EFFECTS OF FATTY ACIDS ON GENE EXPRESSION AND LIPID METABOLISM IN BOVINE INTRAMUSCULAR AND SUBCUTANEOUS ADIPOSE TISSUES

A Dissertation

by

DAVID TYRONE SILVEY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2011

Major Subject: Nutrition

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Approved by:

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ABSTRACT

Effects of Fatty Acids on Gene Expression and Lipid Metabolism in Bovine Intramuscular and Subcutaneous Adipose Tissues. (August 2011)

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Chair of Advisory Committee: Dr. Stephen B. Smith

Pasture feeding depresses adipose tissue development in beef cattle whereas grain feeding, enhances adipogenesis. Therefore, we hypothesized that specific fatty acids would differentially affect lipogenesis in explants of bovine subcutaneous (SC) and intramuscular (IM) adipose tissues. Angus steers were harvested at 12, 14, and 16 mo of age, and IM and SC adipose tissue explants from the 8-11th thoracic rib region were dissected and cultured in media. Media contained no supplemental fatty acids or 40 μM of five fatty acids, stearic acid (18:0), oleic acid (18:1 n-9), *trans*-11 vaccenic acid (18:1 *trans*-11), conjugated linoleic acid (CLA, 18:2 *trans*-10, *cis*-12), or α-linolenic acid (18:3 n-3). After 48 h of culture, lipogenesis using [U-^{14}C]glucose and [1-^{14}C]acetate was measured. Lipogenesis from glucose decreased between 12 and 16 mo of age in SC adipose tissue (from 8.9 to 4.0 nmol per 2 h per 100 mg; $P = 0.001$) and IM adipose tissue (from 4.4 to 2.7 nmol per 2 h 100 mg ; $P = 0.08$). Lipogenesis from acetate did not change over time in SC adipose (approximately 56 nmol per

2 h per 100 mg; $P = 0.23$), but increased over time in IM adipose tissue (from from 11.3 to 17.1 nmol per 2 h 100 mg; $P = 0.02$). Oleic acid increased lipid synthesis from glucose 125% ($P = 0.04$) in IM adipose tissue, whereas stearic acid and *trans*-vaccenic acid increased lipogenesis from glucose in SC adipose tissue by approximately 50% ($P = 0.04$). In SC adipose tissue only, *trans*-vaccenic and increased, lipogenesis from glucose ($P < 0.02$). Lipogenesis from acetate was depressed by CLA nearly 50% in SC adipose tissue. PPARγ gene expression increased between 14 and 16 mo of age in control IM and SC adipocytes. The increase in activity was also observed in AMPK gene expression. C/EBPβ and SCD gene expression did not increase in control samples until 16 mo of age. SC adipose tissue responded to stearic acid by increased GPR43 and AMPK gene expression at 12 mo of age. We conclude that fatty acids differentially affect lipid synthesis in IM and SC adipose tissues, which may account for the effects of pasture and grain feeding on adiposity.

DEDICATION

I dedicate this dissertation and all the work that went into it to my loving family; Bruce Silvey, Lark Silvey, Amy Silvey, Katelyn Silvey, David Silvey II, Ashley Silvey, Savannah Silvey-Holik, David Holik, Brooklyn Holik, Landon Holik, and Holden Holik. It is their encouragement, support, commitment, love, and faith in me that has kept me going every day.

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NOMENCLATURE

ACC	Acetyl CoA carboxylase
AMPK	AMP-activated protein kinase
ATP	Adenosine Triphosphate
C/EBP-β	CCAAT-enhancer-binding protein-β
CLA	(*cis* 9, *trans* 12) Conjugated linoleic acid
CPT1	Carnitine palmitoyltransferase 1
FAME	Fatty acid methyl ester
FFA	Free fatty acids
GPR43	G-protein receptor 43
HDL	High density lipoprotein
IM	Intramuscular
MUFA	Monounsaturated fatty acid
MUFA:SFA	Monounsaturated:saturated fatty acid ratio
LDL	Low density lipoprotein
PPARγ	Peroxisome proliferator-activated receptor gamma
PUFA	Polyunsaturated fatty acids
SC	Subcutaneous
SCD	Stearoyl-CoA desaturase

SFA	Saturated fatty acids
SV	Stromal vascular cells
TVA	*Trans*-11 vaccenic acid
VFA	Volatile fatty acids
VLDL	Very low density lipoprotein

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INTRODUCTION

The ultimate goal of beef producers is to produce a high-quality product that maximizes their profits. The United States Department of Agriculture (USDA) classifies the quality of beef into four basic categories: Prime, Choice, Select, and Standard (the lowest quality). The two criteria for quality grade are marbling (intramuscular adipose tissue) and degree of maturity (physiological age of the animal). Ultimately, the single most important factor to the consumer is taste (1). The savory taste of a high-quality beef comes from the intramuscular adipose tissue depots (1). American's consume 39.3 kg and annually spend an average of $235 on beef per capita (2). Ultimately, beef producers want to preserve the intramuscular adipose depot (to maintain high-quality beef) while reducing the amount of subcutaneous adipose tissue because of its effect on profitability. Insufficient intramuscular adipose tissue and excessive subcutaneous adipose tissue are among the top beef quality challenges (1). In 2009, 26.5 million steers were slaughtered in the United States. Lower quality beef could potentially cost the producers between $21 and $27 per head resulting in a $1.3 billion dollar loss.

Fat deposition in finishing animals is the result of both hyperplasia and hypertrophy (3). Fatty acids deposited in the adipocyte tissues have two origins: from the diet or from de novo fatty acid synthesis. Finishing system can dramatically alter fat deposition and composition, indicating that enzymes involved in lipogenesis are responsive to both dietary energy level

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This dissertation follows the style and format of The Journal of Nutrition.

and source.

Altering a diet can dramatically alter fat deposition and fatty acid composition in, indicating that enzymes involved in lipogenesis are reactive to the diet. Studies have compared (4) the effects of calf and yearling feeding on adiposity, gene expression, and fatty acid composition in Angus steers. Research has found that at the 12th rib, fat thickness and marbling scores were not statistically different (4). However, there were profound differences in the monounsaturated:saturated fatty acid (MUFA:SFA) ratio and SCD gene expression. Keeping cattle on a basal diet of forage the reduced oleic acid (18:1 n-9) content. Greater amounts of oleic acid had a stimulatory effect on IM and SC adipocytes differentiation. Finishing cattle on high-grain such as corn based diets increases lipid deposition rates and alters the fatty acid composition of adipose tissues (5). Monounsaturated fatty acid (MUFA) concentrations increase in a linear manner with increasing time on a corn-based diet. High-concentrate diets and diets supplemented with corn oil have been found to increase carcass fatness (6) in finished steers. High-concentrate diets can also alter the composition of the fatty acids in the adipose tissues (7) compared to cattle that were pasture fed to finishing weight. Studies have (8) found that feeding cattle α-linolenic acid (18:3 n-3) (ALA) or CLA depressed marbling scores. High-concentrate diets especially diets that contain seed oils are abundant in polyunsaturated fatty acids (PUFA). The two most abundant PUFA found in high- concentrate diets are ALA and linoleic acid. Studies (9) found that increasing concentrations of corn oil and forage resulted in increased

biohydrogenation concentrations of ALA in cattle fed feedlot diets to *trans*-11 vaccenic acid (TVA). A portion of these PUFA bypasses the rumen and are deposited directly in adipocytes. Pasture fed cattle usually have lower marbling scores which could be attributed to increased lipolytic gene (i.e. SCD) activity.

The majority (70%) of fat that accumulates during postnatal growth is due to adipocyte hypertrophy (10). Research (3) has suggested that SC adipose tissue is an earlier developing depot and that hyperplasia is nearly complete by approximately 8 mo of life. Research (3) has found that the processes of developing adipose tissue occurs early on cattle development. The greater amount of total fatty acids (per gram of subcutaneous adipose tissue) suggests that adipocytes hypertrophy occurs during the finishing diet and increased dietary energy stimulated SC adipocyte filling (11).

The exact mechanisms and stimulatory factors that support lipid filling are not fully understood but current work on lipogenic genes is increasing our knowledge. Within the last decade, fatty acids have gained significant attention in the arenas of energy intake and glucose disposal with obvious implications for the treatment of insulin resistant diabetes mellitus and obesity. For example, grain-fed cattle have been shown to increase the expression of SCD gene expression (8) which increases the amount of MUFA (i.e., oleic acid) in bovine adipose tissue. Increased oleic acid will increase the healthiness of the final consumer product and have increased marbling score of the steer. Steers with higher marbling scores will grade higher and the fat deposited will contain a healthier profile of fatty acids.

REVIEW OF LITERATURE

IM vs SC depots

Multiple scientific studies indicate that SC and IM adipose tissue are different in metabolic function and processes. Subcutaneous adipose tissue synthesizes fatty acids *de novo* at a higher rate than IM adipose tissue (12,13), even at a constant volume (13). Glucose is utilized at a higher rate for fatty acid biosynthesis in IM adipose tissue than in SC adipose tissue (12,13). Subcutaneous adipose tissue contains larger adipocytes and a higher rate of fatty acid esterification, except for palmitate, than IM adipose tissue (14). The rate of exogenous fatty acid incorporation into adipose tissue is dependent on the fatty acid concentration in medium (14). We wanted to investigate the effect individual fatty acids had on IM and SC adipose tissue. For example, as cattle consume a diet higher in PUFA, the amount of PUFA deposited into the tissue is increased. Increasing SCD gene expression in the steers diet such as grain feeding increased the amount of oleic acid in the IM adipose tissue (8). Increasing the amount of MUFA deposited in the adipose tissue decreases the amount of saturated fatty acid (SFA) deposited in the adipose tissue. Producing a lower amount of palmitate, IM tissue may have to rely on exogenous sources of palmitate to make various cellular products. To date, there is no mechanistic information about the effect of specific fatty acids on lipogenesis and gene expression of IM and SC adipose tissue from growing steers.

Carnitine palmitoyltransferase-1

Carnitine palmitoyltransferase-1 (CPT1) is a rate-limiting enzyme in fatty acid β-oxidation. The primary function of CPT1 is regulating the entrance of long-chain fatty acids into the mitochondria for ATP production (15). Beta oxidation occurs primary in the mitochondria where long-chain fatty acids have to cross the mitochondrial membranes through the carnitine palmitoyltransferase system (16). The location of CPT1 is on the outer membrane of the mitochondria, where it catalyzes the transfer of the acyl group from acylcoenzyme A complexes to carnitine, producing acylcarnitine. Controlling the activity of CPT1 could provide one mechanism for regulating fatty acid β-oxidation. Being able to alter fatty acid β-oxidation shows the important role CPT1 plays in a feedback loop associated with the energy status of the animal (17). Inhibiting CPT1 with compounds such as etomoxir caused intracellular lipid accumulation and insulin resistance in rats (18) demonstrating another mechanism for controlling the metabolism of fatty acids. Studies (19) have reported increased CPT1 activity leads to improved insulin sensitivity in high-fat overfed rats. Increasing or decreasing the activity of CPT1 in young steers is another potential means for altering energy homeostatsis.

CCAAT-enhancer binding proteins

CCAAT-enhancer binding protein (C/EBP) are involved in adipocyte proliferation, metabolism, and differentiation. C/EBP-β binds to DNA-sequences (known as CCAAT boxes) that are promoters of genes involved in adipogenesis (19). In rats, C/EBP-β is known

to regulate acetyl-CoA carboxylase (ACC) activity (20). ACC is the rate-limiting enzyme in fatty acid synthesis (21). Other studies have shown that following differentiation, expression and activation of C/EBP-β is upregulated (22).

AMP-activated protein kinase

AMP-activated protein kinase (AMPK) is a critical regulator of mitochondrial biogenesis and once activated oxidative metabolism is amplified (23). AMPK is regulator of energy homeostasis that is activated in response to energy deprivation. Reducing ATP production could be a side effect of metabolic syndrome (24). AMPK allows cells to increase ATP production (25). Therefore, AMPK functions as a cellular energy sensor, and inactivates energy-consuming processes such as lipogenesis and cholesterol biosynthesis, while activating energy producing processes such as fatty acid oxidation (26). AMPK is specifically activated by increases in ATP utilization or decreases in ATP production caused by metabolic stress (27). In addition to increases in the AMP to ATP ratio, AMPK is activated by rises in intracellular Ca^{2+} (28). AMPK regulates the synthesis and utilization of fatty acids by phosphorylating ACC and modifying gene transcription. The decrease in malonyl-CoA that occurs in muscle during exercise or in response to electrical stimulation is accompanied by a decrease in activity of ACC, the enzyme that synthesizes malonyl-CoA (29). The re-esterification of long chain fatty acids that occurs during lipolysis leads to a reduction of ATP and an increase in the AMP:ATP ratio, which activates AMPK (30). Further studies have demonstrated that fatty acids themselves can lead to an increased

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activation of AMPK (31). An activation of AMPK was demonstrated in cultured rat cardiomyocytes that were treated with palmitate and oleate (32). Studies in rodents show that dietary PUFA (sterol-free fish oil) attenuate the decrease in hepatic AMPK activity observed with fat-free feeding (33). Indicating a direct role PUFA plays in AMPK activity.

G protein-coupled receptor 43

G protein-coupled receptor 43 (GPR43) a receptor for short-chain fatty acids. Shortchain fatty acids also know as volatile fatty acids (VFA) are produced by microbial fermentation in the gastrointestinal tract (34). Acetic, propionic, and butyric acids constitute approximately 95% of total VFA produced in the rumen by microbial fermentation (34). VFA are the major source of energy and substrate for fatty acid synthesis in ruminants. Approximately 60 to 70% of the energy requirement of cattle and sheep is provided by rumen VFA (35). GPR43 is expressed in a number of tissues including adipocytes, and the activity of GPR43 can be enhanced in adipocytes during adipocyte differentiation and high fat feed in rats (36). Research has found (36) found GPR43 activity to be highly expressed in isolated adipocytes but minimally expressed in stromal vascular (SV) cells. Once activated by compounds such as acetate, GPR43 stimulates adipogenesis and inhibits lipolysis (36,37). Using adipocytes from GPR43 acetate and propionate are mediated through the activation of GPR43. GPR43 couples to the Gi pathway in adipocytes, and activation of GPR43 in adipocytes leads to inhibition of lipolysis and suppression of plasma FFA levels. Reducing lipolysis in IM adipose tissue would likely increase marbling. Therefore, activation of GPR43 is not only a

potential controlling point of marbling but also has the potential to change plasma lipid profiles, which could have positive effects on other lipogenic genes. Increasing or decreasing activity of lipogenic genes will ultimately have an effect on lipid accumulation in tissues such as IM. The complete role of GPR43 on lipid accumulation and disposal has not been fully elucidated.

Peroxisome proliferator-activated receptor γ

Activation of PPARγ results in increased whole body insulin sensitivity although the precise mechanisms involved are not completely understood (38). PPARγ is expressed abundantly in bovine adipose tissue (39) and is required for proper adipose tissue development. Differentiation of preadipocytes is transcriptionally initiated by glucocorticoids (40) and a ligand-activated nuclear receptor PPARγ (39). The primary purpose of glucocortoids in the differentiation of the preadipocytes is up-regulating or activating PPARγ (40). Glucocortoids (such as dexamethasone) increase differentiation of bovine stromal-vascular (SV) cells (preadipocytes) (41), and increase arachidonic acid metabolism in preadipocytes (42). During adipocytes differentiation, which develops from PPARγ's activation, expression of numerous genes specific for fatty acid metabolismis induced. A good example of PPARγ relationship to adipogenesis comes from recent observations in PPARγ knock-out mice. PPARγ -/- mice are completely absent of adipose tissue and PPARγ +/- mice have decreased adipose tissue (43,44). Accumulation of adipose tissue leads to obesity, whereas its absence is associated with lipodystrophic syndromes. Increasing adipose tissue in humans is not

favored, however, in cattle adipogenesis is encouraged.

Fatty acids have been shown to activate PPARγ (45), including oleic acid and α-linolenic acid (46). Research (47) has found a difference between the differentiation of preadipocytes from SC and omental fat when treated with a triacylglycerol mixture (intralipid). This difference in adipocyte depot response to fatty acids demonstrates how distinctly different fat depots behave. Once activated, PPARγ increases transcription of adipogenic genes (48). In the case of a bound ligand, PPARγ dimerizes with a retinoid X receptor. The PPARγ – retinoid X receptor dimer stimulates differentiation of adipocytes by binding to the promoters of adipogenic genes (45). PPARγ ligand-activated transcription factors are implicated in such diverse pathways as lipid and glucose homeostasis, control of cellular proliferation, and differentiation. An example of one of these ligands that has a high affinity to PPARγ is troglitazone (a PPARγ agonist) (49). Research Grant (41) has found inherent differences between the differentiation of preadipocytes from SC and IM adipocytes treated with dexamethosone and troglitazone. Once bound to the PPARγ ligand, troglitazone was found to enhance differentiation of bovine SV cells (41). PPARγ also has been shown to increase differentiation of IM SV cells from Japanese Black cattle (50).

Stearoyl-CoA desaturase

SCD plays a major role in adipocyte hypertrophy (51-53). SCD is a delta-9 fatty acid desaturase that converts SFA into MUFA. This oxidative reaction is catalyzed by the iron containing SCD and involves cytochrome b5, NADH (P)-cytochrome b5reductase, and

molecular oxygen. The preferred substrates are palmitoyl-CoA and stearoyl-CoA, which are desaturated to palmitoleoyl-CoA and oleoyl-CoA, respectfully. Palmitic and oleic acid are the most abundant fatty acids in phospholipids, cholesterol esters, and wax esters (54).

Steers fed identical high-roughage diets, Wagyu SC lipids had a greater MUFA:SFA ratio than Angus adipose tissue lipids (55). Even with the increased MUFA:SFA ratio, SCD enzyme activity and gene expression were similar in the SC adipose tissues of the Wagyu and Angus steers (56). Increased SCD activity and changes in the balance between SFA and MUFA have been implicated in various diseases including cancer, diabetes, atherosclerosis, and obesity (57). Increases in cellular SCD activity have been found to influence fatty acid partitioning by promoting fatty acid synthesis while decreasing oxidation (58). Research (58) has reported that the SCD gene is also highly expressed in skeletal muscle from extremely obese humans and from obese insulin- resistant Zucker diabetic fatty rats (59). In the human study, (58) observed elevated SCD expression associated with decreased fatty acid oxidation, increased triacylglycerol synthesis and increased monounsaturation of muscle saturated fatty acids. In fact, studies have shown that the reduced MUFA synthesis occurs in SCD -deficient mice and these mice attain protection from obesity, cellular lipid accumulation and insulin resistance (60). SCD gene expression can be used as a marker for terminal differentiation (i.e., when preadipocytes leave the proliferative phase, express lipogenic enzymes, and begin lipid filling (61, 62).

In bovine adipose tissue, lipogenic enzyme activities and lipogenesis from acetate are

almost undetectable at weaning, despite extensive lipid filling of adipocytes (11). SCD levels are elevated by both dietary and hormonal factors, such as glucose, fructose, SFA and insulin, but repressed by PUFA (60). Research (63) demonstrated that the MUFA:SFA ratio increased from approximately 0.9 to 1.3 in bovine SC adipose tissue during the first 8 mo post-weaning. The increase in bovine SC adipose tissue suggests that desaturase enzyme activity increased with age during this period. Research (64) found SC adipocytes experience hyperplasia between 4 and 7 mo of age and between 13 and 16 mo of age. Pasture versus corn diets both has yielded much different SCD gene expression (8). For example, SCD activity was found to be strongly depressed in SC andIM of cattle finished on pasture diets. Grain diets increased SCD expression and the increased expression of SCD gives the adipose a higher amount of MUFA (8). Stearic acid is one of the main substrates for SCD activity. The 18-carbon fatty acids (i.e. α-linolenic acid) present in pasture and the high-concentrate cattle diets would have been hydrogenated largely to stearic acid in the rumen (65). Research (51) has found elevated SCD mRNA in SC adipose tissue from post-weaning calves was caused by up-regulation of SCD gene expression in response to dietary 18-carbon fatty acids. In preadipocytes, SCD mRNA is virtually undetectable during the proliferative phase; upon differentiation, SCD mRNA levels increase 30-fold as the result of increased SCD gene transcription (66, 67). Inhibiting SCD gene activity in adipose tissue causes *trans*-11 vaccenic acid (TVA) to accumulate. The accumulation of TVA may be the compound that inhibits adipocytes differentiation via interacting with PPARγ resulting in less marbling.

Hypothesis and objectives

We hypothesized that fatty acids would differentially affect gene expression, cellularity and lipid metabolism in bovine IM and SC adipose tissues. The exact mechanisms and genetic differences between IM adipose depot and SC adipose depots are unknown. However, there is a significant amount of evidence that both depots respond differently to various treatments. Based upon our results we have observed different responses to treatments between IM and SC adipose tissues. The main goal of this study was to gain a better understanding of how fatty acids can influence IM and SC adipose depots. One of the greatest strengths of our research model system is that we can test the effects of individual fatty acids on tissues that contain a mixture of differentiated adipocytes. Being able to look at individual fatty acids is a useful tool allowing us to see what effects individual can have on explants adipose tissue. From this knowledge, effective feed programs can be implemented that increase IM adipose depot for a high quality grade while decreasing the wasteful SC adipose depot. For example, increasing SCD gene expression is consistent with the elevation in total MUFA and the SCD index observed in both IM and SC adipose tissue with age (4). More MUFA means the product will be healthier for consumers and more marketable. And increasing the IM adipose tissue in finished steers will cause the steers to grade higher, therefore, the producer will make more money per head of cattle. In conclusion, increasing the amount of IM adipose adipose tissue in steers and reducing SC adipose tissue will ultimately help producers make a better product for consumers and earn higher profits.

MATERIALS AND METHODS

Animals and diets

Procedures for this research were approved by the Texas A&M University Institutional Animal Care and Use Committee, Office of Research Compliance. Experimental procedures were approved by the Texas A&M University Animal Care and Use Committee, Office of Research Compliance. Twelve Angus steers were purchased as calves at weaning (approximately 8 mo of age; 210 kg) and then transported to Texas A&M University Research Center at McGregor. Steers were fed a corn-based diet (Table 1) until the appropriate age was achieved (12, 14, and 16 mo of age). Steers were harvested at Texas A&M University Rosenthal Meat Science and Technology Center.

Sample collection

At 12, 14, and 16 mo of age, IM and SC adipose tissues were collected the 8^{th} to 11^{th} rib section immediately after the hide was removed and placed in 37°C, oxygenated Kreb-Henseleit buffer (KHB) containing 5 mM glucose and transported to the laboratory.

Isolation and culturing of adipose tissues

Intramuscular and SC adipose explants were isolated under sterile conditions and placed immediately into six 35-mm welled culture dishes pre-filled with 3 mL of 37°C differentiation medium (41). The differentiation medium consisted of base medium [DMEM (Invitrogen 31600-034), antibiotic-antimycotic [100 units of penicillin, 0.1 mg streptomycin,

and 0.25 μg amphotericn B per mL, 50 μg/mL gentamicin sulfate, 33 μM biotin, 17 μM pantothenate, 100 μM ascorbate], and 1% bovine serum albumin (fatty acid free), 280 nM bovine insulin, 0.25 μM ciglitizone, 5 mM glucose. Treatment medium was without (control) or with addition of fatty acids. Stearic acid (18:0), oleic acid (18:1 n-9), α-linolenic acid (18:3 n-3), *trans*-11 vaccenic acid (18:1 *trans*-11), and 18:2 *trans*-10, *cis*-12 (CLA) were purchased from Matreya, Inc. (Pleasant Gap, PA). Fatty acids were solubilized in 100% ethanol and then dissolved in differentiation medium containing 5% fatty acid-free bovine serum albumin by stirring for at least 2 h. Dissolved fatty acids were diluted to 40 μM with differentiation medium. Adipose tissues were incubated (NuAire Water-Jacketed Incubator, Model NU-4750) for 48 h at 39°C and 5% CO_2. The differentiation media was changed after the first 24 h to ensure the tissue had fresh reagents and fatty acids. Treatments were applied to IM and SC adipose tissue in each of six, 35-mm diameter wells.

Effects of fatty acids on bovine adipose tissue lipid accumulation

At sample collection and after 48 h explants culture, 2 h *in vitro* incubations were conducted with SC and IM adipose tissue (~100 mg) as described previously (68). Flasks contained 5 mM glucose, 5 mM acetate, 10 mM HEPES buffer and 1 μCi [U-^{14}C]glucose or 1 μCi [1-^{14}C]sodium acetate (American Radiolabeled Chemicals, Inc) in KHB buffer. Vials were gassed for 1 min with 95% O_2:5% CO_2 and incubated for 2 h in a shaking water bath at 37°C. At the end of the incubation period, reactions were terminated by addition of 1 M of

trichloroacetic acid. Flasks were shaken for an additional 2 h. Neutral lipids in tissues were extracted using the (69) procedure, evaporated to dryness, resuspended in 10 mL of scintillation cocktail, and radioactivity was counted with the scintillation counter (Beckman Instruments, Palo Alto, CA).

Fatty acid composition

Adipose tissue lipid was extracted by the modification of the Folch (69) method. Approximately 100 mg of tissue was homogenized with 5.0 mL of chloroform:methanol (2:1, v/v) in a homogenizer (Brinkmann Instruments, Westbury, NY) was stoppered with 5.0 mL of chloroform:methanol. Total volume of homogenate was adjusted to 15 mL by adding chloroform:methanol solution. After sitting at room temperature for 30-60 min, the homogenate was vacuum-filtered through a sintered glass filter funnel fitted with a Whatman filter (Whatman Ltd., Maidstone, England) into a glass test tube containing 8 mL of 0.74% KCl (w/v). The filtered sample was vortexed for 30 sec and then centrifuged at 2,000 *x g* for 15 min for separation. After discarding upper aqueous phase, lower phase was evaporated at 60°C with a nitrogen flushing evaporator. The total extracted lipid was used for analysis of fatty acid composition.

Fatty acids were methylated by the modification of (70) method. Approximately 100 mg of total lipid extract was mixed with 1 mL of 0.5 N of KOH in MeOH and heated in water bath at 70°C for 10 min. After cooling to room temperature, 1 mL of 14% BF_3 in MeOH (w/v) was added to sample and then heated in water bath at 70°C for 30 min.

Samples were cooled to room temperature, and 2 mL of HPLC grade hexane and saturated NaCl solution were added and vortexed for 30 sec. Samples were then centrifuged at 2,000 × *g* for 10 min for separation, transferred to 15 mL glass tube containing anhydrous Na_2SO_4 to remove aqueous molecule. Fatty acid methyl ester (FAME) analyzed by GC-FID (model CP-3800 equipped with a CP-8200 auto-sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 (100 m x 0.25 mm ID) (Chrompack Inc., Middleberg, The Netherlands) with helium as carrier gas (flow rate = 1.2 mL/min). One microliter of sample was injected with the split ratio of 100:1 at 270°C. Oven temperature was set at 165°C for 65 min and increased to 235°C (2°C/min) and held for 15 min. Flame ionization detector detected the signal at 270°C. Standard (GLC 68-D, Nu-chek Prep, MN) was used to identify each peak. For calculation of the SCD ratio, *trans*-vaccenic acid was included in total SFA because it is a substrate for the Δ^9 desaturase reaction. Similary, 18:2 *cis*-9, *trans*-11 CLA was used to calculate MUFA because it is a product of Δ^9 desaturase.

Cellularity

SC and IM adipose tissue were collected from the steers and frozen at 4°C for determination of cellularity by osmium fixation, counting, and sizing (71). Tissue was sliced into sections 1 mm thick and placed in 20-mL scintillation vials. Frozen samples were rinsed three times with 37°C 0.154 M NaCl at 1 h intervals to remove free lipid. After the last rinse, 0.6 mL of 50 mM collidine-HCl buffer (pH 7.4) was added to each sample,

resuspended with 0.01% Triton in 0.154 M NaCl, were used for determination of cell size, volume, and cells/100mg tissue, using bright-field microscopy followed by 1.0 mL of 3% osmium tetroxide in collidine. After incubation for 96 h at 37°C, the osmium solution was removed and the tissue rinsed three times with 0.154 M NaCl until clear. Samples were incubated in 10 mL of 8 M urea at 25°C for 96 h. After degradation of connective tissue with urea, cells were rinsed three times with 0.154 M NaCl. Cells were (Olympus Vanox ABHS3, Olympus, Tokyo, Japan), CCD Color Video Camera (DXC-960MD, Sony, Japan).

RNA isolation

Total RNA was isolated from tissue as described previously (72) using Tri-reagent (Sigma Chemical Co., St. Louis, MO). Approximate 200 mg of tissue was homogenized with 2 mL Tri-reagent. After sitting at room temperature for 5 min, 200 μL chloroform was added and vortexed. Samples were centrifuged (12,000 × *g* for 15 min). The upper clear layer was transferred into new tube and inverted gently with 500 μL isopropanol. After sitting at 4°C for 5 min, samples were centrifuged (12,000 × *g* for 10 min) and dried. Samples were washed with 70% EtOH and dried. The pellet was dissolved with 20 μL of nuclease-free H_2O and stored at - 80°C until further analysis. The concentrations and abundance of total RNA were measured with Nanodrop (NanoDrop Technologies Inc., Wilmington, DE) and the quality of total RNA was determined by 1% agarose gel electrophoresis. One microgram of RNA was used for reverse transcription to produce the first-strand complementary DNA (cDNA) using TaqMan Transcription Reagent and MultiScribe

reverse transcription (Applied Biosystem, Foster City, CA) with the following temperature ramp: 25°C for 10 min, 37°C for 60 min, and 95.5°C for 5 min.

Real-time PCR

Real-time PCR on the cDNA produced from AMPK, C/EBPβ, PPARγ, SCD, CPT1, and GPR43 was performed by collaboration at Texas Tech University with the GeneAmp 7900H Sequence Detection System (Applied Biosystems). The GeneAmp 7900H system used thermal cycling parameters recommended by the manufacture (40 cycles of 15 s at 95°C and 1 min at 60°C).

Statistics

Data will be analyzed using the Mixed Model procedure (PROC MIXED) of SAS (SAS Institute, Cary, NC) as appropriate for completely randomized designs. Main effects were age and concentration of fatty acid, and the age x fatty acid interaction was tested. Means were separated and statistically measured to see what interactions were $P < 0.05$.

RESULTS

Growth performance and carcass traits

Quality grade increased from low Choice to medium Choice by 16 mo of age (Table 2). SC fat thickness over the 12th thoracic rib increased between 12 and 16 mo of age. Carcass weight increased with age of the steers, although ribeye area was unchanged. USDA yield grade increased with animal age.

Cellularity of IM and SC adipose tissue

The 12 and 14 mo of age SC adipocyte volumes were increased by all media fatty acids (Table 3). By 16 mo of age, SC adipocyte volume was decreased by all media fatty acids. SC control adipocyte volume more than doubled from 12 to 16 mo of age. By 16 mo of age, media fatty acids decreased SC adipocytes volume. IM adipocyte volume increased with age. Overall, fatty acids increased IM adipocyte volume. The largest increase in IM adipocytes volume was seen in adipocytes incubated with ALA. In the 12-mo-old steers control, IM adipocyte volume was much larger than the volume of the control SC adipocytes. The 16 mo SC adipocyte volume for the control was three-fold larger than the volume of the IM adipocytes.

Fatty acid composition

Subcutaneous adipose SFA (14:0, 16:0, and 18:0) ranged from 41-44% from 12 mo of age to 16 mo of age (Table 4). The concentration of MUFA and individual fatty acids ranged from

41-45%. A greater amount of stearic acid was the primary reason for the 12 mo steers having a higher concentration of SFA than the older steers. For IM and SC adipose depots, MUFA represented a higher percentage than any other fatty acid group. The SCD index was not different between IM and SC and did not change with age. In general, SFA decreased and MUFA increased in IM adipose tissue, but SFA and MUFA were unchanged in SC adipose tissue.

Incorporation of glucose and acetate into lipids

Rates of incorporation of glucose and acetate into lipids were higher in SC adipose tissue than in IM adipose tissue (Table 5). The depot x age interaction was highly significant (P = 0.005) for glucose incorporation indicating that the decline in incorporation over time was great in SC than IM adipose tissue.

Fatty acids synthesis from acetate increased between 12 and 16 mo of age in IM adipose tissue (Table 5). Conversely, fatty acid synthesis in control IM adipose tissue from glucose decreased from 12 to 16 mo of age. Incorporation of glucose into IM lipids was stimulated by oleic acid in IM adipose tissue of 12 and 16 mo of age. Stearic acid and ALA stimulated lipogenesis from acetate in IM adipose tissue at 14 mo of age in SC adipose tissue. At 16 mo of age, both ALA and oleic acid stimulated lipid synthesis from acetate in IM adipose tissue.

Lipogenesis from glucose decreased from 12 to 16 mo of age in control IM adipose tissue (Table 5). *Trans*-11 vaccenic acid increased lipogenesis from glucose at 12 mo of age in SC adipose tissue. Lipogenesis from acetate did not change with age. There were no

significant effects (P > 0.28) of fatty acids on glucose or acetate incorporation into fatty acids.

Expression of genes related to substrate oxidation and lipid synthesis

IM adipose tissue expression of PPARγ did not change (Table 6). ALA increased PPARγ gene expression in IM adipose tissue at 12 mo of age. SC adipose tissue expression of PPARγ significantly decreased over time, and stearic acid increased PPARγ gene expression in SC adipose tissue at 12 mo of age. SC adipose tissue had peak expression of AMPK and GPR43 at 14 mo of age, and stearic acid increased GPR43 and AMPK gene expression at 12 mo of age. In IM adipose tissue, ALA increased GPR43 gene expression at 12 of age.

Oleic acid decreased C/EBP-β gene expression at 16 mo of age in IM adipose tissue. CPT1β gene expression did not change with age in either depot. In the 12 mo steers, ALA doubled CPT1β gene expression in both IM and SC adipose tissues. The highest expression of all genes tested was SCD at 16 mo of age in SC adipose tissues. Overall, media fatty acids depressed SCD gene expression. SCD gene expression was five-fold higher in IM adipose tissue than in SC adipose tissue at 12 and 14 mo of age, but SCD gene expression increased more rapidly over time in IM adipose tissue than in SC adipose tissue.

DISCUSSION

An important goal of the beef industry is producing beef that is healthy for consumers, which will increase the reputation of beef products and provide opportunities for niche markets. The current practice is to feed steers to get the highest marbling scores possible at reasonable yield grades. Producers want to implement feed programs that would achieve better feed efficiency with cattle and still achieve high marbling scores.

The goal of this research project was to utilize an adipose tissue explant culture system to investigate the effects of specific fatty acids on markers of adipose tissue differentiation. One of the strengths of this experimental model system was the ability to test the effects of individual fatty acids on tissues that contained a mixture of differentiated adipocytes and SV cells. Converting SV cells to adipocytes in IM adipose tissue would give growing steers more IM adipose tissue for lipid filling, hence, higher marbling scores at time of slaughter.
The cattle used in this study demonstrated a doubling in the amount of SC adipose tissue over the longissimus muscle from age 12 mo to 16 mo, indicating active adipogenesis in SC adipose tissue. We chose to measure AMPK, C/EBPβ, PPARγ, SCD, CPT1, and GPR43, which represent lipogenic and lipolytic genes involved in adipose tissue metabolism. PPARγ gene expression exhibited a large increase between 14 and 16 mo of age in control IM and SC adipose tissues, indicating that some portion of the SV cells were differentiating into adipocytes in both adipose tissue depots.

One gene crucial to the regulation of glucose metabolism in adipose cells is AMPK. In this study we observed a peak at 14 mo of age for AMPK gene expression; this would suggest elevated glucose metabolism at that age. In both IM and SC adipose tissues, glucose utilization and lipogenesis were maximal at 14 mo of age. Glucose is more important carbon source for lipogenesis in IM adipose tissue than in SC adipose tissue (12).

C/EBP-ß and SCD are additional markers of differentiation that regulate fatty acid composition of adipose tissues (4). The increase in SCD gene expression in IM is consistent with the elevation in total MUFA IM adipose tissues with age. Ultimately, an increase in SCD activity in IM adipose tissue helps increase oleic acid in the final product.

The most consistent effect of individual media fatty acids was to depress gene expression, although this varied with age of animal, basal level of expression, and specific fatty acid. There were a few observed instances of stimulation of gene expression by media fatty acids. In SC adipose tissue, stearic acid increased GPR43 and AMPK gene expression at 12 mo of age. Our results are consistent with previous research that has also found at 12 mo of age, cattle absorb and deposit a great deal of stearic acid (8), and our data suggest that this could have profound effects on glucose metabolism. The VFA produced in the rumen bind to the GPR receptors, hence, activating not only GPR43 but other genes involved in energy homeostasis. GPR membrane receptors transmit extracellular signals across the plasma membrane, activating cellular responses through a variety of second messenger cascades such as AMPK. AMPK activation enhances fatty acid beta oxidation for the main purpose of

providing ATP.

One unexpected result was the increase in lipogenesis and lipid filling caused by ALA. ALA is enriched in beef from pasture-fed cattle. The volume of the IM adipocytes treated with ALA was over two-fold larger than for the control adipocytes. *Trans*-11 vaccenic acid, stearic acid, oleic acid, and *trans*-10, *cis*-12 (CLA) also increased adipoctye volume in IM adipose tissue. IM adipose tissue treated with oleic acid increased in acetate and glucose incorporation into total lipids. TVA, ALA, and *trans*-10, *cis*-12 CLA also stimulated lipogenesis from acetate.

Cattle strictly on pasture feed are leaner indicating that pasture feeding depresses carcass adiposity, but ALA does not appear to be the reason for this suppression adiposity. In SC adipose tissue, all five media fatty acids reduced adipocyte cell volume. In cattle 14 mo of age, stearic acid, oleic acid, and *trans*-10, *cis*-12 CLA caused a profound increase in adipocyte cell volume. The observation that CLA increased the size of adipocyte size contradicts the findings from (8) who demonstrated that *trans*-10, *cis*-12 CLA depressed differention of bovine preadipocytes.

In our study, the size differences between IM and SC adipocyte volume were profound and both IM and SC tissues responded differently in lipid production, cell size, and gene activity. The young steers lipid filled IM adipocytes months before they started filling SC adipocytes. The difference between IM and SC adipose depot development could be because of an underlying genetic trigger that acts upon IM adipocytes before it acts on SC adipocytes.

An important finding of this research was the demonstration of tissue-specific effects of age and media fatty acids. The differences may allow for differential regulation of the deposition of IM and SC adipose tissue at different stages of growth. Our research indicated that the fatty acids derived from pasture forage or ruminal metabolism did not inhibit the differentiation of IM adipocytes. In fact, these data indicate that fatty acids may have promoted an enrichment of the beef with CLA or n-3 fatty acids without specifically inhibiting the deposition of marbling

CONCLUSIONS

Developing strategies to alter the deposition of fat in cattle is the ultimate goal of the beef cattle industry. An estimated $1.3 billion is lost annually due to insufficient marbling scores. Any strategies to increase IM adipose tissue will help reduce this billion dollar loss to producers. Increasing the amount of MUFA in the IM adipose tissue will help consumers consume a healthier product and increase the reputation of beef products. To date, there is no published mechanistic information about the effects of specific fatty acids on the differentiation of IM and SC adipose tissue in cattle. Therefore, the goal of animal science research is elucidating the mechanisms that regulate the deposition of fat in various anatomical locations. In particular, the beef industry is mainly concerned with feeding programs that enhance the IM adipose tissue in steers that would increase the profitability of beef products.

In our experiment, we investigated the effects specific fatty acids (stearic acid, oleic acid, α-linolenic acid, *trans*-11 vaccenic acid, and *trans*-10, *cis*-12 (CLA)) would have on cellularity, lipogenesis, and lipogenic and lipolytic gene (AMPK, C/EBPβ, PPARγ, SCD, CPT1, and GPR43) expression. We hypothesized that feeding cattle high-concentrate diets would stimulate marbling (increasing IM adipose tissue) via the effects of oleic acid. The increased oleic acid will increase the healthfulness of the beef. The increase in IM adipose tissue would increase the marbling score of the steer. Both results would have a positive

impact on the profit margin for producers and enhance the reputation of grain fed cattle.

Diets containing PUFA (i.e., pasture diets) are hydrogenated to a number of CLA isomers and TVA. A portion of the PUFA will bypass the rumen and are deposited directly to the IM and SC adipocytes. The model we used for our experiment allowed us to test the effects of fatty acids on tissues that contain a mixture of differentiated and undifferentiated adipocytes. We also investigate if SC adipose tissue was in fact less sensitive than IM adipose tissue to the inhibitory effects of specific fatty acids.

The most consistent effect of the fatty acids used was to depress gene expression, although the expression varied with age, basal level of expression, and specific fatty acid. All fatty acids increased the adipocyte cell volume in both SC and IM depots as the age of the steers increased. In both control IM and SC adipose tissues, maximum glucose utilization for metabolism, including lipid synthesis, was measured at 14 mo of age. The largest increase in cell volume occurred in both IM and SC between 14 and 16 mo of age. The largest increase in SC adipocyte volume by a single fatty acid was seen in the 14 mo samples treated with CLA. This again contradicts previous research showing that CLA depresses differentiation. The largest increase in IM adipocytes volume by a single fatty acid was caused by ALA. There were few instances of stimulated gene expression from the media fatty acids. SC adipose tissue responded to stearic acid by increased GPR43 and AMPK gene expression at 12 mo of age. Steers 12 mo of age absorb a large about of stearic acid (8) and based upon our findings we can conclude that the absorption of stearic acid could influence glucose

metabolism. Increased AMPK activity would affect cellular energy homeostasis due to the important role AMPK plays in regulating glucose and lipid metabolism in adipose tissue.

Pasture-fed cattle have lower levels of adiposity and the cause of the depressed lipogenesis is not due to the presence of ALA, based upon our research. We demonstrated the tissue-specific effects of time and feed on media fatty acids. The different effects allow for differential regulation of the production of IM and SC adipose tissue in growing steers. With further research, producers can implement feeding programs that promote differential regulation of the IM and SC adipose tissue in growing and finishing steers. Feeding programs that promote an increase in IM adipose tissue will have a positive impact on profitability for the cattle producers.

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APPENDIX

TABLE 1 Ingredients and chemical composition of the high-energy, corn-based diet

Item	g/100 g corn-based diet
Ground sorghum	20.00
Ground corn	48.05
Cottonseed meal	6.00
Cottonseed hulls	15.00
Molasses	7.50
Limestone	0.96
Trace mineralized salt[a]	0.56
Dicalcium phosphate	0.23
Potassium chloride	0.16
Zinc oxide	0.01
Ammonium sulfate	0.25
Vitamin premix[b]	0.08
R-1500[c]	1.20
Total	100.0
Nutrient composition[d]	
Dry matter, %	89.13
Crude Protein, %	11.16
NEm (Mcal/kg)	1.81
NEg (Mcal/kg)	1.19
Acid detergent fiber, %	14.12
Calcium, %	0.52
Phosphorus, %	0.36

[a] Trace mineralized salt: NaCl, 98%; Zn, 0.35%; Mn 0.28%; Fe, 0.175%; Cu, 0.035%; I, 0.007%; Co, 0.0007%.
[b] Vitamin Premix: vitamin A, 2,200,000 IU/kg; vitamin D, 1,100,000 IU/kg; vitamin E, 2, 200 IU/kg.
[c] R-1500: 1.65g monensin sodium (Rumensin) per kg.
[d] Percentage of dry matter. Calculated values based on NRC (1996).

TABLE 2 Carcass characteristics of Angus steers at different times on a high-energy, corn-based diet

Item	Age, mo			SEM	P-values
	12	14	16		
Marbling scores	SM48	SM13	MT48	13.1	0.571
Quality grade	CH16	CH10	CH49	11.1	0.147
Actual fat thickness, in	0.50[b]	0.91[a]	1.10[a]	0.10	0.006
Adjusted fat thickness, in	0.56[b]	0.99[a]	1.13[a]	0.09	0.007
Ribeye area, in^2	11.2	11.1	11.5	0.62	0.846
KPH, %	2.38	3.25	3.00	0.23	0.064
Carcass weight, lb	571[b]	622[b]	718[a]	18.1	0.009
Yield grade	2.96[b]	4.44[a]	4.94[a]	0.42	0.023

TABLE 3 Cellularity of intramuscular and subcutaneous depots

Tissues	Age, mo	Fatty acid	Area	Radius	Diameter	Volume	Cell ($x10^7$)
Intramuscular adipose tissue	12	Control	10,474	57	115	6,455,881	173.20
		$C_{18:0}$	8,708	52	105	4,971,140	193.13
		cis 9-$C_{18:1}$	7,852	50	99	4,258,504	203.45
		trans 11-$C_{18:1}$	9,363	54	108	5,638,512	188.56
		α-$C_{18:3}$	9,708	55	111	5,851,559	183.34
		trans 10, *cis* 12 - CLA	9,015	53	107	5,175,875	187.52
	14	Control	7,793	49	99	4,151,201	201.25
		$C_{18:0}$	9,203	54	108	5,402,430	187.81
		cis 9-$C_{18:1}$	10,997	59	118	7,071,498	171.80
		trans 11-$C_{18:1}$	9,861	55	111	6,058,625	184.39
		α-$C_{18:3}$	11,176	60	119	7,131,780	168.06
		trans 10, *cis* 12 - CLA	9,121	54	108	5,256,216	186.02
	16	Control	7,177	47	95	3,675,351	209.98
		$C_{18:0}$	12,060	62	124	8,009,489	162.12
		cis 9-$C_{18:1}$	10,464	58	115	6,459,166	173.65
		trans 11-$C_{18:1}$	12,877	64	128	8,807,030	156.36
		α-$C_{18:3}$	13,500	65	130	9,638,197	155.68
		trans 10, *cis* 12 - CLA	11,605	61	121	7,557,940	165.24
		SEM	1,014	2.82	5.65	950,970	9.29
	Effects	Age	0.027	0.003	0.003	0.026	0.007
		Fatty acid	0.057	0.046	0.046	0.039	0.075
		Age x Fatty acid	0.102	0.809	0.809	0.133	0.059
Subcutaneous adipose tissue	12	Control	8,570	52	104	4,791,035	191.97
		$C_{18:0}$	13,262	65	129	9,306,561	156.03
		cis 9-$C_{18:1}$	12,936	64	128	8,976,063	158.01
		trans 11-$C_{18:1}$	12,327	63	125	8,284,255	160.51
		α-$C_{18:3}$	12,479	62	125	8,648,417	165.28
		trans 10, *cis* 12 - CLA	12,890	64	128	8,840,310	156.66
	14	Control	10,350	57	114	6,339,354	174.20
		$C_{18:0}$	16,395	72	144	12,676,936	138.84
		cis 9-$C_{18:1}$	15,839	71	142	12,058,671	141.45
		trans 11-$C_{18:1}$	14,378	68	135	10,424,058	148.40
		α-$C_{18:3}$	13,763	66	132	9,728,699	151.18
		trans 10, *cis* 12 - CLA	16,680	73	146	12,977,701	137.31
	16	Control	19,810	79	158	16,800,312	126.00
		$C_{18:0}$	15,328	70	139	11,579,193	145.05
		cis 9-$C_{18:1}$	15,147	69	138	11,425,568	146.91
		trans 11-$C_{18:1}$	16,374	72	144	12,705,426	139.58
		α-$C_{18:3}$	16,251	72	143	12,576,282	140.25
		trans 10, *cis* 12 - CLA	15,758	71	141	11,982,158	142.03
		SEM	1.182	2.82	5.73	1.267.323	7.63
	P-value	Age	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
		Fatty acid	0.325	0.394	0.182	0.516	0.055
		Age x Fatty acid	0.003	0.004	0.004	0.002	0.011
P-value	Age		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Fatty acid		0.043	0.018	0.017	0.113	0.007
	Location		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Age x Fatty acid		0.261	0.417	0.417	0.148	0.640
	Age x Location		0.331	0.080	0.080	0.012	0.277
	Fatty acid x Location		0.346	0.349	0.349	0.358	0.399
	Age x Fatty acid x Location		0.001	0.001	0.001	0.001	0.002

Table 4 Fatty acid profiles of intramuscular and subcutaneous adipose tissues of Angus steers (g/100g)

Fatty acids	Intramuscular adipose tissue (age, mo)						Subcutaneous adipose tissue (age, mo)						P-values		
	9	12	14	16	SEM	P-value	9	12	14	16	SEM	P-value	Tissue (T)	Month (M)	TxM
$C_{12:0}$	0.07	0.05	0.05	0.07	0.01	0.45	0.07	0.07	0.07	0.08	0.01	0.76	0.24	0.53	0.63
$C_{14:0}$	4.10	3.35	3.40	3.34	0.26	0.26	2.97	4.06	3.41	3.59	0.29	0.07	0.83	0.72	0.02
$C_{14:1}$	1.01	0.82	1.10	1.24	0.15	0.37	0.57	1.15	0.90	0.66	0.17	0.06	0.07	0.57	0.04
$C_{16:0}$	28.1	26.5	26.2	26.3	0.71	0.30	25.7	27.0	26.5	27.0	0.77	0.59	0.77	0.88	0.17
$C_{16:1}$	3.74	3.47	4.09	4.22	0.40	0.27	3.08	3.80	4.05	3.02	0.44	0.50	0.20	0.48	0.30
$C_{18:0}$	15.0	13.3	11.7	10.1	1.09	0.02	14.0	12.6	13.0	14.7	1.18	0.65	0.19	0.22	0.07
cis-9 $C_{18:1}$	34.8	37.3	39.2	41.6	1.40	0.04	40.1	37.4	38.2	37.6	1.53	0.51	0.91	0.38	0.03
cis-11 $C_{18:}$1	1.46	1.64	1.73	1.83	0.17	0.19	1.60	1.52	1.85	1.42	0.18	0.63	0.59	0.50	0.38
trans-11 $C_{18:1}$	3.29	5.44	3.51	3.11	0.57	0.10	3.42	2.61	3.29	3.97	0.62	0.12	0.23	0.67	0.02
$C_{18:2}$	1.73	1.89	1.90	1.67	0.14	0.58	1.97	1.97	1.85	1.88	0.15	0.91	0.25	0.77	0.74
cis-9, *trans*-11 $C_{18:2}$	0.47	0.34	0.41	0.31	0.04	0.20	0.29	0.39	0.32	0.25	0.05	0.09	0.04	0.17	0.14
trans-10, *cis*-12 $C_{18:2}$	0.01	0.02	0.01	0.00	0.01	0.30	0.03	0.02	0.03	0.02	0.01	0.77	0.01	0.48	0.45
$C_{18:3}$	0.13	0.09	0.09	0.05	0.01	0.01	0.07	0.10	0.08	0.07	0.01	0.26	0.28	0.01	0.07
$C_{20:0}$	0.09	0.06	0.07	0.03	0.01	0.02	0.09	0.08	0.08	0.09	0.01	0.71	0.09	0.05	0.05
$C_{20:1}$	0.16	0.19	0.25	0.28	0.04	0.22	0.20	0.23	0.25	0.18	0.04	0.53	0.84	0.38	0.25
$C_{20:2}$	0.01	0.00	0.01	0.00	0.01	0.77	0.00	0.02	0.02	0.01	0.01	0.13	0.05	0.29	0.12
$C_{20:4}$	0.05	0.05	0.04	0.05	0.01	0.27	0.07	0.05	0.05	0.06	0.01	0.15	0.04	0.17	0.10
$C_{24:0}$	0.02	0.04	0.04	0.05	0.01	0.01	0.06	0.03	0.05	0.05	0.01	0.02	0.03	0.01	0.01
SCD index[1)]	0.84	0.91	1.04	1.16	0.09	0.03	1.00	0.97	1.03	0.88	0.09	0.79	0.73	0.49	0.11

$$\text{SCD index} = \frac{(C14{:}1n{-}5+C16{:}1n{-}7+C18{:}1\,n{-}9+cis\,11\,C18{:}1+cis{-}9,trans\,11\,C18{:}2)}{(C14{:}0+C16{:}0+C18{:}0+trans\,11\,C18{:}1)}$$

TABLE 5 Effects of age and media fatty acids on acetate or glucose conversion to total lipid of adipose tissues of Angus steers

Tissue	Age, mo	Fatty acids	Glucose	Acetate
Intramuscular adipose tissue	12	Control	3.44 ± 0.89	10.65 ± 4.41
		$C_{18:0}$	5.42 ± 0.63	5.16 ± 2.43
		cis-9 $C_{18:1}$	4.89 ± 2.81	14.01 ± 10.47
		trans-11, $C_{18:1}$	4.06 ± 0.98	19.56 ± 5.28
		$C_{18:3}$	3.76 ± 1.07	8.19 ± 2.33
		Conjugated linoleic acid	3.32 ± 0.69	8.53 ± 6.18
	14	Control	2.20 ± 0.43	8.65 ± 2.05
		$C_{18:0}$	2.94 ± 0.26	28.64 ± 10.10
		cis-9 $C_{18:1}$	3.34 ± 0.69	7.10 ± 0.80
		trans-11, $C_{18:1}$	1.95 ± 0.03	8.73 ± 7.33
		$C_{18:3}$	2.77 ± 0.65	13.70 ± 10.46
		Conjugated linoleic acid	1.71 ± 0.52	8.81 ± 6.55
	16	Control	2.38 ± 0.22	16.96 ± 1.76
		$C_{18:0}$	1.95 ± 0.48	13.15 ± 1.60
		cis-9 $C_{18:1}$	1.59 ± 0.16	26.58 ± 13.06
		trans-11, $C_{18:1}$	1.95 ± 0.48	18.75 ± 1.06
		$C_{18:3}$	3.03 ± 1.21	31.40 ± 14.76
		Conjugated linoleic acid	1.38 ± 0.33	19.66 ± 1.80
	Effects	Age	0.071	< 0.0001
		Fatty acid	0.037	0.107
		Age x Fatty acid	0.059	0.002
Subcutaneous adipose tissue	12	Control	7.77 ± 2.44	52.69 ± 26.01
		$C_{18:0}$	7.55 ± 1.12	50.44 ± 63.89
		cis-9 $C_{18:1}$	7.79 ± 2.00	70.86 ± 10.90
		trans-11, $C_{18:1}$	14.05 ± 0.62	51.49 ± 32.56
		$C_{18:3}$	13.61 ± 2.87	47.68 ± 31.50
		Conjugated linoleic acid	5.71 ± 0.97	18.29 ± 20.71
	14	Control	6.97 ± 0.57	60.09 ± 15.22
		$C_{18:0}$	7.53 ± 1.80	61.86 ± 4.35
		cis-9 $C_{18:1}$	6.15 ± 0.13	57.16 ± 3.42
		trans-11, $C_{18:1}$	5.90 ± 0.05	50.51 ± 10.15
		$C_{18:3}$	7.41 ± 0.32	55.33 ± 0.57
		Conjugated linoleic acid	7.13 ± 0.26	59.03 ± 12.64
	16	Control	3.17 ± 0.53	47.46 ± 3.75
		$C_{18:0}$	3.63 ± 0.47	48.42 ± 0.44
		cis-9 $C_{18:1}$	3.35 ± 0.37	44.30 ± 2.45
		trans-11, $C_{18:1}$	3.04 ± 0.40	42.18 ± 10.90
		$C_{18:3}$	1.65 ± 0.11	51.82 ± 5.98
		Conjugated linoleic acid	2.99 ± 0.00	42.85 ± 3.76
	Effects	Age	0.003	0.662
		Fatty acid	0.898	0.284
		Age x Fatty acid	0.917	0.825
Effects (*P*-values)	Fatty acid		0.100	0.549
	Tissues		<0.001	<0.001
	Age		0.004	0.368
	Fatty acid x tissue		0.977	0.643
	Fatty acid x age		0.953	0.401
	Location x age		0.005	0.036
	Fatty acid x location x age		0.604	0.764

TABLE 6 Effects of fatty acids and age on adipogenic gene expression in intramuscular or subcutaneous adipose tissue of Angus steers

Tissues	Age, mo	Fatty acid	AMPKα Mean	AMPKα SE	C/EBPβ Mean	C/EBPβ SE	CPT1β Mean	CPT1β SE	GPR43 Mean	GPR43 SE	PPARγ Mean	PPARγ SE	SCD Mean	SCD SE
Intramuscular adipose tissue	12	Control	0.65	0.50	1.41	0.70	2.39	0.70	0.45	0.47	0.87	0.53	0.68	0.74
		$C_{18:0}$	0.79	0.82	0.76	1.17	1.98	1.16	0.65	0.78	1.05	0.89	0.63	1.22
		cis 9-$C_{18:1}$	0.53	0.82	0.98	1.17	1.28	1.16	0.35	0.78	0.84	0.89	0.91	1.22
		trans 11-$C_{18:1}$	0.47	0.82	1.33	1.17	2.82	1.16	0.39	0.78	0.70	0.89	0.65	1.22
		α-$C_{18:3}$	2.20	0.82	1.69	1.17	5.24	1.16	3.04	0.78	3.07	0.89	0.46	1.22
		trans 10, *cis* 12 - CLA	0.23	1.16	0.48	1.65	4.41	1.64	0.10	1.10	0.26	1.25	0.38	1.73
	14	Control	5.45	0.82	0.73	1.17	2.23	1.16	3.82	0.78	3.61	0.89	0.91	1.22
		$C_{18:0}$	0.57	0.82	0.50	1.17	2.18	1.16	0.22	0.78	0.59	0.89	1.64	1.22
		cis 9-$C_{18:1}$	0.62	0.82	0.51	1.17	0.63	1.16	0.34	0.78	0.76	0.89	1.90	1.22
		trans 11-$C_{18:1}$	0.31	0.82	1.32	1.17	1.40	1.16	0.05	0.78	0.51	0.89	1.21	1.22
		α-$C_{18:3}$	0.23	0.95	0.53	1.35	0.34	1.34	0.04	0.90	0.33	1.02	1.29	1.41
		trans 10, *cis* 12 - CLA	1.55	0.82	0.99	1.17	2.47	1.16	0.36	0.78	0.47	0.89	1.29	1.22
	16	Control	0.36	1.16	4.79	1.65	6.17	1.64	0.10	1.10	2.24	1.25	7.70	1.73
		$C_{18:0}$	0.33	0.95	4.45	1.35	4.42	1.34	0.10	0.90	1.41	1.02	3.62	1.41
		cis 9-$C_{18:1}$	0.37	0.95	2.08	1.35	2.16	1.34	0.19	0.90	1.03	1.02	2.51	1.41
		trans 11-$C_{18:1}$	0.92	0.95	3.49	1.35	2.99	1.34	0.40	0.90	1.54	1.02	2.24	1.41
		α-$C_{18:3}$	0.28	1.16	7.24	1.65	5.19	1.64	0.05	1.10	1.90	1.25	3.81	1.73
		trans 10, *cis* 12 - CLA	0.69	0.95	2.17	1.35	2.51	1.34	0.47	0.90	1.85	1.02	2.33	1.41
	Effects	Age	0.359		0.080		0.338		0.282		0.131		0.011	
		Fatty acid	0.291		< 0.001		0.018		0.367		0.374		< 0.001	
		Age x Fatty acid	0.065		0.201		0.531		0.011		0.058		0.002	
Subcutaneous adipose tissue	12	Control	0.71	0.48	3.19	0.67	1.87	0.67	0.65	0.45	1.94	0.51	4.15	0.71
		$C_{18:0}$	5.70	1.16	6.80	1.65	0.65	1.64	7.78	1.10	13.07	1.25	2.15	1.73
		cis 9-$C_{18:1}$	2.49	0.95	2.71	1.35	3.17	1.34	2.79	0.90	3.97	1.02	2.99	1.41
		trans 11-$C_{18:1}$	0.84	0.82	2.27	1.17	2.71	1.16	0.53	0.78	2.17	0.89	3.90	1.22
		α-$C_{18:3}$	0.41	0.95	4.04	1.35	4.94	1.34	0.70	0.90	2.43	1.02	3.05	1.41
		trans 10, *cis* 12 - CLA	1.07	0.95	4.37	1.35	2.38	1.34	0.98	0.90	3.07	1.02	4.40	1.41
	14	Control	6.45	1.16	0.64	1.65	3.21	1.64	8.21	1.10	7.50	1.25	4.00	1.73
		$C_{18:0}$	0.65	0.82	1.48	1.17	1.73	1.16	0.30	0.78	1.70	0.89	7.61	1.22
		cis 9-$C_{18:1}$	0.34	0.82	1.91	1.17	2.10	1.16	0.06	0.78	1.61	0.89	7.29	1.22
		trans 11-$C_{18:1}$	0.35	0.82	2.75	1.17	1.95	1.16	0.07	0.78	1.80	0.89	5.97	1.22
		α-$C_{18:3}$	0.63	0.82	2.47	1.17	1.81	1.16	0.24	0.78	1.63	0.89	6.85	1.22
		trans 10, *cis* 12 - CLA	0.39	0.82	2.50	1.17	1.56	1.16	0.11	0.78	1.81	0.89	5.15	1.22
	16	Control	0.33	0.95	6.14	1.35	7.76	1.34	0.03	0.90	2.12	1.02	6.44	1.41
		$C_{18:0}$	0.18	0.95	9.96	1.35	5.72	1.34	0.02	0.90	1.92	1.02	3.73	1.41
		cis 9-$C_{18:1}$	0.31	0.82	3.85	1.17	4.72	1.16	0.23	0.78	1.94	0.89	6.57	1.22
		trans 11-$C_{18:1}$	0.59	0.95	1.38	1.35	2.39	1.34	0.28	0.90	0.76	1.02	1.38	1.41
		α-$C_{18:3}$	0.41	0.95	6.91	1.35	4.09	1.34	0.47	0.90	2.91	1.02	7.71	1.41
		trans 10, *cis* 12 - CLA	0.44	0.82	5.58	1.17	4.85	1.16	0.59	0.78	2.40	0.89	6.12	1.22
	Effects	Age	0.002		0.076		0.375		0.001		0.002		0.007	
		Fatty acid	0.002		0.001		< 0.0001		0.002		0.002		0.034	
		Age x Fatty acid	< 0.001		0.337		0.108		<0.001		<0.001		0.515	
Effects	Location		0.297		< 0.001		0.378		0.014		< 0.001		< 0.001	
	Fatty acid		0.007		0.033		0.163		0.001		0.001		0.446	
	Age		0.016		< 0.001		< 0.001		0.002		0.025		< 0.001	
	Location x Fatty acid		0.481		0.095		0.628		0.055		0.011		0.573	
	Location x Age		0.216		0.272		0.396		0.170		0.002		0.019	
	Fatty acid x Age		< 0.001		0.081		0.035		< 0.001		< 0.001		0.118	
	Location x Fatty acid x Age		0.301		0.684		0.927		0.001		0.001		0.540	

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