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ORIGINAL ARTICLE

Abomasal infusion of arginine stimulates *SCD* and *C/EBPβ* gene expression, and decreases *CPT1β* gene expression in bovine adipose tissue independent of conjugated linoleic acid

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Abstract Based on previous research with bovine preadipocytes, we hypothesized that infusion of arginine into the abomasum of Angus steers stimulates stearoyl-CoA desaturase (*SCD*) gene expression in bovine subcutaneous (s.c.) adipose tissue, and that this would be attenuated by conjugated linoleic acid (CLA). Growing Angus steers were infused abomasally with L-arginine 50 g/day; $n = 13$; provided as L-arginine HCl) or L-alanine (isonitrogenous control, 100 g/day; $n = 11$) for 14 days. For the subsequent 14 days, half of the steers in each amino acid group were infused with CLA (100 g/day). Body weight gain and average daily gain were unaffected ($P > 0.15$) by infusion of arginine or CLA into the abomasum. The plasma concentrations of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA were increased CLA infusion ($P = 0.001$) and infusion of arginine increased plasma arginine ($P = 0.01$). Compared with day 0, fatty acid synthase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase enzyme activities in s.c. adipose tissue increased by day 14 in steers infused with either alanine or arginine (all $P < 0.01$). NADP-MDH activity was higher ($P = 0.01$) in steers infused with arginine than in steers infused with arginine plus CLA by day 28, but lipid synthesis in vitro from glucose and acetate was unaffected by infusion of either arginine or CLA ($P > 0.40$). By day 28, *C/EBPβ* and *SCD* gene expression was higher, and *CPT1β* gene expression was lower, in s.c. adipose tissue of steers infused with arginine than in steers infused with alanine (±CLA) ($P = 0.05$). CLA decreased adipose tissue oleic acid (18:1n-9) in alanine- or arginine-infused steers ($P = 0.05$), although CLA had no effect on *SCD* gene expression. The data indicate that supplemental arginine promotes adipogenic gene expression and may promote lipid accumulation in bovine adipose tissue. L-Arginine may beneficially improve beef quality for human consumption.

Keywords Adipose tissue · Adipogenesis · Arginine · Beef cattle · Conjugated linoleic acid

Abbreviations

AMPKα	AMP-activated protein kinase-α
C/EBPβ	CCAAT/enhancer-binding protein-β
CLA	Conjugated linoleic acid
CPT1β	Carnitine palmitoyltransferase-1ß
FAME	Fatty acid methyl esters
FAS	Fatty acid synthase
G6PDH	Glucose-6-phosphate dehydrogenase
GPR43	G-coupled protein receptor-43
NADP-MDH	NADP-malate dehydrogenase
6PGDH	6-Phosphogluconate dehydrogenase
PPARγ	Peroxisome proliferator-activated receptor-γ
qPCR	Quantitative real-time polymerase chain reaction
RPS9	40S ribosomal protein S9
SCD	Stearoyl-coenzyme A desaturase
s.c.	Subcutaneous

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Introduction

Conjugated linoleic acid (CLA) and arginine individually have been shown to decrease adiposity in various animal species, depress preadipocyte proliferation, and modify lipid metabolism in cell lines (Adams et al. 2005; Fu et al. 2005; Jobgen et al. 2009a, b; Nall et al. 2009; Park et al. 1999; Smith et al. 2002). The *trans*-10, *cis*-12 CLA isomer may reduce the uptake of lipid by adipocytes by inhibiting lipoprotein lipase and stearoyl-CoA desaturase (*SCD*) enzyme activities (Park et al. 2000; Smith et al. 2002). Arginine, a semi-essential amino acid, stimulates fatty acid oxidation (Jobgen et al. 2009b) and is converted to nitric oxide (NO) by NO synthase (*NOS*) in all mammalian cells (Alderton et al. 2001). Accordingly, feeding the NOS inhibitor, L-N^{ω} nitro-arginine, to rats increased total body fat, with a concomitant reduction in serum nitrate (Khedrara et al. 1999). Addition of 4 % arginine to the diet reversed this effect. Khedrara et al. (1999) provided the earliest evidence that dietary arginine, via NO, may reduce adiposity in rats, possibly by increasing the expression of *NOS* and AMPK-activated protein kinase (reviewed in Wu et al. 2012; Wu 2013).

Arginine also enhances fatty acid oxidation, partly via NO-mediated changes in expression of genes including *SCD*, AMP-activated protein kinase-alpha (*AMPK*α), and peroxisome proliferator-activated receptor-γ (*PPAR*γ) co-activator-1α (*PGC1*α) (McKnight et al. 2010). NO from arginine modulated expression of *AMPK*α (Lira et al. 2007), increased carnitine palmitoyltransferase-1α (*CPT1*α) and *PGC1*α expression in liver, and increased hepatic substrate oxidation compared with alanine-supplemented rats (see Jobgen et al. 2006 for review). However, we were unable to demonstrate any effects of 4-week arginine supplementation on glucose or fatty acid conversion to CO_2 in rat liver (Nall et al. 2009). Arginine did increase palmitate oxidation in epididymal adipose tissue, but had no effect on epididymal fat mass and actually caused a small increase in epididymal adipocyte volume in rats (Nall et al. 2009). In other studies, supplemental arginine suppressed the expression of adipose tissue acetyl CoA carboxylase, fatty acid synthase (*FAS*), and *SCD*, and decreased body fat mass in rats (Jobgen et al. 2006, 2009a, b). Similarly, Fu et al. (2005) demonstrated that dietary arginine reduced adiposity in Zucker diabetic fat (ZDF) rats.

Only two studies have evaluated the effects of arginine and CLA provided in combination on adipose tissue lipid metabolism (Nall et al. 2009; Go et al. 2012). In rats, arginine supplementation decreased lipid synthesis from palmitic acid and CLA increased glucose and palmitic acid conversion to CO_2 in vitro in epididymal adipose tissue (Nall et al. 2009). However, our previous study with pigs fed arginine and/or CLA suggested that the combination of arginine and CLA would promote fatter carcasses (Go et al. 2012). In addition, exposure of bovine preadipocytes to arginine stimulated *SCD* and *PPAR*γ gene expression, which was attenuated by co-incubation with *trans*-10, *cis*-12 CLA. Increasing *SCD* gene expression and thereby the production of monounsaturated fatty acids in beef would be considered beneficial, as the consumption of ground beef enriched with oleic acid (18:1n-9, the primary product of stearoyl-CoA desaturase activity) in randomized control trials consistently increases high-density lipoprotein cholesterol in human populations (Adams et al. 2010; Gilmore et al. 2011, 2013).

Based on our previous studies with bovine preadipocytes (Chung et al. 2006) and pigs (Go et al. 2012), we hypothesized that supplementing beef cattle with arginine may promote *SCD* gene expression, and may actually promote carcass adiposity. If this was the case, then cattle and pigs, as livestock species, respond quite differently to arginine supplementation. We also included CLA as a treatment, as it should attenuate the effects of arginine on *SCD* gene expression, as well as on adipogenic gene expression.

Materials and methods

Animal procedures for this study were approved by the Texas A&M University Institutional Animal Care and Use Committee, Office of Research Compliance (Animal Use Protocol #2010-210). L-Arginine-HCl and L-alanine were provided by Ajinomoto Inc. (Tokyo, Japan) and CLA (mixed isomers) was provided as a free fatty acid preparation by Lipid Nutrition B. V. (A-80; Wormerveer, The Netherlands). The A-80 CLA preparation contains approximately 40 % each *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers. Unless otherwise stated, all chemicals and biochemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and diets

Twenty-six ruminally cannulated Angus steers were stratified by body weight and then assigned to one of four treatment groups arranged as a modified 2 × 2 factorial design. All steers were fed corn-based basal diet (Table 1) (Smith et al. 2012). The experimental period was 28 days, divided into 2 periods. During the first period (day 0–day 14), L-alanine (100 g/day; isonitrogenous control; $n = 13$) or arginine (50 g/day; $n = 13$; provided as L-arginine-HCl) were infused into the abomasum via anchored infusion lines (Wickersham et al. 2009). Two steers from the alanine infusion group lost their ruminal cannulas during this period and had to be excluded from the study. During the second period (day 15–day 28), steers remained on their treatment from period 1; however, 6 (alanine-infused) or 7 (arginine-

Table 1 Ingredients and chemical composition of the basal (control) diet

Item	
Ground milo	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 percentages	100.00
Nutritional composition, % as fed[d]	
Dry matter	89.13
Crude protein	11.16
Calcium	0.52
Phosphorous	0.36
Fatty acids (% total lipids in basal diet)	
Myristic, 14:0	1.01
Palmitic, 16:0	21.58
Palmitoleic, 16:1n-7	1.04
Stearic, 18:0	8.94
18:1 *trans*-fatty acids	0.86
Oleic, 18:1n-9	27.49
cis-Vaccenic, 18:1n-7	1.04
Linoleic, 18:2n-6	32.77
α-Linolenic, 18:3n-3	2.62

[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: 1.65 g monensin sodium (Rumensin) per kg

[d] Calculated values based on NRC (2000)

infused) steers were abomasally infused with CLA (100 g/day), while the remaining steers in each amino acid group received no supplemental fatty acids. All solutions (L-alanine, arginine, and CLA) were infused into the abomasum as a single, pulse dose immediately before feeding. Steers were housed individually and allowed free access to feed and water, and were weighed on day 0 and day 28.

Sample collection

Subcutaneous (s.c.) adipose tissue was obtained by biopsy technique as described previously (Martin et al. 1999). Adipose tissue samples were obtained just before amino acid infusion (day 0); after 14 days of amino acid infusion (just before CLA infusion); and 14 days after the initiation of CLA infusion (day 28). Before each biopsy, blood (10 mL) was withdrawn from the jugular vein into heparinized vacutainer tubes. After blood samples were withdrawn, an area of approximately 200 cm^2 was sheared closely and then alternately scrubbed three times each with ChlorHex Scrub (Vedco, St. Joseph, mO) and 70 % isopropyl alcohol. Lidocaine HCl (Western Veterinary Supply, Porterville, CA; 2 %; 1.5–2.0 mL; 30–40 mg) was injected in three locations, at the tips and apex of the projected V-shaped incision. After a minimum of 5 min, a V-shaped incision, approximately 4 cm on each side, was made through the hide with a sterile scalpel, between the ischium and coccygeal vertebrae in the caudal portion of the tailhead region. If the steers showed any sign of discomfort, the area was reinjected with an additional 1 mL of 2 % lidocaine at each of the same three previous injection sites.

After the incision was made, the flap of skin was lifted by gently scraping the underlying connective tissues. All underlying adipose tissue within the biopsy site was removed, but the underlying muscle was left unharmed. After removal of the adipose tissue samples, the area was blotted with sterile gauze, the incision was stapled closed (Royal AutoSuture Skin Stapler, U.S. Surgical Corp., Norwalk, CT), and the area of the incision was sprayed with isopropyl alcohol.

One portion of the adipose tissue sample was used fresh for the measurement of glucose and acetate incorporation into total lipids (described below). The remainder was frozen in liquid nitrogen and transported to the Texas A&M University laboratory for subsequent measurement of specific adipogenic mRNA, lipogenic enzyme activities, and fatty acid composition. Fatty acid and amino acid composition was measured in plasma samples.

Plasma amino acid concentrations

Amino acids in diets and plasma amino acids were analyzed using HPLC; column conditions and pre-column derivatization of amino acids with *o*-phthaldialdehyde were as previously described (Wu et al. 2007). Amino acids were quantified on the basis of authentic standards (Sigma-Aldrich) using the Millennium Workstation (Waters, Inc.) (Wu et al. 2007).

Fatty acid composition of plasma and s.c. adipose tissue

Lipids were extracted in chloroform:methanol (2:1, vol/vol) from 5 g of feed samples, 1 mL of plasma, and 100 mg of adipose tissue by the method of Folch et al. (Folch et al. 1957). Fatty acid methyl esters (FAME) were prepared from 50 mg lipid from each sample as described

by Morrison and Smith (1964), modified to include an additional saponification step (Archibeque et al. 2005). The FAME were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m × 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with helium as the carrier gas (flow rate = 1.2 mL/min). After 32 min at 180 °C, oven temperature was increased at 20 °C/min to 225 °C and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270 and 300 °C, respectively. Individual fatty acids were identified using genuine external standards (Nu-Chek Prep, Inc., Elysian, MN).

Lipid synthesis in vitro

Acetate and glucose incorporation into total lipids was measured as described previously (Smith and Crouse 1984). Flasks contained Krebs-Henseleit Buffer (pH 7.4), 5 mM glucose, 5 mM acetate, 1 μCi/mL [U-^{14}C]glucose or [1-^{14}C]acetate, and 10 mM HEPES (3 mL total volume). Adipose tissue explants were incubated at 37.5 °C in a shaking water bath for 2 h, after which reactions were terminated by the addition of 3 mL of 1 M trichloroacetic acid to each flask. Adipose tissue samples were rinsed with 0.154 M NaCl and placed in 5 mL of chloroform:methanol (2:1, vol/vol), homogenized, and the lipid extracted (Folch et al. 1957). The total lipid extract was transferred to a scintillation vial, 10 mL of scintillation cocktail (Scintiverse) was added, and samples counted in a Beckman Coulter LS 6500 Multi-purpose Scintillation Counter (Beckman Coulter, Brea, CA).

Enzyme activities

Frozen subcutaneous adipose tissue samples were homogenized in three volumes of 0.25 M sucrose, 0.01 M potassium phosphate (pH 7.4), 1 mM EDTA, and 1 mM DTT. The homogenate was centrifuged at 5,000×*g* for 15 min at 4 °C. The supernate was centrifuged for 30 min at 17,300×*g* at 4 °C. Activities of fatty acid synthase (FAS), NADP-malate dehydrogenase (NADP-MDH), glucose-6-phosphate dehydrogenase (G6PDH), and 6-phosphogluconate (6PGDH) were determined in the cytoplasmic supernates as described previously (Smith and Prior 1981; Smith and Crouse 1984). Substrates and cofactors were purchased from Boehringer Mannheim (Indianapolis, IN) and all other chemicals were from Sigma Chemical (St. Louis, MO). Rates of change in absorbance at 339 nm were recorded on a Beckman DU-7 spectrophotometer (Beckman Instruments, Irvine, CA). Activity was determined as the amount of enzyme that reduced 1 nmol of nicotinamide adenine dinucleotide phosphate ($NADP^+$)/(min·mg of protein) for G6PDH, 6PGDH and NADP-MDH as the amount of enzyme that oxidized 1 nmol of NADPH/(min·g of protein) (FAS).

RNA isolation and qRT-PCR analysis

Total RNA was extracted from previously frozen s.c. adipose tissue with Tri Reagent (Sigma Chemicals, St. Louis, MO), as reported previously (Brooks et al. 2011). The concentration of RNA was quantified with a NanoDrop ND-100 Spectrophotometer (Thermo Scientific, Washington, DE). The 260:280 ratio for all samples was greater than 1.85. *AMPKα*, CCAAT/enhancer-binding protein-*β* (*C/EBPβ*), carnitine palmitoyl transferase-1ß (*CPT1β*), G-coupled protein receptor-43 (*GPR43*), *PPARγ*, and *SCD* genes were cloned into the vector of PCRII-TOP (Invitrogen) using RT-PCR and primers as described previously (Smith et al. 2012) (Table 2).

Complementary DNA was produced from 1 μg RNA using TaqMan Reverse Transcriptase Reagents (Applied Biosystems, Foster City, CA) and the protocol recommended by the manufacturer. Random hexamers were used as primers in cDNA synthesis. Measurement of the relative quantity of the cDNA of interest was carried out using TAMRA PCR Master Mix (Applied Biosystems, Foster City, CA), appropriate forward and reverse primers, and 1 μL of the cDNA mixture. Assays were performed in duplicate in the GeneAmp 5700 Sequence Detection System (Applied Biosystems) using thermal cycling parameters recommended by the manufacturer (40 cycles of 15 s at 95 °C and 1 min at 60 °C). Cycle threshold (Ct) values were means of duplicate measurements. The comparative Ct values were employed to determine expression levels for target genes; fold change was determined as $2^{\Delta\Delta CT}$ with *RPS9* as the endogenous control. Titration of the target mRNA primers against increasing amounts of cDNA gave linear responses with slopes between −2.8 and −3.0. To reduce the effect of assay-to-assay variation in the PCR assay, all values were calculated relative to a calibration standard run on every real-time PCR assay. The ABI Prism 7000 detection system (Applied Biosystems) was used to perform the assay utilizing the thermal cycling variables recommended by the manufacturer (50 cycles of 15 s at 95 °C and 1 min at 60 °C).

Commercially available eukaryotic 40S ribosomal protein S9 (*RPS9*) RNA (Applied Biosystems; GeneBank Accession #X03205) was used as the endogenous control. The mean ± SEM for *RPS9* cycles over depot and age was 22.41 ± 0.12. *RPS9* qPCR cycles did not vary with animal age ($P = 0.22$), nor were they different across adipose tissue depot ($P = 0.45$). Other studies in bovine adipose tissue explants (Hosseini et al. 2010) and bovine liver (Janovick-

Table 2 Forward and reverse primers and probes used for quantitative real-time polymerase chain reaction

Maker gene	Gene No.		Sequence (5′ to 3′)
RPS9	DT860044	Forward	GAGCTGGGTTTGTCGCAAAA
		Reverse	GGTCGAGGCGGGACTTCT
		Taqman probe	6FAM-ATGTGACCCCGCGGAGACCCTTC-TAMRA
AMPK-α	NM_001109802	Forward	ACCATTCTTGGTTGCTGAAACTC
		Reverse	CACCTTGGTGTTTGGATTTCTG
		Taqman probe	6FAM-CAGGGCGCGCCATACCCTTG-TAMRA
C/EBPβ	NM_176788	Forward	CCAGAAGAAGGTGGAGCAACTG
		Reverse	TCGGGCAGCGTCTTGAAC
		Taqman probe	6FAM-CGCGAGGTCAGCACCCTGC-TAMRA
CPT1β	NM_001034349	Forward	ACACATCTACCTGTCCGTGATCA
		Reverse	CCCCTGAGGATGCCATTCT
		Taqman probe	6FAM-TCCTGGAAGAAACGCCTGATTCGC-TAMRA
GPR43	FJ_562212	Forward	GGCTTTCCCCGTGCAGTA
		Reverse	ATCAGAGCAGCGATCACTCCAT
		Taqman probe	6FAM-AAGCTGTCCCGCCGGCCC-TAMRA
PPARγ	NM_181024	Forward	ATCTGCTGCAAGCCTTGGA
		Reverse	TGGAGCAGCTTGGCAAAGA
		Taqman probe	6FAM-CGCGAGGTCAGCACCCTGC-TAMRA
SCD	AB075020	Forward	TGCCCACCACAAGTTTTCAG
		Reverse	GCCAACCCACGTGAGAGAAG
		Taqman probe	6FAM-CCGACCCCCACAATTCCCG-TAMRA

Table 3 Body weights and rates of gain of steers 28 days post-amino acid infusion/14 days post-CLA infusion

Item[a]	Alanine		Arginine		SEM	*P* values
	None ($n = 5$)	CLA ($n = 6$)	None ($n = 6$)	CLA ($n = 7$)		
Initial body weight (kg)	414.6	404.7	411.6	404.6	12.26	0.55
Final body weight (kg)	454.4	451.2	446.8	456.8	7.19	0.45
Gain (kg)	39.8	46.5	35.2	52.2	7.32	0.17
Average daily gain (kg/day)	1.42	1.66	1.26	1.87	0.26	0.18
Average daily intake (kg/day)	14.6	15.1	16.2	14.2	0.44	0.09
Gain:feed	0.084	0.111	0.078	0.123	0.017	0.11

[a] Data are overall means for the 28-day experiment

Guretzky et al. 2007) demonstrated that *RPS9* mRNA expression was stable and suitable as a housekeeping gene under their conditions. In addition, *RPS9* was used as a housekeeping gene for analyzing the expression of genes in bovine muscle (Baxa et al. 2010; Chung et al. 2012).

Statistical analysis

Data for the first 14 days, which compared post-infusion of amino acids (L-alanine or L-arginine) to baseline values, were analyzed by one-way analysis of variance (SuperANOVA, Abacus Concepts, Inc, San Diego, CA). Data for day 14–day 28 were analyzed by two-way analysis of variance, with amino acids (L-alanine or arginine) and CLA (L-absence or presence) as the main effects; the model also tested the amino acid × CLA interaction. Means were separated by Fisher's Protected LSD if the F-test indicated significant differences ($P < 0.05$).

Results

Growth performance

Abomasal infusion of CLA caused a slight decrease in average daily feed intake ($P = 0.09$) and gain:feed

($P = 0.11$) (Table 3). Infusion or arginine or CLA had no effect on final body weight ($P = 0.45$) or total gain over the experimental period ($P = 0.17$).

Plasma amino acid composition

Plasma arginine and alanine increased by day 14 in arginine- and alanine-infused steers, respectively (Fig. 1). Plasma arginine continued to increase between day 14 and day 28 (Fig. 1a), but plasma alanine returned to baseline values by day 28 (Fig. 1b). Plasma glycine and lysine decreased, but histidine and phenylalanine increased by day 14 in both arginine- and alanine-infused steers (Table 4). In the alanine-infused steers, ornithine increased and serine decreased by day 14. By day 28, the plasma concentration of ornithine was higher in arginine-infused steers than in alanine-infused steers ($P = 0.02$), whereas threonine was higher in alanine-infused steers than in arginine-infused steers ($P = 0.02$) (Table 5). There were no effects of CLA infusion on plasma amino acid concentrations; nor were any of the amino acid × CLA effects significant, although co-infusion of arginine and CLA tended ($P = 0.07$) to increase plasma glutamate.

Plasma and adipose tissue fatty acid composition

Plasma palmitoleic acid (16:1n-7), 18:1*trans* isomers, and *cis*-vacennic acid (18:1n-11) decreased by day 14 in arginine- and alanine-infused steers (Table 6) (P values < 0.03). Palmitoleic acid decreased in s.c. adipose tissue of alanine-infused steers ($P = 0.03$), and *cis*-vacennic acid increased ($P = 0.01$) in adipose tissue of both arginine- and alanine-infused steers by day 14. The *cis*-9, *trans*-11 CLA isomer increased in adipose tissue of arginine- and alanine-infused steers by day 14 ($P = 0.05$). Oleic acid was not different between day 0 and day 14 ($P = 0.24$), but was increased by day 28 in adipose tissue from both arginine- and alanine-steers ($P = 0.01$) (Fig. 2a). Oleic acid was decreased by CLA by day 28 in adipose tissue of both alanine- and arginine-infused steers ($P = 0.01$). By day 28, plasma palmitic acid (16:0) was higher in steers infused with arginine (±CLA) than in steers infused with alanine ($P = 0.04$) (Table 7). Co-infusion of CLA decreased α-linolenic acid (18:3n-3) ($P = 0.01$) and increased both CLA isomers ($P = 0.001$) in both arginine- and alanine-infused steers.

There were no differences between alanine- and arginine-infused steers in any subcutaneous adipose tissue fatty acids at day 28 (Table 7) (all $P \geq 0.010$). Co-infusion of CLA with both amino acids increased adipose tissue palmitic acid and *trans*-10, *cis*-12 CLA ($P = 0.01$). There were no amino acid × CLA interactions, although co-infusion of alanine and CLA tended ($P = 0.06$) to increase adipose tissue palmitic acid.

Lipid synthesis and lipogenic enzyme activities

Neither amino acid infusion nor CLA infusion affected the incorporation of glucose or acetate into total lipids in s.c. adipose tissue by day 14 ($P \geq 0.42$) (Table 8). The

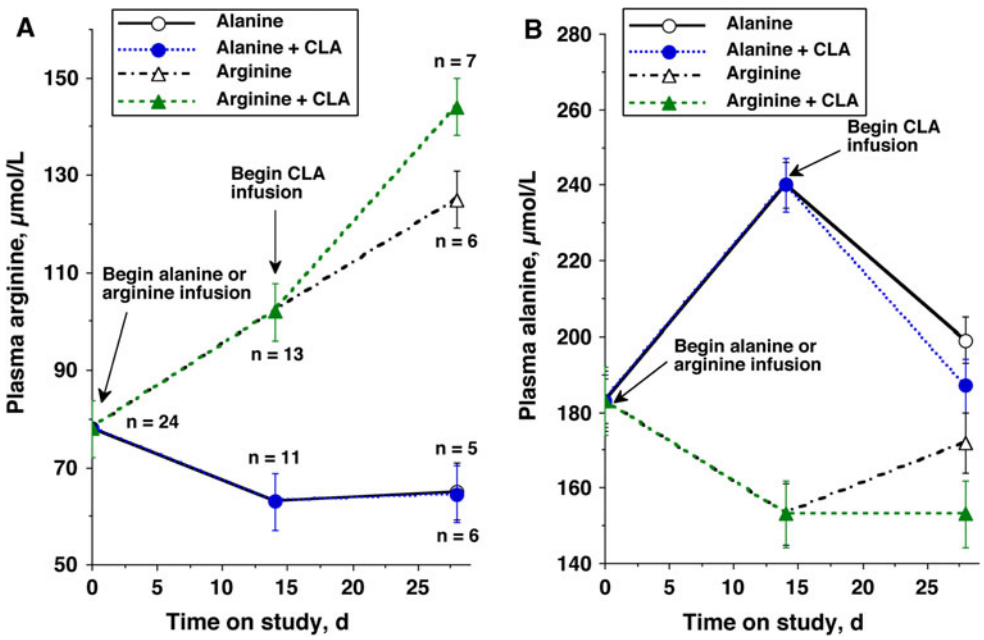

Fig. 1 Plasma arginine (**a**) and alanine (**b**) in cattle infused with L-arginine (50 g/day) or L-alanine (100 g/day) in the absence or presence of infused CLA (100 g/day). **a** The plasma concentration of arginine was higher in arginine-infused steers by day 14 ($P = 0.02$) than in steers infused with alanine. The amino acid × CLA interaction was not significant for arginine ($P = 0.71$). **b** The plasma concentration of alanine was greater in alanine-infused steers than in arginine-infused steers at day 14 ($P = 0.002$) and at day 28 ($P = 0.03$). The amino acid × CLA interaction was not significant for alanine ($P = 0.78$). Numbers of steers at each time point are indicated in **a**, and pooled SEM are affixed to the symbols

Table 4 Amino acid concentrations in the plasma of steers at baseline and 14 days post-amino acid infusion

Amino acid concentration, μmol/L	Day 0	Day 14		SEM[A]	*P* values
	Baseline ($n = 24$)	Alanine ($n = 11$)	Arginine ($n = 13$)		
Asparagine	25	21	20	1.4	0.32
Aspartate	10	22	20	2.9	0.17
β-Alanine	79	95	79	11.1	0.84
Citrulline	47	47	50	2.0	0.72
Glutamine	200	196	190	6.1	0.79
Glutamate	56	68	57	2.7	0.20
Glycine	243[a]	182[b]	170[b]	8.1	0.001
Histidine	45[b]	53[a]	53[a]	1.4	0.01
Isoleucine	62	71	77	3.0	0.09
Leucine	131	120	122	3.7	0.45
Lysine	57[a]	37[b]	41[b]	2.7	0.002
Methionine	27	33	32	1.5	0.16
Ornithine	72[b]	64[b]	108[a]	4.8	0.005
Phenylalanine	47[b]	65[a]	72[a]	4.2	0.03
Serine	72[a]	67[a]	57[b]	2.7	0.05
Taurine	31	35	33	1.5	0.58
Threonine	41	41	39	1.8	0.82
Tyrosine	48	51	53	2.0	0.51
Valine	54	54	60	2.4	0.56

[A] Pooled SEM from one-way analysis of variance. Data are means for the indicated number of animals per treatment group. Data for alanine and arginine are illustrated in Fig. 1

[a,b,c] Means with common superscripts are not different ($P > 0.05$)

Table 5 Interaction means for plasma amino acid concentrations in steers at 28 days post-amino acid infusion/14 days post-CLA infusion

Amino acid	Alanine ($n = 5$)	Alanine + CLA ($n = 6$)	Arginine ($n = 6$)	Arginine + CLA ($n = 7$)	SEM[A]	*P* values		
						AA	CLA	AA × CLA
Asparagine	14	17	17	17	1.6	0.60	0.65	0.45
Aspartate	7	7	8	9	0.5	0.19	0.69	0.90
β-Alanine	96	80	83	71	1.6	0.75	0.67	0.95
Citrulline	48	44	51	51	2.1	0.30	0.68	0.64
Glutamine	195	211	224	199	13.8	0.76	0.87	0.49
Glutamate	69	62	57	70	2.8	0.77	0.62	0.07
Glycine	194	210	181	203	6.8	0.48	0.18	0.82
Histidine	54	51	51	50	2.8	0.64	0.74	0.85
Isoleucine	55	52	51	51	2.4	0.68	0.70	0.82
Leucine	98	91	96	93	4.1	0.97	0.59	0.85
Lysine	46	49	51	51	2.1	0.39	0.71	0.75
Methionine	25	19	24	15	2.2	0.57	0.12	0.72
Ornithine	143	113	188	207	15.5	0.02	0.85	0.39
Phenylalanine	43	34	36	36	2.1	0.61	0.28	0.34
Serine	61	67	62	64	2.3	0.90	0.37	0.68
Taurine	37	30	31	28	2.3	0.38	0.33	0.67
Threonine	36	35	28	30	1.4	0.02	0.95	0.57
Tyrosine	46	37	38	35	3.0	0.42	0.36	0.65
Valine	45	40	41	41	2.5	0.80	0.68	0.67

AA amino acid (alanine or arginine), *CLA* absence or presence of CLA, *AA* × *CLA* amino acid × CLA interaction

[A] Pooled SEM from two-way analysis of variance. Values, expressed as μmol/L, are means for the indicated number of animals per treatment group. Data for alanine and arginine are illustrated in Fig. 1

Table 6 Fatty acid composition of plasma and subcutaneous adipose tissue of steers at baseline and 14 days post-amino acid infusion

Fatty acid	Day 0	Day 14		SEM[A]	*P* values
	Baseline ($n = 24$)	Alanine ($n = 11$)	Arginine ($n = 13$)		
Plasma, g/100 total fatty acids					
Palmitic	12.0	11.7	12.0	0.50	0.74
Palmitoleic	0.75[a]	0.61[b]	0.55[b]	0.03	0.03
Stearic	18.9	19.4	20.5	0.21	0.15
18:1*trans*[B]	2.45[a]	1.10[b]	0.93[b]	0.04	0.001
Oleic acid	10.0	8.8	8.8	0.44	0.14
cis-Vaccenic	1.01[a]	0.80[b]	0.69[b]	0.04	0.001
Linoleic	42.1	45.0	44.3	0.64	0.13
α-Linolenic	0.64	0.68	0.60	0.05	0.82
18:2*cis*-9, *trans*-11	0.03	0.01	0.01	0.01	0.44
18:2*trans*-10, *cis*-12	0.04	0.01	0.01	0.02	0.49
Subcutaneous adipose tissue, 100 g total fatty acids					
Palmitic	28.3	28.0	27.6	0.29	0.61
Palmitoleic	4.19[a]	3.35[b]	3.92[a]	0.13	0.03
Stearic	15.0	15.8	14.9	0.25	0.38
18:1*trans*[B]	2.54	2.52	2.26	0.14	0.69
cis-Vaccenic	1.18[a]	0.88[b]	0.86[b]	0.06	0.01
Linoleic	1.46	1.47	1.55	0.07	0.84
α-Linolenic	0.14	0.12	0.13	0.02	0.84
18:2*cis*-9, *trans*–11	0.05[b]	0.11[a]	0.12[a]	0.01	0.05
18:2 *trans*-10, *cis*-12	0.09	0.07	0.04	0.02	0.57

[A] Pooled SEM from one-way analysis of variance. Data are means for the indicated number of animals per treatment group. Data for s.c. adipose tissue oleic acid are illustrated in Fig. 2

[B] Total 18:1 *trans*-isomers

[a,b] Means with common superscripts are not different ($P > 0.05$)

activities of G6PDH, 6PGDH, and FAS were greater by day 14 than at baseline, whereas the activity of NADP-MDH was less at day 14 than at baseline in adipose tissue of alanine- and arginine-infused steers. At day 28, NADP-MDH activity was greater in adipose tissue of steers infused with arginine in the absence of CLA (Table 9). Acetate and glucose incorporation into lipids and all other enzyme activities were not different in adipose tissue from alanine- and arginine-infused steers in the absence or presence of CLA (all $P \geq 0.31$).

Adipose tissue gene expression

By day 14, alanine infusion increased *AMPK* gene expression in s.c. adipose tissue when compared with day 0 ($P = 0.005$) (Table 8). Arginine infusion (±CLA) increased *SCD* gene expression by day 14 ($P = 0.05$) (Fig. 2b). By day 28, *SCD* gene expression in adipose tissue was higher in arginine-infused steers than in adipose tissue of steers infused with alanine plus CLA (±CLA). The amino acid × CLA interaction was not significant for *SCD* gene expression ($P = 0.58$). *C/EBPβ* expression was higher ($P = 0.05$), and *CPT1β* expression was lower in arginine-infused steers than in alanine-infused steers. The expression of the genes measured in adipose tissue in this study was not affected by CLA ($P \geq 0.34$).

Discussion

There is growing interest in the important roles for L-arginine and NO in regulating lipid metabolism in mammals (Dai et al. 2013; Satterfield et al. 2012; Tan et al. 2012). Results of the present study indicated that similar changes in adipose tissue lipogenesis and gene expression occurred in both alanine- and arginine-infused steers. After 14 days of infusion, FAS, G6PDH, and 6PGDH enzyme activities increased, indicating increased lipogenic capacity. We recently reported that acetate incorporation into lipids increases between 12 and 16 months of age in cattle of the same breed type (Angus) and age as the cattle of this study (Choi et al. 2013), and the increases in FAS, G6PDH, and 6PGDH enzyme activities is consistent with our previous results. The increase in *AMPKα* gene expression observed in alanine-infused steers should antagonize lipid biosynthesis, but this is not consistent with known increase in adiposity in beef cattle steers typically observed during this period of time. However, glucose incorporation into lipids decreases with age in beef cattle (Choi et al. 2013), similar to the decline in NADP-MDH activity we observed. Thus, *AMPKα* may selectively influence substrate incorporation into lipids in bovine adipose tissue.

Our laboratory has published extensively on the effects of feeding CLA to pigs (Adams et al. 2005; Demaree et al. 2002; Go et al. 2012; King et al. 2004; Smith et al. 2002, 2010). Feeding CLA to pigs depressed adipose tissue SCD activity (Smith et al. 2002) and monounsaturated fatty acids (Adams et al. 2005; Demaree et al. 2002; Smith et al. 2002), which has been confirmed recently (Tous et al. 2012). DNA synthesis was depressed in s.c. adipose tissue of pigs fed 1.5 % CLA, compared to pigs fed corn oil or beef tallow (Adams et al. 2005), consistent with the depression caused by CLA in 3T3-L1 preadipocyte proliferation that we reported previously (Satory and Smith 1999). However, s.c. adipocyte volume was only marginally reduced by CLA (Adams et al. 2005; Demaree et al. 2002; Smith et al. 2002). Feeding 1 % CLA to finishing

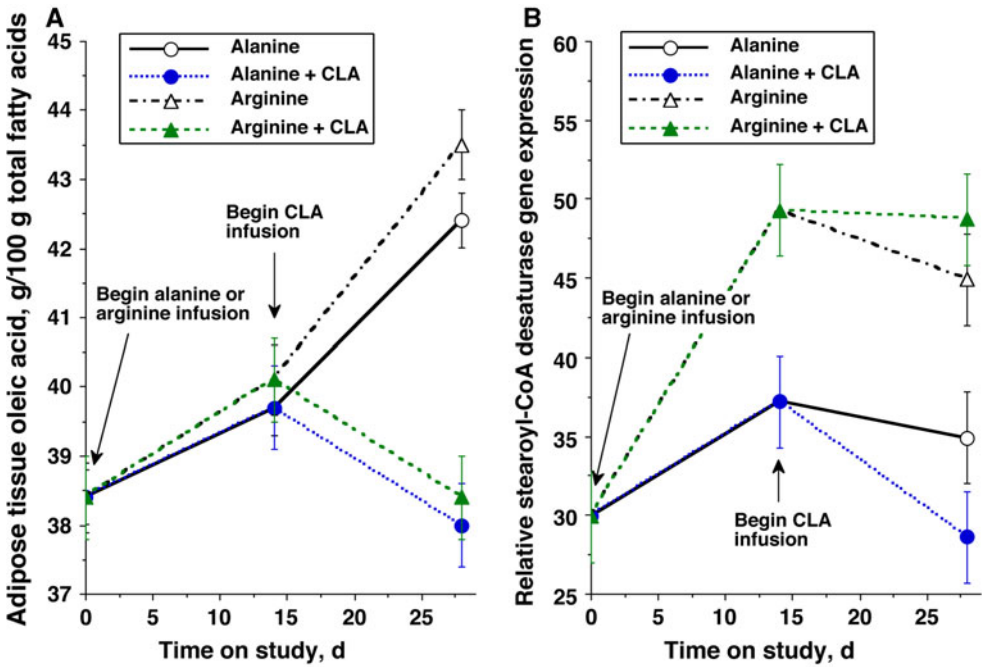

Fig. 2 Subcutaneous adipose tissue oleic acid (18:1n-9) (**a**) and subcutaneous adipose tissue stearoyl-CoA desaturase-1 gene expression (*SCD*) (**b**) in cattle infused with L-arginine (50 g/day) or L-alanine (100 g/day) in the absence or presence of infused CLA (100 g/day). **a** Oleic acid was not different between day 0 and day 14 ($P = 0.24$), but was increased by day 28 in adipose tissue from both arginine- and alanine-steers ($P = 0.01$). Oleic acid was decreased by CLA by day 28 ($P = 0.01$). **b** Arginine increased *SCD* gene expression by day 14 ($P = 0.05$), and day 28 ($P = 0.05$). *SCD* gene expression was not affected by CLA at day 28 ($P = 0.89$). The amino acid × CLA interaction was not significant for adipose tissue oleic acid ($P = 0.83$) or *SCD* gene expression ($P = 0.58$). Pooled SEM are affixed to the symbols

pigs for 4 weeks had no effect on s.c. fat thickness (Go et al. 2012), although others have demonstrated that feeding CLA for longer periods or feeding higher levels of CLA decreased pig carcass adiposity (Barnes et al. 2012; Dugan et al. 1997; Ostrowska et al. 1999; Wiegand et al. 2002). In rodents, CLA caused a reduction in fat mass in (Park et al. 1997; Sisk et al. 2001; Takahashi et al. 2002), and we demonstrated that CLA increased body weight gain and gain:feed and decreased retroperitoneal fat mass in rats (Nall et al. 2009).

Paradoxically, CLA increased retroperitoneal and s.c. adipocyte volumes of pigs and also increased intramuscular lipid (Go et al. 2012). This is consistent with a recent study that demonstrated that CLA increased marbling scores and marbling adipocyte volume in pigs (Barnes et al. 2012). Mixed isomers of CLA stimulated lipid synthesis in 3T3-L1 preadipocytes (Satory and Smith 1999) and Ding et al. (2002) subsequently demonstrated that CLA increases porcine adipocyte differentiation. Thus, under some conditions, CLA actually promotes adiposity and this effect may be specific to individual adipose tissue depots or cell lines.

CLA isomers are hydrogenated when fed to beef cattle, so there are very few reports of the effects of CLA on bovine adipogenesis. Schlegel et al. (2012a) demonstrated that feeding rumen-protected CLA (4.3 g *cis*-9, *trans*-11 CLA and 3.8 g *trans*-10, *cis*-12 CLA per day) to dairy cattle had no discernable effect on genes involved in hepatic lipid metabolism. Schlegel et al. (2012b) fed protected CLA supplements containing up to 5.5 g *cis*-9, *trans*-11 CLA and 4.8 g *trans*-10, *cis*-12 CLA per day to beef cattle and demonstrated that only the *trans*-10, *cis*-12 CLA isomer was increased in carcass fat, similar to results of the current study. As in the current study, the CLA supplement decreased oleic acid in s.c. adipose tissue, even though the supplementation level was much lower than in the current study.

The relative proportion of plasma *trans*-10, *cis*-12 CLA in the CLA-infused steers was several fold higher (0.96 g/100 g total plasma fatty acids) than in CLA-fed pigs (0.32 g/100 g fatty acids; Go et al. 2012) or CLA-fed rats (undetectable; Nall et al. 2009). In spite of this, the adipose tissue concentration of *trans*-10, *cis*-12 CLA was lower (0.20 g/100 g fatty acids) than in rats (1.6 g/100 fatty acids) or pigs (0.73 g/100 g fatty acids), so that the effective dose of *trans*-10, *cis*-12 CLA was lower in bovine adipose tissue than in pigs or rats.

We recently reported that *GPR43* gene expression increased markedly between 14 and 16 months of age (Smith et al. 2012), and the level of *GPR43* gene expression was equivalent to the level of *PPARγ* expression in

Table 7 Interaction means for fatty acid composition of plasma and subcutaneous adipose tissue of steers at 28 days post-amino acid infusion/14 days post-CLA infusion

Fatty acid	Alanine ($n = 5$)	Alanine + CLA ($n = 6$)	Arginine ($n = 6$)	Arginine + CLA ($n = 7$)	SEM[A]	*P* values		
						AA	CLA	AA × CLA
Plasma, g/100 g total plasma fatty acids								
Palmitic	10.3	10.6	11.4	10.8	0.16	0.04	0.50	0.15
Palmitoleic	0.57	0.55	0.55	0.45	0.03	0.30	0.29	0.50
Stearic	20.1	19.6	20.9	18.9	0.68	0.99	0.39	0.61
18:1*trans*[B]	1.34	1.68	1.20	1.51	0.19	0.71	0.44	0.97
Oleic acid	9.6	8.9	8.1	7.9	0.33	0.06	0.50	0.69
cis-Vaccenic	0.78	0.80	0.85	0.70	0.03	0.81	0.38	0.27
Linoleic	44.2	44.1	44.2	45.7	0.99	0.73	0.75	0.70
α-Linolenic	0.79	0.59	0.82	0.48	0.05	0.67	0.01	0.42
18:2*cis*-9, *trans*-11	0.01	1.35	0.01	2.09	0.24	0.25	0.001	0.25
18:2*trans*-10, *cis*-12	0.01	0.96	0.01	1.68	0.21	0.26	0.005	0.26
Subcuteanous adipose tissue, g/100 g total fatty acids								
Palmitic	27.0	29.8	28.2	28.7	0.35	0.87	0.01	0.06
Palmitoleic	3.18	3.47	4.25	3.92	0.22	0.10	0.96	0.49
Stearic	15.7	16.8	14.3	15.7	0.56	0.32	0.31	0.94
18:1*trans*[B]	1.87	1.55	0.66	1.82	0.28	0.42	0.46	0.21
cis-Vaccenic	0.92	0.57	0.49	0.60	0.11	0.40	0.62	0.34
Linoleic	1.35	1.31	1.37	1.57	0.08	0.38	0.64	0.46
α-Linolenic	0.07	0.06	0.03	0.06	0.06	0.48	0.34	0.31
18:2*cis*-9, *trans*-11	0.17	0.29	0.22	0.29	0.03	0.71	0.18	0.74
18:2*trans*-10, *cis*-12	0.07	0.20	0.02	0.14	0.03	0.20	0.01	0.93

AA amino acid (alanine or arginine), *CLA* absence or presence of CLA, *AA* × *CLA* amino acid × CLA interaction

[A] Pooled SEM from two-way analysis of variance. Data are means for the indicated number of animals per treatment group. Data for adipose tissue oleic acid are illustrated in Fig. 2

[B] Total 18:1 *trans*-isomers

this study. *GPR43* encodes a cell-surface receptor that is activated by the volatile fatty acids, acetate and propionate (Brown et al. 2003), and works via *AMPKα* to inhibit fatty acid biosynthesis in adipose tissue (Hardie 2007). The elevation of *GPR43* gene expression and concomitant depression of NADP-MDH activity caused by CLA suggest that CLA depressed de novo fatty acid synthesis, especially from glucose (Smith and Prior 1981; Smith and Smith 1995). However, lipid synthesis in vitro from glucose and acetate was not affected by CLA or arginine infusion.

To our knowledge, this is the first report of the effects of arginine on lipid metabolism in a ruminant species, infused either singly or in combination with CLA. The plasma arginine concentration was doubled by abomasal arginine infusion, similar to results observed in pigs (Go et al. 2012) and rats (Nall et al. 2009) supplemented with arginine. Supplemental arginine enhanced adipose tissue fatty acid oxidation in rats (Jobgen et al. 2009b; Nall et al. 2009), consistent with elevated expression of *AMPKα* and *PPARγ* co-activator 1(*PGC1α)* (Jobgen et al. 2009a). However, arginine had no effect on fatty acid oxidation and similarly did not affect the expression of *AMPKα* or *PGC1α* in porcine adipose tissue (Go et al. 2012). Although *PGC1α* was not measured in the current study, abomasal arginine infusion had no effect on *AMPKα* gene expression. Thus, there are species differences among mammals in response to L-arginine supplementation.

We previously demonstrated that arginine supplemented at 5 mM increased *SCD* gene expression in bovine preadipocytes (Choi et al. 2013; Chung et al. 2006). Consistent with the findings with bovine preadipocytes, *SCD* gene expression was significantly higher after 14 days of arginine infusion, relative to day 0 levels of expression. Arginine infusion also increased *C/EBPβ* gene expression, which would promote adipocyte differentiation (Tang and Lane 2012). This is consistent with the elevated *SCD* expression elicited by arginine. Also, the depressed *CPT1β* gene expression caused by arginine infusion would lead to reduced lipid turnover and thereby elevated lipid

Table 8 Total lipid synthesis from glucose and acetate, lipogenic enzyme activities, and gene expression in subcutaneous adipose tissue from steers at baseline and 14 days post-amino acid infusion

Item	Day 0	Day 14		SEM[A]	*P* values
	Baseline ($n = 12$)	Alanine ($n = 6$)	Arginine ($n = 6$)		
Glucose incorporation[B]	395	402	339	35.2	0.78
Acetate incorporation[B]	4,763	4,156	3,233	463	0.42
FAS[C]	25[b]	155[a]	136[a]	16.5	0.001
NADP-MDH[C]	123[a]	82[b]	67[b]	7.3	0.001
G6PDH[C]	502[b]	879[a]	969[a]	79	0.01
6PGDH[C]	211[b]	328[a]	356[a]	24	0.01
Gene expression[D]					
C/EBPβ	3.52	1.91	2.66	0.35	0.24
PPARγ	1,556	1,881	1,577	139	0.63
CPT1β	0.34	0.40	0.34	0.04	0.77
AMPKα	2.26[b]	5.51[a]	2.25[b]	0.44	0.005
GPR43	1,923	2,592	2,832	382	0.57

[A] Pooled SEM from one-way analysis of variance. Data are means for the indicated number of animals per treatment group

[B] Glucose and acetate incorporation into total lipids: nmol substrate incorporated/(2 h·100 mg adipose tissue)

[C] Enzyme activities: nmol substrate converted to product/(min·100 mg protein). *FAS* fatty acid synthase, *NADP-MDH* NADP-malate dehydrogenase, *G6PDH* glucose-6-phosphate dehydrogenase, *6PGDH* 6-phosphogluconate dehydrogenase

[D] Gene expression was normalized to *RSP9* gene expression. Data for *SCD* are illustrated in Fig. 1

[a,b] Means with common superscripts are not different ($P > 0.05$)

Table 9 Interaction means total lipid synthesis from glucose and acetate, lipogenic enzyme activities, and gene expression in subcutaneous adipose tissue for steers at 28 days post-amino acid infusion/14 days post-CLA infusion

Item	Alanine	Alanine + CLA	Arginine	Arginine + CLA	SEM[A]	*P* values		
						AA	CLA	AA × CLA
Lipogenesis	($n = 3$)	($n = 3$)	($n = 3$)	($n = 3$)				
Glucose incorporation[B]	453.9	434.7	521.4	508.1	43.9	0.50	0.87	0.97
Acetate incorporation[B]	4,949	4,717	5,805	5,502	510	0.49	0.82	0.97
Fatty acid synthase[C]	48	66	51	48	13.2	0.81	0.81	0.73
NADP-MDH[C]	73[b]	53[b]	126[a]	51[b]	10.6	0.07	0.005	0.05
G6PDH[C]	1,047	902	699	993	95	0.54	0.72	0.31
6PGDH[C]	252	206	211	187	22	0.55	0.48	0.82
Gene expression[D]	($n = 5$)	($n = 6$)	($n = 6$)	($n = 7$)				
C/EBPβ	1.42	2.42	4.09	3.78	0.53	0.05	0.74	0.53
PPARγ	1,224	1,621	1,592	1,264	152	0.98	0.91	0.27
CPT1β	0.44	0.41	0.19	0.31	0.04	0.05	0.56	0.36
AMPKα	3.36	5.44	2.36	2.95	0.68	0.21	0.34	0.59
GPR43	2,022	6,627	2,069	3,003	452	0.94	0.34	0.98

AA amino acid (alanine or arginine), *CLA* absence or presence of CLA, *AA* × *CLA* amino acid × CLA interaction

[A] Pooled SEM from one-way analysis of variance. Data are means for the indicated number of animals per treatment group

[B] Glucose and acetate incorporation into total lipids: nmol substrate incorporated/(2 h·100 mg adipose tissue)

[C] Enzyme activities: nmol substrate converted to product/(min·100 mg protein). *FAS* fatty acid synthase, *NADP-MDH* NADP-malate dehydrogenase, *G6PDH* glucose-6-phosphate dehydrogenase, *6PGDH* 6-phosphogluconate dehydrogenase

[D] Gene expression was normalized to *RSP9* gene expression. Data for *SCD* are illustrated in Fig. 1

[a,b] AA × CLA interaction means with common superscripts are not different ($P > 0.05$)

assimilation in adipose tissue. Taken together with the increase in NADP-MDH activity, these results suggest that arginine promotes adiposity in beef cattle, similar to results in pigs fed arginine (Go et al. 2012). Likewise, intramuscular adipose tissue increases in growing pigs fed an arginine-supplemented diet for 2 months (Tan et al. 2009). Thus, arginine may beneficially improve the quality of beef and pork in animal production. This is in contrast to the ability of arginine to depress adiposity in rodents and humans (McKnight et al. 2010; Wu 2013). The basis for the difference in effects of arginine on adiposity between humans, rats, pigs, and cattle may lie in the fact that, in livestock species, adipose tissue is the primary site of de novo fatty acid biosynthesis (Mersmann et al. 1973; Hanson and Ballard 1967).

CLA infusion did not depress *SCD* gene expression, although it decreased in adipose tissue oleic acid. The intracellular concentration of *trans*-10, *cis*-12 CLA may have been too low to depress *SCD* gene expression, but in bovine preadipocytes, concentrations as low as 20 μM CLA caused a 50 % reduction in *SCD* gene expression (Chung et al. 2006). Although CLA had no effect on *SCD* gene expression, it may have depressed SCD catalytic activity (Park et al. 2000).

In summary, abomasal infusion of arginine promoted some aspects of adipogenesis, such as *SCD* gene expression and NADP-MDH activity. Conversely, CLA decreased NADP-MDH activity and increased *GPR43* gene expression, which would depress adipogenesis. Although carcass data were not recorded for these cattle, the data suggest that supplemental arginine would increase the concentration of oleic acid in beef, whereas CLA would depress oleic acid in beef. We suggest that L-arginine mays beneficially improve beef quality for human consumption.

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Conflict of interest The authors declare that there is no conflict of interest.

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