human 91.2% (83/91)<br>Human 91.2% (83/91) |
| | Nephroblastoma overexpressed gene | *121* | *NM_002514*<br>BC015028 | Human 87.7% (100/114)<br>Human 87.7% (100/114) |
| | mRNA adenylate cyclase-stimulation, G-protein alpha | *238* | *X03404*<br>*M13006*<br>*X63893* | Bovine 100% (238/238)<br>Bovine 99.6% (237/238)<br>Pig 94.1% (223/227) |
| | Mitochondrion* | *207* | *J01394* | Bovine 98.9% (89/90) |
| | Actin* | *551* | *K00623*<br>X52815<br>AK056683 | Bovine 98.0% (497/507)<br>Rat 92.6% (338/365)<br>Human 91.4% (349/382) |
| | Osteonectin mRNA | 676 | J03233<br>BC004974<br>NM_003118 | Bovine 99.1% (525/530)<br>Human 89.4% (237/265)<br>Human 89.4% (237/265) |
| | G3PDH mRNA | *190* | *AF140546*<br>X94251 | Ovine 93.4% (71/76)<br>Pig 95.8% (46/48) |
| | Ferritin heavy chain mRNA | 488 | AF540563<br>AB003903<br>U54800 | Bovine 98.2% (386/393)<br>Bovine 97.9% (385/393)<br>Ovine 96.2% (379/394) |
| | Decorin mRNA/Bone proteoglycan II | 797 | Y00712<br>AF125041<br>AF125537 | Bovine 98.5% (645/655)<br>Ovis 98.2% (640/652)<br>Pig 94.3% (615/652) |
| NR | Pancreatic anionic trypsinogen* | 417 | AF453325 | Bovine 93.2% (218/234) |
| | Clone RP42-354B6 | 474 | AC091660 | Bovine 90.1% (109/121) |
| | Satellite DNA fragment | 360 | V0012J.0026<br>V00123<br>V00121 | Bovine 89.4% (219/245)<br>Bovine 88.6% (217/245)<br>Bovine 85.1% (212/249) |

** indicates transcripts with multiple clones sequenced*



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Table 2. A sub set of genes identified as up-regulated during adipocyte differentiation

| Clone ID | Accession Number | Gene Name |
|---|---|---|
| g4h03 | NM_173991 | *Bos taurus* Apolipoprotein E |
| g1a05 | NM_174077 | *Bos taurus* Glutathione Peroxidase (GPx plasma isoform) |
| g4j03 | NM_001014860 | *Bos taurus* Heterogeneous Nuclear Ribonucleoprotein F |
| f8k22 | S65367 | *Bos taurus* Leucine Aminopeptidase |
| a1g09 | NM_181016 | *Bos taurus* serum amyloid A protein |
| f6k21 | NM_001034627 | *Bos taurus* similar to Gelsolin precursor |
| f6m23 | NM_001038125 | *Bos taurus* similar to WD-repeat protein |
| g3i21 | g3i21 | g3i21 |
| f8p01 | NM_001034398 | Homo sapiens Nucleoside transporter, member 1 |
| f5n06 | XM_591873 | Predicted: *Bos taurus* Complement factor B precursor |
| f6c15 | XM_616962 | Predicted: *Bos taurus* L-type amino acid transporter 2 |
| f5o05 | XM_874332 | Predicted: *Bos taurus* Mid-1-related Chloride Channel 1, variant 4 |
| g2d01 | Unknown | Unknown |



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Table 3. A subset of genes down-regulated during adipogenesis

| Clone ID | Accession Number | Gene Name |
|---|---|---|
| f7n10 | AV665945 | *Bos taurus* Adipocyte cell line *Bos taurus* cDNA clone |
| f7b07 | BC104601 | *Bos taurus* cDNA clone IMAGE:8121028 |
| a2b12 | AC089993 | *Bos taurus* clone RP42-553M7 |
| f5n03 | NM_001024562 | *Bos taurus* Epithelial membrane protein 3 |
| f7g23 | NM_176610 | *Bos taurus* Milk fat globule-EGF factor 8 protein |
| g4j17 | NM_174137 | *Bos taurus* Serpine1 |
| f5m03 | BTU84139 | *Bos taurus* Structure-specific recognition protein 1 |
| g3c21 | g3c21 | g3c21 |
| g3k05 | g3k05 | g3k05 |
| g3m21 | g3m21 | g3m21 |
| g4g20 | AL139128 | Human gene for a novel protein |
| f8d19 | NM_001013593 | Mitochondrial branched chain aminotransferase precursor |
| f5e20 | BC043319 | Mus musculus Microtubule-actin crosslinking factor 1 |
| g1b04 | BC021481 | Mus musculus Myosin IC |
| f7g24 | XM_882815 | Predicted: *Bos taurus* Calcyphosine isoform b, transcript variant 2 |
| f8g13 | XM_589036 | Predicted: *Bos taurus* calmodulin 2 |
| f8k01 | XM_876108 | Predicted: *Bos taurus* Heterogeneous nuclear ribonucleoprotein G |
| a2b11 | AY160683 | Predicted: *Bos taurus* hypothetical protein |
| g4j21 | XM_614379 | Predicted: *Bos taurus* insulin-like growth factor-binding protein 6 |
| f5g22 | XM_581318 | Predicted: *Bos taurus* Procollagen, type V, alpha 2 |
| a2a10 | XM_580302 | Predicted: *Bos taurus* similar to Y+L amino acid transporter 1 |
| f7c06 | XM_594151 | Predicted: *Bos taurus* Sodium-dependent vitamin transporter |
| g4o16 | XM_874741 | Predicted: *Bos taurus* Thioredoxin interacting protein isoform 2 |
| a2a12 | NM_001034039 | Pro alpha 1(I) collagen [Bos taurus] |
| g1o20 | D84482 | Rattus norvegicus PMSG-induced ovarian mRNA |
| g4k20 | NM_174175 | Seryl-tRNA synthetase [*Bos taurus*] |
| g4d21 | XM_874313 | Similar to Proprotein convertase subtilisin/kexin type 7 precursor |
| g4i16 | CX954053 | UMC-bof_0A01-009-h08 Ovarian Follicle bof *Bos taurus* |
| a2a08 | Unknown | Unknown |
| f5n02 | Unknown | Unknown |



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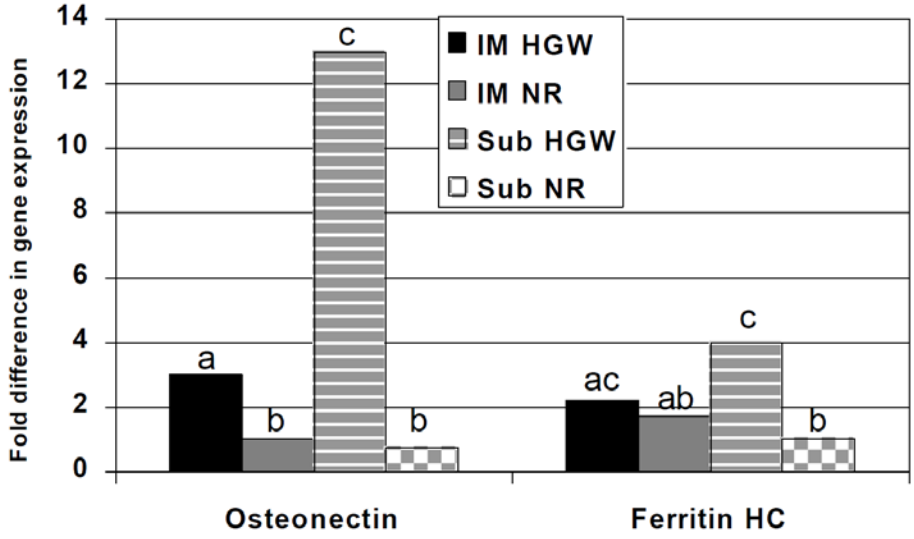
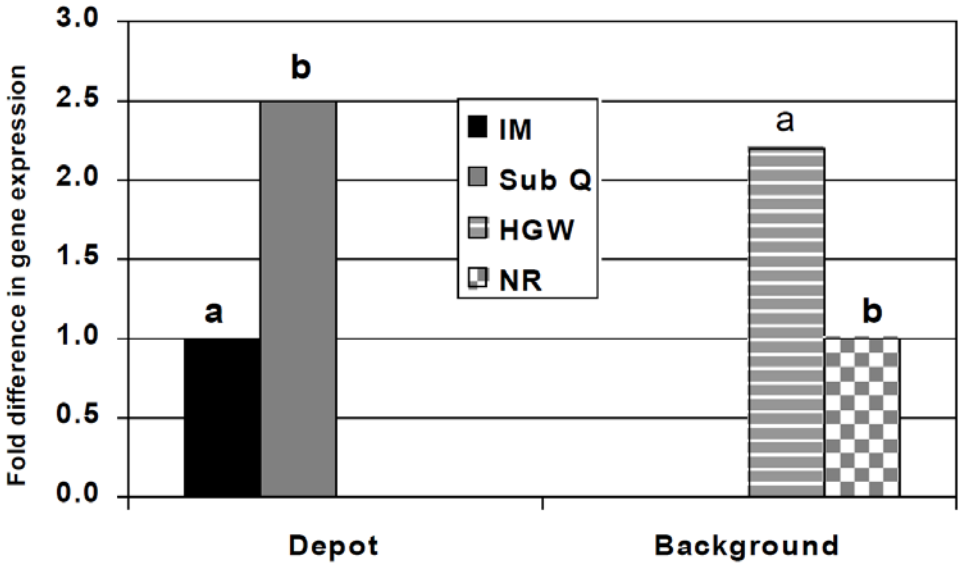
Figure 1. Effect of depot and background on gene expression in adipose tissue from steers after Grazing




[a,b,c] Means within gene with different superscripts differ (P < 0.05)

Figure 2. Effect of depot and background on decorin gene expression in adipose tissue from steers after grazing




[a,b] Means within category with different superscripts differ (P < 0.05)



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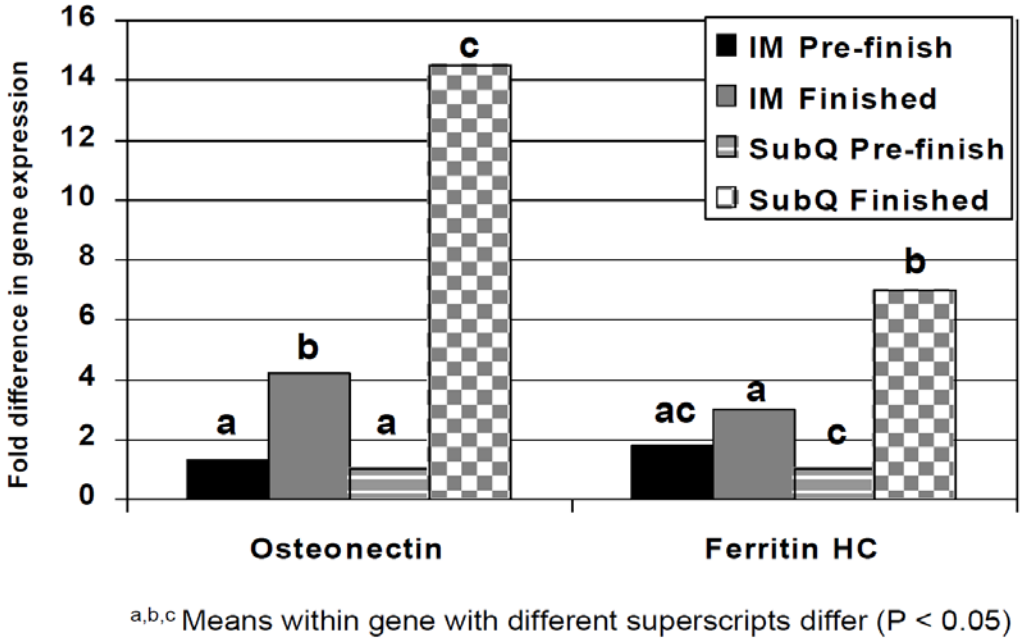
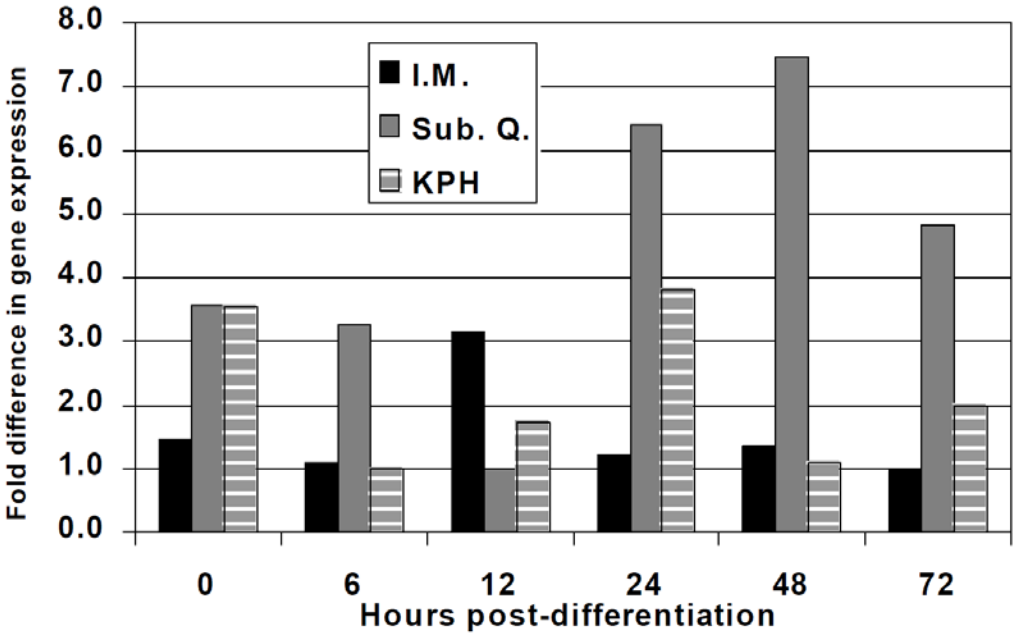
Figure 3. Effect of depot and harvest on gene expression in adipose tissue from native range steers




[a,b,c] Means within gene with different superscripts differ (P < 0.05)

Figure 4. Comparison of primary cultured bovine adipocyte PPARγ gene expression




Depot, Time, and Depot x Time P < 0.001



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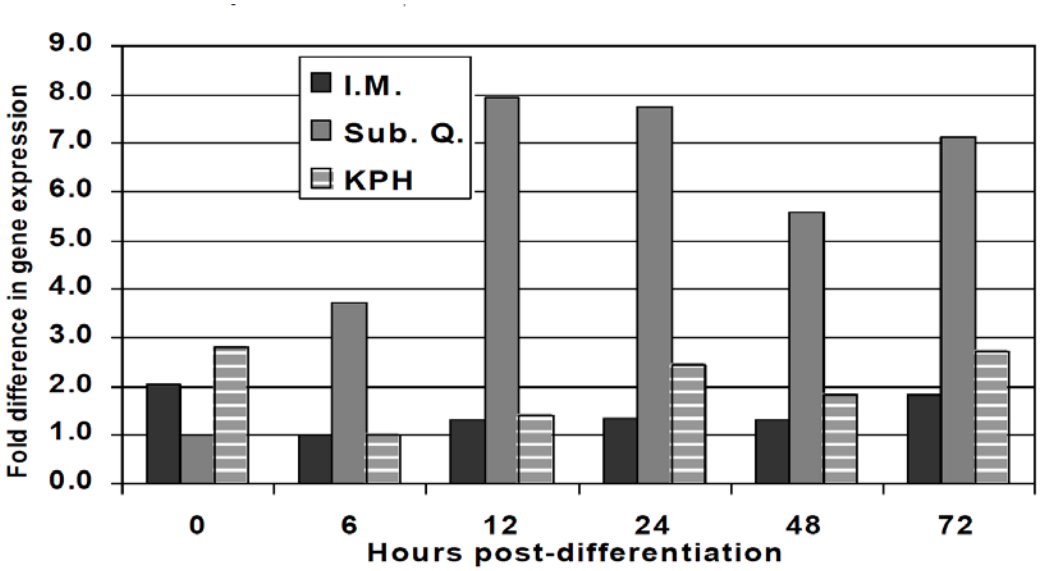
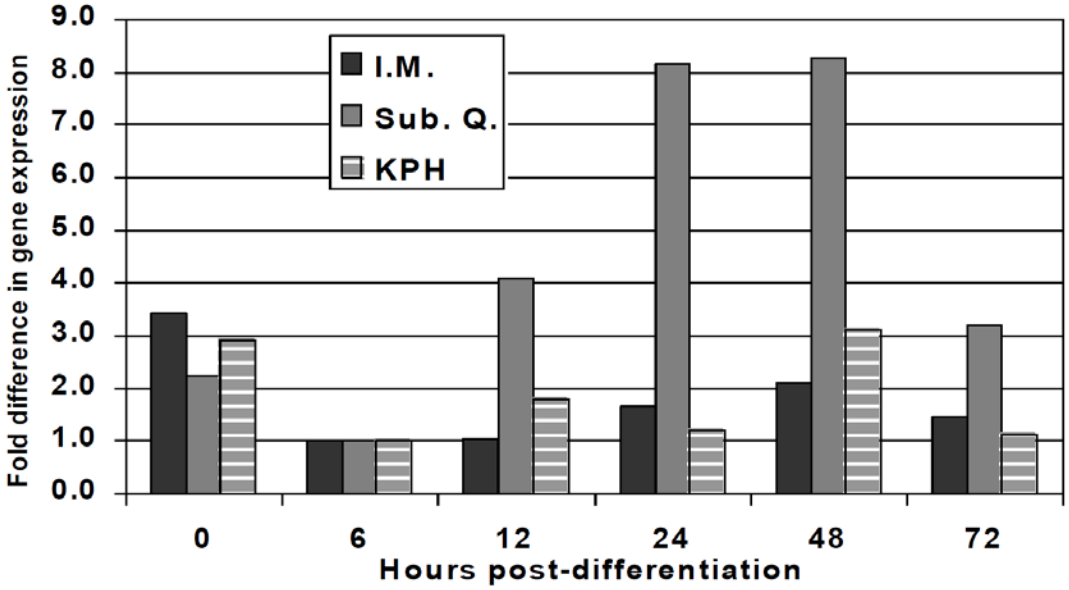
Figure 5. Comparison of primary cultured bovine adipocyte stearoyl-CoA desaturase gene expression




Depot P = 0.11, Time P = 0.17, and Depot x Time P = 0.04

Figure 6. Comparison of primary cultured bovine adipocyte lipoprotein lipase gene expression




Depot P = 0.82, Time P < 0.001, and Depot x Time P < 0.001



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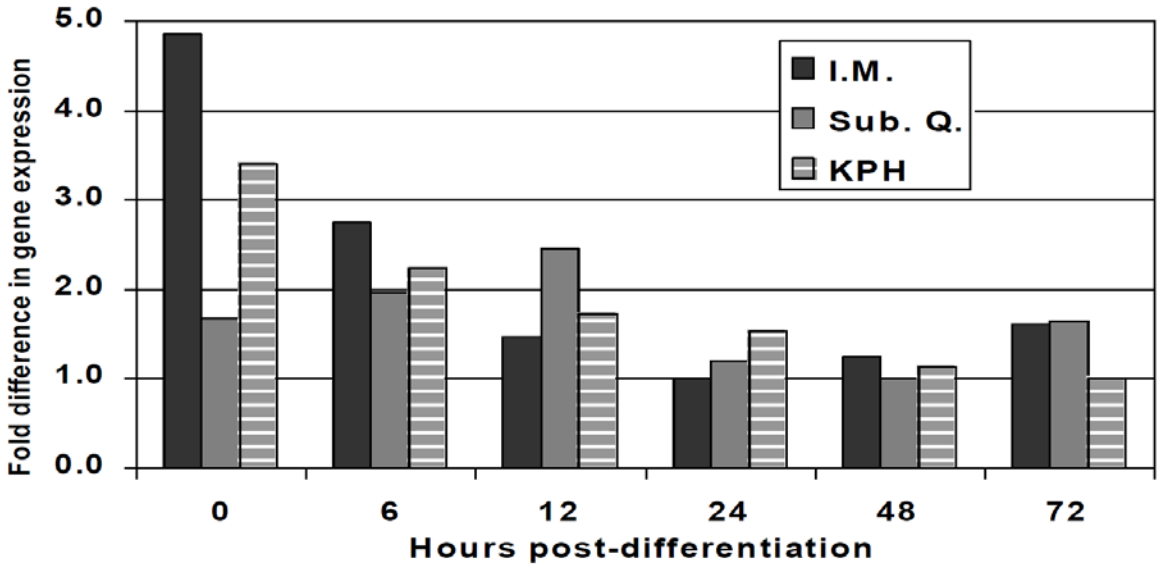
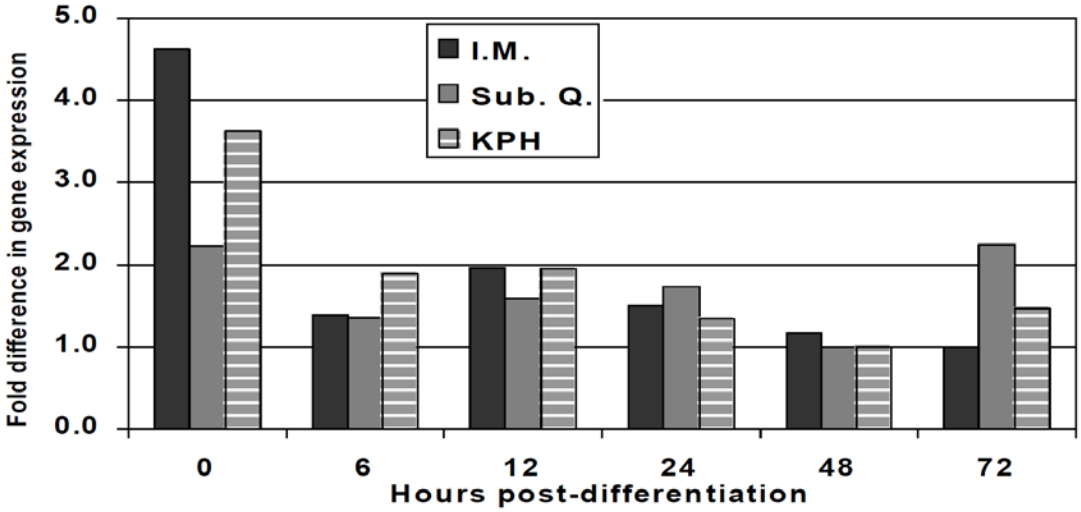
Figure 7. Comparison of primary cultured bovine adipocyte fatty acid synthase gene expression




Depot P = 0.16, Time P < 0.001, and Depot x Time P < 0.001

Figure 8. Comparison of primary cultured bovine adipocyte acyl-CoA synthetase gene expression




Depot P < 0.001, Time P < 0.001, and Depot x Time P = 0.002



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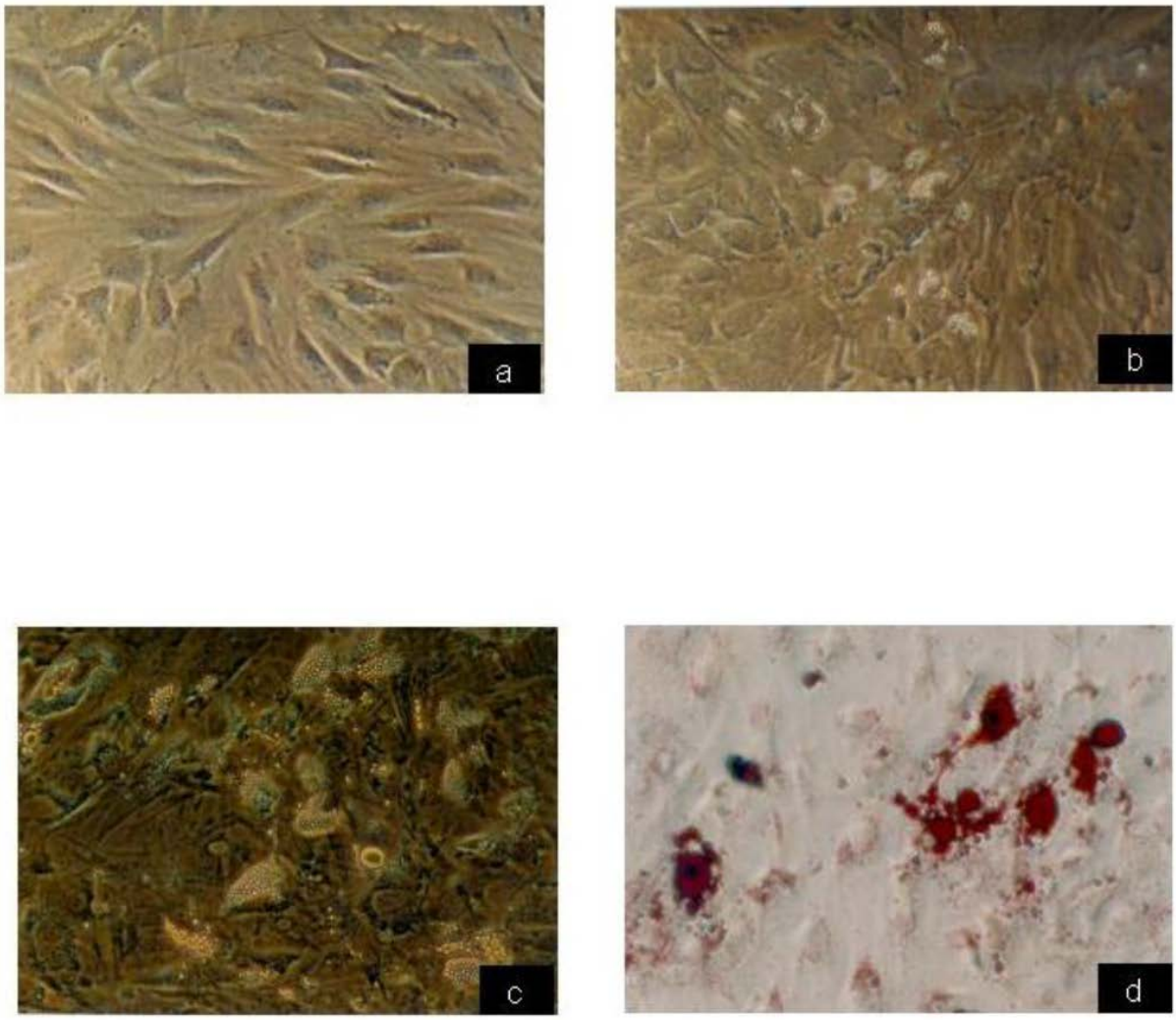
Figure 9. Microscopic images of Stromal Vascular Cells at different stages of differentiation




(a) Confluent SVCs morphologically similar to fibroblasts. (b) Differentiating adipocytes 48 hours, cells started showing change in morphology by 12 hours and the lipid droplets were apparent by 48 hours post induction. (c) Differentiating adipocytes 120 hours post induction, cytoplasm of the cells were filled with lipid droplets by 120h of differentiation. (d) Oil Red O stained lipid droplets in the cytoplasm (120 hours).



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