LIPID METABOLISM, GENE EXPRESSION, SUBSTRATE OXIDATION, AND MEAT QUALITY OF GROWING-FINISHING PIGS SUPPLEMENTED WITH CONJUGATED LINOLEIC ACID AND ARGININE

A Dissertation

by

GWANG-WOONG GO

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

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Nutrition

Lipid Metabolism, Gene Expression, Substrate Oxidation, and Meat Quality of

Growing-finishing Pigs Supplemented with Conjugated Linoleic Acid and Arginine

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December 2010

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ABSTRACT

Lipid Metabolism, Gene Expression, Substrate Oxidation, and Meat Quality of Growing-finishing Pigs Supplemented with Conjugated Linoleic Acid and Arginine.

(December 2010)

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Chair of Advisory Committee: Dr. Stephen B. Smith

We hypothesized that supplementation of dietary conjugated linoleic acid (CLA) and arginine singly or in combination would increase animal performance and meat quality by decreasing adiposity and increasing lean mass in growing-finishing pigs. Sixteen pigs (80 kg) were assigned to four treatments in a 2 x 2 factorial design, differing in dietary fatty acid and amino acid composition [control: 2.05% alanine (isonitrogenous control) plus 1% canola oil (lipid control); CLA: 2.05% alanine + 1% CLA; arginine: 1% arginine + 1% canola oil; arginine + CLA: 1% arginine + 1% CLA]. Preliminary tests indicated that up to 2% arginine was acceptable without interfering with lysine absorption. Pigs were allowed to feed free choice until reaching 110 kg. There were no significant differences across treatments in feed intake, weight gain, or feed efficiency. CLA tended to decrease carcass length ($P = 0.06$), whereas backfat thickness tended to be greater in pigs supplemented with arginine ($P = 0.08$). Arginine decreased muscle pH

at 45 min postmortem (*P* = 0.001) and tended to increase lightness of muscle at 24 h postmortem (*P* = 0.07). CLA supplementation increased the concentrations of *trans*-isomers of 18:1 (*P* = 0.001) and SFA (*P* = 0.01) in s.c. and r.p. adipose tissue. CLA supplementation increased palmitate incorporation into total lipids in *longissimus* muscle (*P* = 0.01). Glucose oxidation to CO_2 in r.p. and s.c. adipose tissue were greater in pigs supplemented with CLA in the absence or presence of arginine (*P* = 0.03 and *P* = 0.04, respectively). The volume of s.c. adipocytes in s.c. and r.p. adipose tissues was greater in pigs supplemented with CLA, arginine, or CLA plus arginine than in control pigs (*P* = 0.001). Neither CLA nor arginine affected the expression of PGC-1α, AMPK, mTOR, CPT-1A, FAS, or SCD (*P* > 0.05) in any tissues. We conclude that there was no significant interaction between arginine and CLA. Supplementary CLA or arginine to finishing-growing pigs did not modulate growth performance and did not reduce adiposity. CLA increased intramuscular fat content without deteriorating meat quality traits and increased saturated fatty acids and substrate oxidation in adipose tissues. In the presence of 1% of canola oil or CLA in the diet, arginine has the potential to deteriorate meat quality by reducing early postmortem pH and by increasing carcass fatness.

DEDICATION

I dedicate this dissertation and all the work that went into it to my mother, Yeonsuk Yoon, who has been the single most important and influential person in my life. My mother would do whatever was needed for me to be successful in my entire life. My mother has supported me every step of my educational journey and for that I will always be thankful. My mother's unconditional encouragement, support, commitment, love, and faith in me have made my Ph.D. possible.

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NOMENCLATURE

ACC	Acyl-CoA carboxylase
ACO	Acetyl CoA oxidase
AMPK	AMP-activated protein kinase
C/EBP	CAAT/enhancer-binding protein
CLA	Conjugated linoleic acid
CPT-1A	Carnitine palmitoyltransferase 1
ERK	Extracellular signal-related kinase
FAME	Fatty acid methyl ester
FAS	Fatty acid synthase
HDL	High density lipoprotein
ILP	Intermediate lipoprotein
LDL	Low density lipoprotein
mTOR	Mammalian target of rapamycin
MUFA	Monounsaturated fatty acid
NRF-1	Nuclear respiratory factor 1
NFκB	Nuclear factor-κB
NO	Nitric oxide
NOS	Nitric oxide synthase
PGC-1	Peroxisome proliferator-activated receptor gamma coactivator 1
PPAR	Peroxisome proliferator-activated receptor
PSE	Pale, soft, and exudative

RLP	Remnant lipoprotein
r.p.	Retroperitoneal
s.c.	Subcutaneous
SCD	Stearoyl-CoA desaturase
SFA	Saturated fatty acid
TNF	Tumor necrosis factor
UCP	Uncoupling protein
VLDL	Very low density lipoprotein
ZDF rat	Zucker diabetic fatty rat

TABLE OF CONTENTS

	Page
ABSTRACT	iii
DEDICATION	v
ACKNOWLEDGEMENTS	vi
NOMENCLATURE	viii
TABLE OF CONTENTS	x
LIST OF TABLES	xii
INTRODUCTION	1
REVIEW OF LITERATURE	3
General aspects of CLA and arginine	3
Increasing energy expenditure by CLA and arginine	4
Inhibition of lipid synthesis and adipocyte differentiation by CLA and arginine	4
The effects of CLA and arginine on growth performance and meat quality characteristics	5
Hypothesis and objectives	7
MATERIALS AND METHODS	8
Animals, experimental diet, and sampling	8
Carcass traits and meat quality characteristics	9
Lipogenesis and CO_2 production *in vitro*	10
Fatty acid composition	11
Plasma amino acids	12
Cellularity	12
RNA isolation and qRT-PCR analysis	13
Statistical analysis	14
RESULTS	15
Growth performance and carcass traits	15

Page

Meat quality traits 15
Fatty acid composition 15
Incorporation of glucose and palmitate into CO_2 or lipid *in vitro* 17
Expression of genes related to substrate oxidation and lipid synthesis 18
Metabolic syndrome straits and lipoprotein profiles in plasma 19
Plasma amino acids profiles 19

DISCUSSION 20

CONCLUSIONS 28

LITERATURE CITED 31

APPENDIX 44

VITA 65

LIST OF TABLES

TABLE		Page
1	Calculated nutrient content of the basal diet	44
2	Composition of experimental diet	45
3	Fatty acid profiles of test diet	46
4	Amino acid profiles of test diets	47
5	Growth performance of pigs fed CLA, arginine, or arginine plus CLA	48
6	Carcass traits of pigs fed CLA, arginine, or CLA plus arginine	49
7	Meat quality characteristics of pigs fed CLA, arginine, or CLA plus arginine	50
8	Fatty acid composition of liver from pigs fed diets containing CLA, arginine, CLA plus arginine	51
9	Fatty acid composition of *longissimus* muscle from pigs fed diets containing CLA, arginine, or CLA plus arginine	52
10	Fatty acid composition of intestinal duodenal mucosal cells from pigs fed diets containing CLA, arginine, or CLA plus arginine	53
11	Fatty acid composition of r.p. adipose tissue from pigs fed diets containing CLA, arginine, or CLA plus arginine	54
12	Fatty acid composition of s.c. adipose tissue from pigs fed diets containing CLA, arginine, or CLA plus arginine	55
13	Fatty acid composition of plasma from pigs fed diets containing CLA, arginine, or CLA plus arginine	56
14	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	57

TABLE Page

15 Incorporation of glucose and palmitate carbon into CO_2 and lipids *in vitro* and cellularity in r.p. adipose tissue in pigs fed CLA, arginine, or CLA plus arginine 58

16 Incorporation of glucose and palmitate carbon into CO_2 and lipids *in vitro* and cellularity in s.c. adipose tissue in pigs fed CLA, arginine, or CLA plus arginine 59

17 Expression of genes in liver, longissimus muscle, and intestinal duodenal mucosal cells in pigs fed CLA, arginine, or CLA plus arginine 60

18 Expression of genes in r.p. adipose tissue and s.c. adipose tissue in pigs fed CLA, arginine, or CLA plus arginine 61

19 Metabolic syndrome traits and lipoprotein profiles of plasma in pigs fed CLA, arginine, or CLA plus arginine 62

20 Essential amino acids in plasma of pigs fed CLA, arginine, and CLA plus arginine 63

21 Nonessential amino acids in plasma of pigs fed CLA, arginine, and CLA plus arginine 64

INTRODUCTION

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid. CLA is known for reducing body fat mass by increasing energy expenditure (fatty acid oxidation and phosphorylation) and decreasing lipogenesis (adipocyte differentiation and fatty acid synthesis). Arginine, a semi-essential amino acid, also enhances fatty acid oxidation, partly via NO-mediated change in expression of genes including SCD, AMPK, and PGC-1α.

There is constant demand for improving animal performance and meat quality among producers and consumers. Breeding systems to increase animal performance have resulted in quality problems such as PSE meat and reduced intramuscular fat. Novel approaches with compounds considered as safe are needed to increase animal performance and meat quality via increasing lean mass and decreasing adiposity. Reducing fat by CLA appears not to deteriorate pork meat quality, including pH, color, water holding capacity, marbling, and tenderness. The effects of arginine (in single or combination with CLA) on meat quality have not yet been determined. Furthermore, there is no information on the interaction between CLA and arginine in the pig.

Taken together, both CLA and arginine seem to modulate energy expenditure and lipogenesis at the molecular level, lowering fat mass without meat quality deterioration. We hypothesized that arginine and CLA, singly or in combination, would improve animal performance and meat quality, reducing adiposity and enhancing

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

leanness in finishing weight pigs. This research provided an integrated study of the role of CLA and arginine in modulating lipogenesis, substrate oxidation, and meat quality.

REVIEW OF LITERATURE

General aspects of CLA and arginine. CLA is a group of geometric and positional isomers of linoleic acid, having conjugated double bonds (1), originating from biohydrogenation and isomerization of linoleic acid by rumen bacteria (2) and delta-9 dehydrogenation of *trans*-11 vaccenic acid in mammalian tissues (3, 4). The most predominant isomer in nature is *cis-9, trans-11* CLA (*c9*, *t11* CLA, >80%), which is mainly ruminant origin including meat and dairy, while *trans-10, cis-12* CLA *(t10, c12* CLA*)* is found in negligible amounts in food. Since CLA from beef was shown to be anti-carcinogenic (5), numerous studies demonstrated the physical, biochemical, and physiological properties of CLA: 1) decreasing severity of atherosclerosis (6), 2) modulation of immune function (7, 8), 3) antioxidative action (9), 4) growth promotion (10), 5) reduction of body fat and modulating lipid metabolism (11-15). These functions of CLA are isomer dependent. The *t10, c12* CLA is in particular active for its anti-adipogenic property (16-19).

Arginine is a semi-essential amino acid, playing a multiple roles as a precursor for NO, which modulates energy expenditure and lipogenesis. There are two main pathways of synthesis of NO: arginine-NOS pathway (20) and nitrate-nitrite-NO pathway (21). Arginine-NOS pathway is more dominant with isoforms: 'endothelial' (eNOS), 'neuronal' (nNOS), and ‘inducible’ (iNOS) (22). According to Bryan et al. (23), mammalian tissues generate NO to modify proteins at heme and sulfhydryl sites, thereby

increasing substrate oxidation. NO from mitochondria has been established the basis for a novel regulatory pathway of energy metabolism and oxygen consumption (24, 25).

Increasing energy expenditure by CLA and arginine. Dietary CLA enhances fatty acid oxidation by activating CPT-1B in skeletal muscle and increases ACO (rate limiting enzyme in peroximal oxidation) in liver (26). Supplementing 0.5% of CLA in the diet effectively increased energy expenditure in Balb-C mice during exercise (27), and 0.93% of CLA supplemented to Balb-C mice increased energy expenditure (28). 1% of *c9, t11* CLA supplementation for 8 wk to Sprague-Dawley rats increased PPARα, ACO, and UCP, and protected mitochondria against oxidative stress (29). Furthermore, 4 g/d of dietary CLA supplementation for 6 mo increased substrate oxidation and energy expenditure in over weight adults (30).

NO from arginine modulates expression of AMPK (31), which is necessary for mitochondrial biogenesis in response to oxidation of substrate. Hela cells, expressing eNOS, displayed increases in mitochondrial DNA content, cytochrome c oxidase, and the mRNA of PGC-1α and NRF-1 (32). Arginine increased CPT-1A and PGC-1α expression in liver, and increased hepatic energy expenditure compared with alanine-supplemented rats (33). Arginine also depressed lipogenesis from glucose and palmitate in liver when supplemented to rats, and also increased palmitate oxidation to CO_2 (34).

Inhibition of lipid synthesis and adipocyte differentiation by CLA and arginine. CLA, given at a dose of 3.2 g/d, caused modest weight loss in body fat in humans (35), and depressed SCD gene expression and catalytic activity in adipocytes (36). Research with SCD gene knockout mice showed that a SCD mutation caused increased AMPK in liver

and skeletal muscle, which inhibited lipogenesis and enhanced fatty acid oxidation (37). Decreased ACC caused by AMPK increased CPT-1A activity by decreasing malonyl CoA in scd -/- mice liver (38). CLA prevented lipid filling by decreasing PPARγ in rodent preadipocytes (39) and bovine preadipocytes (40). The *t10, c12* CLA isomer supplementation decreased expression of C/EBPα in 3T3-L1 adipocyte (41). Both PPARγ and C/EBPα control lipid metabolism, adipocyte differentiation, proliferation, and lipogenesis in adipose tissue (42). CLA affected PPARγ via NFκB, which regulates mitogen-activated protein kinases and TNF-α (43). CLA activated NFκB in stromal-vascular cells, resulting in secretion of interleukin-6, -8, and TNF-α. These cytokines activated NFκB and ERK, which phosphorylated NFκB and other transcription factors including PPARγ, which resulted in reduced adipogenic gene expression (44). Dietary 0.5% CLA for 5 wk decreased adipose tissue cell size in Sprague-Drawley rats (45).

Arginine, via NO, suppressed the expression of ACC, FAS, and SCD, decreasing body fat mass in rat (33, 46, 47). NO down-regulated triacylglycerol synthesis and increased lipolysis. The addition of 4% arginine to the diet decreased total body fat (48). Fu et al. (49) demonstrated that dietary arginine reduced adiposity in Zucker diabetic fat (ZDF) rats and Nall et al. (34) indicated that arginine depressed r.p. adipose tissue in Sprague-Dawley rats. After 10 wk of treatment, those rats fed arginine had 25% less epididymal fat and 45% less abdominal fat than control rats.

The effects of CLA and arginine on growth performance and meat quality characteristics. Supplemental arginine to growing-finishing pigs for 2 mo had no effect on weight gain or feed intake (50), and body weight also was not affected by dietary

arginine supplementation in pregnant gilts (51). On the other hand, other investigators have demonstrated that dietary arginine supplementation enhanced growth performance of neonatal pigs. Weight gains were increased in piglets supplemented with arginine (52). Similarly, average daily weight gain and body weight increased in milk-fed piglets receiving arginine treatment (53).

Dietary CLA supplementation to pigs had no effect on daily weight gain, feed intake, or feed efficiency (54). Likewise, CLA supplementation to growing-finishing pigs did not affect on average daily gain (55). However, weight gain, feed intake, and feed efficiency were greater in rats supplemented with 1% CLA (34).

In piglets , oral administration of N-carbamylglutamate, an analogue of endogenous N-acetylglutamate (an activator of arginine synthesis), increased weight gain an the rate of protein synthesis in skeletal muscle (56). Similarly, CLA increased mTOR signaling activity in human preadipocytes (44), and dietary arginine supplementation increased mTOR signaling of muscle in neonatal pig (57). According to Nall et al. (34), dietary arginine and CLA supplementation to rats caused a reduction in serum branched-chain amino acids (BCAA), which was caused by depression of muscle protein turnover. Thus, CLA or arginine supplementation to pigs could increase lean mass by depression of protein turnover via stimulation of mTOR signaling pathway in skeletal muscle.

Other investigators demonstrated that arginine and CLA reduced fat mass. Total carcass fat decreased in pigs fed arginine for 2 mo, but arginine had no effect on average backfat thickness or carcass length (58). Similarly, arginine supplementation reduced

the weight of r.p. and epididymal adipose tissue in ZDF rats (49). Dietary arginine supplementation increased expression of genes, including PGC-1α and AMPK. Moreover, arginine increased lipolysis in rat adipose tissue and inhibited lipogenesis in liver and adipose tissue (33).

Early postmortem muscle pH is an indicator of the rate of postmortem metabolism and the degree of protein denaturation (59). The rate of pH decline and ultimate pH in muscle are highly related to drip loss and meat color parameters (60, 61). As pH declines, drip loss and lightness increases (62). Other investigators have demonstrated that CLA supplementation to pigs slightly increased or had no change in pH (54, 55, 63-65). Dietary 0.5% CLA supplementation increased muscle pH and glycogen concentrations and tended to lower lactate content (66).

Hypothesis and objectives. We hypothesized that co-supplementation of dietary CLA and arginine would have additive effects on animal performance and meat quality by decreasing adiposity and increasing lean mass in growing-finishing pigs. This study will determine the effects of CLA and arginine supplementation on growth performance, carcass traits, and meat quality characteristics in growing-finishing pigs. We will establish the relative role of liver, muscle, small intestine, and s.c. and r.p. adipose tissues metabolism in contributing to the single and combined effects of arginine and CLA on reducing adiposity and modulating gene expression of growing-finishing pigs.

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.

Animals, experimental diet, and sampling. Sixteen pigs were purchased from the Texas A&M University Animal Science Teaching/Research Center (ASTREC) at approximately 80 kg body weight. Two gilts and two castrated males were randomly assigned per group. Pigs were allotted to four treatments; 2 x 2 factorial design with two lipids and two amino acids (Ajinomoto Inc., Tokyo, Japan). Treatment groups (n = 4) were: 1) control (2.05% L-alanine plus 1% canola oil); 2) CLA (2.05% L-alanine plus 1% CLA); 3) arginine (1.0% L-arginine plus 1.0% canola oil); and 4) arginine plus CLA (1.0% arginine plus 1.0% CLA). Mixed isomers of CLA were used (Lipid Nutrition G-80; triacylglycerol preparation, Clarinol). Canola oil used as a lipid control, because it is food grade and has a similar melting point as the triacylglycerol preparation of CLA. L-alanine was used as an iso-nitrogenous control for arginine. Blood samples were obtained by vein puncture with 1 mL syringes and plasma was analyzed for dose response of plasma arginine concentrations to dietary level of arginine. Growing-finishing pigs tolerate 1% of chronic supplementation of arginine and plasma arginine concentrations returned to baseline levels within 4-5 h administration (67). Pigs were allowed to free access to feed and water until they reached a projected weight of 110 kg. Pig weight and feed consumption data were collected weekly.

When the average pen weight was 110 kg, approximately 12 h after last feeding, pigs were transported to the Texas A&M University Rosenthal Meat Science and Technology Center for sampling. Pigs were harvested by standard industry procedures. Plasma samples were collected, and portion of blood samples was analyzed for plasma amino acid and fatty acid profiles. Lipoprotein profiles and metabolic syndrome traits, including homocysteine, insulin, and triacylglycerol, were analyzed by SpectraCell Laboratory (Houston, TX). Portions of the fresh liver, *longissimus* muscle, s.c. adipose tissue, peritoneal adipose tissue, and small intestine placed immediately in Kreb-Henseleit buffer (KHB) containing 5 mM glucose (oxygenated and 37°C) and transported immediately to the laboratory for lipogenesis *in vitro* and CO_2 production measurement. Other portions were snap frozen in liquid N_2 for other experiment, including fatty acid composition, cellularity, gene expression, and substrate oxidation.

Carcass traits and meat quality characteristics. When the average pen weight was 110 kg, pigs were harvested by standard industry procedures. 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. Loineye area was measured by using plotting paper at the 10th rib. Dressing percentage was calculated with the proportion of carcass weight relative to its live slaughter weight.

Longissimus muscle between the 9th and 11th thoracic rib from left carcass side was sampled at 45 min for meat quality and sensory evaluation. Both at 1 h and 24 h,

meat temperature and pH were measured in triplicate (IQ150, IQ Scientific Instrument) and mat color criteria were measured in triplicate (Chroma meter CR-300, Minolta) 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.

Lipogenesis and CO_2 production in vitro. Two-hour *in vitro* incubations conducted with fresh liver, *longissimus dorsi*, s.c. adipose tissue, r.p. adipose tissue, and small intestine samples (≈ 100 mg) as described previously (68, 69). 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 well with fluted filter paper for the measurement of CO_2 (Hamby et al., 1986). Vials were gassed for 1 min with 95% O_2:5% CO_2 and incubated for 2 h in a shaking water bath at 37°C. At the end of the incubation period, reactions terminated by 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. The neutral lipids in tissues were extracted using the Folch et al. (70) procedure, evaporated to dryness, resuspended in 10 mL of scintillation cocktail, and radioactivity counted with the scintillation counter (Beckman Instruments, Palo Alto, CA). The hanging center wells were transferred to 20 mL scintillation vials, and 2 mL of distilled ionized water added. After another 30 min, 10 mL of a commercial scintillation fluid added and dpm were counted by scintillation spectrometry.

Fatty acid composition. Lipid was extracted by the modification of Folch method (70). Approximately 1.0 g of tissue was homogenized with 5.0 mL of chloroform:methanol (2:1, v/v) in a homogenizer (Brinkmann Insruments, Westbury, NY), or approximately 1 mL of plasma was stoppered with with 5.0 mL of chloroform:methanol. Total volume of homogenate was adjusted to 15 mL by adding chloroform:methanol solution. After sitting in room temperature for 30-60 min, the homogenate was vacuum filtered through a sintered glass filter funnel fitted with a Whatman filter (Whatman Ltd., Maidstone, England) into a glass test tube containing 8 mL of 0.74% KCl (w/v). The filtered sample was vortexed for 30 sec and centrifuged at 2,000 g for 15 min for separation. After discarding upper aqueous phase, lower phase was evaporated at 60°C with a nitrogen flushing evaporator. Liquid was total extracted lipid and used for fatty acid composition and slip point.

Fatty acids were methylated by the modification of Morrison and Smith method (71). Approximately 100 mg of total lipids extract was taken into another glass tube. Lipid was mixed with 1 mL of 0.5 N of KOH in MeOH and heated in water bath at 70°C for 10 min. After cooling in room temperature, 1 mL of 14% BF3 in MeOH (w/v) was added to sample, then heated in water bath at 70°C for 30 min and sit in room temperature. Two milliliter of HPLC grade hexane and saturated NaCl solution were added and vortexed for 30 sec. Samples were then centrifuged at 2,000 × g for 10 min for separation, then transferred to 15 mL glass tube containing anhydrous Na_2SO_4 to remove aqueous molecule. Total volume of hexane was determined for optimal FAME concentrations. FAME were analyzed by GC-FID (model CP-3800 equipped with a CP-

8200 auto-sampler, Varian Inc, CA). Separation of FAME was accomplished on a fused silica capillary column (100 m x 0.25 mm ID) (model CP-7420, Varian Inc, CA) with the helium as carrier gas (flow rate = 1.7 mL/min). One microliter of sample was injected with the split ratio of 100:1 at 270°C. Oven temperature set up 165°C for 65 min and increased to 235°C (2°C/min) and held for 15 min. FID detected the signal at 270°C. Standard (GLC 68-D, Nu-chek Prep, MN) was used to identify each peak.

Plasma amino acids. Plasma amino acids were analyzed using HPLC. The HPLC apparatus and pre-column derivatization of amino acids with *o*-phthaldialdehyde were as previously described (72). Amino acids were quantified on the basis of authentic standards (Sigma-Aldrich) using the Millemmium workstation (Waters) (72).

Cellularity. s.c. adipose tissue and r.p. adipose tissue were collected by immediately postmortem from pigs for determination of cellularity by osmium fixation, counting, and sizing (69). Tissue was sliced into sections 1 mm thick and placed in 20 mL scintillation vials. Tissues were rinsed three times with 37°C 0.154 M NaCl at 1 h intervals to remove free lipid. After the last rinse, 0.6 mL of 50 mM collidine-HCl buffer (pH 7.4) was added to each sample, followed by 1.0 mL of 3% osmium tetroxide in collidine. After incubation for 96 h at 37°C, the osmium solution was removed and the tissue rinsed three times with 0.154 M NaCl until clear. Samples were incubated in 10 mL of 8 M urea at 25°C for 96 h. After degradation of connective tissue with urea, tissues were rinsed three times with 0.154 M NaCl. Tissues, resuspended with 0.01% Triton in 0.154 M NaCl, were used for determination of cell size, volume, and cells/100mg tissue, using bright-field microscope (Olympus Vanox ABHS3, Olympus, Tokyo, Japan), CCD Color

Video Camera (DXC-960MD, Sony, Japan)

RNA isolation and qRT-PCR analysis. Total RNA was isolated from tissue as described previously (73) using Tri-reagent (Sigma Chemical Co., St. Louis, MO). Approximate 200 mg of tissue was homogenized with 2 mL Tri-reagent. After sitting at room temperature for 5 min, 200 μL chloroform was added and vortexed. Samples were centrifuged (12,000 × g for 15 min). The upper clear layer was transferred into new tube and inverted gently with 500 μL isopropanol. After sitting at 4°C for 5 min, samples were centrifuged (12,000 × g for 10 min) and dried. Samples were washed with 70% EtOH and dried. Pellet was dissolved with 20 μL of nuclease-free H_2O and stored at -80°C until further analysis.

The concentrations and abundance of total RNA was measured with Nanodrop (NanoDrop Technologies Inc., Wilmington, DE) and the quality of total RNA was determined by 1% agarose gel electrophoresis. One microgram of RNA was used for reverse transcription to produce the first-strand complementary DNA (cDNA) using TaqMan Transcription Reagent and MultiScribe reverse transcription (Applied Biosystem, Foster City, CA) with the following temperature ramp: 25°C for 10 min, 37°C for 60 min, and 95.5°C for 5 min.

Quantitative PCR was used to analyze the quantity of gene expression including AMPK, PGC-1α, PPARγ, FAS, SCD, ACC, mTOR, and CPT-1. Eukaryotic 18S rRNA was used as an endogenous gene expression control. Measurement of the relative quantity of cDNA was performed using TaqMan Universal PCR mixer, Assays-on-demand Gene Expression Products (Applied Biosystem), and 1 μg of cDNA mixture.

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 Duncan method if their respective *F*-test indicated significant differences ($P < 0.05$).

RESULTS

Growth performance and carcass traits. Neither arginine nor CLA dietary supplementation affected total gain, total feed intake, average daily gain, average daily feed intake, or feed efficiency (feed/gain) in pigs. Neither arginine nor CLA affected slaughter weight, carcass weight, dressing percentage, or carcass quality grade. Loineye area and muscle score were not affected by arginine or CLA. However, carcass length was 2.4% shorter in pigs supplemented with CLA ($P = 0.06$). Total fat thickness (9.5% greater) and backfat thickness (9.6% greater) tended to be greater in pigs fed arginine ($P = 0.09$ and $P = 0.08$, respectively).

Meat quality traits. *Longissimus* muscle pH at 45 min postmortem was lower by 0.36 pH units in pigs supplemented with arginine than in pigs receiving alanine ($P = 0.001$). Muscle color (L*, a*, and b*) at 45 min postmortem was not affected by arginine or CLA. Lightness of muscle at 24 h postmortem tended to be increased by arginine ($P = 0.07$) and tended to be decreased by CLA ($P = 0.07$). CLA supplementation increased intramuscular fat ($P = 0.01$) and tended to decrease moisture ($P = 0.06$).

Fatty acid composition. Selected fatty acid profiles from liver, *longissimus* muscle, intestinal duodenal mucosal cells, s.c. adipose tissue, and r.p. adipose tissue are indicated. Canola added diets had higher concentrations of 18:1 *c9*, 18:1 *c11*, 18:2 n6, and 18:3 n3 and CLA added diets had higher 18:2 *c9, t11* and 18:2 *t10, c12*. However, there were no differences in saturated fatty acids, including 12:0, 14:0, 16:0, 18:0, and 20:0, among experimental diets.

CLA supplementation increased concentrations of *c9, t11* CLA (*P* = 0.001), *t10, c12* CLA (*P* = 0.001), SFA (*P* = 0.01), and *trans*-isomers of 18:1 in liver (*P* = 0.001). Arginine increased oleic acid (*P* = 0.05), MUFA (*P* = 0.06), and the MUFA:SFA ratio (*P* = 0.06) in liver. In *longissimus* muscle, MUFA, SFA, and the MUFA:SFA ratio were not affected by CLA or arginine. *Trans*-isomers of 18:1 (*P* = 0.001, palmitoleic acid, and myristoleic acid were greater in pigs supplemented with CLA in the absence or presence of arginine (all *P* = 0.001) in *longissimus* muscle. The concentrations of oleic acid was decreased (*P* = 0.005) and the concentrations of *c9, t11* CLA (*P* = 0.001) and *t10, c12* CLA (*P* = 0.001) were increased in *longissimus* muscle in pigs fed CLA. CLA supplementation increased the concentrations of *trans*-isomers of 18:1 (*P* = 0.001), *c9, t11* CLA (*P* = 0.001), and *t10, c12* CLA (*P* = 0.001), and decreased the MUFA:SFA ratio in intestinal duodenal mucosal cells. Neither CLA nor arginine affected stearic acid, oleic acid, MUFA, or SFA in intestinal duodenal mucosal cell in pigs.

CLA supplementation increased the concentrations of *trans*-isomers of 18:1 (*P* = 0.001), *c9, t11* CLA (*P* = 0.003), *t10, c12* CLA (*P* = 0.003), and SFA (*P* = 0.01) and decreased oleic acid (*P* = 0.001), MUFA (*P* = 0.001), and the MUFA:SFA ratio (*P* = 0.001) in r.p. adipose tissue. Arginine did not affect any s.c. adipose tissue fatty acids. CLA supplementation significantly increased myristic acid, palmitoleic acid, stearic acid, *trans*-isomers of 18:1, and CLA (all *P* = 0.001) in s.c. adipose tissue. Oleic acid (*P* = 0.001), α-linolenic acid (*P* = 0.001), arachidonic acid (*P* = 0.001), and MUFA (*P* = 0.001) were reduced in s.c. adipose tissue in pigs fed CLA in the absence or presence of arginine.

No fatty acids were affected by arginine in plasma. CLA treatment increased the concentrations of myristic acid (P = 0.02), *c9, t11* CLA (P = 0.02), and *t10, c12* CLA (P = 0.02) in plasma. SFA tended to be increased (P = 0.07) and oleic acid tended to be depressed (P = 0.06) by CLA in plasma.

Incorporation of glucose and palmitate into CO_2 or lipid in vitro. The oxidation of glucose to CO_2 was approximately 10-fold higher than the rate of palmitate oxidation in liver. The rate of palmitate incorporation into total lipids was approximately 20-fold higher than lipid synthesis from glucose in liver. Neither CLA nor arginine affected CO_2 production from glucose or palmitate in liver. CLA tended to reduce hepatic lipid synthesis from glucose (P = 0.10). The rate of palmitate incorporation was greater in pigs supplemented with arginine than that of alanine-fed pigs (P = 0.02).

The oxidation of glucose was approximately 10-fold greater than the rate of palmitate oxidation in *longissimus* muscle. The oxidation rates from glucose or palmitate were not affect by CLA or arginine in *longissimus* muscle. Palmitate was more dominantly used for lipid synthesis than glucose in *longissimus* muscle. CLA supplementation in the absence of arginine increased palmitate incorporation into total lipids in *longissimus* muscle (P = 0.01). CO_2 production from glucose was approximately 10-fold greater than that from palmate and was affected by neither CLA nor arginine in intestinal duodenal mucosal cells. The oxidation of palmitate to CO_2 tended to be greater in pigs fed CLA than in pigs fed arginine (P = 0.07).

Glucose oxidation to CO_2 in r.p. adipose tissue was approximately 15-fold higher than palmitate oxidation to CO_2 and was greater in pigs supplemented with CLA in the

absence or presence of arginine (*P* = 0.03). Arginine did not affect CO_2 production from glucose or palmitate in r.p. adipose tissue. Neither CLA nor arginine affected lipid synthesis from glucose or palmitate in r.p. adipose tissue. CLA, arginine, and CLA plus arginine increased the volume of adipocytes (*P* = 0.001). Glucose oxidation to CO_2 in s.c. adipose tissue was approximately 15-fold higher than palmitate oxidation to CO_2 and was greater in pigs supplemented with CLA than canola-treated pigs (*P* = 0.04). Arginine did not affect the oxidation of glucose or palmitate to CO_2 in s.c. adipose tissue. Arginine increased glucose incorporation into total lipids in s.c. adipose tissue (*P* = 0.06). Lipid synthesis from glucose or palmitate was not affected by CLA treatment. The volume of s.c. adipocytes was greater in pigs supplemented CLA, arginine, or CLA plus arginine than that of control pigs (*P* = 0.001).

Expression of genes related to substrate oxidation and lipid synthesis. Neither CLA nor arginine affected gene expression, including PGC-1α, AMPK, mTOR, CPT-1A, FAS, and SCD, in liver. In *longissimus* muscle, both CLA and arginine supplementation increased PGC-1α mRNA level but the effect was not detected when pigs were supplemented the combination of CLA plus arginine (*P* = 0.07). AMPK, mTOR, CPT-1B, FAS, and SCD were not affected by CLA or arginine in *longissimus* muscle.

Arginine did not affect gene expression in intestinal duodenal mucosal cells. CLA supplementation tended to depress lipogenic expression of FAS (*P* = 0.07) and SCD (*P* = 0.07) in intestinal duodenal mucosal cells. In intestinal duodenal mucosal cells, AMPK mRNA tended to be reduced by CLA supplementation (*P* = 0.10) and

mTOR mRNA was less (P = 0.04) in pigs fed CLA. Arginine had no effects on gene expression in intestinal duodenal mucosal cells.

Neither CLA nor arginine affected mRNA levels, including PGC-1α, AMPK, mTOR, CPT-1B, FAS, and SCD, in r.p. or s.c. adipose tissues.

Metabolic syndrome straits and lipoprotein profiles in plasma. Plasma insulin was significantly reduced in pigs supplemented with CLA, compared to canola-fed pigs (P = 0.05). Homocysteine concentrations tended to be increased by arginine supplementation (P = 0.07), but there was no interaction with CLA. The concentrations of VLDL, LDL, and HDL were not affected by CLA or arginine. Arginine supplementation tended to increase the concentrations of intermediate lipoproteins (P = 0.07) and remnant lipoproteins (P = 0.07) in plasma.

Plasma amino acid profiles. Neither CLA nor arginine affected the concentrations of most essential amino acids, including histidine, isoleusine, leusine, lysine, methionine, threonine, tryptophan, and valine. CLA tended to increase the concentrations of phenylananine (P = 0.06). There were no significant differences in the concentrations of nonessential amino acids except serine, which tended to increase in pigs supplemented with CLA compared with the other treatment groups (P = 0.06).

DISCUSSION

Neither arginine nor CLA supplementation for 4 wk affected growth performance in the current study. Similarly, 0.5 and 1.0% of arginine supplementation from 60 kg to 110 kg finishing weight did not affect weight gain or feed intake (50). Body weight also was not affected by 1% of dietary arginine supplementation in pregnant gilts (51). However, other investigators have demonstrated that arginine supplementation to neonatal pigs enhanced growth performance. Average daily weight gain by d 28 and body weight by d 15 were greater in milk-fed piglets received 0.2 and 0.5% arginine than in control piglets (53). Similarly, weight gains were increased in piglets fed 0.2% and 0.4% of arginine supplementation to 7 to 21 d (52). Furthermore, CLA or arginine supplementation to pigs could increase lean mass by depression of protein turnover via stimulation of mTOR signaling pathway in skeletal muscle. CLA increased mTOR signaling activity in human preadipocytes (44), and dietary arginine supplementation increased mTOR signaling of muscle in neonatal pigs (57). Oral administration of N-carbamylglutamate, an analogue of endogenous N-acetylglutamate (an activator of arginine synthesis), for 7 d increased weight gain an the rate of protein synthesis in skeletal muscle in piglets (56). However, there was no significant difference in mTOR levels in different tissues in this study. Therefore, longer-term administration, higher doses of arginine, or treatment of piglets may be necessary in finishing-growing pigs to enhance animal performance or protein synthesis.

Dietary 2% CLA supplementation to pigs ranging in weight from 23.5 – 110 kg had no effect on daily weight gain, feed intake, or feed efficiency in pigs (54). Dietary 0.75% CLA supplementation to growing-finishing pigs did not affect average daily gain (55). However, we previously demonstrated that weight gain, feed intake, and feed efficiency was greater in rats supplemented with 1% CLA (34). These results indicate that there may be species differences in CLA effects on growth performance between rats and pigs.

Contrary to previous results with rats receiving 10 wk arginine supplementation, neither arginine nor CLA decreased s.c. fat mass. Drinking 1.51% arginine for 10 wk reduced the weight of r.p. and epididymal adipose tissue in Zucker diabetic fatty (ZDF) rats (49). Arginine supplementation increased expression of genes centrally responsible for substrate oxidation, including PGC-1α and AMPK. Moreover, arginine increased lipolysis in rat adipose tissue and inhibited lipogenesis in liver and adipose tissue (33). A recent reported that total carcass fat decreased in pigs fed arginine for 2 mo, but arginine had no effect on average backfat thickness or carcass length (58). Thus, a lack of evidence in the current study for a fat-reducing effect of arginine in the presence of canola oil or CLA was unusual in light of previous reports. However, we previously demonstrated that four week dietary arginine supplementation did not affect epididymal fat mass in young rats (34).

The statistical lack of effect of arginine in adiposity may have been related to the use of canola oil as a lipid control. However, earlier studies demonstrated that average fat thickness and marbling score were not affected by 10 or 20% canola oil

supplementation in pigs (74). Similarly, dietary supplementation of canola oil up to 10% had no effect on backfat thickness in growing-finishing pigs (75). Supplementation of 2% CLA to pigs from 23.5 kg to 110 kg decreased backfat thickness by 11% (54). Similarly, backfat depth decreased in pigs fed 0.75% CLA supplementation from 28 kg to 115 kg of weight without affecting loineye area (55). Our laboratory previously reported that r.p. adipose tissue:body weight ratio was less in rats fed 1% dietary CLA supplementation (34). However, we also demonstrated that feeding CLA for 35 d to postweanling piglets did not affect the mass of adipose tissue (76). These indicate that longer-term treatment with CLA may be necessary for reducing fat mass and that there are species differences between rats and pigs. Thus, feeding 1% of arginine or CLA supplementation to pigs 80 kg to 110 kg of weight may not be practical for reducing fat mass in finishing-growing pigs. Earlier initiation of treatment and a longer period of supplementation may be required for arginine to exert its effect on reducing white fat in growing-finishing pigs.

The rate of pH decline and ultimate pH in muscle are highly related to drip loss and meat color parameters (60, 61). Early postmortem muscle pH is an indicator of the rate of postmortem metabolism and the degree of protein denaturation (59). As pH declines, drip loss and lightness increases (62). Other investigators have demonstrated that CLA supplementation to pigs slightly increased or had no change in pH (54, 55, 63-65). Dietary 0.5% CLA supplementation increased muscle pH and glycogen concentrations and tended to lower lactate content (66). In the current study, pH at 45 min postmortem was lower, although ultimate pH was not affected in pigs supplemented

with arginine. Arginine supplementation tended to increase the lightness of muscle at 24 h postmortem. Dietary arginine supplementation to ZDF rats increased AMPK gene expression (49). AMPK activation indirectly increases glycolysis via activating glycogen phosphorylase (promoting glycogenolysis) and 2-phosphofructokinase, thus AMPK may be potent modulator of postmortem glycolysis. Lower early postmortem pH in pigs supplemented with arginine may be explained by AMPK activation by arginine. Therefore, arginine supplementation with canola oil or CLA could enhance glycolysis and lactate content in meat, which is deteriorative to pork meat quality.

Our results demonstrated that dietary CLA supplementation increased intramuscular fat and tended to decrease water content. Previously, investigators demonstrated that 2% CLA supplementation increased marbling scores and intramuscular fat in finishing-growing pigs (63), and 5% CLA supplementation increased intramuscular fat in the loin of pigs (65). Intramuscular fat content in *longissimus* muscle is highly related to consumer perception meat quality aspects. As intramuscular fat content increases in pork loin up to 3.25%, the perception of texture and taste increases (77). In the current study, arginine increased lightness of meat but did not affect the concentrations of intramuscular fat. Lack of effect on intramuscular fat by arginine is unusual in light of previous reports. Dietary 1% arginine supplementation to finishing pigs for 2 mo in the absence of canola oil or CLA increased intramuscular fat and antioxidative capacity via total antioxidant capacity and glutathione peroxidase (50). Similarly, lipid content in *longissimus* muscle increased in pigs supplemented with 1% arginine (58). Thus, increased lightness by arginine up to 50 and intramuscular fat

content by CLA up to 3.25% would not cause adverse effects in pork meat quality. Intramuscular fat could increase lightness of meat. However, in this study, there is lack of evidence that CLA or arginine, singly or in combination, increased lightness via increasing intramuscular fat content.

In the current study, fatty acid profiles differed between treatments in a manner that was consistent with differences in dietary lipid composition. CLA-added diets increased CLA isomers and decreased oleic acid, linoleic acid, and α-linolenic acid compared to canola-added diets. There were no differences in the concentrations of saturated fatty acids, including palmitic acid and stearic acid. However, dietary CLA supplementation increased the total saturated fatty acids liver, in s.c. adipose tissue, and r.p. adipose tissue. This effect can be explained by depressed Δ^9-desaturase by CLA and degradation of CLA to 18:1 *trans*-isomers. Stearoyl-CoA desaturase enzyme activity and gene expression decreased dose-dependently by CLA treatment in 3T3-L1 adipocytes (78). We previously demonstrated that dietary CLA supplementation to pigs reduced stearoyl-CoA desaturase enzyme activity and the Δ^9-desaturase index in s.c. adipose tissue (36). Unlike our previous results that CLA supplementation did not affect saturated fatty acids in liver in rats (34), CLA supplementation enhanced hepatic saturated fatty acids in liver in growing-finishing pigs. Furthermore, dietary CLA supplementation increased 18:1 *trans*-isomers across tissues, including liver, *longissimus* muscle, intestinal duodenal mucosal cell, s.c. adipose tissue, and r.p. adipose tissue. This suggests that dietary CLA can be metabolized to 18:1 *trans*-isomers, including 18:1 *t10* and 18:1 *t11*, in these tissues.

In s.c. adipose tissue, increased concentrations of saturated fatty acids can cause firmer bellies, fewer problems with sausage making, bacon slicing, and lipid oxidation. Thiobarbituric acid-reactive substance value was lower (55, 65) and belly firmness increased (64) in pigs fed CLA.

There was a lack of effect of arginine or an interaction between CLA and arginine on fatty acid profiles among the tissues sampled. Similarly, we previously demonstrated that arginine did not affect fatty acid composition in epididymal adipose tissue and plasma fatty acids in young rats (34). Thus, arginine supplementation for 4 wk has little or no effect on modulating fatty acid composition of liver, plasma, and peripheral tissues.

Unlike previous results (46, 79), there was the lack of effect of CLA or arginine on glucose or palmitate oxidation to CO_2 in liver and *longissimus* muscle, which is consistent with our previous study that neither CLA nor arginine affected hepatic substrate oxidation in rats (34). Also, neither CLA nor arginine supplementation modulated mRNA levels of related genes, including PGC-1α, AMPK, CPT-1A, and CPT-1B. However, CO_2 production in intestinal duodenal mucosal cells increased by 101% in pigs supplemented with CLA, despite a lack of changes in gene expression. Likewise, dietary CLA supplementation, with or without arginine, increased energy utilization from glucose in s.c. and r.p. adipose tissue despite changes in mRNA levels.

It is well known that there are dominant species differences in major lipogenic sites; liver for mouse and rats, and adipose tissue for pigs. The results obtained in this study indicate that CLA or arginine supplementation enhanced hepatic lipogenesis from

palmitate. Likewise, other investigators demonstrated that CLA supplementation to mice enhanced triacylglycerol accumulation and steatosis in liver, via increasing mitochondrial citrate carrier activity and cytosolic lipogenic enzymes (80). Thus, despite lack of evidence of genes expression, including SCD and FAS, the data suggest that CLA or arginine, known for lipodystrophy, could inhibit lipid uptake by adipose tissues, which either could lead to the hepatic accumulation of dietary fat, or depress substrate oxidation in liver.

CLA supplementation also enhanced lipogenesis from palmitate in *longissimus* muscle by 109%, which is consistent with increased intramuscular fat by CLA singly or in combination with arginine. However, there was no significant change in gene expression in muscle, including FAS and SCD. Therefore, we demonstrated that CLA or arginine supplementation for 4 wk to pigs may increase hepatic lipogenesis from palmitate and CLA supplementation to pigs may enhance the lipid synthesis in *longissimus* muscle.

Other investigators demonstrated that CLA treatment reduced body fat mass in different models, including human (15), mice (81), rat (34), and pigs (54, 55). CLA supplementation decreased the size of 3T3-L1 preadipocytes (82) and rat adipocytes (45). However, in current study, CLA supplementation, singly or in combination with arginine, did not depress lipid synthesis in r.p. adipose tissue. Unlike a previous study in which arginine supplementation to young rats reduced lipid synthesis in epididymal adipose tissue without affecting adiposity (34), lipogenesis and related gene expression were not

affected, but adipocytes were enlarged in r.p. adipose tissue by CLA or arginine supplementation.

In s.c. adipose tissue, CLA treatment, singly or with arginine, increased adipocyte volume without modulating mRNA levels of related genes. We previously reported similar results that mixed isomers of CLA supplementation to 3T3-L1 preadipocytes promoted lipid filling via *de novo* lipogenesis from glucose, which may lead to accumulation of fat in growing animals (83). Similarly, CLA supplementation to rats increased lipogenesis in adipose tissue (84). Likewise, lipogenesis from glucose in s.c. adipose tissue tended to increase in pigs fed arginine singly without change in related genes expression. However, we previously demonstrated that arginine supplementation decreased lipid synthesis from palmitate in rat epididymal adipose tissue (34). We concluded that, relative to control pigs fed a canola-enriched diet, arginine promoted adipogenesis and adiposity.

CONCLUSIONS

Neither arginine nor CLA supplementation affected growth performance, including weight gain and feed efficiency. There were no significant differences in mTOR levels in the tissues examined in this study. Neither arginine nor CLA decreased s.c. or r.p. fat mass in growing-finishing pigs. The lack of effect of arginine in reducing adiposity may have been related to the use of canola oil as a lipid control. Therefore, longer-term administration, higher doses of arginine, or treatment of piglets may be necessary in finishing-growing pigs to enhance animal performance, protein synthesis, or reducing adiposity.

In the current study, CLA supplementation increased intramuscular fat, which may increase consumer perception. pH at 45 min postmortem was lower in pigs supplemented with arginine, although ultimate pH was not affected. Arginine supplementation tended to increase the lightness of muscle at 24 h postmortem. Therefore, arginine supplementation with canola oil or CLA could enhance glycolysis or lactate content via activating AMPK, which is deteriorative to pork meat quality.

Fatty acid profiles differed between treatments in a manner that was consistent with differences in dietary lipid composition. CLA-added diets increased CLA isomers and decreased oleic acid, linoleic acid, and α-linolenic acid compared to canola-added diets. Even though there were no differences in the concentrations of dietary saturated fatty acids, including palmitic acid and stearic acid, dietary CLA supplementation increased the total saturated fatty acids in liver, s.c. and r.p. adipose tissues. This effect

can be explained by depressed Δ^9-desaturase by CLA or degradation of CLA to 18:1 *trans*-isomers.

CLA supplementation enhanced hepatic saturated fatty acids in liver. Dietary CLA supplementation increased 18:1 *trans*-isomers across tissues, including liver, *longissimus* muscle, intestinal duodenal mucosal cell, s.c. adipose tissue, and r.p. adipose tissue. This suggests that dietary CLA can be metabolized to 18:1 *trans*-isomers, including 18:1 *t10* and 18:1 *t11*, in these tissues. In s.c. adipose tissue, increased concentrations of saturated fatty acids can cause firmer bellies, fewer problems with sausage making, bacon slicing, and lipid oxidation.

There was the lack of effect of CLA or arginine on glucose or palmitate oxidation to CO_2 in liver and *longissimus* muscle. Also, neither CLA nor arginine supplementation modulated mRNA levels of related genes, including PGC-1α, AMPK, and CPT-1A or CPT-1B. However, CO_2 production in intestinal duodenal mucosal cells increased in pigs supplemented with CLA, despite a lack of changes in gene expression. Likewise, dietary CLA supplementation, with or without arginine, increased energy utilization from glucose in s.c. and r.p. adipose tissue despite changes in mRNA levels.

CLA or arginine supplementation to pigs may increase hepatic lipogenesis from palmitate and CLA supplementation to pigs may enhance the lipid synthesis in *longissimus* muscle. In s.c. adipose tissue, CLA supplemetation increased adipocyte volume without modulating mRNA levels of related genes. Our results indicate that both dietary CLA or arginine supplementation to pigs affects lipogenesis in different manner depend on tissues. In adipose tissues, CLA or arginine supplementation to pigs

significantly increased adiposity even though CLA supplementation increased substrate utilization. And there was little evidence of interaction between CLA and arginine.

In conclusion, our novel hypothesis, that 1% of CLA and arginine would have synergetic effects on improving growth performance and meat quality by reducing adiposity and increasing substrates utilization, proved to be incorrect, in that 1) there was little evidence in reducing adiposity, substrate oxidation, and related gene expression; 2) arginine with canola or CLA accelerated pH drop in meat quality characteristics and increased backfat; and 3) CLA and arginine worked independently. Longer-term administration of arginine or treatment of younger pigs (e.g. 40 kg body weight) may be necessary to enhance animal performance, protein synthesis, or reducing adiposity in growing-finishing pigs. However, we demonstrate consistent effects of CLA that 1) CLA increased intramuscular fat content in *longissimus* muscle for consumer perception; 2) CLA increased the capacity of bacon processing via increasing saturated fatty acids; 3) CLA increased substrate oxidation in adipose tissues; and 4) CLA decreased the concentrations of insulin in plasma.

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APPENDIX

TABLE 1 Calculated nutrient content of the basal diet[1-3]

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

[1]Commercial diet prepared by Producers Cooperation Association, Bryan, TX., 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.
[2]As fed basis
[3]Vitamin and trace minerals content of diet exceeded requirements established by the National Research Council (1998).

TABLE 2 Composition of experimental diet

Item, %	Treatment			
	Control	CLA	Arg	CLA + Arg
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

TABLE 3 Fatty acid profiles of test diets (g/100g total lipids)

	Treatment[1]			
Fatty acid	Control	CLA	Arg	CLA + Arg
C12:0	nd[3]	nd	nd	nd
C14:0	0.1	0.1	0.1	0.1
C14:1 n5	nd	nd	nd	nd
C16:0	12.3	12.5	12.3	12.4
C16:1 n7	0.3	0.3	0.3	0.3
C18:0	1.9	2.0	2.0	2.0
C18:1 *trans*[2]	nd	nd	nd	nd
C18:1 *c9*	33.7	23.7	33.9	23.3
C18:1 *c11*	1.7	1.2	1.7	1.2
C18:2 n6	43.2	39.5	43.7	39.5
C18:2 *c9, t11*	0.0	7.5	0.0	7.7
C18:2 *t10, c12*	0.0	7.6	0.0	7.8
C18:3 n3	3.9	2.3	3.9	2.4
C20:0	0.3	0.3	0.3	0.2
C20:1 n11	0.5	0.5	0.6	0.5
C20:4 n6	nd	nd	nd	nd
C20:5 n3	nd	nd	nd	nd
C22:6 n3	nd	nd	nd	nd

[1]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.
[2]Sum of amount of 18:1 *t9*, 18:1 *t10*, and 18:1 *t11*.
[3]nd = not detectable.

TABLE 4 Amino acid profiles of test diets[1-3]

	Treatment[1]			
Amino acid, %	Control	CLA	Arg	CLA + Arg
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

[1]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.
[2]As-fed basis
[3]Molecular weights of intact amino acids were used for calculation of amino acids in diet.

TABLE 5 Growth performance of pigs fed CLA, arginine, or arginine plus CLA

	Treatment[1]					*P*-value		
Item	Control	CLA	Arg	CLA + Arg	SEM	AA	FA	AA × FA[2]
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/d	0.78	0.70	0.79	0.82	0.07	0.38	0.74	0.69
Average daily feed, kg/d	2.88	2.95	2.90	2.89	0.07	0.79	0.68	0.91
Feed efficiency[3]	3.77	4.23	3.74	3.55	0.27	0.27	0.65	0.45

[1]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.
[2]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.
[3]Feed efficiency = feed:gain.

TABLE 6 Carcass traits of pigs fed CLA, arginine, or CLA plus arginine

	Treatment[1]					*P*-value		
Item	Control	CLA	Arg	CLA + Arg	SEM	AA	FA	AA × FA[2]
Slaughter weight, kg	111	105	109	110	4.02	0.69	0.65	0.76
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
Fat thickness, cm								
Backfat thickness	2.49	2.50	2.74	2.78	0.15	0.08	0.83	0.38
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
Muscle score	2.00	2.00	2.00	2.00	-	-	-	-
Loineye area, cm^2	38.7	35.5	38.2	41.0	2.82	0.39	0.93	0.60

[1]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.

[2]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

TABLE 7 Meat quality characteristics of pigs fed CLA, arginine, or CLA plus arginine

	Treatment[1]					*P*-value		
	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
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*[4]	42.7	45.0	46.0	45.3	1.80	0.33	0.67	0.60
a*[5]	6.89	8.58	8.66	8.30	0.82	0.37	0.42	0.39
b*[6]	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[ab]	46.8[b]	52.2[a]	48.7[ab]	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[ab]	0.26	0.10	0.01	0.03

[a-b]Means in rows not bearing a common superscript differ, $P < 0.10$.
[1]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.
[2]Largest SEM among treatments.
[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.
[4]L* = lightness
[5]a* = redness
[6]b* = yellowness

TABLE 8 Fatty acid composition of liver from pigs fed diets containing CLA, arginine, CLA plus arginine (g/100g total lipids)

	Treatment[1]					*P*-value		
Fatty acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
C14:0	0.21[b]	0.43[a]	0.46[a]	0.56[a]	0.08	0.02	0.04	0.02
C16:0	12.1[b]	15.7[a]	15.6[a]	15.2[a]	0.50	0.03	0.01	0.001
C16:1 n7	0.51	0.72	0.91	0.82	0.11	0.04	0.57	0.10
C18:0	23.0	23.2	21.3	21.6	1.00	0.11	0.83	0.46
C18:1 *trans*[4]	0.37[b]	0.88[a]	0.36[b]	0.88[a]	0.05	0.91	0.001	0.001
C18:1 *c9*	14.5	14.0	18.3	16.0	1.35	0.05	0.31	0.16
C18:1 *c11*	1.63	1.50	1.69	1.59	0.11	0.51	0.31	0.70
C18:2 n6	10.0[b]	14.3[a]	14.2[a]	14.9[a]	0.89	0.02	0.01	0.002
C18:2 *c9, t11*	0.05[b]	1.17[a]	0.11[b]	1.23[a]	0.11	0.57	0.001	0.001
C18:2 *t10, c12*	nd	0.30[a]	0.01[b]	0.30[a]	0.04	0.86	0.001	0.001
C18:3 n3	0.27[b]	0.26[b]	0.50[a]	0.39[ab]	0.05	0.001	0.19	0.005
C20:4 n6	21.3[a]	15.7[b]	15.3[b]	15.0[b]	1.14	0.01	0.02	0.001
C20:5 n3	0.57[a]	0.38[bc]	0.49[ab]	0.34[c]	0.04	0.18	0.001	0.001
C22:6 n3	2.07[a]	1.09[b]	1.03[b]	1.25[b]	0.20	0.08	0.10	0.004
MUFA	17.2	17.8	21.4	19.9	1.58	0.06	0.80	0.25
SFA	38.2[b]	42.5[a]	39.6[b]	40.2[ab]	0.86	0.62	0.01	0.01
MUFA:SFA[5]	0.45	0.42	0.55	0.51	0.05	0.06	0.56	0.30

[a-c]Means in rows not bearing a common superscript differ, $P < 0.05$.
[1]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.
[2]Largest SEM among treatments.
[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.
[4]Sum of amount of 18:1 *t9*, 18:1 *t10*, and 18:1 *t11*.
[5]MUFA:SFA = (14:1 n5 + 16:1 n7 + 18:1 *c9* + 18:1 *c11* + 18:2 *c9, t11*) / (14:0 + 16:0 + 18:0 + 18:1 *trans*)
[6]nd = not detectable.

TABLE 9 Fatty acid composition of *longissimus* muscle from pigs fed diets containing CLA, arginine, or CLA plus arginine (g/100g total lipids)

	Treatment[1]					*P*-value		
Fatty acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
C14:0	1.47	1.51	1.30	1.35	0.22	0.45	0.82	0.89
C14:1 n5	0.02[c]	0.05[a]	0.02[bc]	0.03[b]	0.004	0.14	0.001	0.001
C16:0	24.8	24.9	24.0	25.0	1.34	0.78	0.67	0.94
C16:1 n7	2.95[c]	5.04[a]	3.41[c]	4.24[b]	0.26	0.55	0.001	0.001
C18:0	11.3	10.9	10.5	11.6	0.39	0.99	0.42	0.24
C18:1 *trans*[4]	0.20[b]	0.35[a]	0.15[b]	0.35[a]	0.03	0.38	0.001	0.001
C18:1 *c9*	41.3[a]	36.1[b]	39.8[ab]	37.2[b]	1.26	0.87	0.005	0.03
C18:1 *c11*	3.83	4.59	4.22	4.28	0.29	0.90	0.18	0.35
C18:2 n6	7.42	8.15	7.92	8.45	0.69	0.56	0.36	0.75
C18:2 *c9, t11*	0.03[b]	0.51[a]	0.05[b]	0.46[a]	0.06	0.83	0.001	0.001
C18:2 *t10, c12*	nd[6]	0.18	nd	0.17	0.03	0.87	0.001	0.001
C18:3 n3	0.37[a]	0.26[b]	0.30[b]	0.29[b]	0.02	0.34	0.03	0.03
C20:4 n6	1.35	1.80	2.25	1.54	0.30	0.32	0.69	0.20
C20:5 n3	0.04	0.05	0.07	0.04	0.01	0.26	0.36	0.16
C22:6 n3	0.05	0.06	0.07	0.06	0.01	0.38	0.64	0.51
MUFA	49.0	47.1	48.3	47.0	1.61	0.80	0.30	0.76
SFA	38.5	38.4	36.7	39.0	1.71	0.73	0.54	0.80
MUFA:SFA[5]	1.32	1.24	1.35	1.22	0.09	0.94	0.23	0.67

[a-c]Means in rows not bearing a common superscript differ, $P < 0.05$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

[4]Sum of amount of 18:1 *t9*, 18:1 *t10*, and 18:1 *t11*.

[5]MUFA:SFA = (14:1 n5 + 16:1 n7 + 18:1 *c9* + 18:1 *c11* + 18:2 *c9, t11*) / (14:0 + 16:0 + 18:0 + 18:1 *trans*)

[6]nd = not detectable.

TABLE 10 Fatty acid composition of intestinal duodenal mucosal cells from pigs fed diets containing CLA, arginine, or CLA plus arginine (g/100g total lipids)

	Treatment[1]					*P*-value		
Fatty acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
C14:0	0.63	0.90	0.69	0.81	0.12	0.91	0.11	0.41
C14:1 n5	0.01	0.02	0.01	0.01	0.01	0.39	0.32	0.63
C16:0	21.5	24.1	21.9	22.8	1.15	0.69	0.12	0.38
C16:1 n7	1.38	1.29	1.19	1.21	0.14	0.31	0.80	0.73
C18:0	15.7	18.8	17.7	17.7	0.98	0.63	0.13	0.15
C18:1 *trans*[4]	0.29[b]	0.63[a]	0.29[b]	0.72[a]	0.08	0.58	0.001	0.001
C18:1 *c9*	25.0	24.1	26.4	21.3	1.95	0.72	0.12	0.28
C18:1 *c11*	2.81[a]	2.23[b]	2.61[a]	2.11[b]	0.11	0.15	0.00	0.001
C18:2 n6	12.3	10.4	9.85	12.0	1.52	0.79	0.93	0.59
C18:2 *c9, t11*	0.01[b]	0.66[a]	0.04[b]	0.75[a]	0.09	0.50	0.001	0.001
C18:2 *t10, c12*	nd[6]	0.24	nd	0.26	0.06	0.78	0.001	0.001
C18:3 n3	0.47[a]	0.27[b]	0.33[b]	0.25[b]	0.04	0.06	0.001	0.001
C20:4 n6	7.03	5.10	6.69	7.30	1.04	0.37	0.52	0.44
C20:5 n3	0.10	0.05	0.10	0.07	0.02	0.51	0.03	0.13
C22:6 n3	0.28[a]	0.16[b]	0.18[b]	0.25[ab]	0.03	0.80	0.47	0.04
MUFA	29.9	28.9	31.0	25.9	2.04	0.62	0.13	0.30
SFA	40.2	46.3	42.7	44.3	1.95	0.92	0.05	0.15
MUFA:SFA[5]	0.74[a]	0.63[b]	0.72[a]	0.58[b]	0.03	0.16	0.001	0.001

[a-b]Means in rows not bearing a common superscript differ, $P < 0.05$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

[4]Sum of amount of 18:1 *t9*, 18:1 *t10*, and 18:1 *t11*.

[5]MUFA:SFA = (14:1 n5 + 16:1 n7 + 18:1 *c9* + 18:1 *c11* + 18:2 *c9, t11*) / (14:0 + 16:0 + 18:0 + 18:1 *trans*)

[6]nd = not detectable.

TABLE 11 Fatty acid composition of r.p. adipose tissue from pigs fed diets containing CLA, arginine, or CLA plus arginine (g/100g total lipids)

	Treatment[1]					*P*-value		
Fatty acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
C14:0	1.71^{bc}	2.75^{a}	1.47^{c}	2.23^{ab}	0.22	0.09	0.001	0.001
C16:0	30.1	34.0	28.0	32.0	1.78	0.25	0.03	0.12
C16:1 n7	1.65	1.89	1.53	1.86	0.14	0.59	0.05	0.25
C18:0	17.7	18.1	17.2	17.5	0.78	0.49	0.61	0.87
C18:1 *trans*[4]	0.49^{bc}	0.86^{a}	0.33^{c}	0.60^{b}	0.09	0.02	0.001	0.002
C18:1 *c9*	31.3^{ab}	25.2^{c}	34.5^{a}	28.8^{bc}	1.57	0.04	0.001	0.002
C18:1 *c11*	1.82^{a}	1.56^{b}	1.88^{a}	1.79^{a}	0.08	0.08	0.03	0.03
C18:2 n6	11.2	10.8	11.6	10.8	0.94	0.81	0.55	0.93
C18:2 *c9, t11*	0.55^{bc}	1.31^{a}	0.12^{c}	0.86^{ab}	0.23	0.07	0.003	0.01
C18:2 *t10, c12*	0.30^{bc}	0.73^{a}	0.05^{c}	0.48^{ab}	0.13	0.07	0.003	0.01
C18:3 n3	0.68^{ab}	0.48^{c}	0.79^{a}	0.56^{bc}	0.07	0.18	0.003	0.02
C20:4 n6	0.24	0.18	0.22	0.18	0.02	0.59	0.03	0.17
MUFA	36.0^{ab}	30.5^{c}	38.7^{a}	33.9^{bc}	1.49	0.04	0.002	0.005
SFA	50.7^{ab}	56.5^{a}	47.6^{b}	53.1^{ab}	1.94	0.10	0.01	0.02
MUFA:SFA[5]	0.73^{ab}	0.55^{c}	0.82^{a}	0.66^{bc}	0.05	0.06	0.002	0.01

$^{a\text{-}c}$Means in rows not bearing a common superscript differ, $P < 0.05$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

[4]Sum of amount of 18:1 *t9*, 18:1 *t10*, and 18:1 *t11*.

[5]MUFA:SFA = (14:1 n5 + 16:1 n7 + 18:1 *c9* + 18:1 *c11* + 18:2 *c9, t11*) / (14:0 + 16:0 + 18:0 + 18:1 *trans*)

[6]nd = not detectable.

TABLE 12 Fatty acid composition of s.c. adipose tissue from pigs fed diets containing CLA, arginine, or CLA plus arginine (g/100g total lipids)

	Treatment[1]					*P*-value		
Fatty acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
C14:0	1.04^{b}	1.97^{a}	1.15^{b}	1.75^{a}	0.10	0.54	0.001	0.001
C14:1 n5	0.003	0.005	nd[6]	0.003	0.002	0.15	0.20	0.29
C16:0	21.5^{b}	26.3^{a}	22.2^{b}	26.0^{a}	0.69	0.77	0.001	0.001
C16:1 n7	1.98	2.13	1.81	1.95	0.11	0.13	0.19	0.26
C18:0	11.3^{b}	15.5^{a}	12.8^{b}	16.5^{a}	0.67	0.07	0.001	0.001
C18:1 *trans*[4]	0.34^{b}	0.82^{a}	0.36^{b}	0.72^{a}	0.05	0.41	0.001	0.001
C18:1 *c9*	42.2^{a}	32.4^{b}	41.6^{a}	32.4^{b}	1.25	0.78	0.001	0.001
C18:1 *c11*	2.78^{a}	2.41^{b}	2.59^{ab}	2.28^{b}	0.12	0.19	0.01	0.04
C18:2 n6	14.3	12.7	13.1	12.8	0.64	0.43	0.16	0.33
C18:2 *c9, t11*	0.05^{b}	1.33^{a}	0.13^{b}	1.29^{a}	0.13	0.88	0.001	0.001
C18:2 *t10, c12*	nd	0.73^{a}	0.05^{b}	0.72^{a}	0.09	0.82	0.001	0.001
C18:3 n3	0.85^{a}	0.51^{b}	0.77^{a}	0.53^{b}	0.04	0.44	0.001	0.001
C20:4 n6	0.27^{a}	0.17^{c}	0.23^{ab}	0.19^{bc}	0.02	0.62	0.002	0.01
MUFA	48.1^{a}	39.3^{b}	47.3^{a}	38.8^{b}	1.34	0.62	0.001	0.001
SFA	34.9^{b}	45.2^{a}	37.2^{b}	45.7^{a}	1.31	0.33	0.001	0.001
MUFA:SFA[5]	1.39^{a}	0.89^{b}	1.28^{a}	0.86^{b}	0.06	0.29	0.001	0.001

$^{a-c}$Means in rows not bearing a common superscript differ, $P < 0.05$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

[4]Sum of amount of 18:1 *t9*, 18:1 *t10*, and 18:1 *t11*.

[5]MUFA:SFA = (14:1 n5 + 16:1 n7 + 18:1 *c9* + 18:1 *c11* + 18:2 *c9, t11*) / (14:0 + 16:0 + 18:0 + 18:1 *trans*)

[6]nd = not detectable.

TABLE 13 Fatty acid composition of plasma from pigs fed diets containing CLA, arginine, or CLA plus arginine (g/100g total lipids)

	Treatment[1]					*P*-value		
Fatty acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
C14:0	0.16	0.50	0.30	0.52	0.10	0.44	0.02	0.11
C16:0	15.4	17.2	16.3	16.9	0.61	0.59	0.08	0.26
C16:1 n7	0.70	0.74	0.82	0.79	0.12	0.48	0.98	0.90
C18:0	14.9	15.3	14.3	14.7	0.58	0.30	0.46	0.64
C18:1 *trans*[4]	0.20	0.79	0.36	0.49	0.23	0.85	0.14	0.37
C18:1 *c9*	20.6	18.8	24.0	19.8	1.37	0.13	0.06	0.10
C18:1 *c11*	1.62	1.30	1.62	1.51	0.15	0.53	0.16	0.41
C18:2 n6	26.5	26.9	25.8	25.7	1.81	0.61	0.95	0.96
C18:2 *c9, t11*	nd	0.90	nd	0.90	0.33	1.00	0.02	0.13
C18:2 *t10, c12*	nd	0.32	nd	0.34	0.12	0.95	0.02	0.14
C18:3 n3	0.53	0.38	0.57	0.19	0.13	0.60	0.07	0.25
C20:4 n6	12.7	10.2	10.1	11.4	0.79	0.37	0.43	0.13
C20:5 n3	0.27	0.15	0.30	0.10	0.09	0.94	0.11	0.45
C22:6 n3	0.61	0.39	0.33	0.52	0.14	0.57	0.85	0.54
MUFA	22.9	21.8	26.6	23.2	1.52	0.12	0.18	0.19
SFA	32.9	36.2	33.5	34.7	1.15	0.78	0.07	0.26
MUFA:SFA[5]	0.70	0.61	0.80	0.68	0.06	0.16	0.10	0.21

[1]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.
[2]Largest SEM among treatments.
[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.
[4]Sum of amount of 18:1 *t9*, 18:1 *t10*, and 18:1 *t11*.
[5]MUFA:SFA = (14:1 n5 + 16:1 n7 + 18:1 *c9* + 18:1 *c11* + 18:2 *c9, t11*) / (14:0 + 16:0 + 18:0 + 18:1 *trans*)
[6]nd = not detectable.

TABLE 14 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

	Treatment[1]					*P*-value		
Tissue/substrate	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
Liver metabolism, nmol substrate converted to product/(100mg × 2h)								
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[ab]	56.2[a]	48.5[ab]	6.04	0.02	0.98	0.07
Longissimus muscle metabolism, nmol substrate converted to product/(100mg × 2h)								
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/(100mg × 2h)								
CO_2 production								
Glucose	186	189	106	151	38.6	0.11	0.53	0.35
Palmitate	16.0[ab]	32.1[a]	13.8[b]	18.1[ab]	5.50	0.15	0.07	0.10

[a-b]Means in rows not bearing a common superscript differ, $P < 0.10$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

TABLE 15 Incorporation of glucose and palmitate carbon into CO_2 and lipids *in vitro* and cellularity in r.p. adipose tissue in pigs fed CLA, arginine, or CLA plus arginine

	Treatment[1]					*P*-value		
Tissue/substrate	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
r.p. adipose tissue metabolism, nmol substrate converted to product/(100mg × 2h)								
CO_2 production								
Glucose	58.3	100	57.8	110	23.0	0.83	0.03	0.20
Palmitate	5.72	7.14	5.80	5.99	1.11	0.63	0.47	0.79
Lipid synthesis								
Glucose	64.5	59.6	33.7	83.8	22.6	0.89	0.32	0.48
Palmitate	112	107	120	117	11.2	0.41	0.72	0.85
r.p. adipose tissue metabolism, nmol substrate converted to product/(10^{-9} × cell × 2h)								
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
r.p. adipose tissue cellularity								
Adipocyte volume, pL	666[b]	811[a]	805[a]	814[a]	8.63	0.001	0.001	0.001
Adipocytes/100mg × 10^{-9}	1.00[a]	0.94[d]	0.96[c]	0.97[b]	0.01	0.001	0.001	0.001

[a-d]Means in rows not bearing a common superscript differ, $P < 0.10$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

TABLE 16 Incorporation of glucose and palmitate carbon into CO_2 and lipids *in vitro* and cellularity in s.c. adipose tissue in pigs fed CLA, arginine, or CLA plus arginine

	Treatment[1]					*P*-value		
Tissue/substrate	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
s.c. adipose tissue metabolism, nmol substrate converted to product/(100mg × 2h)								
CO_2 production								
Glucose	52.4	89.0	73.0	88.4	14.1	0.50	0.06	0.21
Palmitate	4.66	5.29	4.57	5.03	0.94	0.86	0.56	0.95
Lipid synthesis								
Glucose	33.7[b]	33.9[b]	80.8[a]	39.5[ab]	14.9	0.09	0.18	0.09
Palmitate	97.5	93.9	107	104	11.0	0.38	0.79	0.84
s.c. adipose tissue metabolism, nmol substrate converted to product/(10^{-9} × cell × 2h)								
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[ab]	15.2	0.06	0.20	0.07
Palmitate	91.1	92.9	110	109	11.0	0.12	0.97	0.49
s.c. adipose tissue cellularity								
Adipocyte volume, pL	597[d]	735[c]	789[b]	875[a]	11.0	0.001	0.001	0.001
Adipocytes/100mg × 10^{-9}	1.07[a]	1.01[b]	0.97[c]	0.96[c]	0.01	0.001	0.001	0.001

[a-d]Means in rows not bearing a common superscript differ, $P < 0.10$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

TABLE 17 Expression of genes in liver, longissimus muscle, and intestinal duodenal mucosal cells in pigs fed CLA, arginine, or CLA plus arginine

	Treatment[1]					*P*-value		
	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
Liver								
PGC-1α	1.00	0.75	0.50	0.66	0.23	0.27	0.80	0.56
AMPK	1.00	1.40	0.64	1.06	0.45	0.43	0.37	0.71
mTOR	1.00	0.47	0.52	0.59	0.26	0.58	0.37	0.50
CPT-1A	1.00	1.51	1.18	1.20	0.67	0.95	0.72	0.97
FAS	1.00	0.91	0.74	0.44	0.35	0.33	0.60	0.70
SCD	1.00	0.98	0.99	0.65	0.39	0.66	0.72	0.91
Longissimus muscle								
PGC-1α	1.00[b]	1.63[a]	1.52[a]	1.01[b]	0.19	0.85	0.80	0.07
AMPK	1.00	1.14	0.93	0.68	0.35	0.43	0.88	0.81
mTOR	1.00	1.44	1.69	1.17	0.59	0.72	0.94	0.85
CPT-1B	1.00	1.21	1.39	0.96	0.38	0.85	0.78	0.84
FAS	1.00	1.29	1.01	1.18	0.39	0.90	0.55	0.94
SCD	1.00	1.61	0.56	0.83	0.55	0.27	0.42	0.59
Intestinal duodenal mucosal cells								
PGC-1α	1.00	0.16	0.60	0.28	0.24	0.64	0.18	0.41
AMPK	1.00	0.38	2.03	0.31	0.51	0.62	0.10	0.38
mTOR	1.00[a]	0.14[b]	0.57[ab]	0.31[ab]	0.14	0.83	0.04	0.08
CPT-1B	1.00	0.69	1.08	0.32	0.26	0.75	0.13	0.37
FAS	1.00	0.38	2.81	0.65	0.36	0.27	0.07	0.16
SCD	1.00	0.19	0.85	0.17	0.25	0.79	0.07	0.29

[a-b]Means in rows not bearing a common superscript differ, $P < 0.10$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

TABLE 18 Expression of genes in r.p. adipose tissue and s.c. adipose tissue in pigs fed CLA, arginine, or CLA plus arginine

	Treatment[1]					*P*-value		
	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
r.p. adipose tissue								
PGC-1α	1.00	1.02	0.67	0.95	0.72	0.75	0.81	0.98
AMPK	1.00	0.81	1.74	0.92	1.10	0.70	0.61	0.92
mTOR	1.00	1.67	0.71	1.30	0.48	0.51	0.22	0.60
CPT-1B	1.00	0.97	0.39	0.51	0.47	0.25	0.93	0.71
FAS	1.00	1.10	2.19	1.01	1.01	0.50	0.61	0.71
SCD	1.00	0.62	1.83	1.42	0.95	0.30	0.62	0.69
s.c. adipose tissue								
PGC-1α	1.00	1.53	2.71	1.57	1.16	0.43	0.86	0.74
AMPK	1.00	1.29	2.66	2.05	1.09	0.27	0.88	0.70
mTOR	1.00	1.05	2.21	1.71	0.57	0.11	0.68	0.41
CPT-1B	1.00	2.46	5.72	4.00	2.13	0.19	0.91	0.52
FAS	1.00	0.98	2.06	1.39	0.61	0.24	0.57	0.59
SCD	1.00	0.96	0.50	0.16	0.68	0.25	0.75	0.70

[1]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.
[2]Largest SEM among treatments.
[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

TABLE 19 Metabolic syndrome traits and lipoprotein profiles of plasma in pigs fed CLA, arginine, or CLA plus arginine

	Treatment[1]					*P*-value		
	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
Metabolic syndrome traits								
TG, mg/dL	22.7	36.3	32.7	35.5	5.46	0.46	0.14	0.29
Total cholesterol, CEQ/dL[4]	89.2	89.7	84.0	99.7	6.57	0.59	0.22	0.36
Insulin, µIU/mL	64.4	20.0	51.5	30.5	16.4	0.97	0.05	0.23
Homocysteine, µmol/L	13.9	11.4	17.8	18.0	2.99	0.07	0.69	0.30
Lipoprotein profiles								
VLDL, nmol	24.3	24.5	24.7	24.8	1.28	0.80	0.91	0.99
LDL, nmol	405	407	374	445	35.0	0.81	0.30	0.53
HDL, nmol	5605	5454	5106	5952	431	0.87	0.42	0.54
ILP, nmol	6.10	6.05	6.83	8.83	1.03	0.07	0.34	0.17
RLP, nmol	33.0	33.0	36.7	44.8	4.55	0.07	0.36	0.20

[1]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.
[2]Largest SEM among treatments.
[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

[4]Cholesterol equivalents per dL

TABLE 20 Essential amino acids in plasma (nmol/mL) of pigs fed CLA, arginine, and CLA plus arginine

	Treatment[1]					*P*-value		
Amino acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
Histidine	74.9	84.4	81.9	80.0	5.92	0.83	0.53	0.71
Isoleucine	80.2	90.7	88.1	86.1	8.97	0.85	0.63	0.86
Leucine	154	169	156	149	22.1	0.68	0.85	0.93
Lysine	78.2	98.4	107	104	25.0	0.49	0.74	0.85
Methionine	32.6	36.2	32.4	30.3	2.75	0.29	0.80	0.52
Phenylalanine	64.1[b]	86.8[a]	76.7[ab]	72.6[ab]	5.25	0.90	0.16	0.06
Threonine	65.8	87.0	88.0	90.3	7.16	0.11	0.14	0.11
Tryptophan	37.8	42.6	46.3	45.7	5.89	0.33	0.72	0.73
Valine	203	222	208	202	18.4	0.69	0.73	0.87

[a-b]Means in rows not bearing a common superscript differ, $P < 0.10$.

[1]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.

[2]Largest SEM among treatments.

[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

TABLE 21 Nonessential amino acids in plasma (nmol/mL) of pigs fed CLA, arginine, and CLA plus arginine

	Treatment[1]					*P*-value		
Amino acid	Control	CLA	Arg	CLA + Arg	SEM[2]	AA	FA	AA × FA[3]
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	43.3	50.4	44.5	49.3	3.89	0.99	0.13	0.51
Aspartate	10.5	11.0	11.4	14.0	1.70	0.26	0.38	0.51
Citrulline	52.8	66.6	60.5	59.3	8.87	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	66.1	59.7	56.0	57.7	11.2	0.59	0.83	0.93
Serine	84.1[b]	106[a]	101[ab]	90.4[ab]	5.55	0.91	0.44	0.06
Taurine	82.2	119	118	156	27.5	0.19	0.18	0.35
Tyrosine	42.3	60.3	54.4	58.4	10.8	0.63	0.31	0.65

[1]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.
[2]Largest SEM among treatments.
[3]AA, amino acid effect (alanine or arginine); FA, fatty acid effect (canola or CLA); AA × FA, interaction effect.

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Gwang-woong Go was born in 1979 and grew up in Seoul, South Korea. Gwang-woong is the son of Yeonsook Yoon and Dongsam Go. In October of 2005, Gwang-woong married Gyoungok. Gwang-woong and Gyoungok has a son, Jaewon David Go, who was born in Bryan, Texas in 2009.

Gwang-woong obtained a BS degree in 2005 and MS degree in 2007 from Korea University, under the advice of Byoung-chul Kim. Gwang-woong began his Ph.D. program at Texas A&M University as a graduate student in the Intercollegiate Faculty of Nutrition in 2007. Gwang-woong conducted his Ph.D. research in the direction of Dr. Stephen B. Smith.

Gwang-woong will continue his research career as a postdoctoral fellow at University of Maryland School of Medicine studying Gaucher's disease using induced pluripotent stem cell (iPSC) under Dr. Ricardo A. Feldman.