ARGININE AND CONJUGATED LINOLEIC ACID REDUCE FAT MASS IN RATS

A Thesis

by

JENNIFER LYNN NALL

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

MASTER OF SCIENCE

May 2008

Major Subject: Nutrition

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Approved by:

Chair of Committee, Stephen B. Smith
Committee Members, John Bauer
Guoyao Wu
Chair of Nutrition Faculty, Stephen B. Smith

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ABSTRACT

Arginine and Conjugated Linoleic Acid Reduce Fat Mass in Rats.

(May 2008)

Jennifer Lynn Nall, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Stephen B. Smith

This experiment tested the hypothesis that the combination of dietary conjugated linoleic acid (CLA) plus arginine will decrease fat accumulation and increase muscle mass. Twenty-four male Sprague Dawley rats were assigned to treatments in a 2 x 2 factorial design with six rats per treatment group (control: 2.55% alanine in drinking water + 1.5% canola oil; arginine: 1.25% arginine-HCl in drinking water + 1.5% canola oil; conjugated linoleic acid (CLA; mixed isomers): 2.55% alanine in drinking water + 1.5% CLA; arginine + CLA: 1.25% arginine-HCl in drinking water + 1.5% CLA). Rats fed arginine plus CLA had a higher daily intake of food ($P = 0.02$). Rats fed arginine plus CLA had a higher average daily gain than rats fed only arginine or CLA. Arginine and CLA increased final body weights ($P = 0.04$,0.009). Abdominal fat mass ($P = 0.08$) and abdominal fat:lean body mass ratio ($P = 0.03$) were decreased by CLA but were unaffected by arginine. Epididymal fat pad weight was unaffected by arginine or CLA ($P > 0.55$). CLA tended ($P < 0.07$) to decrease the plasma oleic/stearic acid ratio. Conversion of ^{14}C-labeled glucose and palmitic acid to total lipids and CO_2 in epididymal adipose tissue *in vitro* was unaffected by CLA. Arginine decreased ($P =$

0.09) palmitic acid incorporation into total lipids in epididymal adipose tissue. CLA plus arginine supplementation also reduced glucose incorporation into total lipid (P = 0.20). Arginine stimulated lipid synthesis from glucose in the absence of CLA, but had no effect in the presence of CLA. In liver, arginine increased the rate of conversion of glucose to CO_2 (P = 0.06). Singly, arginine depressed plasma concentrations of all measured amino acids except serine. CLA depressed all amino acids except cysteine. The combination of arginine plus CLA depressed the concentration of plasma arginine (P = 0.05). The data indicate that arginine plus CLA decreases the fat/lean mass ratio, which may be due to effects on the metabolism of muscle, liver, and adipose tissue.

DEDICATION

This thesis is dedicated to my parents, who have provided me with unconditional love, guidance, and support.

ACKNOWLEDGEMENTS

I would like to thank Dr. Stephen B. Smith for allowing me the opportunity to be his graduate student. He has been a wonderful mentor and has provided guidance and encouragement my entire graduate career. I would also like to thank Dr. Guoyao Wu and Dr. John Bauer for being on my committee and for providing guidance throughout my project.

I would also like to express my appreciation to my fellow graduate students in the Nutrition Department for their friendship. I would like to thank Scott Jobgen for his assistance with dissection of adipose and muscle tissues and Xilong Li for assisting in amino acid analysis. Finally, I would like to thank my fellow labmates, Matt Brooks, Anne Ford, Stacey Turk, Gwang-woong Go, and Lindsay Ward, for their friendship and support.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Obesity in humans is now considered endemic. Accumulation of fat mass in humans and animals depends on balancing caloric intake and energy expenditure. Although there currently is no effective treatment for obesity, studies have provided convincing evidence that arginine and conjugated linoleic acid (CLA) independently reduce fat mass in animal species (1,2). Because, individually, both arginine and CLA have been shown to reduce fat mass, our objective was to document the interaction between arginine and CLA on carcass composition in rodent species. This has never been documented before, and the information gained from this study will be used in future studies with bovine and porcine species and humans. This study will give us the information needed to produce livestock efficiently and to produce a high-quality product. For humans, this information will help us begin to treat obesity and associated diseases such as type-II diabetes and cardiovascular disease.

The objectives for this study were: 1) to determine how rat adipose tissue responds to arginine and/ or CLA treatments during growth; 2) establish if arginine supplementation will affect apparent hepatic stearoyl CoA desaturase-1 (SCD1) activity; and 3) document the effects of arginine and CLA on adiposity and adipose tissue metabolism.

In rat species, brown adipocytes can be found within white adipose tissue. Promoting mitochondrial biogenesis in brown adipocytes may be the mechanism by

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This thesis follows the Journal of Nutrition format.

which dietary arginine works to reduce fat mass in rodent species. Based on what is known about arginine and CLA, we hypothesized that adding dietary arginine and CLA will reduce fat mass and increase lean muscle gain in rats.

LITERATURE REVIEW

Nitric oxide (NO)

Previous studies have shown that dietary supplementation of arginine increases brown fat mass (1). Nitric oxide is an important regulator of metabolism. Because NO is a small, hydrophilic gas molecule, it is able to diffuse into cells and act on its target molecules via cGMP-dependent pathways. NO regulates vascular tone, neurotransmission, immunity, and overall homeostasis. L-Arginine is the precursor for NO and is converted to NO by nitric oxide synthase (NOS). NOS has three isoforms: nNOS, iNOS, and eNOS. nNOS was first identified in neuronal tissue (3,4,5). iNOS has been shown to be induced by both cytokines and lipopolysaccharides (4,5), and eNOS was identified in vascular endothelial cells (4,5). Although nNOS and eNOS produce relatively small amounts of NO (4,5), iNOS can be induced to produce high amounts of NO (4,5). All of the NOS isoforms may be induced under stimuli via transcriptional and translational pathways (6).

Several studies have suggested that insulin-sensitive tissues are key sites for NO synthesis. These include skeletal muscle, heart, kidneys, liver, and the brain. The NOS isoforms are mainly found in the sarcolemma, sarcoplasm, sarcoplasmic reticulum, and mitochondria of muscle fibers (7). In myocytes, the expression of NOS is regulated by factors such as fiber type, contraction, developmental stage, and the pathological state (8,9,10). Two of the isoforms, eNOS and iNOS, are expressed in brown adipose tissue. However, these two isoforms appear to be absent in white adipose tissue (11,12). In the liver, eNOS is distributed uniformly. On the other hand, iNOS is primarily found in the

cytoplasm of periportal hepatocytes (13). The compartmentalization of these NOS isoforms may be one way in which NO synthesis is regulated.

NOS synthesis by iNOS and obesity

An increase in the expression of iNOS and an overproduction of NO has been linked to obesity. In genetically obese rats, for example, iNOS expression in adipose tissue and skeletal muscle was higher than lean, non-obese wild-types (14,15). By treating the obese rodents with an iNOS inhibitor, insulin sensitivity was improved, thus preventing diet-induced obesity.

Recently, it was demonstrated that dietary arginine reduced adiposity in Zucker diabetic fat (ZDF) rats (16). These rats genetically resemble human Type II diabetics. After a 10-wk treatment period, the rats fed 1.25% arginine in drinking water had 25% less epididymal fat and 45% less abdominal fat than those rats given alanine (isonitrogenous control) in their water. In addition, serum levels of arginine and NO derivatives increased significantly in the arginine-fed rats. These results suggested that dietary arginine was effectively metabolized to citrulline, yielding NO. This study also showed that the inhibition of NO synthesis resulted in increased triglyceride levels and body fat.

Arginine has been shown to enhance several genes that are responsible for fatty acid and glucose oxidation, such as PGC-1α. Arginine increases the expression of PGC-1α (16), which is a regulator of oxidative phosphorylation. Therefore, an increase in the expression of PGC-1α could have caused the decrease in fat deposition. Arginine decreased stearoyl CoA desaturase (SCD1) gene expression in the liver as well.

Stearoyl CoA desaturase

Stearoyl CoA desaturase (SCD) is a Δ9 desaturase that catalyzes the synthesis of monounsaturated fatty acids from saturated fatty acyl-CoA. The favored substrates of SCD are palmitate (16:0) and stearate (18:0). Their respective products, especially oleate (18:1 n-9), are favored substrates for triglyceride synthesis. By decreasing hepatic SCD 1 expression, very low density lipoprotein (VLDL) output from the liver will also decrease, potentially resulting in a decrease in adipose tissue accumulation.

Conjugated linoleic acid

Studies also have provided convincing evidence that conjugated linoleic acid (CLA) decreases body fat. CLA decreases uncoupling protein-1 (UCP 1) expression in brown adipose tissue (17). UCP 1 is an uncoupling protein found only in brown adipose tissue. A UCP 2 isoform can be found in white adipose tissue (17). Like NO, CLA decreases SCD gene expression in the liver and decreases PPARγ2 expression in adipose tissue (18,19,20). PPARγ2 is a master gene that is found in brown and white adipose tissue. It acts to increase SCD expression and increase fatty acid synthesis (18,19,20). By decreasing PPARγ expression, SCD expression and fatty acid synthesis also is decreased, which results in a decrease in fat deposition.

Recently, Corino et al. (21) demonstrated that CLA reduced adiposity in market weight pigs. The pigs fed CLA had a lower adipocyte area (i.e., smaller adipocytes) than the control group. Dietary CLA, especially trans-10, cis-12 (t10,c12) CLA reduces carcass adiposity in several species (22,23). In addition, mixed isomers of CLA depressed 3T3-L1 preadipocyte proliferation and [^{3}H] thymidine incorporation into

DNA (24). Adams et al. (25) and Corino et al. (21) similarly reported that CLA reduced the percentage of proliferating cells in subcutaneous adipose tissue of pigs. Although CLA caused only small reductions in adipocyte volume, it strongly depressed SCD gene expression and catalytic activity (26). The pigs used by Smith et al. (26) and Adams et al. (25) were weanling pigs; CLA has been shown to more strongly depress adiposity in market-weight pigs (21, 27). The t10,c12 CLA isomer prevents lipid filling of preadipocytes by decreasing PPARγ gene expression (18,19,20,28).

Several studies have addressed the effects of CLA on brown adipose tissue gene expression. Feeding mixed isomers of CLA to rats had no effect on expression of any of the UCPs in brown or white adipose tissues, although it reduced adipose tissue weights (17). Conversely, mice fed CLA had increased expression of UCP 1 in brown adipose tissue, and they had increased UCP 2 gene expression in both brown and white adipose tissues (17). Thus, in rats at least, the expression of UCPs is not necessary for the reduction in adiposity caused by CLA. Takahasi et al. (2) also demonstrated that feeding mixed isomers of CLA depressed the weights of white and interscapular brown adipose tissues. CLA decreased PPARγ mRNA, but it increased UCP 2 in brown adipose tissue. CLA also depressed PPARγ mRNA in white adipose tissue of one strain of mice (2). Rodriguez et al. (29) reported that t10, 12 CLA depressed UCP 1 gene expression in cultured brown adipocytes, whereas c9,t11 CLA had the opposite effect. This presents the possibility that feeding mixed isomers of CLA may actually reduce adiposity by stimulating thermogenesis, but this has not been documented in an animal system.

Observations from these studies led to the possibility that NO and CLA can have either complementary or antagonistic effects on adiposity. Several investigators have demonstrated an interaction between CLA and NO. Yu et al. (30) and Iwakiri et al. (31) reported that CLA decreased iNOS gene expression in murine macrophages. Yang and Cook (32) demonstrated that both c9,t11 and t10,c12 CLA isomers depressed NO synthesis in macrophages from CLA-fed mice, and Eder et al. (33) demonstrated that both CLA isomers decreased iNOS gene expression in human aortic endothelial cells. To date, there are no published reports of the interaction between CLA isomers and NO (or arginine) on mitochondrial biogenesis in adipose tissue metabolism.

Recently, t10,c12 CLA was shown to reduce lipoprotein lipase, the enzyme responsible for the breakdown of triglycerides, while increasing lipogenic enzyme activities in hamsters (34). In addition, the transcriptional factors that regulate lipogenic enzymes were also studied. These include SREBP-1a, SREBP-1c, and PPARγ. CLA was shown to increase the expression of all three transcriptional factors, even though there was a reduction in acetyl CoA carboxylase and fatty acid synthase levels (34).

There is also convincing evidence that CLA differentially alters fatty acid composition in porcine adipose tissue (26,35,36). Dietary supplementation of CLA causes a significant increase in myristic (14:0) and palmitic acids while reducing arachidonic acid. This suggests that CLA alters the activities of the Δ^5, Δ^6, and Δ^9 – desaturases.

Based on what is currently known about CLA and arginine, we hypothesized that feeding a dietary combination of CLA and arginine would reduce fat mass by increasing

oxidation of metabolic substrates while depressing synthesis of lipids in adipose tissue and liver.

MATERIALS AND METHODS

The overall objective of this study was to document the interrelationships between adipose tissue and liver metabolism and adipogenesis in rat species. Therefore, the response of rat liver and epididymal adipose tissue to exposure to arginine and mixed isomers of CLA during growth was characterized. Specifically, we wanted to determine whether arginine treatment would stimulate the rate of oxidation of glucose and palmitate in rat adipose tissue. The effects of arginine and/or CLA on plasma and hepatic fatty acids were measured to provide an estimate of hepatic SCD1 activity.

Animals and diets

For this study, 24 male Sprague Dawley rats (8 wk old) were obtained from a commercial source and were growing rats. Beginning at 9 wk of age, the rats received drinking water containing 2.55% alanine or 1.25% arginine and fed a casein-based semi-purified diet containing 1.5% canola or mixed isomers of CLA for 5 wk (Table 1, 2). The dietary treatment lasted for 5 wk. The study was a 2 x 2 factorial design, with two levels of arginine (0 and 1.5%) and two levels of CLA (0 and 1.5%). The treatment groups were:

- Control rats (n = 6), fed 2.55% L-alanine (isonitrogenous control) plus 1.5% canola (lipid control).
- Arginine rats (n = 6), fed 1.25% L-arginine plus 1.5% canola.
- CLA rats (n = 6), fed 2.55% L-alanine plus 1.5% mixed isomers of CLA (triacylglycerol preparation).

•Arginine plus CLA rats (n = 6), fed 1.25 % L-arginine plus 1.5% mixed isomers of CLA.

Canola was used as a lipid control because it is food grade and its melting point is similar to the triacylglycerol preparation of CLA. Both diets had 20% protein, 70% carbohydrate, and 4% fat (Table 1). Food and water intake was measured on a daily basis and body weight was measured every 3-4 d.

Sample collection

After 5 wk of treatment and approximately 12 h after last feeding, the rats were terminated using a CO_2 gas chamber. Final body weights were taken prior to termination and empty carcass weight was taken following termination. The liver, adipose tissue, and viscera were all dissected and weighed. Samples of liver and adipose tissue were stored at -80ºC. The soleus and extensor digitorum longus muscles were also dissected, weighed, and stored at -80ºC. Fresh liver and epididymal tissues were collected at harvest to measure metabolism *in vitro*. Blood was collected by exsanguination and plasma was separated and stored at -80ºC for analysis of amino acid and fatty acid concentrations.

Lipogenesis

Lipogenesis was measured using the fresh adipose tissues and liver collected at harvest. Two-hour *in vitro* incubations were conducted in culture flasks with liver or epididymal adipose tissue samples (37,38). Samples (approximately 100 mg) were incubated in flasks containing 5 mM glucose, 0.75 mM palmitate, 10 mM Hepes buffer, 50 ng/mL insulin, and either 1 μCi [U-^{14}C]glucose or 0.5 μCi [1-^{14}C]palmitate in Krebs-

Henseleit buffer. Vials were gassed for 1 min with 95% O_2:5% CO_2 and incubated for 2 h in a shaking water bath at 37°C. At the end of the incubation period, reactions were terminated by addition of 0.5 mL of 2 N H_2SO_4, and flasks were shaken for an additional 2 h.

Neutral lipid extraction

Neutral lipids in the adipose tissues were extracted using the Folch et al. (39) procedure, evaporated to dryness, and resuspended in 10 mL of scintillation cocktail. The radioactivity in the lipid was counted with a liquid scintillation counter (Beckman Instruments, Palo Alto, CA).

Substrate oxidation

The production of CO_2 was used as an indicator of glucose and fatty acid oxidation. Liver and epididymal adipose tissues were collected at harvest and incubated as described above. Hanging center wells were suspended in 25-mL flasks containing 3 mL medium. Filter paper was placed inside the hanging center well and after 2 h incubation, the filter paper was soaked with NaOH. At the same time, 0.5 mL of 2N H_2SO_4 was injected into the medium. The flasks were shaken for an additional 2 h to allow the release of CO_2 from the medium. The NaOH trapped the CO_2 released from the medium. The filter paper was then placed in scintillation vials, 1 mL water and 10 mL scintillation fluid were added, and the samples were counted on a liquid scintillation counter.

Fatty acid analysis

Fatty acid composition in liver and plasma was determined using a fatty acid methylation procedure (FAME). Fatty acids were extracted using Folch et al (38) methods. Methylation was done as described by Morrison and Smith (39). FAME were analyzed using a Varian Gas Chromatograph (model CP-3800 fixed with a CP-8200 autosampler, Varian Inc., Walnut Creek, CA). Identification of sample fatty acids were made by comparing the relative retention times of FAME peaks from samples with those of standards (reference standard GLC-68B, Nu-Chek Prep, Elysian, MN). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m x 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands). Helium was the carrier gas (1.2 mL/min). After 32 min at 180ºC, oven temperature was increased at 20ºC/min to 225ºC and held for 13.75 min. Total run time was 48 min. Injector and detector temperatures were at 270 and 300ºC, respectively.

Amino acids

Plasma amino acids were analyzed using high performance liquid chromatography. (The HPLC apparatus and precolumn derivatization of amino acids with *o*-phthaldialdehyde were as previously described (40). Amino acids (except proline and cysteine) were separated on a Supelco 3-µm reversed-phase C18 column (4.6 x 150 mm, I.D.) guarded by a Supelco 40-µm reversed-phase C18 column (4.6 x 50 mm, I.D.). The HPLC mobile phase consisted of solvent A (0.1 mmol/L sodium acetate-0.5% tetrahydrofuran-9% methanol; pH 7.2) and solvent B (methanol), with a combined total flow rate of 1.1 mL/min. A gradient program with a total running time of 49 min

(including the time for column regeneration) was developed for satisfactory separation of amino acids (0 min, 14% B; 15 min, 14% B; 20 min, 30% B; 24 min, 35% B; 26 min, 47% B; 34 min, 50% B; 38 min, 70% B; 40 min, 100% B; 42 min, 100% B; 42.1 min, 14% B; 48.5 min, 14% B). Proline was measured by an HPLC method involving oxidation of proline to 4-amino-1-butanol and precolumn derivatization with *o*-phthaldialdehyde. For cysteine analysis, 100 µL sample was mixed with 50 µL of 50 mM iodoacetic acid (an alkylating agent) for 5 min at room temperature, to convert cysteine to S-carboxymethylcysteine. The latter then reacts with *o*-phthaldialdehyde to form a highly fluorescent derivative. For cystine analysis, 100 µL sample was mixed with 100 µL of 28 mM 2-mercaptoethanol (a reducing agent) for 5 min at room temperature, to convert cystine to cysteine, and the latter was then analyzed as described above. Amino acids were quantified on the basis of authentic standards (Sigma Chemicals, St. Louis, MO) using the Millennium^{TM} workstation (Waters Inc., Milford, MA) (40).

Adipose tissue cellularity

Approximately 25 mg of frozen adipose tissue was thinly sliced using a scalpel blade. The exact weights of the tissue samples were recorded. Samples were kept on ice at all times. The samples were transferred to scintillation vials and rinsed three times with 0.154 mol/L NaCl. In the hood, 0.6 mL of 50 mM collidine-HCl buffer was added. One milliliter of 3% osmium tetraoxide was added, and samples incubated in a water bath at 37ºC for 72 h. The osmium was removed and the samples were rinsed with 0.7%

NaCl. Triton X-100 was added and samples were swirled gently to separate the adipocytes. Adipocytes were counted with a two-chamber Neubauer hemocytometer.

Statistical analysis

Data was analyzed using the SuperNova (Abacus concepts, Berkeley, CA) statistical analysis program. Two way analysis of variance was used at a significance level of $P < 0.05$, and the main effects of fatty acid, amino acid, and their interaction were analyzed.

RESULTS

Food and water intake

Rats that were fed arginine plus CLA had a higher daily intake of food ($P = 0.02$, Table 3). Rats fed arginine plus CLA had a higher average daily gain than rats fed only arginine or CLA (Table 3), but the difference was not significant. As expected, supplemented intake of alanine was significantly higher in the alanine-fed rats, whereas arginine intake was greater in the arginine-fed rats.

Final tissue weights

Final body weight was measured just prior to termination (Table 3). Although there was no significant arginine x CLA interaction ($P = 0.83$), arginine and CLA individually increased final body weight ($P = 0.04$ and 0.009). CLA tended to reduce abdominal fat mass ($P = 0.08$), but arginine had no significant effect on abdominal fat mass ($P = 0.34$; Table 4). Ratios of abdominal fat:final body weight and abdominal fat:empty carcass weight also were calculated. For the abdominal fat:final body weight ratio, there was a significant CLA effect ($P = 0.05$; Fig.1). However, there was no significant arginine x CLA interaction ($P = 0.14$) on the abdominal fat:final body weight ratio. The ratio of abdominal fat:empty carcass weight showed a similar trend, with CLA decreasing the ratio ($P = 0.03$). Ratios of epididymal fat:final body weight and epdidymal fat:shrunken body weight showed no significant arginine x CLA interaction ($P = 0.49$ and 0.52, respectively). There also were no CLA or arginine effects on these ratios ($P = 0.52$ and 0.51; Fig. 2). There was no significant arginine x CLA effect on final soleus and EDL muscle weights ($P = 0.58$ and 0.68, respectively; Table 5). Finally,

there was no significant arginine x CLA interaction on final liver or viscera weight (P = 0.68 and 0.80, respectively). CLA tended to increase liver weight (P = 0.09; Table 5).

Liver fatty acids

CLA significantly increased saturated fatty acids such as myristic acid and palmitic acid (P = 0.03 and 0.04, respectively; Table 6). There was no significant arginine x CLA effect (P = 0.50, 0.54) on hepatic myristic and palmitic acid concentrations. CLA significantly decreased monounsaturated fatty acids such as myristoleic acid and palmitoleic acid (P = 0.009, 0.007). There was not a significant arginine x CLA effect (P = 0.56, 0.24) on myristoleic and palmitoleic acid concentrations. CLA did decrease the ratio of hepatic palmitoleic acid:palmitic acid (Fig.4). CLA did not have a significant effect on stearic acid (P = 0.59). However, CLA significantly decreased the concentration of oleic acid (P = 0.0001). CLA also significantly decreased the oleic/stearic acid ratio (P = 0.0005). As expected, CLA supplementation also significantly increased cis-9,trans-11 CLA concentrations (P = 0.0001; Table 6).

Plasma fatty acids

Although there was no arginine x CLA interaction observed for plasma fatty acids, CLA significantly changed saturated and unsaturated fatty acid concentrations (Table 7). CLA significantly increased palmitic acid and stearic acid (P = 0.03 and 0.005, respectively). CLA did not have a significant effect on plasma oleic acid concentration (P = 0.49). However, CLA did tend to decrease the plasma oleic/stearic acid ratio (P = 0.07). CLA also significantly decreased eicosapentaenoic

(EPA) acid (P = .01). As predicted, CLA significantly increased cis-9,trans-11 CLA concentration (P = .04; Table 7).

Lipid synthesis in liver and epididymal adipose tissue

Lipid synthesis was measured using glucose and palmitate as substrates. Glucose can be used to synthesize the glycerol backbone of triacylglycerols and phospholipids or for fatty acid synthesis. Palmitate can be used in the synthesis of neutral lipids or phospholipids. In the liver, neither arginine nor CLA supplementation significantly affected conversion of glucose to fatty acids (P = 0.44). There also were no significant arginine or CLA effects on the conversion of palmitate to lipids (P = 0.13).

In epididymal adipose tissue, arginine plus CLA supplementation decreased the rate of lipid synthesis from glucose (P = 0.20). In addition, arginine tended to decrease the rate of lipid synthesis from palmitate (P = 0.09). In this study, epididymal adipose tissue was the major site of lipid synthesis from glucose and palmitate (Table 8).

Adipose tissue cellularity

Cellularity was measured using the osmium fixation method as an indicator of the effects of arginine and/or CLA supplementation on adipocyte size and number. A larger number of cells/area correlates to a decrease in cell size. There were no significant arginine or CLA effects on adipocytes/100 mg adipose tissue (P = 0.63; Table 9).

CO_2 production

The production of CO_2 was used as an indicator of glucose and fatty acid oxidation (Table 9). In liver tissue with glucose as the substrate, there was no significant arginine x CLA interaction ($P = 0.48$). Arginine supplementation tended to increase hepatic CO_2 production from glucose ($P = 0.06$). With palmitate as the substrate, there was also no significant arginine x CLA interaction ($P = 0.33$). For epididymal adipose tissue CO_2 production, there was no significant arginine x CLA interaction with either glucose or palmitate ($P = 0.99$ and 0.92, respectively). When rates were expressed as nmol $CO_2/10^6$ cells, there also was no significant arginine x CLA interaction with either glucose or palmitate as substrate ($P = 0.56$ and 0.78, respectively; Table 9).

Amino acids

A significant arginine x CLA interaction was seen for reducing arginine, alanine, and ornithine levels ($P = 0.05$, 0.002, and 0.04 respectively). Interestingly, these amino acids behaved in the same manner across treatment groups. The decreased plasma levels of these amino acids suggest that arginine plus CLA strongly stimulated protein synthesis. The levels of branched chain amino acids valine, isoleucine, and leucine were all depressed in the treatment groups that included CLA. This suggests that protein turnover in the skeletal muscle was reduced, leading to an increase in lean muscle mass. CLA also reduced plasma levels of glutamine, citrulline, tyrosine, and phenylalanine.

DISCUSSION

Researchers have been trying to find ways to effectively treat obesity and prevent diseases associated with obesity such as type II diabetes and cardiovascular disease. This study examined the effects of arginine and CLA supplementation on lipid metabolism in rats. The results from this study indicated that arginine plus CLA supplementation increased lean muscle mass, decreased abdominal fat mass, and increases glucose oxidation in the liver. Arginine also doubled glucose and palmitate oxidation in epididymal adipose tissue, but this effect was not statistically significant. These findings suggest that arginine plus CLA supplementation may be an effective method for treating obesity while concomitantly increasing lean muscle mass.

The findings from this study with growing rats showed that arginine supplementation did not affect EDL or soleus muscle mass. There are differences in muscle fiber type in these two muscles. Bain et al. (41) reported that the rat soleus muscle has more oxidative fibers than the EDL muscle. It also demonstrated a higher catalase activity in the soleus muscle of rats (41). Arginine may have different effects on different muscles, depending on the muscle fiber type. In myocytes, the expression of NOS is regulated by factors such as fiber type, contraction, developmental stage, and the pathological state (6,7,8). The increase in lean muscle mass with arginine plus CLA supplementation also may be, in part, due to the activation of the mammalian target of rapamycin (mTOR) pathway. Chung et al. (42) found that supplementation of CLA altered human adipocyte morphology through the activation of the mTOR pathway, p70 S6 ribosomal protein kinase, and S6 ribosomal protein. Through the activation the

mTOR pathway and these ribosomal proteins, CLA supplementation may provide translational control of the expression of adipose differentiation-related protein (ADRP) and the transcriptional control of perilipin A. Perilipin A is found on the outer surface of lipids and functions as a protective protein coat, and in order for lipolysis to occur, this protein must be removed. With CLA supplementation, there was an increase in the removal of perilipin A to the cytosol, allowing the lipid droplet to undergo lipolysis (42). In addition, CLA also activates the mitogen-activated protein kinase kinase/extracellular signal-related kinase (MEK/ERK) pathway. The activation of this pathway led to a decrease in mRNA levels of PPARγ, perilipin, glucose transporter 4, ADRP, lipoprotein lipase, and adiponectin (42). These effects led to a decrease in the uptake of fatty acids and glucose, resulting in a decrease in cellular triglycerides (42). Arginine has also been shown to activate the mTOR pathway (43). These finding suggest that arginine and CLA may decrease fat mass and increase lean mass through activation of the mTOR pathway.

Previous studies have shown that dietary supplementation of arginine increases brown fat mass (1). Nitric oxide is an important regulator of metabolism. Because NO is a small, hydrophilic gas molecule, it is able to diffuse into cells and act on its target molecules via cGMP-dependent pathways. NO regulates vascular tone, neurotransmission, immunity, and overall homeostasis. L-Arginine is the precursor for NO and is converted to NO by nitric oxide synthase (NOS). NOS has three isoforms: nNOS, iNOS, and eNOS. nNOS was first identified in neuronal tissue (3,4,5). iNOS has been shown to be induced by both cytokines and lipopolysaccharides (4,5), and

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Several studies have suggested that insulin-sensitive tissues are key sites for NO synthesis. These include skeletal muscle, heart, kidneys, liver, and the brain. The NOS isoforms are mainly found in the sarcolemma, sarcoplasm, sarcoplasmic reticulum, and mitochondria of muscle fibers (7). Two of the isoforms, eNOS and iNOS, are expressed in brown adipose tissue. However, these two isoforms appear to be absent in white adipose tissue (11,12). In the liver, eNOS is distributed uniformly. On the other hand, iNOS is primarily found in the cytoplasm of periportal hepatocytes (13). The compartmentalization of these NOS isoforms may be one way in which NO synthesis is regulated.

Fu et al. (16) demonstrated that arginine supplementation significantly reduced epididymal and abdominal adipose tissue mass in ZDF rats. Although there was a reduction in abdominal adipose tissue weight with arginine supplementation in our study, arginine did not have a significant effect on reducing epididymal adipose tissue. These different results could be due to the differences in lean vs. obese rats. Fu et al (16) used Zucker diabetic rats, which genetically resemble type II diabetes. Sprague Dawley rats, which are not genetically obese, were used in this study. The rats used by Fu et al. (16) were fed for a longer period of time (10 wk), while the rats in this study were fed for 5 wk. A longer feeding period may have resulted in more significant

arginine effects, especially in epididymal adipose tissue. Finally, the Zucker diabetic rats were fed a higher fat diet (6%), while the rats in this study were fed a diet that was 4% fat. The differences in diet also may have contributed to the different results seen in these two studies.

With arginine plus CLA supplementation, plasma arginine concentrations decreased. In addition, the plasma concentrations of branched chain amino acids also were decreased by the supplementation of arginine and/or CLA. Because branched chain amino acids are deaminated in skeletal muscle (44), these findings suggest that arginine and CLA increase protein synthesis and/or decrease protein turnover in skeletal muscle, which would explain the increase in lean mass that was observed. Gluatamine and alanine, which is produced by the skeletal muscle, decreased with arginine and CLA supplementation. This indicates less nitrogen flux from muscle and/or the intestine.

Ntambi et al. (45) reported that supplementation of CLA reduced hepatic SCD1 gene expression in mice. With a decrease in hepatic SCD1 activity, liver and plasma monounsaturated fatty acid concentrations are reduced. This is consistent with our findings, as rats that were fed CLA had decreased liver monounsaturated fatty acid:saturated fatty acid ratios (Fig. 3,4) and plasma oleic:stearic acid ratios. This ratio is used as an index of hepatic SCD1 activity (22,36). With a decrease in hepatic SCD1 activity, there also is a decrease in triglyceride levels, a decrease in VLDL output from the liver, and a decrease in abdominal fat mass. Arginine also has been shown to decrease hepatic SCD1 activity (16). Our findings are consistent with these studies, because rats that were fed arginine plus CLA had the largest decrease in

monounsaturated/saturated fatty acid ratio and the largest decrease in abdominal fat mass.

Glucose oxidation was increased in the liver with arginine supplementation. The production of CO_2 from glucose is a measure of mitochondrial activity, specifically pentose cycle and TCA cycle activity. There was not, however, a significant effect of arginine or CLA supplementation on palmitate oxidation. The measurement of CO_2 production from palmitate is an indicator of TCA cycle activity. With palmitate, there was no apparent increase in mitochondrial activity. In epididymal adipose tissue, there was no significant effect of arginine or CLA on oxidation of glucose or palmitate, even though rates of CO_2 production were twice as high in arginine rats. We cannot rule out the possibility that mitochondrial activity in epididymal adipose tissue is elevated by arginine supplementation. Clearly, CLA had no effect on glucose or palmitate oxidation.

Further studies on arginine plus CLA supplementation are needed in order to better understand the effects of arginine and CLA effects on lipid metabolism across different species. This study did provide exciting evidence that arginine plus CLA supplementation has an effect on hepatic and adipose tissue lipid metabolism and may prove beneficial in future studies.

CONCLUSION

In conclusion, the results from this study show that both arginine and CLA have significant effects on lipid metabolism in both liver and adipose tissues. Surprisingly, the combination of arginine plus CLA also promoted lean muscle mass. The results from this study may be beneficial in developing a treatment for obesity in both humans and animals. This could help improve overall human health and increase productivity in livestock production. Further studies should be done in order to better understand effects of arginine and CLA on adiposity in livestock species and humans.

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APPENDIX

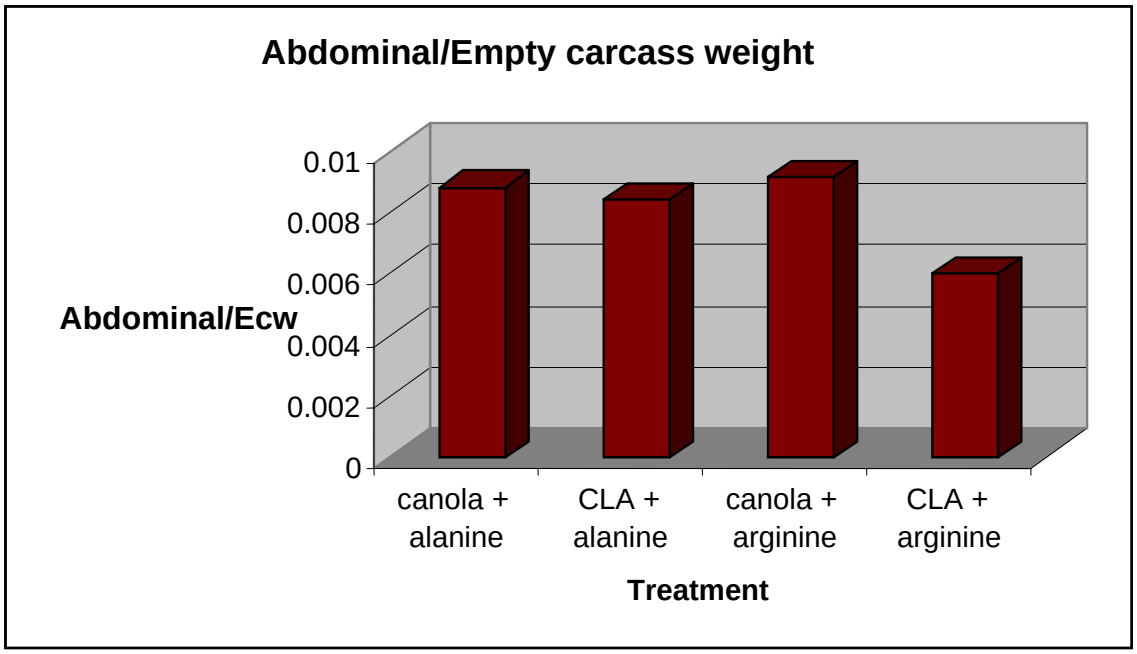

Figure 1. Abdominal adipose tissue:empty carcass weight ratios of rats fed arginine CLA, or a combination of arginine and CLA over a 5 wk period.

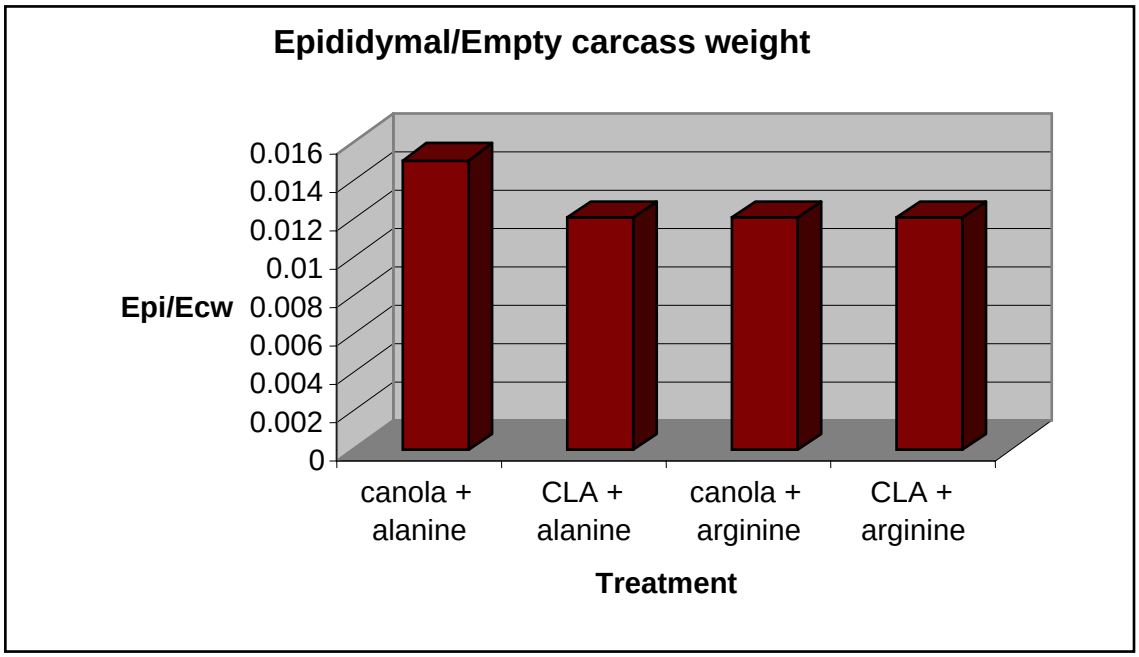

Figure 2. Epididymal adipose tissue:empty carcass weight ratios of rats fed arginine, CLA, or a combination of arginine and CLA over a 5 wk period.

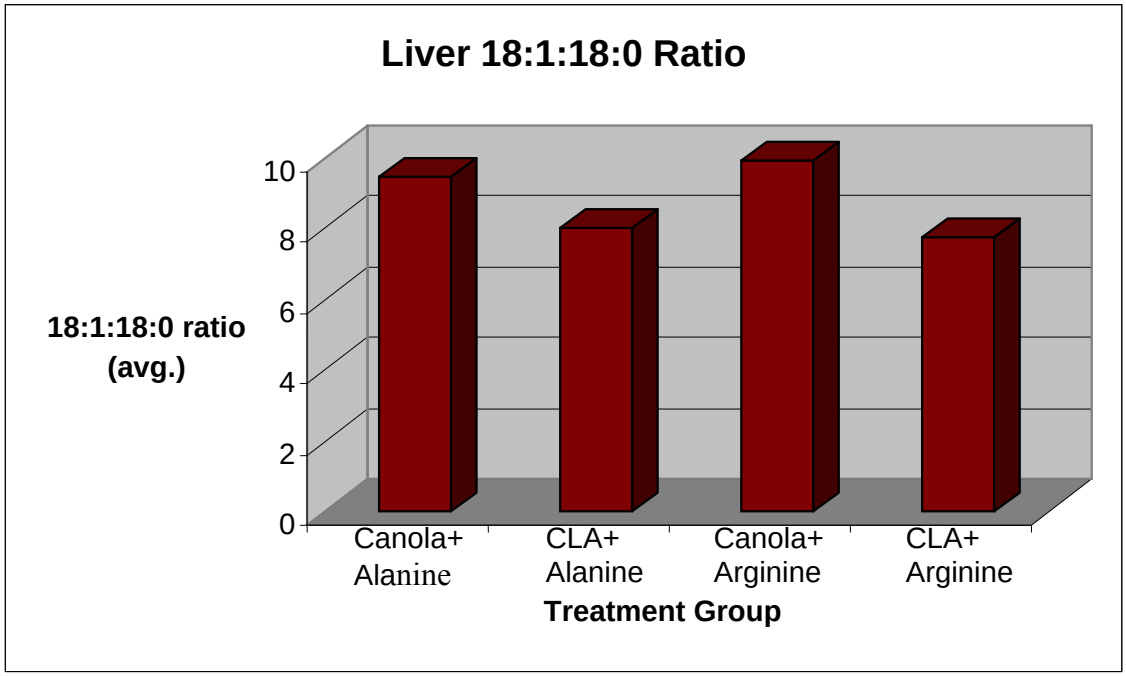

Figure 3. Ratio of hepatic oleic acid:stearic acid of rats fed arginine, CLA, or a combination of arginine and CLA over a 5 wk period.

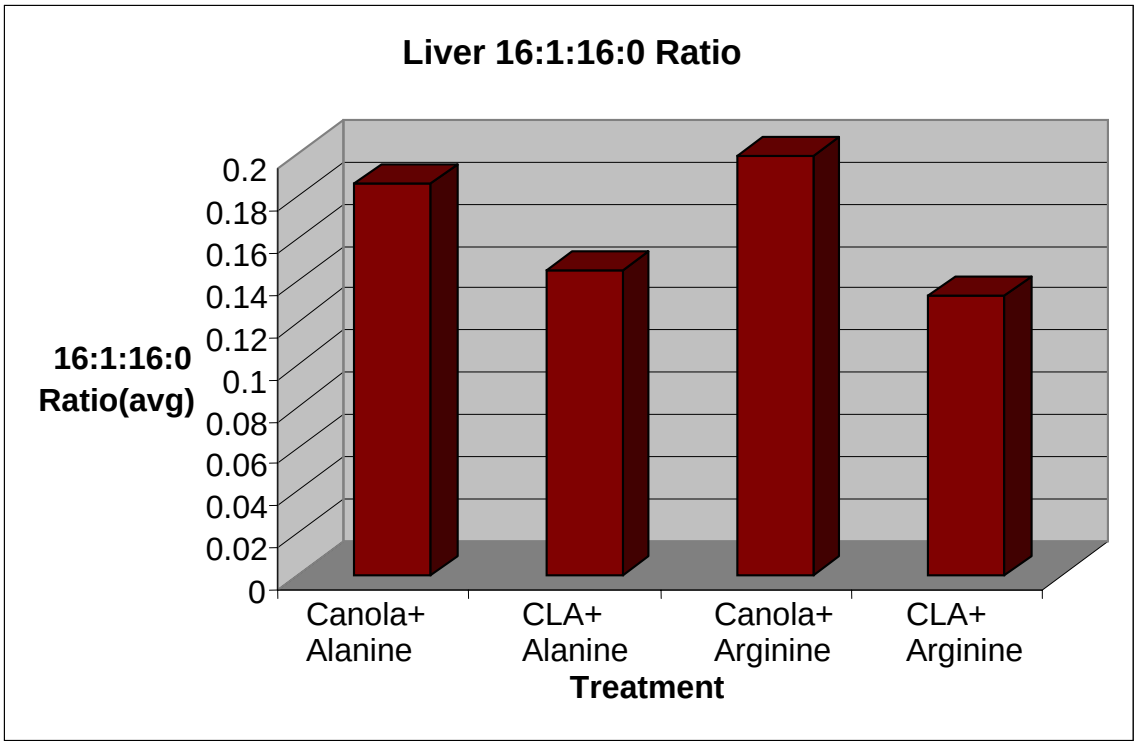

Figure 4. Ratio of hepatic palmitoleic acid:palmitic acid in rats fed arginine, CLA, or a combination of arginine and CLA over 5 wk period.

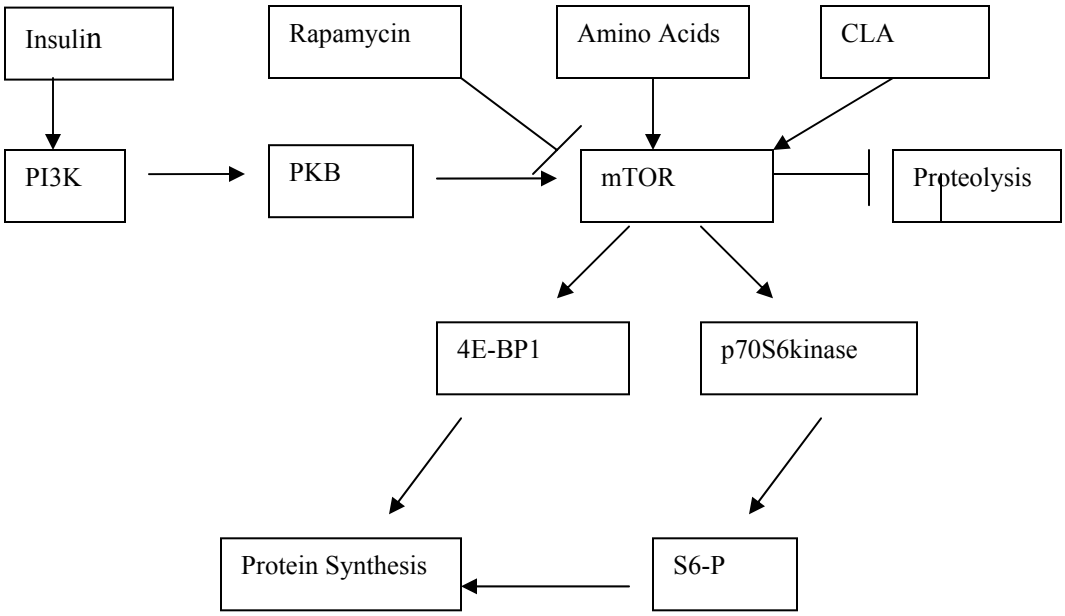

Figure 5. mTOR pathway.

Table 1. Composition of diets fed to rats over a 5 wk period.

					D12450B and D06101401-02	
	D12450B		D06101401	(CLA diet)	DO6101402 (Canola diet)	
%	gm	kcal	gm	kcal	gm	kcal
Protein	19.2	20	19	20	19	20
Carbohydrate	67.3	70	66.3	70	66.3	70
Fat	4.3	10	4.2	10	4.2	10
Total	90.8	100	89.4	100	89.4	100
kcal/gm	3.85		3.79		3.79	
Ingredient	gm	kcal	gm	kcal	gm	kcal
Casein, 80 Mesh	200	800	200	800	200	800
L-Cystine	3	12	3	12	3	12
Corn Starch	315	1260	315	1260	315	1260
Maltodextrin 10	35	140	35	140	35	140
Sucrose	350	1400	350	1400	350	1400
Cellulose, BW200	50	0	50	0	50	0
Soybean Oil	25	225	25	225	25	225
Lard	20	180	20	180	20	180
Mineral Mix S10026	10	0	10	0	10	0
DiCalcium Phosphate	13	0	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0	5.5	0
Potassium Citrate, 1H20	16.5	0	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0
CLA, Calrinol G-80	0	0	16	0	0	0
Canola Oil	0	0	0	0	16	0
Total	1055.05	4057	1071.05	4057	1071.05	4057

Table 2. Fatty acid composition of diets fed to rats over 5 wk period.

Diet	14 0	16 0	16 1	18 0	18 1	18 1 c11	18 2	18 3	c9t11	t10c12	20 1	20.4
Canola	0.6516	14.5997	0.7172	5.635	37.7938	2.2418	33.6919	4.6505				
CLA	0.4367	13.141	0.6801	6.4218	26.445	1.6133	30.9271	2.7718	5.5246	5.5279	6.1021	.4091

Table 3. Weekly body weights, food intake, and alanine/arginine intake of rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Item	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA xAA
Final body weight, g	424.7	442.1	437.3	457.5	3.79	0.009	0.04	0.83
Shrunken body weight, g	365.9	372.8	371.6	387.9	3.19	0.06	0.09	0.42
Body weight gain, g/d	1.74	1.40	1.17	1.73	0.17	0.74	0.72	0.20
Food intake, g/d	34.0	35.4	35.8	37.9	0.44			0.02
Supplemented alanine intake, g/d	0.87	0.90	0	0				
Supplemented arginine intake, g/d	0	0	0.54	0.58				

[1]Data are means for 3 cages per treatment group, 2 rats per cage. Alanine and arginine intakes were based on calculated water intake.

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

Table 4. Tissue weights and weight ratios from rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Tissue	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA xAA
Abdominal adipose tissue, g	3.22	3.13	3.38	2.40	0.16	0.08	0.34	0.14
Abdominal/final body weight	0.007	0.007	0.008	0.005	0.0004	0.05	0.26	0.14
Abdominal/shrunken body weight	0.009	0.008	0.009	0.006	0.0004	0.03	0.19	0.12
Epididymal adipose tissue, g	5.35	4.53	4.53	4.73	0.32	0.66	0.66	0.46
Epididymal/final body weight	0.009	0.010	0.010	0.010	0.36	0.52	0.42	0.49
Epididymal/shrunken body weight	0.014	0.012	0.012	0.012	0.004	0.51	0.53	0.52

[1]Data are means for 6 animals per treatment group.

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

Table 5. Final tissue weights of rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Tissue/	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA x AA
Liver, g	15.2	15.9	14.7	15.8	0.251	0.09	0.56	0.68
Viscera, g	28.0	29.5	28.4	29.3	0.566	0.32	0.94	0.80
Soleus[3], g	0.343	0.327	0.306	0.307	0.008	0.64	0.89	0.58
EDL[3], g	0.329	0.314	0.340	0.335	0.006	0.42	0.19	0.68

[1]Data are means for 6 animals per treatment group units are in grams.

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

[3]Weights are two muscles

Table 6. Liver fatty acids (g/100 g total fatty acids) of rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Fatty Acid	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA x AA
Myristic acid	0.89	1.05	0.98	1.06	0.028	0.03	0.36	0.50
Myristoleic acid	0.073	0.056	0.073	0.05	0.004	0.009	0.60	0.56
Palmitic acid	18.36	20.08	19.17	20.12	0.329	0.04	0.50	0.54
Palmitoleic acid	3.41	2.91	3.82	2.66	0.158	0.007	0.77	0.24
Margaric acid	0.229	0.227	0.208	0.227	0.006	0.48	0.37	0.37
trans-10 Heptadecenioic acid	0.159	0.152	0.152	0.153	0.005	0.78	0.82	0.75
Stearic acid	3.08	3.19	3.03	3.42	0.079	0.12	0.59	0.40
trans Vaccenic acid	0.14	0.40	0.13	0.42	0.033	0 .0001	0.90	0.75
Oleic acid	28.99	25.06	29.94	25.84	0.480	0.0001	0.08	0.85
Cis-11 Oleic acid	3.74	3.27	3.95	3.48	0.069	0.0001	0.04	0.98
Linoleic acid	34.70	33.11	32.63	31.79	0.536	0.26	0.12	0.72
Cis-9, trans11 CLA	0.07	2.46	0.11	2.98	0.293	0.0001	0.19	0.26
trans-10,cis12 CLA	0.008	1.56	0.02	1.93	0.199	0.0001	0.27	0.29
Linolenic acid	2.41	2.06	2.39	1.86	0.062	0.0001	0.20	0.29
Arachidonic acid	0.615	0.535	0.438	0.407	0.049	0.58	0.14	0.80
Eicosapentaenoic acid	0.039	0.020	0.025	0.02	0.004	0.11	0.32	0.46
Docosahexanoic acid	0.15	0.103	0.083	0.057	0.015	0.20	0.05	0.71
Oleic/stearic acid	9.46	8.01	9.93	7.73	0.281	0.0005	0.84	0.40

[1]Data are means for 6 animals per treatment group.

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

Table 7. Plasma fatty acids (g/100 g total fatty acids) from rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Fatty Acid	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA x AA
Myristic	0.938	1.04	0.805	0.653	0.13	0.94	0.37	0.65
Myristoleic	0.169	0.164	0.145	0.068	0.05	0.69	0.57	0.73
Palmitic acid	21.3	23.2	20.8	21.9	0.35	0.03	0.14	0.52
Palmitoleic acid	2.79	2.10	2.57	2.34	0.21	0.33	0.98	0.61
Stearic acid	11.5	13.2	10.9	12.2	0.30	0.005	0.12	0.71
trans-Vaccenic acid	0.27	0.922	0.153	0.232	0.182	0.34	0.29	0.45
cis-9 Oleic acid	19.3	18.9	18.7	16.9	0.77	0.54	0.48	0.69
cis-Vaccenic acid	3.16	2.33	3.18	2.96	0.14	0.62	0.24	0.26
Oleic acid	19.88	17.51	19.40	17.51	0.77	0.49	0.49	0.67
Linoleic acid	20.1	18.4	20.9	21.2	0.72	0.66	0.25	0.54
cis-9,trans-11 CLA	0.0	0.686	0.326	0.876	0.14	0.04	0.36	0.81
Linolenic acid	0.0	1.38	0.345	0.954	0.21	0.02	0.92	0.33
Arachidonic acid	16.8	15.2	16.9	17.2	0.78	0.71	0.54	0.60
Eicosapentaenoic acid	0.382	0.0	0.352	0.104	0.06	0.01	0.75	0.57
Docasahexaenoic	1.59	1.30	1.71	1.52	0.09	0.19	0.35	0.78
Oleic/stearic acid	1.69	1.44	1.72	1.40	0.07	0.07	0.99	0.80

[1]Data are means for 6 animals per treatment group.

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

Table 8. Lipogenesis in epididymal adipose tissue and liver from rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Tissue/substrate	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA x AA
Liver								
Glucose/100 mg	1.34	1.34	1.26	1.12	0.19	0.87	0.71	0.87
Palmitate/100 mg	87.2	63.1	48.3	21.5	9.2	0.39	0.10	0.65
Epididymal adipose tissue								
Cells/100 mg	565,922	550,427	438,893	499,786	38,189	0.78	0.27	0.63
Glucose/100 mg	17.6	18.3	14.9	14.2	1.41	0.99	0.25	0.81
Glucose/10^6 cells	35.4	34.3	63.1	28.7	6.1	0.14	0.35	0.16
Palmitate/100 mg	230.6	171.3	97.6	106.7	16.8	0.34	0.001	0.20
Palmitate/10^6 cells	416.9	279.2	279.5	236.7	31.4	0.22	0.09	0.58

[1]Data are means for 6 animals per treatment group. Rates are nmol substrate converted to lipid per 100 mg tissue or per 10^6 cells (epididymal adipose tissue only).

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

Table 9. CO_2 in epididymal adipose tissue and liver from rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Tissue/substrate	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA x AA
Liver								
Glucose/100 mg	3.15	2.74	7.50	4.75	0.87	0.34	0.06	0.48
Palmitate/100 mg	7.33	17.11	6.61	7.93	1.20	0.21	0.26	0.33
Epididymal								
Glucose/100 mg	7.34	10.05	8.75	11.48	1.38	0.38	0.65	0.99
Glucose/10^6 cells	13.09	17.70	26.95	22.70	3.53	0.98	0.23	0.56
Palmitate/100 mg	4.55	8.26	7.88	12.19	1.81	0.21	0.25	0.92
Palmitate/10^6 cells	8.68	16.22	22.34	25.71	2.56	0.46	0.13	0.78

[1]Data are means for 6 animals per treatment group. Rates are nmol substrate converted to CO_2 per 100 mg tissue or per 10^6 cells (epididymal adipose tissue only).

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

Table 10. Cells/epididymal adipose tissue depot in rats fed arginine, CLA, or a combination of arginine and CLA over a 5 wk period.

Treatment Group	Cells/Epididymal adipose tissue
Canola + alanine	30,300,000
CLA + alanine	24,900,000
Canola + arginine	19,900,000
CLA + arginine	23,600,000

Table 11. Plasma amino acids of rats fed arginine, conjugated linoleic acid (CLA) or a combination of arginine plus CLA for 5 wk.[1]

	Alanine		Arginine		Pooled	*P*-values		
Amino Acid	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA x AA
ASN	69.8	54.5	61.2	41.9	3.93	0.03	0.18	0.80
SER	210.4	161.3	211.9	143.2	11.44	0.01	0.72	0.67
GLN	787.9	535.7	517.5	369.4	48.10	0.03	0.02	0.57
GLY	192.3	145.3	171.9	128.3	8.84	0.01	0.28	0.92
THR	243.4	176.7	238.7	166.0	12.52	0.007	0.75	0.90
ALA	680.0	422.1	424.8	288.6	35.45	0.002	0.002	0.31
TYR	92.7	59.9	72.3	48.9	4.96	0.004	0.09	0.61
TRP	103.1	75.9	69.4	51.4	5.82	0.04	0.01	0.67
MET	65.2	46.4	45.8	36.9	3.36	0.03	0.03	0.44
VAL	226.3	148.7	136.7	107.3	11.29	0.008	0.001	0.21
PHE	68.4	49.0	51.5	42.8	3.39	0.04	0.08	0.42
ILE	111.3	71.6	71.4	50.6	5.84	0.004	0.004	0.36
LEU	192.6	122.6	121.7	92.4	10.5	0.01	0.01	0.28
CYS	188.5	191.0	169.2	182.0	6.74	0.59	0.32	0.72

Table 11 continued

	Alanine		Arginine		Pooled	*P*-values		
Amino Acid	Canola	CLA	Canola	CLA	SEM	FA[2]	AA	FA x AA
GLU	212.2	129.5	135.9	112.2	11.64	0.02	0.03	0.17
HIS	109.7	77.6	93.0	72.5	3.97	0.0005	0.13	0.41
CIT	74.4	58.8	73.3	52.8	3.83	0.02	0.64	0.75
ARG	87.5	100.6	251.7	172.6	14.38	0.15	0.0001	0.05
Beta-AlA	8.5	5.1	5.6	4.2	0.49	0.01	0.04	0.25
TAU	400.4	262.8	263.1	268.3	20.70	0.10	0.10	0.08
ORN	136.3	53.0	126.5	120.4	9.77	0.01	0.11	0.04
ASP	21.6	14.04	19.9	17.7	1.62	0.15	0.76	0.42
LYS	424.3	313.9	315.4	254.7	22.7	0.06	0.06	0.57

[1]Data are means for 6 animals per treatment group. Units are nmol/mL

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

VITA

Jennifer Lynn Nall was born in Orange County, California. Jennifer is the daughter of Marie Stiles and John Nall, Jr. Jennifer has one sister, Lisa, two stepsisters, Nicole and Jenna, and two stepbrothers, Sean and Scott. Jennifer received her bachelors' degree in biomedical science from Texas A&M University in May 2006. Jennifers' future academic goals include attending veterinary school beginning in fall 2008.

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