Dietary Supplementation of L-Arginine and Conjugated Linoleic Acid Reduces Retroperitoneal Fat Mass and Increases Lean Body Mass in Rats \(^1,2\)

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**Abstract**

We hypothesized that L-arginine and conjugated linoleic acid (CLA) would have additive effects in decreasing adiposity. Sprague Dawley rats were assigned to the following dietary groups (\(n = 6/\text{group; 5 wk total})

1) control (2.55% L-alanine plus 1.5% canola oil); 2) arginine (1.25% L-arginine plus 1.5% canola oil); 3) CLA (2.55% L-alanine plus 1.5% CLA); and 4) arginine plus CLA (1.25% L-arginine plus 1.5% CLA). Supplemental amino acids were provided in drinking water and CLA was incorporated into the food pellets. Daily weight gain, food intake, arginine intake, and final body and eviscerated body weights were greater in rats fed supplemental CLA than in rats fed canola oil. The retroperitoneal adipose tissue:body weight ratio was less in rats fed supplemental CLA than in rats fed canola oil, but epididymal adipose tissue, liver, and soleus and extensor digitorum longus muscle weights were unaffected by arginine or CLA. CLA decreased epididymal adipose tissue concentrations of palmitoleic, oleic, and cis-vaccenic acid. CLA and arginine increased palmitate oxidation to CO\(_2\) in epididymal adipose tissue in vitro relative to control rats. Glucose and palmitate incorporation into total lipids in epididymal adipose tissue was lower in rats fed supplemental arginine than in alanine-fed rats. Arginine increased plasma glycerol relative to alanine-fed rats and CLA and arginine independently decreased most serum essential amino acids and alanine, glutamate, glutamine, and ornithine. We conclude that CLA and arginine modulated adipose tissue metabolism by separate, but not additive, effects. Also, CLA and arginine may have depressed muscle protein turnover. J. Nutr. 139: 1279–1285, 2009.

**Introduction**

Previous studies have demonstrated that arginine and conjugated linoleic acid (CLA) \(^5\) independently reduce fat mass in animal species \(^1–3\). We demonstrated previously that dietary arginine reduced adiposity in Zucker diabetic fatty (ZDF) rats \(^1\) and in diet-induced obese rats \(^3\). After a 10-wk treatment period, ZDF rats receiving 1.25% supplemental arginine had 25% less epididymal adipose tissue mass and 45% less retroperitoneal (RP) adipose tissue mass than those rats supplemented with alanine (isonitrogenous control). Arginine stimulated glucose and fatty acid oxidation and glycerol release in both RP and epididymal adipose tissues. Similarly, after 12 wk of supplemental arginine, RP adipose tissue mass in rats fed low-fat or high-fat diets was reduced 30–40% and epididymal adipose tissue mass was reduced by ~20% relative to rats receiving supplemental alanine \(^3\).

Adipose tissue mass also is depressed by supplemental CLA in mice \(^4,5\), hamsters \(^6\), rabbits \(^7\), and ZDF and Sprague Dawley rats [reviewed in \(^8\)]. Treatment of 3T3-L1 preadipocytes with mixed isomers of CLA depressed 3T3-L1 preadipocyte proliferation but increased glucose incorporation into total lipids \(^9\). Pigs fed CLA had a lower adipocyte area (i.e. smaller adipocytes) than control pigs \(^10\). Although CLA caused only small reductions in adipocyte volume \(^10,11\), it strongly depressed stearoyl-CoA desaturase (SCD) gene expression and catalytic activity in porcine adipose tissue \(^12\). The \(\text{trans}-10, \text{cis}-12\) CLA isomer apparently prevents lipid filling of adipocytes by depressing PPAR\(\gamma\) gene expression in human preadipocytes \(^13\), 3T3-L1 preadipocytes \(^14\), and mice \(^15\), whereas arginine may reduce adiposity by stimulating fatty acid oxidation \(^1,3\). Therefore, we hypothesized L-arginine plus CLA
Sigma-Aldrich or Fischer Scientific.

and, unless otherwise indicated, all other chemicals were obtained from

Materials and Methods

would have independent and additive effects in decreasing adiposity by modulating adipose tissue and liver metabolism.

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Radioisotopes were obtained from American Radiolabeled Chemicals and, unless otherwise indicated, all other chemicals were obtained from Sigma-Aldrich or Fischer Scientific.

TABLE 1 Composition of the canola oil and CLA pelleted diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Canola oil</th>
<th>CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>18.7</td>
<td>18.7</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Corn starch</td>
<td>29.4</td>
<td>29.4</td>
</tr>
<tr>
<td>Maltodextrin-10</td>
<td>3.27</td>
<td>3.27</td>
</tr>
<tr>
<td>Sucrose</td>
<td>32.7</td>
<td>32.7</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4.67</td>
<td>4.67</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>2.33</td>
<td>2.33</td>
</tr>
<tr>
<td>Lard</td>
<td>1.87</td>
<td>1.87</td>
</tr>
<tr>
<td>Canola oil</td>
<td>1.50</td>
<td>—</td>
</tr>
<tr>
<td>Canola oil CLA</td>
<td>—</td>
<td>1.50</td>
</tr>
<tr>
<td>Other</td>
<td>5.61</td>
<td>5.61</td>
</tr>
<tr>
<td>Energy content, kJ/100 g pellets, as fed</td>
<td>90.6</td>
<td>90.6</td>
</tr>
</tbody>
</table>

Fatty acids, g/100 g total pellet fatty acids

Myristic, 14:0        0.65  0.44
Palmitic, 16:0        14.6  13.1
Palmitoleic, 16:1(n-7)| 0.72  0.68
Stearic, 18:0         5.64  6.42
Oleic, 18:1(n-9)      | 37.8  26.4
cis-Vaccenic, 18:1(n-7)| 2.24  1.61
Linoleic, 18:2(n-6)   | 33.7  30.9
α-Linolenic, 18:3(n-3)| 4.65  2.77
CLA, 18:2(cis-9, trans-11)| ND  5.52
CLA, 18:2(trans-10, cis-12)| ND  5.53
Arachidonic, 20:4(n-6)| ND  0.41

1 Research Diets.
2 Lipid Nutrition.
3 Other: dicalcium phosphate, 1.21 g/100 g; calcium carbonate, 0.51 g/100 g; potassium citrate, 1.54 g/100 g; choline bitartrate, 0.19 g/100 g; mineral mix, 0.93 g/100 g [composition in g/kg mineral mix: magnesium oxide, 41.9; magnesium sulfate·7H2O, 257.6; sodium chloride, 269; chromium KSO4·12H2O, 1.925; cupric carbonate, 1.05; potassium iodate, 0.035; ferric citrate, 21; manganese carbonate, 12.25; sodium selenite, 0.035; zinc carbonate, 5.6; sodium fluoride, 0.20; ammonium molybdate·4H2O, 0.30; sucrose, 399.1]; vitamin mix, 0.93 g/100 g [composition in g/kg vitamin mix: retinyl palmitate, 0.80; cholecalciferol, 1.0; all-rac-a-tocopherol acetate, 10; menadione sodium bisulfite, 0.08; biotin (1.0%), 2.0; cyanocobalamin (0.1%), 1.0; folic acid, 0.20; nicotinic acid, 3.0; calcium pantothenate, 1.6; pyridoxine-HCl, 0.70; riboflavin, 0.60; thiamin-HCl, 0.60; and sucrose, 978.4].
4 ND, not detectable.

Rats and diets. This experiment was approved by the Texas A&M University laboratory animal use committee (Animal Use Protocol no. 2004–75) for the ethical treatment of the rats. Twenty-four male Sprague Dawley rats (8 wk old) were purchased from Harlan Laboratories and acclimated to their cages (2 rats per cage). At 9 wk of age, the rats received drinking water containing L-alanine or L-arginine and were fed a casein-based semipurified diet containing canola oil or mixed isomers of CLA (Table 1). The dietary treatment lasted for 5 wk.

The treatment groups (n = 6) were as follows: 1) control [2.55% L-alanine (isonitrogenous control) plus 1.5% canola oil (lipid control)]; 2) arginine (1.23% L-arginine plus 1.5% canola oil); 3) CLA [2.55% L-alanine plus 1.5% mixed isomers of CLA (Lipid Nutrition G-80; triacylglycerol preparation)]; and 4) arginine plus CLA (1.25% L-arginine plus 1.5% CLA). Canola oil was used as a lipid control, because it is food grade and its melting point is similar to the triacylglycerol preparation of CLA. The lipids in the diets (Research Diets) varied primarily in the concentrations of oleic acid, o-linolenic acid, and the concentrations of the CLA isomers (Table 1). Food and water intakes were measured on a daily basis and body weight was measured every 3–4 d.

Sample collection. Approximately 12 h after last feeding, rats were killed using a CO2 gas chamber. Final body weights were measured prior to killing and empty carcass weights (i.e., completely eviscerated carcass weights) were measured immediately following termination. The liver, epididymal, and RP adipose tissues, viscera (containing associated adipose tissue), and soleus and extensor digitorum longus (EDL) muscles were dissected and weighed. Fresh liver and epididymal adipose tissue were collected at harvest to measure metabolism in vitro. Blood was collected by exsanguination and plasma and serum were separated and stored at −80°C for analysis of amino acids, fatty acids, glycerol, and triglycerides.

Glucose and palmitate carbon incorporation into CO2 and total lipids. Two-hour in vitro incubations were conducted with fresh liver and epididymal adipose tissue as described (16). Samples (~100 mg) were incubated for 2 h in flasks containing 5 mM/L glucose, 0.75 mM/L palmitate, 300 mg/mL bovine serum albumin, 10 mM/L HEPES buffer, 8.6 mM/L insulin, and either 6.12 GBq/L [U-14C]glucose or 3.06 GBq/L [1-14C]palmitate in Krebs-Henseleit buffer (pH 7.35–7.40). After the 2-h incubation, CO2 was collected as described (17). Total lipids in the adipose tissues were extracted using the Folch et al. (18) procedure, completely evaporated, and reconstituted in 10 mL of scintillation cocktail (Budget-Solve, Research Products International). The radioactivity in the lipid was counted with a liquid scintillation counter (Beckman Instruments).

Fatty acids. Diet, plasma, and epididymal adipose tissue lipids were extracted (18) and saponification of lipids and methylation of fatty acids was performed (19), modified as described previously (20). FAME were analyzed using a Varian gas chromatograph (model CP-3800) equipped with a fused silica capillary column CP-Sil 88 (100 m × 0.25 mm i.d.) (Chrompack) (20). Identification of fatty acids was accomplished by comparison to authentic standards (Nu-Chek Prep).

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TABLE 2 Daily gain and daily intake of amino acids and arginine of rats fed alanine, arginine, alanine plus CLA, or arginine plus CLA for 5 wk

<table>
<thead>
<tr>
<th>Item</th>
<th>Alanine</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Canola</td>
<td>CLA</td>
</tr>
<tr>
<td>Daily weight gain, g/d</td>
<td>1.05</td>
<td>1.35</td>
</tr>
<tr>
<td>Daily food intake, g/d</td>
<td>17.0</td>
<td>17.7</td>
</tr>
<tr>
<td>Gain:food intake</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Alane from water, g/d</td>
<td>1.01</td>
<td>1.03</td>
</tr>
<tr>
<td>Arginine from water, g/d</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Data are averages for each rat (2 rats per cage, 3 cages per treatment group).
2 FA, Fatty acid effect (canola or CLA); AA, amino acid effect (alanine or arginine); FA × AA, interaction effect.
Glycerol and triglycerides. Plasma glycerol and triglycerides were measured with a commercial assay kit (Sigma-Aldrich, TR0100) as per the manufacturer’s specifications. Assays were conducted with a Beckman spectrophotometer at 540 nm.

Amino acids. Serum amino acids were analyzed using HPLC. The HPLC apparatus and precolumn derivatization of amino acids with \( o\)-phthaldialdehyde were as previously described (21). Amino acids were quantified on the basis of authentic standards (Sigma-Aldrich) using the Millennium workstation (Waters) (21).

Adipose tissue cellularity. Epididymal adipocytes were osmium-fixed for sizing as described previously (16). Adipocytes were counted with a 2-chamber Neubauer hemocytometer. Diameter of adipocytes was determined visually with a micrometer.

Statistical analysis. Data were analyzed using the SuperNova (Abacus Concepts) statistical analysis program. Two-way ANOVA was used at a significance level of \( P \leq 0.05 \) and the main effects of fatty acid, amino acid, and their interaction were analyzed. When the fatty acid × amino acid interaction was significant, means were separated by the Fishers protected least significant difference method.

Results

Food intake and body and tissue weights. Weight gain, food intake, and weight gain:food intake ratios were greater in rats receiving supplemental CLA than in canola oil-fed rats (Table 2). Supplemental arginine intake via drinking water also was higher in rats fed arginine plus CLA than in rats fed arginine plus canola oil. Arginine and CLA independently increased final body weight and CLA increased eviscerated body weight (Table 3). Neither arginine nor CLA affected RP adipose tissue mass, but CLA reduced the RP adipose tissue:body weight ratio. Epididymal adipose tissue mass and the epididymal adipose tissue:body weight ratio were not affected by arginine or CLA. There were differences in visceral and skeletal muscle weights (Table 3).

Table 3: Absolute body and eviscerated body weights and relative tissue weights of rats fed alanine, arginine, alanine plus CLA, or arginine plus CLA for 5 wk

<table>
<thead>
<tr>
<th>Item</th>
<th>Canola oil</th>
<th>CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weights, g</td>
<td>424.7</td>
<td>442.1</td>
</tr>
<tr>
<td>Eviscerated body weights, g</td>
<td>385.9</td>
<td>372.8</td>
</tr>
<tr>
<td>RP adipose tissue, g</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td>RP final body weight x 100</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Epididymal adipose tissue, g</td>
<td>5.3</td>
<td>4.5</td>
</tr>
<tr>
<td>Epididymal:final body weight x 100</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Liver, g</td>
<td>15.2</td>
<td>15.9</td>
</tr>
<tr>
<td>Liver:final body weight x 100</td>
<td>3.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Viscera, g</td>
<td>28.0</td>
<td>29.5</td>
</tr>
<tr>
<td>Viscera:final body weight x 100</td>
<td>6.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Soleus, g</td>
<td>0.034</td>
<td>0.033</td>
</tr>
<tr>
<td>Soleus:final body weight x 100</td>
<td>0.008</td>
<td>0.007</td>
</tr>
<tr>
<td>EDL, g</td>
<td>0.033</td>
<td>0.031</td>
</tr>
<tr>
<td>EDL:final body weight x 100</td>
<td>0.007</td>
<td>0.007</td>
</tr>
</tbody>
</table>

1 Data are means, \( n = 6 \).
2 FA, Fatty acid effect (canola or CLA); AA, amino acid effect (alanine or arginine); FA × AA, interaction effect.
3 Viscera weight includes all thoracic and abdominal organs (including liver) plus mesenteric adipose tissue.
4 Weights are muscles from both legs.

Table 4: Selected epididymal adipose tissue and plasma fatty acids, and plasma glycerol and triglycerides of rats fed alanine, arginine, alanine plus CLA, or arginine plus CLA for 5 wk

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Canola oil</th>
<th>CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitoleic, 16:1(n-7)</td>
<td>3.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Oleic, 18:1(n-9)</td>
<td>29.0</td>
<td>25.1</td>
</tr>
<tr>
<td>cis-Vaccenic, 18:1(n-7)</td>
<td>3.7</td>
<td>3.3</td>
</tr>
<tr>
<td>CLA, 18:2(cis-9, trans-11)</td>
<td>0.1</td>
<td>2.5</td>
</tr>
<tr>
<td>CLA, 18:2(trans-10, cis-12)</td>
<td>0.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Plasma fatty acids, g/100 g total fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Oleic</td>
<td>19.3</td>
<td>18.9</td>
</tr>
<tr>
<td>cis-Vaccenic</td>
<td>3.2</td>
<td>2.3</td>
</tr>
<tr>
<td>CLA, 18:2(cis-9, trans-11)</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Glycerol, mmol/L</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.7</td>
<td>3.2</td>
</tr>
</tbody>
</table>

1 Data are means, \( n = 6 \).
2 FA, Fatty acid effect (canola or CLA); AA, amino acid effect (alanine or arginine); FA × AA, interaction effect.
no effects of arginine or CLA on liver, total viscera, or EDL or soleus muscle weights.

Fatty acids, triglycerides, and glycerol. Selected fatty acids from epididymal adipose tissue and plasma are indicated (Table 4). CLA supplementation increased concentrations of \(\text{cis-9, trans-11 CLA and trans-10, cis-12 CLA}\) and decreased concentrations of palmitoleic, oleic, and \(\text{cis-vaccenic acids}\) in epididymal adipose tissue compared with control rats. Arginine did not affect any epididymal adipose tissue fatty acids.

Neither CLA nor arginine had any effect on plasma mono-unsaturated fatty acids (Table 4). The plasma concentration of \(\text{cis-9, trans-11 CLA}\) was increased by supplementary CLA compared with rats receiving supplementary canola oil, but plasma \(\text{trans-10, cis-12 CLA}\) was below the level of detection in rats from all treatment groups. Neither CLA nor arginine affected the concentration of plasma triglycerides, but arginine tended \((P = 0.07)\) to increase plasma glycerol concentrations.

\[
\begin{array}{cccccc}
\text{Tissue/substrate} & \text{Canola oil} & \text{CLA} & \text{Canola oil} & \text{CLA} & \text{Pooled SEM} \\
\hline
\text{Liver metabolism, nmol substrate converted to product/(100 mg C12 h)} \\
\text{CO2 production} \\
\text{Glucose} & 2.5 & 1.4 & 3.3 & 2.1 & 0.4 & 0.19 0.36 0.94 \\
\text{Palmitate} & 5.8 & 10.5 & 6.1 & 6.6 & 1.2 & 0.32 0.48 0.42 \\
\text{Lipid synthesis} \\
\text{Glucose} & 0.9 & 0.4 & 0.7 & 0.7 & 0.1 & 0.13 0.95 0.19 \\
\text{Palmitate} & 108.2 & 83.8 & 57.3 & 50.5 & 10.6 & 0.22 0.13 0.36 \\
\text{Epididymal adipose tissue metabolism, nmol substrate converted to product/(100 mg C12 h)} \\
\text{CO2 production} \\
\text{Glucose} & 5.8 & 10.5 & 5.5 & 10.0 & 1.2 & 0.08 0.85 0.57 \\
\text{Palmitate} & 3.2 & 5.3 & 5.2 & 12.0 & 1.2 & 0.04 0.04 0.25 \\
\text{Lipid synthesis} \\
\text{Glucose} & 13.9 & 21.1 & 12.9 & 12.5 & 1.4 & 0.18 0.06 0.13 \\
\text{Palmitate} & 216.9 & 173.8 & 90.8 & 102.7 & 16.9 & 0.56 0.002 0.31 \\
\end{array}
\]

\[
\begin{array}{cccccc}
\text{Adipocyte volume, pl} & 229.6 & 238.6 & 278.0 & 222.1 & 13.4 & 0.39 0.58 0.24 \\
\text{Adipocytes/100 mg, \(10^3\)} & 5.7 & 5.5 & 6.4 & 6.4 & 0.4 & 0.37 0.92 0.47 \\
\text{Adipocytes/fat pad, \(10^3\)} & 3.3 & 2.5 & 2.8 & 2.4 & 0.3 & 0.42 0.65 0.80 \\
\end{array}
\]

\[
1 \text{ Data are means, } n = 6. \]

\[
2 \text{ FA, fatty acid effect (canola or CLA); AA, amino acid effect (alanine or arginine); FA \(\times\) AA, interaction effect.}
\]

TABLE 6 Serum concentrations of essential amino acids in rats fed alanine, arginine, alanine plus CLA or arginine plus CLA for 5 wk\(^1\)

\[
\begin{array}{ccccccc}
\text{Amino acid} & \text{Canola} & \text{CLA} & \text{Canola} & \text{CLA} & \text{Pooled SEM} & \text{FA}^2 & \text{AA} & \text{FA \(\times\) AA} \\
\hline
\text{Histidine} & 109.7 & 77.6 & 93.0 & 72.5 & 3.9 & 0.01 0.13 0.41 \\
\text{Isoleucine} & 68.4 & 49.0 & 51.5 & 42.8 & 3.4 & 0.04 0.08 0.42 \\
\text{Leucine} & 111.3 & 71.6 & 71.4 & 50.6 & 5.8 & 0.01 0.01 0.36 \\
\text{Lysine} & 136.3^a & 53.0^b & 126.5^a & 120.4^a & 9.8 & 0.01 0.11 0.04 \\
\text{Methionine} & 103.1 & 75.9 & 69.4 & 51.4 & 5.8 & 0.04 0.01 0.67 \\
\text{Phenylalanine} & 226.3 & 148.7 & 136.7 & 107.3 & 11.3 & 0.01 0.01 0.21 \\
\text{Threonine} & 243.4 & 176.7 & 238.7 & 166.0 & 12.5 & 0.01 0.75 0.90 \\
\text{Tryptophan} & 122.3 & 92.7 & 123.7 & 92.7 & 4.9 & 0.01 0.09 0.61 \\
\text{Valine} & 65.2 & 46.4 & 45.8 & 36.9 & 3.4 & 0.03 0.03 0.44 \\
\end{array}
\]

\[
1 \text{ Data are means, } n = 6. \text{ Means without a common letter differ, } P \leq 0.05. \\
2 \text{ FA, fatty acid effect (canola or CLA); AA, amino acid effect (alanine or arginine); FA \(\times\) AA, interaction effect.}
\]
**TABLE 7** Serum concentrations of nonessential amino acids in rats fed alanine, arginine, alanine plus CLA, or arginine plus CLA for 5 wk.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Canola</th>
<th>CLA</th>
<th>Canola</th>
<th>CLA</th>
<th>Pooled SEM</th>
<th>FA</th>
<th>AA</th>
<th>FA × AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>400.4</td>
<td>262.8</td>
<td>263.1</td>
<td>268.3</td>
<td>20.7</td>
<td>0.10</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>Arginine</td>
<td>87.5³</td>
<td>100.6²</td>
<td>251.7³</td>
<td>172.6³</td>
<td>14.4</td>
<td>0.15</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>Asparagine</td>
<td>69.8</td>
<td>54.5</td>
<td>61.2</td>
<td>41.9</td>
<td>3.9</td>
<td>0.03</td>
<td>0.18</td>
<td>0.80</td>
</tr>
<tr>
<td>Aspartate</td>
<td>21.6</td>
<td>14.0</td>
<td>19.9</td>
<td>17.7</td>
<td>1.6</td>
<td>0.15</td>
<td>0.76</td>
<td>0.42</td>
</tr>
<tr>
<td>Citrulline</td>
<td>74.4</td>
<td>58.8</td>
<td>73.3</td>
<td>52.8</td>
<td>3.8</td>
<td>0.02</td>
<td>0.64</td>
<td>0.75</td>
</tr>
<tr>
<td>Cystine</td>
<td>188.5</td>
<td>191.0</td>
<td>169.2</td>
<td>182.0</td>
<td>6.7</td>
<td>0.59</td>
<td>0.32</td>
<td>0.72</td>
</tr>
<tr>
<td>Glutamate</td>
<td>212.2</td>
<td>129.5</td>
<td>135.9</td>
<td>112.2</td>
<td>11.6</td>
<td>0.02</td>
<td>0.03</td>
<td>0.17</td>
</tr>
<tr>
<td>Glutamine</td>
<td>787.9</td>
<td>535.7</td>
<td>517.5</td>
<td>369.4</td>
<td>48.1</td>
<td>0.03</td>
<td>0.02</td>
<td>0.57</td>
</tr>
<tr>
<td>Glycine</td>
<td>192.3</td>
<td>145.3</td>
<td>171.9</td>
<td>128.3</td>
<td>8.0</td>
<td>0.01</td>
<td>0.28</td>
<td>0.92</td>
</tr>
<tr>
<td>Ornithine</td>
<td>192.6</td>
<td>122.6</td>
<td>121.7</td>
<td>92.4</td>
<td>10.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>Serine</td>
<td>210.4</td>
<td>161.3</td>
<td>211.9</td>
<td>143.2</td>
<td>11.4</td>
<td>0.01</td>
<td>0.72</td>
<td>0.67</td>
</tr>
<tr>
<td>Taurine</td>
<td>424.3</td>
<td>313.9</td>
<td>315.4</td>
<td>254.7</td>
<td>22.7</td>
<td>0.06</td>
<td>0.06</td>
<td>0.57</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>680.0</td>
<td>422.1</td>
<td>424.8</td>
<td>288.6</td>
<td>35.5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.31</td>
</tr>
</tbody>
</table>

¹ Data are means, n = 6. Means without a common letter differ, P ≤ 0.05.
² FA, fatty acid effect (canola or CLA); AA, amino acid effect (alanine or arginine); FA × AA, interaction effect.

Serum amino acids. The serum concentrations of all essential amino acids were lower in rats receiving supplemental CLA than in rats fed canola oil (Table 6). CLA also depressed serum concentrations of the nonessential amino acids cystine, glutamate, glutamine, ornithine, taurine, and tyrosine (Table 7) relative to canola oil-fed rats. There was a CLA × arginine interaction for serum lysine and arginine, which were lower in rats fed CLA plus arginine than in the other treatment groups.

Supplemental arginine depressed serum concentrations of all essential amino acids except histidine, lysine, and threonine (Table 6) and also depressed concentrations of glutamate, glutamine, ornithine, taurine, and tyrosine (Table 7) relative to alanine-fed rats. Supplemental arginine increased serum arginine substantially compared with rats fed alanine.

Discussion

Many of the changes in epididymal adipose tissue fatty acid concentrations were consistent with the dietary fatty acid composition. Oleic acid comprised 38% of total dietary fatty acids in the pellets containing canola oil and only 26% of fatty acids in the CLA-enriched pellets, so the lower adipose tissue oleic acid in the CLA-fed rats, relative to canola oil-fed rats, can in part be explained by diet fatty acid composition.

Dietary palmitoleic acid did not differ between the canola oil- and CLA-supplemented diets, so the depression in the concentration of palmitoleic acid in epididymal adipose tissue caused by supplemental CLA indicated that CLA reduced SCD activity in that tissue (12,20,22). However, none of the plasma monounsaturated fatty acids were affected by supplemental CLA or arginine, indicating that neither CLA nor arginine affected hepatic SCD activity under the conditions of this study. Hepatic SCD activity is necessary for hepatic triglyceride synthesis in mice (23) and humans (24). The lack of an effect of arginine or CLA on hepatic SCD activity in the current study was consistent with the lack of effect of these supplements on glucose or palmitate incorporation into total lipids in vitro in liver or plasma triglycerides. We previously demonstrated that feeding arginine for 12 wk to rats reduced plasma triglycerides by nearly 30% (3), so longer term feeding of arginine and/or CLA may depress hepatic SCD activity.

Supplemental arginine increased plasma glycerol; Fu et al. (1) similarly reported that arginine supplementation to rats increased glycerol release from rat adipose tissues in vitro. Therefore, the reduction in RP adipose tissue mass by arginine may have been due in part to an increased rate of lipolysis.

In the current study, arginine did not affect epididymal adipose tissue mass. Fu et al. (1) demonstrated that arginine supplementation reduced epididymal adipose tissue mass and increased glucose and octanoate oxidation to CO₂ in epididymal adipose tissue of obese ZDF rats. In previous studies (1,3), rats received supplemental arginine for 10 or 12 wk rather than 5 wk. Recently (25), we fed ZDF rats a lower concentration of arginine (0.2%) for only 4 wk. As in the current study, arginine supplementation reduced RP adipose tissue mass but did not affect epididymal adipose tissue mass.

Despite a lack of effect of arginine or CLA on epididymal adipose tissue mass or adiposity, arginine and CLA independently doubled the rate of palmitate oxidation. Similarly, CLA doubled glucose oxidation in epididymal adipose tissue in vitro (P = 0.08), whereas arginine did not affect glucose oxidation. Arginine, but not CLA, reduced glucose and palmitate incorporation into total lipids. This indicates that CLA and arginine modify adipose tissue substrate metabolism by separate mechanisms.

The lack of effect of CLA on lipid synthesis in epididymal adipose tissue is unusual in light of previous reports [reviewed in (8)]. However, we reported previously that mixed isomers of CLA increased glucose incorporation into lipids in 3T3-L1 preadipocytes (9). Faulconnier et al. (26) similarly reported that CLA increased adipose tissue lipogenesis in male Wister rats. Thus, although others have demonstrated that CLA reduces adiposity in rats (8), it has not been possible to demonstrate a consistent effect of CLA on lipogenesis and/or adiposity in rats.

Muscle growth and serum amino acids. Unlike previous results (1,3), arginine supplementation did not increase EDL or soleus muscle mass. Expression of nitric oxide synthase has been reported in myocytes in culture (27), nitric oxide synthase isoforms are present in the sarclemma, sarcoplasm, sarcoplasmic reticulum, and mitochondria of muscle fibers (28), and arginine supplementation increases lean mass in growing pigs.
Similarly, eviscerated body mass, which included the skin and head, was increased significantly by the combination of arginine and CLA. We conclude that arginine plus CLA increased axial muscle mass.

Other investigators have demonstrated that CLA increases lean mass, although this is species dependent (8). We have demonstrated for the first time, to our knowledge, that arginine and/or CLA caused a reduction in serum branched-chain amino acids (BCAA) and several nonessential amino acids involved in nitrogen transport from skeletal muscle to liver. The reduction in BCAA could have been caused by increased oxidation of BCAA in skeletal muscle or decreased release of BCAA from skeletal muscle. However, serum concentrations of glutamine and alanine, which are synthesized by the skeletal muscle, decreased in skeletal muscle or decreased release of BCAA from skeletal muscle. However, serum concentrations of glutamine and alanine, which are synthesized by the skeletal muscle, decreased with arginine and CLA supplementation. CLA reduced serum ornithine by over 60% and, in rats receiving supplemental arginine, CLA depressed plasma arginine; both phenomena indicate a depression in nitrogen flux through the urea cycle. Taken together, the data suggest that CLA and arginine increased lean mass by depressing the turnover of muscle proteins (sarcoplasmic or myofibrillar). We demonstrated previously that oral supplementation of piglets with N-carbamylglutamate, an activator of arginine synthesis, increased plasma arginine as well as protein synthesis in the longissimus dorsi and soleus muscles (30). These data suggest that arginine plus CLA supplementation may have activated the mammalian target of rapamycin (mTOR) pathway (31,32). Supplemental arginine increased mTOR signaling activity in neonatal pig muscle (32) and trans-10,cis-12 CLA increased mTOR signaling in human preadipocytes (33). If arginine and CLA work through a common mechanism in skeletal muscle, i.e., stimulation of the mTOR signaling pathway, this could explain the similarity in reductions in plasma amino acids caused by both arginine and CLA.

Our original hypothesis, that arginine and CLA would have additive effects on adipose tissue and liver metabolism, proved to be incorrect, because there were no significant CLA × arginine interactions. However, there were many instances in which arginine and CLA had independent effects, such as on lipid synthesis from palmitate in epididymal adipose tissue. The most novel, and unexpected, effect of both arginine and CLA was the apparent depression of muscle protein turnover, which clearly warrants further investigation.

**Literature Cited**


29.


