Amino Acids
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ORIGINAL ARTICLE

Lipid metabolism in pigs fed supplemental conjugated linoleic acid and/or dietary arginine

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Abstract We proposed that the combination of conjugated linoleic acid (CLA) and arginine would decrease adiposity by depressing lipid synthesis in liver and adipose tissues of growing pigs. Pigs were allotted to treatments in a 2 × 2 factorial design with two lipids (CLA or canola oil) and two amino acids [L-arginine or L-alanine (isonitrogenous control)]; supplements were provided from 80 to 110 kg body weight (approximately 4 weeks). Treatment groups ($n = 4$) were: control (2.05% L-alanine plus 1% canola oil); CLA (2.05% L-alanine plus 1% CLA); arginine (1.0% L-arginine plus 1.0% canola oil); arginine plus CLA (1.0% arginine plus 1.0% CLA). Arginine increased backfat thickness ($P = 0.07$) in the absence or presence of CLA, and arginine supplementation increased subcutaneous and retroperitoneal adipocyte volume, especially in combination with dietary CLA (interaction $P = 0.001$). Arginine increased palmitate incorporation into total lipids by over 60% in liver ($P = 0.07$). Dietary CLA increased palmitate incorporation into lipids in *longissimus* muscle by over 100% ($P = 0.01$), and CLA increased *longissimus* muscle lipid by nearly 20%. CLA increased glucose oxidation to CO_2 by over 80% in retroperitoneal and subcutaneous adipose tissues ($P = 0.04$), and doubled palmitate oxidation to CO_2 in intestinal duodenal mucosal cells ($P = 0.07$). Arginine supplementation decreased muscle pH at 45 min postmortem ($P = 0.001$), indicating elevated early postmortem glycolysis, and CLA and arginine independently increased *PGC-1α* gene expression in *longissimus* muscle. CLA but not arginine depressed *mTOR* gene expression in intestinal duodenal mucosal cells. CLA decreased serum insulin by 50% ($P = 0.02$) but increased serum triacylglycerols by over 40%. CLA supplementation increased ($P \leq 0.01$) total saturated fatty acids in liver and adipose tissue. In conclusion, neither CLA nor arginine depressed tissue lipid synthesis in growing/finishing pigs, and in fact dietary CLA promoted elevated intramuscular lipid and arginine increased carcass adiposity.

Keywords Arginine · Conjugated linoleic acid · Substrate oxidation · Lipogenesis · Gene expression

Abbreviations

AMPK	AMP-activated protein kinase
CLA	Conjugated linoleic acid
CPT-1A	Carnitine palmitoyltransferase 1A
FAS	Fatty acid synthase
HDL	High density lipoprotein
LDL	Low density lipoprotein
PGC-1α	PPARγ co-activator 1α
PPARα	Peroxisome proliferator-activated receptor α
RLP	Remnant lipoprotein
s.c.	Subcutaneous
r.p.	Retroperitoneal
SCD	Stearoyl-CoA desaturase
VLDL	Very low density lipoprotein

Introduction

Arginine and conjugated linoleic acid (CLA) individually have been shown to reduce adiposity in many animal species, depress preadipocyte proliferation, and modulate lipid metabolism in cell lines (Smith et al. 2002; Adams

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et al. 2005; Park et al. 1999; Jobgen et al. 2009b; Nall et al. 2009). CLA supplementation to rats increased the expression of peroxisome proliferator-activated receptor α (*PPARα*), acetyl CoA oxidase, and uncoupling protein (Choi et al. 2007) and also increased substrate oxidation and energy expenditure in overweight adults (Close et al. 2007). CLA caused modest body fat loss in humans (Whigham et al. 2007), depressed stearoyl CoA desaturase (*SCD*) gene expression and catalytic activity in adipose tissue (Smith et al. 2002), and inhibited lipogenesis while enhancing fatty acid oxidation in adipose tissue (Dobrzyn and Ntambi 2005). CLA prevented lipid filling by decreasing *PPARγ* in rodent preadipocytes (Brown et al. 2003) and bovine preadipocytes (Smith et al. 2009), and the *trans*-10, *cis*-12 CLA isomer decreased the expression of CCAAT-enhancer-binding proteins α in 3T3-L1 adipocytes (Kang et al. 2003). CLA may have depressed *PPARγ* gene expression via nuclear factor kappa B, which regulates mitogen-activated protein kinases and tumor necrosis factor α (Brown et al. 2004), resulting in reduced adipogenic gene expression (Chung et al. 2005).

Arginine, a semi-essential amino acid, also enhances fatty acid oxidation, partly via nitric oxide (NO)-mediated changes in expression of genes including *SCD*, AMP-activated protein kinase (*AMPK*), and PPARγ co-activator 1α (*PGC-1α*) (McKnight et al. 2010). NO from arginine modulated expression of *AMPK* (Lira et al. 2007), increased carnitine palmitoyltransferase 1A (*CPT-1A*) and *PGC-1α* expression in liver, and increased hepatic substrate oxidation compared with alanine-supplemented rats (Jobgen et al. 2006). However, in a more recent study, we were unable to demonstrate any effects of arginine 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.

There has been only one report of the effects of the combination of arginine and CLA on adipose tissue and liver metabolism (Nall et al. 2009). Supplementing diets with 1.5% CLA or 1.25% arginine increased body weights and eviscerated body weights, suggesting that both compounds stimulated muscle protein synthesis. Also, CLA depressed retroperitoneal (r.p.) adipose tissue mass in that study (Nall et al. 2009). Based on these results, we hypothesized that co-supplementation of dietary CLA and arginine would have additive effects on reducing adiposity, increasing lean mass, and promoting substrate oxidation in growing–finishing pigs (an excellent animal model for studying human nutrition and metabolism) (Smith 1998).

Materials and methods

Procedures for this research were approved by the Texas A&M University Institutional Animal Care and Use Committee, Office of Research Compliance. Amino acid supplements were provided by Ajinomoto Inc. (Tokyo, Japan) and CLA was provided by Lipid Nutrition B.V. (Wormerveer, The Netherlands) (Clarinol G-80 triacylglycerol preparation of mixed isomers). Canola oil was a food grade product purchased from a local retail outlet.

Preliminary experiment

Four pigs were rotated through one of four dietary arginine concentrations (0, 0.5, 1, or 2%) over a 4-week period in a 4 × 4 Latin square design. The 0% arginine diet contained 4.09% alanine; the 0.5% arginine diet contained 0.5% arginine, 3.07% alanine, and 0.52% cornstarch; the 1% arginine diet contained 1% arginine, 2.04% alanine, and 1.05% cornstarch; the 2% arginine diet contained 2% arginine and 2.09% cornstarch. All diets contained 1% CLA, and all additional CLA, alanine, arginine, or cornstarch replaced equal mass amounts of the basal diet (Table 1).

Table 1 Calculated nutrient content of the basal diet

Item	Amount
Components, % as fed	
Crude protein	13.99
Crude fat	3.04
Crude fiber	3.73
Calcium	0.65
Phosphorus	0.55
Lysine	0.60
Methionine + cysteine	0.41
Tryptophan	0.14
Threonine	0.45
Arginine	0.82
Metabolizable energy, Mcal/kg	3.08

Commercial diet prepared by Producers Cooperation Association, Bryan, TX, USA, closed formula, but diet ingredients were sorghum, wheat middlings, meat and bone meal, soybean meal, salt, limestone, dicalcium phosphate, trace mineral premix, vitamin premix, and lysine–HCl

Test diets were provided after an overnight fast. At 0 min (immediately before feeding) and 30, 60, and 120 min after feeding, blood samples were drawn from an ear vein and plasma amino acids were measured by HPLC (Wu et al. 2007).

Animals, diets, and sampling

Sixteen pigs were obtained from the Texas A&M University Animal Science Teaching/Research Center at approximately 80 kg body weight. Two gilts and two castrated males were assigned randomly to each of four treatment groups. Pigs were allotted to treatments in a 2 × 2 factorial design with two lipids and two amino acids. Treatment groups ($n = 4$) were: control (2.05% L-alanine plus 1% canola oil); CLA (2.05% L-alanine plus 1% CLA); arginine (1.0% L-arginine plus 1.0% canola oil) and arginine plus CLA (1.0% arginine plus 1.0% CLA). Composition of amino acids in diets was determined as described previously (Li et al. 2011).

Canola oil was used as a lipid control because it is food grade and has a similar melting point as the triacylglycerol preparation of CLA. Canola contains approximately 70% 18:1n-9, so the control and arginine diets contained 1.40 g 18:1n-9/100 g diet (Table 2). The CLA and CLA + arginine diets contained 0.95 and 0.94 g 18:1n-9/100 g diet, so these diets contained approximately 0.45 g 18:1n-9/100 g diet less than the control and arginine dietary treatments.

The CLA and CLA + arginine diets contained 0.77–0.80 g *cis*-9, *trans*-11 plus *trans*-10, and *cis*-12 CLA isomers (Table 2). The CLA preparation used in this study contained 80% *cis*-9, *trans*-11 plus *trans*-10, and *cis*-12 CLA, so the amounts of these CLA isomers analyzed in the diets were as expected. There was no detectable CLA in the control and arginine diets.

The supplemental level of arginine was set at 1% (Table 2), as 2% arginine caused a small depression in plasma lysine concentrations between 60 and 120 min after feeding (Fig. 1), probably due to competitive transport of basic amino acids (Wu et al. 2007). L-Alanine was used as an iso-nitrogenous control for arginine, because this amino acid is extensively degraded by pigs (Wu 2009; Li et al. 2010) and does not affect either food intake (Tan et al. 2009, 2011) or endogenous arginine synthesis (Wu et al. 2009).

Pigs were allowed free access to feed and water until they reached a projected body weight of 110 kg (4–5 weeks after initiation of the experiments). Pig weight and feed consumption data were collected weekly. When the average pen body weight was 110 kg, pigs were transported to the Texas A&M University Rosenthal Meat Science and Technology Center and harvested by standard industry procedures. Tissues from liver, *longissimus* muscle, subcutaneous (s.c.) adipose tissue, and r.p. adipose tissue were immediately placed in 37°C KHB buffer containing 5 mM glucose (oxygenated, pH 7.4), and transported to the laboratory for analysis of metabolism in vitro. Portions of each tissue, including intestinal mucosal cells (Wu et al. 2007) were snap-frozen in liquid nitrogen and stored at −80°C for adipose tissue cellularity, fatty acid composition and/or gene expression.

Table 2 Composition of experimental diets

Item	Treatments			
	Control	CLA	Arginine	CLA + arginine
Components, g/100 g diet as fed				
Basal diet	96.95	96.95	98.00	98.00
Canola oil	1.00	–	1.00	–
CLA	–	1.00	–	1.00
Alanine	2.05	2.05	–	–
Arginine	–	–	1.00	1.00
Total	100	100	100	100
Fatty acids, g/100 g diet as fed				
16:0	0.49	0.50	0.49	0.50
16:1n-7	0.01	0.01	0.01	0.01
18:0	0.08	0.08	0.08	0.08
18:1n-9	1.40	0.95	1.40	0.94
18:1n-7	0.07	0.05	0.07	0.05
18:2n-6	1.77	1.59	1.76	1.59
18:2*cis*-9, *trans*-11	0	0.38	0	0.40
18:2*trans*-10, *cis*-12	0	0.39	0	0.40
18:3n-3	0.16	0.09	0.16	0.10
Amino acids, g/100 g diet as fed				
Alanine	2.85	2.88	0.82	0.84
Arginine	0.85	0.84	1.86	1.89
Asparagine	0.45	0.44	0.42	0.44
Aspartate	0.61	0.59	0.60	0.61
Cysteine	0.27	0.28	0.27	0.26
Glutamate	1.18	1.19	1.15	1.17
Glutamine	1.37	1.33	1.35	1.31
Glycine	0.61	0.60	0.59	0.58
Histidine	0.37	0.36	0.35	0.36
Isoleucine	0.61	0.60	0.60	0.61
Leucine	1.31	1.28	1.27	1.24
Lysine	0.76	0.75	0.75	0.74
Methionine	0.30	0.29	0.28	0.30
Phenylalanine	0.72	0.70	0.69	0.71
Proline	1.16	1.12	1.20	1.15
Serine	0.62	0.60	0.59	0.58
Threonine	0.53	0.51	0.52	0.51
Tryptophan	0.15	0.14	0.15	0.15
Tyrosine	0.51	0.48	0.49	0.50
Valine	0.76	0.74	0.74	0.75

Treatments: control, 1% canola oil + 2.05% alanine; CLA, 1% CLA + 2.05% alanine; arginine, 1% canola oil + 1% arginine; CLA + arginine, 1% CLA + 1% arginine

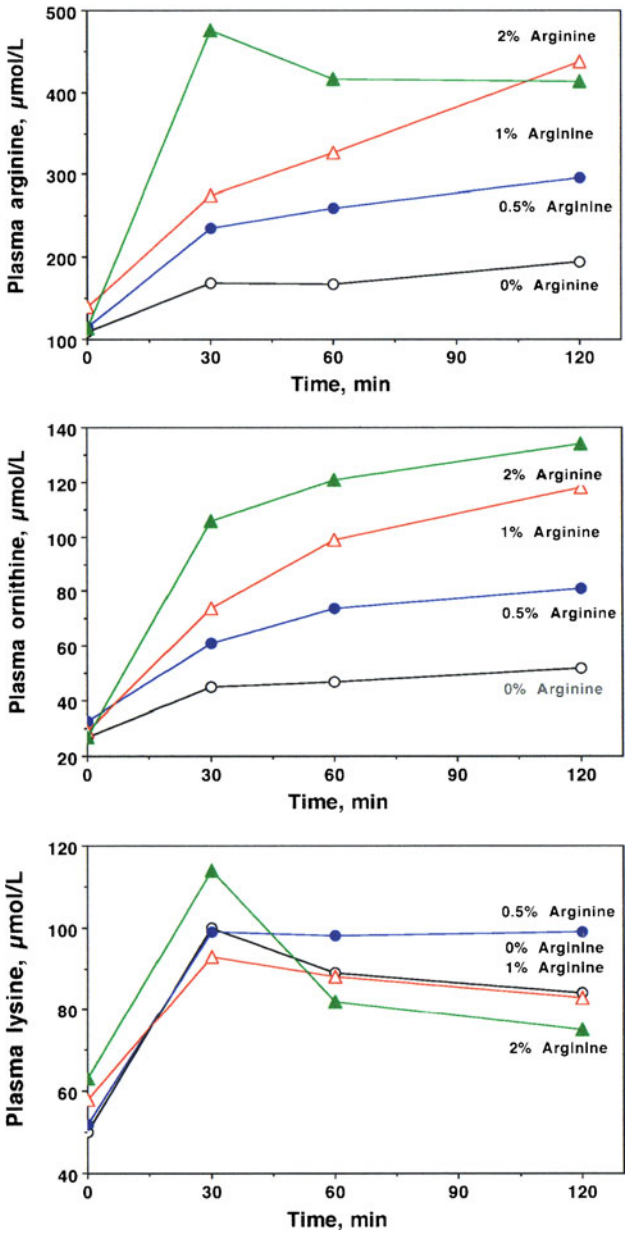

Fig. 1 Plasma arginine (*top panel*), ornithine (*middle panel*) and lysine (*bottom panel*) immediately preceeding and 120 min following consumption of basal diets supplemented with 0, 0.5, 1, or 2% arginine. The 0% arginine diet contained 4.09% alanine and 95.91% basal diet; the 0.5% arginine diet contained 0.5% arginine, 3.07% alanine, 0.52% cornstarch, and 95.91% basal diet; the 1% arginine diet contained 1% arginine, 2.04% alanine, 1.05% cornstarch, and 95.91% basal diet; the 2% arginine diet contained 2% arginine, 2.09% cornstarch, and 95.91% basal diet. All diets contained 1% CLA (80% CLA mixed isomers). Each data point is the mean for four pigs. Pigs were rotated through each dietary arginine concentration over a 4-week period in a 4 × 4 Latin square design

Plasma amino acid concentrations

Blood samples were collected at harvest into EDTA coated Vacutainer tubes. Plasma was separated by centrifugation (5,000×*g* for 15 min) and stored at −80°C until analyzed. 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).

Carcass traits and meat quality characteristics

After chilling at 2°C for 24 h, the right carcass side was weighed and midline backfat thickness was measured at the first rib, 10th rib, last rib, and last lumbar vertebrae. Carcass length was measured as the distance between the bottom of the pubic bone and the bottom of the first rib at the dorsal middle. *Longissimus* muscle cross-sectional area was measured using plotting paper at the 10th rib. Dressing percentage was calculated with the proportion of carcass weight relative to its live slaughter weight. At 45 min and 24 h, muscle temperature and pH were measured in triplicate (IQ150, IQ Scientific Instruments, Loveland, CO, USA) and meat color criteria were measured in triplicate (Minolta Chromameter CR-300, Osaka, Japan) after exposing the surface to the air for 30 min. An average of triplicate measurements was recorded and results were expressed as C.I.E. L*, a*, b* (L*, lightness; a*, redness–greenness; b*, yellowness–blueness). Drip loss was measured by suspending muscle samples standardized for surface area in an inflated plastic bag for 48 h at 2°C and measuring the beginning and final muscle weights. The amount of fat and moisture was measured in duplicate by a fat and moisture analyzer (SMART Trac, CEM, Matthews, NC, USA).

Lipogenesis and CO_2 production in vitro

Two-hour in vitro incubations were conducted with fresh liver, *longissimus* muscle, s.c. adipose tissue, r.p. adipose tissue, and intestinal duodenal mucosal cells (≈100 mg) as described previously (May et al. 1994; Huerta-Leidenz et al. 1996). Flasks contained 5 mM glucose, 0.75 mM palmitate, 10 mM HEPES buffer and 1 µCi [U-^{14}C]glucose or 0.5 µCi [1-^{14}C]palmitic acid in KHB buffer. Flasks also contained hanging center wells with fluted filter paper for the collection of CO_2. 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 2 h incubation period, reactions terminated by the addition of 1 mL of 2 N H_2SO_4, and 0.2 mL of 2 N NaOH is injected into the hanging center well. Flasks were shaken for an additional 2 h. Glucose and palmitate carbon incorporation into

Table 3 Plasma amino acid concentration of pigs fed CLA, arginine, and CLA plus arginine

Amino acid	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
Essential amino acids								
Histidine	75	84	82	80	5.9	0.83	0.53	0.71
Isoleucine	80	91	88	86	8.9	0.85	0.63	0.86
Leucine	154	169	156	149	22.1	0.68	0.85	0.93
Lysine	78	98	107	104	25.0	0.49	0.74	0.85
Methionine	33	36	32	30	2.7	0.29	0.80	0.52
Phenylalanine	64^{b}	87^{a}	77^{ab}	73^{ab}	5.2	0.90	0.16	0.06
Threonine	66	87	88	90	7.2	0.11	0.14	0.11
Tryptophan	38	43	46	46	5.9	0.33	0.72	0.73
Valine	203	222	208	202	18.4	0.69	0.73	0.87
Nonessential amino acids								
Alanine	341	423	322	270	45.8	0.10	0.76	0.18
Arginine	120	298	149	139	90.1	0.48	0.37	0.50
Asparagine	43	50	44	49	3.9	0.99	0.13	0.51
Aspartate	10	11	11	14	1.7	0.26	0.38	0.51
Citrulline	53	67	60	59	8.9	0.99	0.49	0.75
Glutamate	157	149	148	169	37.0	0.89	0.85	0.97
Glutamine	419	436	361	384	58.2	0.35	0.73	0.80
Glycine	708	818	774	758	79.8	0.97	0.56	0.81
Ornithine	66	60	56	58	11.2	0.59	0.83	0.93
Serine	84^{b}	106^{a}	101^{ab}	90^{ab}	5.5	0.91	0.44	0.06
Taurine	82	119	118	156	27.5	0.19	0.18	0.35
Tyrosine	42	60	54	58	10.8	0.63	0.31	0.65

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group), expressed as μmol/L. Means in rows not bearing a common superscript alphabets differ, $P < 0.10$

neutral lipids and CO_2 in tissues was measured as described previously (Nall et al. 2009).

Fatty acid composition

Lipid was extracted from plasma, tissues and diets by the Folch method (Folch et al. 1957), and fatty acids were methylated in 100 mg lipid extract as described by Morrison and Smith (1964). Fatty acid methyl esters were analyzed by gas/liquid chromatography (Varian model CP-3800 equipped with a CP-8200 auto-sampler, Varian Inc, CA, USA). Separation of fatty acid methyl esters was accomplished on a fused silica capillary column (100 m × 0.25 mm ID) (model CP-7420, Varian Inc, CA, USA) with the helium as carrier gas (flow rate = 1.7 mL/min), with a split ratio of 100:1 at 270°C. Oven temperature set up to 165°C for 65 min and increased to 235°C (2°C/min) and held for 15 min. The flame ionization detector was at 270°C. An authentic standard (GLC 68-D, Nu-Chek Prep, MN, USA) was used to identify each peak.

Cellularity

Subcutaneous and r.p. adipose tissues were collected immediately for determination of cellularity by osmium fixation, counting, and sizing as described previously (May et al. 1994) with modifications. Osmium-fixed adipocytes were resuspended with 0.01% Triton X-100 in 0.154 M NaCl and used for determination of cell size, volume, and cells/100 mg tissue, using bright-field microscope (Olympus Vanox ABHS3, Olympus, Tokyo, Japan) and CCD Color Video Camera (DXC-960MD, Sony, Japan). ImageJ software (released by NIH) was used for image processing and analysis.

RNA isolation and qRT-PCR analysis

Total RNA from 200 mg of each tissue was isolated as described previously (Chomczynski and Sacchi 1987) using Tri-reagent (Sigma Chemical Co., St. Louis, MO, USA). The concentrations and abundance of total RNA

were measured with Nanodrop (NanoDrop Technologies Inc., Wilmington, DE, USA) 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 cDNA using TaqMan Transcription Reagent and MultiScribe reverse transcription (Applied Biosystem, Foster City, CA, USA) with the following temperature ramp: 25°C for 10 min, 37°C for 60 min, and 95.5°C for 5 min. Quantitative PCR was used to analyze the quantity of gene expression including *AMPK*, *PGC-1α*, *PPAR*, *FAS*, *SCD*, *mTOR*, and *CPT-1B*. Eukaryotic 18S rRNA was used as an endogenous control. Measurement of the relative quantity of cDNA was performed using TaqMan Universal PCR mixer, Assays-on-demand Gene Expression Products (Applied Biosystem, Foster City, CA, USA), and 1 μg of cDNA mixture.

Serum insulin, triacylglycerol, and lipoprotein fractions

Frozen aliquots of serum were sent to Spectracell Laboratories, Inc. (Houston, TX, USA) for lipid and lipoprotein–lipid analyses using an analytical ultracentrifugation process. A complete Lipoprotein Particle Profile™ test was performed using the lipoprotein subgroup particle number analysis method. This lipoprotein particle separation procedure utilizes a patented method (Patent No.: US 7,856,323 B2) with a continuous gradient generated by analytical ultracentrifugation. The lipoprotein particles were stained with a fluorescent dye and then separated in the gradient over a range of $d = 1.000$–1.300 g cm^3. After separation, the fluorescence of the lipoprotein particles was measured in an HPLC-type flow system and normalized to a cholesterol scale with a proprietary algorithm. Values for each lipoprotein subgroup at their specific densities were determined using a multiple Gaussian fit/integration routine. The total number of VLDL, LDL, remnant lipoprotein (RLP), dense LDL_{III} and LDL_{IV}, HDL, and buoyant HDL_{2b} particles were determined at their specific densities. The coefficient of variation for this analysis using known standards has been reported as 2–3%. Analysis of serum insulin and triaclyglycerol was performed with commercially available kits (Carolina Liquid Chemistries, Brea, CA, and Olympus, Center Valley, PA, USA) using an Olympus AU400e chemistry immune analyzer.

Statistical analysis

Data were analyzed as a 2 × 2 factorial analysis of variance with arginine and CLA as the main effects. The model tested main effects of arginine and CLA, as well as the arginine × CLA interaction. Means were separated the Duncan method, if their respective F test indicated significant differences ($P < 0.05$).

Results

Preliminary trial—plasma amino acids

There were linear increases in plasma arginine and ornithine concentrations as dietary supplemental arginine increased from 0 to 2% (Figs. 1, 2). Plasma lysine peak values were observed at 30 min after feeding, and declined in all supplemental groups thereafter (Fig. 3). Dietary supplementation with 2% arginine caused a small depression in plasma lysine, although this difference was not statistically significant. To avoid interference in intestinal lysine absorption by arginine (Wu et al. 2007), arginine was included at 1% of the diet in this study.

Plasma amino acid profiles

CLA increased the concentrations of phenylalanine and serine in the absence of arginine (arginine × CLA interaction $P = 0.06$; Table 3). No other plasma amino acids, including arginine, were affected by dietary arginine or CLA.

Growth performance, carcass traits, and meat quality

Neither arginine nor CLA dietary supplementation affected total body weight gain, total feed intake, average daily

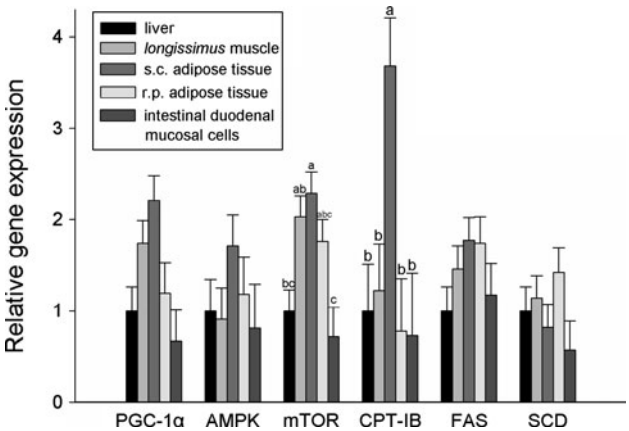

Fig. 2 Gene expression in liver, *longissimus* muscle, intestinal duodenal mucosal cells, and subcutaneous (s.c.) and retroperitoneal (r.p.) adipose tissue in growing–finishing pigs. Data are pooled across treatment groups. *Bars* are mean ± standard error of the mean ($n = 16$ per bar). [abc]Means without common superscripts differ ($P < 0.05$). [ab]CPT-1B gene expression was higher ($P < 0.05$) in s.c. adipose tissue than in liver, *longissimus* muscle, r.p. adipose tissue, or intestinal mucosal cells, and mTOR gene expression was higher in s.c. adipose tissue than in intestinal mucosal cells. Also, PGC-1α and FAS gene expression was higher ($P \leq 0.10$) in s.c. adipose tissue than in intestinal mucosal cells

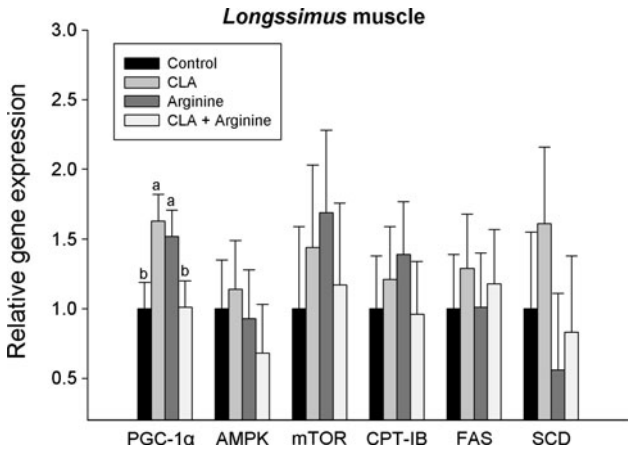

Fig. 3 Gene expression in *longissimus* muscle of growing–finishing pigs supplemented CLA and/or arginine. *Bars* are mean ± standard error of the mean ($n = 4$ per bar). [ab]PGC-1α gene expression was higher ($P < 0.05$) in muscle from CLA- and arginine-fed pigs than in muscle from control pigs or pigs fed CLA + arginine

gain, average daily feed intake, or feed efficiency (feed/gain) (Table 4). Neither arginine nor CLA affected slaughter weight, carcass weight, dressing percentage, or carcass quality grade (Table 6). Muscle scores and loin-eye areas also were not affected by arginine or CLA. However, carcass length was 2.4% shorter in pigs supplemented with CLA ($P = 0.06$). First rib fatness and total backfat thickness were greater in pigs fed arginine than in control pigs ($P = 0.07$ and 0.09, respectively) (Table 4).

CLA increased *longissimus* muscle pH at 45 min postmortem relative to arginine-fed pigs (arginine × CLA interaction $P = 0.001$) (Table 5). Arginine increased ($P = 0.06$) and CLA decreased ($P = 0.07$) muscle lightness (L*). CLA supplementation increased percentage intramuscular fat ($P = 0.01$) and concomitantly decreased percentage moisture ($P = 0.06$) in *longissimus* muscle.

Glucose and palmitate metabolism in vitro and adipose tissue cellularity

In all tissues examined in this study, the rate glucose conversion to CO_2 was 10- to 15-fold greater than the rate of palmitate conversion to CO_2 (Tables 6, 7). Conversely, palmitate incorporation into total lipids was 3- to 40-fold higher than lipid synthesis from glucose.

Neither CLA nor arginine affected CO_2 production from glucose in liver, *longissimus* muscle, or intestinal duodenal mucosal cells (Table 6). CLA tended ($P = 0.10$) to depress hepatic lipid synthesis from glucose, whereas hepatic palmitate incorporation into total lipids was greater in pigs supplemented with arginine than in liver from control pigs (arginine × CLA interaction $P = 0.07$). CLA supplementation in the absence of arginine increased palmitate incorporation into total lipids in *longissimus* muscle (arginine × CLA interaction $P = 0.001$). The conversion of palmitate to CO_2 in intestinal duodenal mucosal cells

Table 4 Growth performance and carcass traits of pigs fed CLA, arginine, or CLA plus arginine

Item	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
Slaughter weight (kg)	111	105	109	110	4.02	0.69	0.65	0.76
Total gain (kg)	43	39	44	45	3.81	0.37	0.73	0.68
Total feed (kg)	184	188	185	184	4.56	0.80	0.69	0.91
Average daily gain (kg/day)	0.78	0.70	0.79	0.82	0.07	0.38	0.74	0.69
Average daily feed (kg/day)	2.88	2.95	2.90	2.89	0.07	0.79	0.68	0.91
Feed:gain	3.77	4.23	3.74	3.55	0.27	0.27	0.65	0.45
Carcass								
Hot weight (kg)	85.0	80.0	83.6	86.5	3.63	0.49	0.78	0.63
Length (cm)	82.7	80.0	81.5	80.6	0.87	0.72	0.06	0.19
Dressing (%)	76.8	76.0	76.8	78.3	0.76	0.16	0.65	0.22
Grade	1.30	1.00	1.30	1.35	0.17	0.32	0.48	0.48
Backfat thickness (cm)	2.49	2.50	2.74	2.78	0.15	0.08	0.83	0.38
Backfat thickness								
First rib	3.62	3.75	4.06	4.19	0.23	0.07	0.58	0.32
Last rib	2.03	1.91	2.10	2.16	0.16	0.32	0.84	0.71
Last lumbar	1.84	1.91	2.10	2.03	0.17	0.28	1.00	0.73
Total	7.49	7.56	8.26	8.38	0.44	0.09	0.83	0.39
Loineye area (cm^2)	38.7	35.5	38.2	41.0	2.82	0.39	0.93	0.60

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group)

Table 5 Meat quality characteristics of pigs fed CLA, arginine, or CLA plus arginine

	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
45 min postmortem								
pH	5.94[a]	6.00[a]	5.59[b]	5.64[b]	0.08	0.001	0.46	0.001
L* (lightness)	42.7	45.0	46.0	45.3	1.80	0.33	0.67	0.60
a* (redness)	6.89	8.58	8.66	8.30	0.82	0.37	0.42	0.39
b* (yellowness)	1.80	3.05	2.79	2.76	0.61	0.57	0.32	0.49
24 h postmortem								
pH	5.65	5.64	5.68	5.63	0.03	0.80	0.20	0.43
L*	48.6[a,b]	46.8[b]	52.2[a]	48.7[a,b]	1.50	0.06	0.07	0.08
a*	8.24	9.01	10.14	8.83	0.73	0.25	0.72	0.33
b*	3.98	4.09	5.07	4.70	0.67	0.21	0.85	0.63
Bag drip loss (%)	5.71	5.12	6.27	6.10	2.29	0.73	0.87	0.98
Moisture (%)	74.1[a]	72.7[b]	73.6[a]	73.8[a]	0.25	0.25	0.06	0.003
Intramuscular fat (%)	2.30[b]	3.17[a]	2.02[b]	2.55[a,b]	0.26	0.10	0.01	0.03

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group). Means in rows not bearing a common superscript alphabets differ, $P < 0.10$

Table 6 Incorporation of glucose and palmitate carbon into CO_2 and lipids in vitro in liver, *longissimus* muscle, and intestinal duodenal mucosal cells in pigs fed CLA, arginine, or CLA plus arginine

Tissue/substrate	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
Liver metabolism, nmol substrate converted to product/(100 mg × 2 h)								
CO_2 production								
Glucose	68.4	49.4	47.5	46.8	7.96	0.22	0.27	0.32
Palmitate	4.88	6.01	5.56	6.54	0.72	0.43	0.17	0.49
Lipid synthesis								
Glucose	2.94	1.72	2.35	2.05	0.43	0.81	0.10	0.27
Palmitate	34.0[b]	41.4[a,b]	56.2[a]	48.5[a,b]	6.04	0.02	0.98	0.07
Longissimus muscle metabolism, nmol substrate converted to product/(100 mg × 2 h)								
CO_2 production								
Glucose	36.1	42.5	46.5	30.5	9.63	0.82	0.74	0.68
Palmitate	4.66	5.27	4.33	6.02	0.79	0.76	0.18	0.50
Lipid synthesis								
Glucose	1.80	1.16	2.41	1.95	1.13	0.52	0.62	0.88
Palmitate	28.6[b]	59.9[a]	28.2[b]	30.1[b]	5.37	0.01	0.01	0.001
Intestinal duodenal mucosal metabolism, nmol substrate converted to product/(100 mg × 2 h)								
CO_2 production								
Glucose	186	189	106	151	38.6	0.11	0.53	0.35
Palmitate	16.0[a,b]	32.1[a]	13.8[b]	18.1[a,b]	5.50	0.15	0.07	0.10

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group). Means in rows not bearing a common superscript alphabets differ, $P < 0.10$

tended to be greater in pigs fed CLA than in pigs fed arginine (arginine × CLA interaction $P = 0.10$) (Table 6).

Arginine did not affect the conversion of glucose or palmitate to CO_2 in either s.c. or r.p. adipose tissue ($P > 0.30$) (Table 7). Arginine increased glucose incorporation into total lipids in s.c. adipose tissue in the absence of CLA (arginine × CLA interaction $P = 0.07$). Adipocyte volume in both adipose tissues was greater in pigs

Table 7 Incorporation of glucose or palmitate carbon into CO_2 and lipids in vitro and cellularity in retroperitoneal and subcutaneous adipose tissue in pigs fed CLA, arginine, or CLA plus arginine

Tissue/substrate	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
Retroperitoneal adipose tissue metabolism, μmol substrate converted to product/(10^{-6} × cell × 2 h)								
CO_2 production								
Glucose	58.3	107	60.4	113	24.2	0.85	0.03	0.18
Palmitate	5.73	7.62	6.06	6.18	1.14	0.63	0.38	0.66
Lipid synthesis								
Glucose	64.5	63.5	35.2	86.4	23.4	0.89	0.29	0.50
Palmitate	112	114	126	121	11.7	0.39	0.91	0.84
Retroperitoneal adipose tissue cellularity								
Adipocyte volume, pL	666^{b}	811^{a}	805^{a}	814^{a}	8.63	0.001	0.001	0.001
Adipocytes/100 mg × 10^{-9}	1.00^{a}	0.94^{d}	0.96^{c}	0.97^{b}	0.01	0.001	0.001	0.001
Subcutaneous adipose tissue metabolism, μmol substrate converted to product/(10^{-6} × cell × 2 h)								
CO_2 production								
Glucose	48.9	88.1	75.2	92.3	14.4	0.30	0.04	0.14
Palmitate	4.35	5.23	4.71	5.25	0.92	0.83	0.44	0.88
Lipid synthesis								
Glucose	31.5^{b}	33.5^{b}	83.3^{a}	$41.2^{a,b}$	15.2	0.06	0.20	0.07
Palmitate	91.1	92.9	110	109	11.0	0.12	0.97	0.49
Subcutaneous adipose tissue cellularity								
Adipocyte volume, pL	597^{d}	735^{c}	789^{b}	875^{a}	11.0	0.001	0.001	0.001
Adipocytes/100 mg × 10^{-9}	1.07^{a}	1.01^{b}	0.97^{c}	0.96^{c}	0.01	0.001	0.001	0.001

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group). Means in rows not bearing a common superscript alphabets differ, $P < 0.10$

supplemented CLA, arginine, or CLA plus arginine than in control pigs ($P = 0.001$). The greatest increase in adipocyte volume was caused by the combination of dietary arginine and CLA (arginine × CLA interaction $P = 0.001$) (Table 7).

Fatty acid composition of plasma and tissues

CLA supplementation increased ($P < 0.02$) concentrations of *cis*-9, *trans*-11 CLA (*c9*, *t11* CLA), *trans*-10, *cis*-12 CLA (*t10*, *c11* CLA), and saturated fatty acids in plasma, *longissimus* muscle and intestinal mucosal cells (Table 8), and in s.c. and r.p. adipose tissues and liver (Table 9). CLA decreased 18:1n-9 in plasma and *longissimus* muscle and in r.p. and s.c. adipose tissues. In the adipose tissue depots, dietary CLA also increased the saturated fatty acids 16:0 and 18:0 and decreased 20:4n-6 ($P < 0.02$) (Table 9).

Expression of genes related to substrate oxidation and lipid synthesis

mTOR gene expression was greater ($P < 0.05$) in s.c. adipose tissue than in liver or intestinal mucosal cells, and *CPT-1B* gene expression was greater in s.c. adipose tissue than all other tissues (Fig. 2). *PGC-1α* and *FAS* gene expression was higher ($P < 0.10$) in s.c. adipose tissue than in intestinal mucosal cells (mean separation for *PGC-1α* and *FAS* is not indicated by superscripts in Fig. 2).

In *longissimus* muscle, both CLA and arginine individually increased *PGC-1α* gene expression ($P < 0.05$), but the effect was not detected when pigs were supplemented with the combination of CLA + arginine (Fig. 3). Expression levels of *AMPK*, *mTOR*, *CPT-1B*, *FAS*, and *SCD* were not affected by CLA or arginine in *longissimus* muscle.

mTOR gene expression was lower ($P < 0.05$) in intestinal mucosal cells from CLA-fed pigs than in cells from control pigs (Fig. 4). Also, *AMPK*, *FAS*, and *SCD* gene expression was lower ($P \leq 0.10$) in intestinal mucosal cells from pigs fed CLA (±arginine) than in control pigs (mean separation for *AMPK*, *FAS*, and *SCD* is not indicated by superscripts in Fig. 4). Neither CLA nor arginine affected gene expression in liver, r.p. adipose tissue, or s.c. adipose tissue (treatment means not shown).

Table 8 Fatty acid composition (g/100 g total fatty acids) of plasma, *longissimus* muscle, and intestinal duodenal cells from pigs fed diets containing CLA, arginine, CLA plus arginine

Fatty acid	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
Plasma								
16:0	15.4	17.2	16.3	16.9	0.61	0.59	0.08	0.26
18:0	14.9	15.3	14.3	14.7	0.58	0.30	0.46	0.64
18:1n-9	20.6	18.8	24.0	19.8	1.37	0.13	0.06	0.10
18:2n-6	26.5	26.9	25.8	25.7	1.81	0.61	0.95	0.96
18:2*cis*-9, *trans*-11	nd	0.90	nd	0.90	0.33	1.00	0.02	0.13
18:2*trans*-10, *cis*-12	nd	0.32	nd	0.34	0.12	0.95	0.02	0.14
20:4n-6	12.7	10.2	10.1	11.4	0.79	0.37	0.43	0.13
Longissimus muscle								
16:0	24.8	24.9	24.0	25.0	1.34	0.78	0.67	0.94
18:0	11.3	10.9	10.5	11.6	0.39	0.99	0.42	0.24
18:1n-9	41.3[a]	36.1[b]	39.8[a,b]	37.2[b]	1.26	0.87	0.005	0.03
18:2n-6	7.42	8.15	7.92	8.45	0.69	0.56	0.36	0.75
18:2*cis*-9, *trans*-11	0.03[b]	0.51[a]	0.05[b]	0.46[a]	0.06	0.83	0.001	0.001
18:2*trans*-10, *cis*-12	nd	0.18	nd	0.17	0.03	0.87	0.001	0.001
20:4n-6	1.35	1.80	2.25	1.54	0.30	0.32	0.69	0.20
Intestinal duodenal mucosal cells								
16:0	21.5	24.1	21.9	22.8	1.15	0.69	0.12	0.38
18:0	15.7	18.8	17.7	17.7	0.98	0.63	0.13	0.15
18:1n-9	25.0	24.1	26.4	21.3	1.95	0.72	0.12	0.28
18:2n-6	12.3	10.4	9.85	12.0	1.52	0.79	0.93	0.59
18:2*cis*-9, *trans*-11	0.01[b]	0.66[a]	0.04[b]	0.75[a]	0.09	0.50	0.001	0.001
18:2*trans*-10, *cis*-12	nd	0.24	nd	0.26	0.06	0.78	0.001	0.001
20:4n-6	7.03	5.10	6.69	7.30	1.04	0.37	0.52	0.44

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group). Means in rows not bearing a common superscript alphabets differ, $P < 0.10$

nd Not detectable

Serum insulin, triacylglycerol, and lipoprotein fractions

CLA depressed serum insulin concentrations by approximately 50% ($P = 0.02$) and increased serum triacylglycerol by over 40% ($P = 0.01$) (Table 10). The elevation in serum triacylglycerol caused by CLA was greater in pigs that also were supplemented with dietary arginine (arginine × CLA interaction $P = 0.08$). Neither CLA nor arginine affected serum total cholesterol, LDL, VLDL, or HDL cholesterol concentrations ($P > 0.21$). CLA depressed LDL_{IV} particle number in the absence of dietary arginine (arginine × CLA interaction $P = 0.05$). LDL particle size was increased by dietary arginine ($P = 0.03$) and CLA ($P = 0.05$). Arginine increased serum RLP concentrations, especially in the presence of arginine (arginine × CLA interaction $P = 0.03$).

Discussion

Although 1% dietary arginine increased plasma arginine concentrations nearly fourfold during the first 2 h postprandially, the elevation in plasma arginine was not apparent in the pigs at harvest (15 h postprandially). In contrast, plasma CLA concentrations remained elevated even after the 15 h fast; this no doubt reflects the postprandial release of CLA from adipose tissue depots, in which CLA was relatively enriched.

The lower early postmortem pH in pigs supplemented with arginine may have been caused by enhanced AMPK activity (McKnight 2010), as dietary arginine supplementation to ZDF rats increased *AMPK* gene expression (Fu et al. 2005). Dietary arginine had no effect on *AMPK* gene expression in the muscle of pigs in this study, but arginine

Table 9 Fatty acid composition (g/100 g total fatty acids) of retroperitoneal and subcutaneous adipose tissue and liver from pigs fed diets containing CLA, arginine, or CLA plus arginine

Fatty acid	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
Retroperitoneal adipose tissue								
16:0	30.1	34.0	28.0	32.0	1.78	0.25	0.03	0.12
18:0	17.7	18.1	17.2	17.5	0.78	0.49	0.61	0.87
18:1n-9	$31.3^{a,b}$	25.2^{c}	34.5^{a}	$28.8^{b,c}$	1.57	0.04	0.001	0.002
18:2n-6	11.2	10.8	11.6	10.8	0.94	0.81	0.55	0.93
18:2*cis*-9, *trans*-11	$0.55^{b,c}$	1.31^{a}	0.12^{c}	$0.86^{a,b}$	0.23	0.07	0.003	0.01
18:2*trans*-10, *cis*-12	$0.30^{b,c}$	0.73^{a}	0.05^{c}	$0.48^{a,b}$	0.13	0.07	0.003	0.01
20:4n-6	0.24	0.18	0.22	0.18	0.02	0.59	0.03	0.17
Subcutaneous adipose tissue								
16:0	21.5^{b}	26.3^{a}	22.2^{b}	26.0^{a}	0.69	0.77	0.001	0.001
18:0	11.3^{b}	15.5^{a}	12.8^{b}	16.5^{a}	0.67	0.07	0.001	0.001
18:1n-9	42.2^{a}	32.4^{b}	41.6^{a}	32.4^{b}	1.25	0.78	0.001	0.001
18:2n-6	14.3	12.7	13.1	12.8	0.64	0.43	0.16	0.33
18:2*cis*-9, *trans*-11	0.05^{b}	1.33^{a}	0.13^{b}	1.29^{a}	0.13	0.88	0.001	0.001
18:2*trans*-10, *cis*-12	nd	0.73^{a}	0.05^{b}	0.72^{a}	0.09	0.82	0.001	0.001
20:4n-6	0.27^{a}	0.17^{c}	$0.23^{a,b}$	0.19^{bc}	0.02	0.62	0.002	0.01
Liver								
16:0	12.1^{b}	15.7^{a}	15.6^{a}	15.2^{a}	0.50	0.03	0.01	0.001
18:0	23.0	23.2	21.3	21.6	1.00	0.11	0.83	0.46
18:1n-9	14.5	14.0	18.3	16.0	1.35	0.05	0.31	0.16
18:2n-6	10.0^{b}	14.3^{a}	14.2^{a}	14.9^{a}	0.89	0.02	0.01	0.002
18:2*cis*-9, *trans*-11	0.05^{b}	1.17^{a}	0.11^{b}	1.23^{a}	0.11	0.57	0.001	0.001
18:2*trans*-10, *cis*-12	nd	0.30^{a}	0.01^{b}	0.30^{a}	0.04	0.86	0.001	0.001
20:4n-6	21.3^{a}	15.7^{b}	15.3^{b}	15.0^{b}	1.14	0.01	0.02	0.001

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group). Means in rows not bearing a common superscript alphabets differ, $P < 0.10$

nd Not detectable

supplementation tended to increase the lightness of muscle at 24 h postmortem, consistent with a more rapid pH decline. Therefore, arginine supplementation may have enhanced the rate of glycolysis in muscle.

We previously demonstrated that weight gain, feed intake, and feed efficiency were greater in rats supplemented with 1% CLA than in control rats (Nall et al. 2009); however, neither arginine nor CLA supplementation for 4 weeks affected growth performance in pigs. This is consistent with previous studies in pigs, in which dietary CLA supplementation (0.5–2%) had no effect on weight gain, feed intake, or feed efficiency in pigs fed to the same end weight as in the current study (approximately 110 kg) (Tischendorf et al. 2002; Wiegand et al. 2002; Mateo et al. 2007; Ma et al. 2010). We previously demonstrated that daily weight gain by day 28 and body weight by day 15 were greater in milk-fed piglets receiving 0.2 and 0.4% arginine (dry matter basis) than in control piglets (Kim and Wu 2004). Similar results were obtained for piglets fed 0.2 and 0.4% of arginine supplementation from 7 to 21 days (Wu et al. 2004). Thus, arginine supplementation to pigs may be more effective in piglets than in finishing–growing pigs, although this has not been demonstrated for CLA.

CLA did not affect backfat thickness, and we previously demonstrated that feeding CLA for 35 days to postweanling piglets did not decrease adipose tissue mass (Demaree et al. 2002). Other investigators demonstrated that supplementation of CLA to pigs from 23.5 to 110 kg (Tischendorf et al. 2002) or from 28 to 115 kg body weight (Wiegand et al. 2002) decreased backfat thickness. Also, CLA depressed preadipocyte proliferation in young pigs (Adams et al. 2005). Thus, we anticipated that CLA would depress adiposity in this study.

Our results demonstrated that dietary CLA supplementation increased intramuscular fat in *longissimus* muscle, consistent with greater rates of lipid synthesis from palmitate

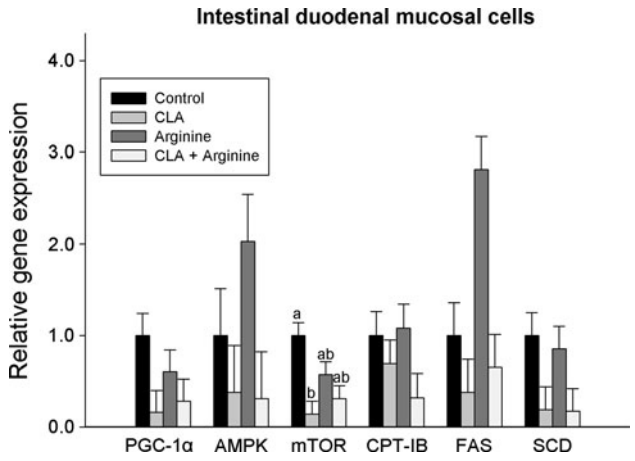

Fig. 4 Gene expression in intestinal duodenal mucosal cells (Fig. 2) of growing–finishing pigs supplemented CLA and/or arginine. *Bars* are mean ± standard error of the mean ($n = 4$ per bar). ^{ab}mTOR gene expression was lower ($P < 0.05$) in intestinal mucosal cells from CLA-fed pigs than in cells from control pigs. Also, AMPK, FAS, and SCD gene expression was lower ($P \leq 0.10$) in intestinal mucosal cells from pigs fed CLA (±arginine) than in control pigs

in vitro in muscle from CLA-fed pigs, relative to control pigs. CLA also increased *PGC-1α* gene expression in muscle, which should have promoted fatty acid oxidation; however, neither CLA nor arginine affected in vitro substrate oxidation in muscle. Other investigators have demonstrated that 2% CLA supplementation increased marbling scores and intramuscular fat in finishing–growing pigs (Dugan et al. 1999), and 5% CLA supplementation increased intramuscular fat in the loin of pigs (Joo et al. 2002). In the current study, arginine did not affect the concentration of intramuscular fat, but in previous reports, dietary 1% arginine supplementation to finishing pigs increased intramuscular fat (Tan et al. 2009; Ma et al. 2010). Similarly, supplementing differentiation media with 5 mM arginine stimulated adipogenic gene expression in bovine preadipocytes (Smith et al. 2009). Differences in experimental conditions between this and previous studies (Tan et al. 2009; Ma et al. 2010) suggest that arginine supplementation must be provided to younger swine and/or for longer duration to elicit changes in intramuscular lipid.

Dietary CLA supplementation significantly increased total saturated fatty acids liver, s.c. adipose tissue, and r.p. adipose tissue. We also reported that dietary CLA supplementation to pigs depressed SCD enzyme activity in pig s.c. adipose tissue (Smith et al. 2002). Likewise, SCD enzyme activity and *SCD* gene expression were decreased by CLA treatment of 3T3-L1 adipocytes (Choi et al. 2000). We previously demonstrated that arginine did not affect fatty acid composition in epididymal adipose tissue and plasma fatty acids in young rats (Nall et al. 2009); consistent with this was the lack of effect of arginine on the relative proportions of saturated and monounsaturated fatty acids in this study. Thus, although arginine promoted *SCD* gene expression in bovine preadipocytes (Smith et al. 2009), it did not have the same effect when provided as a dietary supplement to rats or pigs.

It is apparent that glucose was more effectively oxidized to CO_2 in vitro than palmitate, whereas the rate of palmitate esterification into total lipids in vitro was much greater

Table 10 Insulin, triacylglycerol, and lipoprotein cholesterol and particle number concentrations in plasma of pigs fed CLA, arginine, or CLA plus arginine

	Treatment				SEM	*P* values		
	Control	CLA	Arginine	CLA + arginine		Arginine	CLA	Arg × CLA
Insulin (μIU/mL)	64.9	20.0	46.2	30.5	7.3	0.40	0.02	0.26
Triacylglycerol (mg/dL)	23.3^{d}	36.3^{b}	28.3^{c}	39.5^{a}	2.4	0.20	0.01	0.08
Total cholesterol (mg/dL)	92.0	89.8	89.0	97.0	2.8	0.34	0.30	0.20
VLDL cholesterol (mg/dL)	6.23	6.38	6.30	6.48	0.15	0.39	0.30	0.37
VLDL number (nmol/L)	23.8	24.5	24.0	24.8	0.50	0.41	0.24	0.34
LDL cholesterol (mg/dL)	54.7^{a,b}	52.8^{a,b}	51.0^{a}	60.0^{b}	2.1	0.35	0.21	0.12
Total LDL number (nmol/L)	421.5	407.0	399.3	444.5	14.7	0.40	0.31	0.20
LDL_{III} number (nmol/L)	152.3	146.7	139.0	134.5	7.7	0.21	0.38	0.44
LDL_{IV} number (nmol/L)	65.0^{a}	60.3^{b}	66.0^{a,b}	61.8^{a,b}	1.6	0.36	0.08	0.05
LDL size (nm)	19.94	19.94	19.95	20.11	0.02	0.03	0.05	0.46
RLP number (nmol/L)	32.3^{c}	33.0^{b,c}	36.5^{a,b}	44.8^{a,b}	2.1	0.02	0.15	0.03
HDL cholesterol (mg/dL)	31.0	30.3	31.3	31.5	1.0	0.36	0.45	0.47
Total HDL number (nmol/L)	5,681	5,454	5,442	5,952	178	0.37	0.35	0.22
HDL_{2b} number (nmol/L)	1,040	1,072	1,091	1,114	57	0.27	0.40	0.44

Treatments: control = 1% canola oil + 2.05% alanine, CLA = 1% CLA + 2.05% alanine, arginine = 1% canola oil + 1% arginine, and CLA + arginine = 1% CLA + 1% arginine. Data are means ($n = 4$ per treatment group). Means in rows not bearing a common superscript alphabets differ, $P < 0.10$

than glucose carbon incorporation into total lipids. In rat liver and epididymal adipose tissue, glucose and palmitate were converted to CO_2 at roughly equal rates although, as in pig tissues, palmitate incorporation was much greater than glucose incorporation into lipids (Nall et al. 2009).

CPT-1B gene expression was higher in s.c. adipose tissue than in r.p. adipose tissue, but the rate of palmitate oxidation did not differ between adipose tissue depots. In both rats (Nall et al. 2009) and pigs (current study), we have been unable to demonstrate an effect of arginine or CLA on palmitate conversion to CO_2. In epididymal adipose tissue of rats and both adipose tissues of pigs, CLA increased the oxidation of glucose, and arginine increased lipid synthesis from glucose in rat adipose tissue (Nall et al. 2009) and pig s.c. adipose tissue. In addition, although not significant, arginine caused a small increase in rat epididymal adipocyte volume (Nall et al. 2009), and both arginine and CLA increased adipocyte volume in the current study. Thus, we are able to demonstrate some consistent effects of arginine and CLA on adipose tissue metabolism between rats and pigs. However, arginine depressed palmitate incorporation into lipids in rat adipose tissue (Nall et al. 2009). This effect was not observed in the current study, indicating a possible species difference in response to arginine supplementation.

Some investigators have reported that CLA supplementation to rats increased lipogenic enzymes activity in adipose tissue (Faulconnier et al. 2004), whereas other investigators demonstrated that CLA treatment reduced body fat mass in different models, including human (Blankson et al. 2000), mice (Park et al. 1999), rat (Nall et al. 2009), and pigs (Tischendorf et al. 2002; Wiegand et al. 2002). Unlike studies in which CLA is fed to pigs (Smith et al. 2002; Demaree et al. 2002), CLA supplementation to differentiation media decreased the size of 3T3-L1 preadipocytes (Evans et al. 2001) and rat adipocytes (Azain et al. 2000). Moreover, arginine supplementation not only increased the expression of genes centrally responsible for substrate oxidation but also increased lipolysis in rat adipose tissue and inhibited lipogenesis in liver and adipose tissue (Jobgen et al. 2006). Therefore, a lack of effect of CLA and arginine in depressing lipid synthesis in s.c. and r.p. adipose tissues was unusual in light of previous reports, but was supported by the dramatic increase in s.c. and r.p. adipocyte volumes.

The increased backfat thickness and adipocyte volume caused by dietary supplementation of arginine in this study may have been related to the use of canola oil as a lipid control. The arginine diet fed to the pigs also contained 1% canola oil. However, earlier studies demonstrated that the backfat thickness was not affected by 10 or 20% canola oil supplementation in pigs (St. John et al. 1987; Myer et al. 1992). The use of 1% canola oil in the current study resulted in the control and arginine-supplemented pigs receiving <0.5 g 18:1n-9/100 g diet more than pigs supplemented with CLA or CLA + arginine. In addition, there is no evidence in the literature for any species that oleic acid would depress carcass adiposity. We conclude that, in the pig at least, arginine, as well as CLA, promotes adipocyte lipid filling.

Our initial hypothesis was that arginine and CLA would, through independent mechanisms, additively reduce carcass fatness in growing pigs. Instead, arginine promoted lipid synthesis from palmitate in liver, whereas CLA increased lipid synthesis in muscle, and only in intestinal mucosal cells did CLA enhance palmitate oxidation. The net effect was an increase in backfat thickness (arginine effect) and intramuscular lipid (CLA effect), indicating that combining these compounds as supplements in pig production would promote fatter carcasses. This leads us to conclude that supplementing diets with CLA plus arginine may exacerbate obesity in humans.

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

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