Effect of dietary α-lipoic acid on growth, body composition, muscle pH, and AMP-activated protein kinase phosphorylation in mice


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ABSTRACT: The effects of α-lipoic acid (ALA) on the growth, body composition, postmortem AMP-activated protein kinase (AMPK) activation, and 24-h muscle pH were investigated. Thirty male C57BL/6J mice were fed diets containing 0, 0.5, or 1.0% ALA (DM basis). At the end of the 3-wk feeding trial, carcass weights decreased ($P < 0.05$) 14 and 30% for mice fed 0.5 and 1.0% ALA, respectively, compared with the 0% group, with decreases in BW as the levels of dietary ALA increased. This change in carcass weight occurred because carcass fat content for mice receiving 0.5 and 1.0% ALA was 7.32 and 8.09% lower ($P < 0.05$), respectively, than for the 0% ALA treatment, and because gonadal fat decreased ($P < 0.05$) 85% in mice fed 1.0% ALA compared with those fed 0% ALA. Dietary ALA caused a slight increase ($P = 0.07$) in carcass moisture content, with no effect on protein and ash content. Furthermore, ALA supplement decreased ($P < 0.05$) ADFI (DM basis) from 4.3 g/d for 0% ALA-fed mice to 3.4 g/d for 1.0% ALA-fed mice. At 20 min postmortem, pH was greater ($P < 0.05$) in muscle of mice fed 1.0% ALA than in muscle of mice fed 0% ALA. Ultimate (24-h) pH values differed ($P < 0.05$) among treatments, and mean values were 5.83, 6.08, and 6.29 for 0, 0.5, and 1.0% ALA, respectively. Phosphorylation of AMPK α subunit at Thr$^{172}$, an indicator of AMPK activation, was decreased ($P < 0.05$) in muscle of ALA-treated mice at 20 min postmortem. Because AMPK has a crucial role in the control of glycolysis, the reduction in AMPK activation decreases glycolysis, and thereby increases the ultimate pH of postmortem muscle. In summary, dietary ALA supplement can decrease fat accumulation in mice, and because ALA increased muscle pH at 20 min and 24 h postmortem, these results suggest that dietary ALA supplementation might decrease carcass fatness and prevent the development of PSE pork and poultry. However, further research is required to test the effects of ALA in swine and poultry.

Key Words: AMP-Activated Protein Kinase, Fat, Glycolysis, Mice, Muscle, Pale, Soft, and Exudative Meat

Introduction

Pale, soft and exudative meat is characterized by a pale-colored, very wet, and very soft appearance. The high incidence of PSE pork, turkey, and chicken results in significant losses to the meat industry (Woelfel et al., 2002; Josell et al., 2003). Rapid or excessive glycolysis in postmortem muscle, coupled with high muscle temperatures, is commonly believed to be the cause of PSE in meat (Solomon et al., 1998; van Laack and Kauffman, 1999; Miller et al., 2000a), but the mechanisms associated with abnormal glycolysis in postmortem muscle are largely unclear.

Recent biomedical studies indicated that AMP-activated protein kinase (AMPK) plays a crucial role in the initiation of glycolysis in skeletal and cardiac muscle (Hardie, 2003). Adenosine monophosphate-activated protein kinase is activated when the AMP-to-ATP ratio increases inside myocytes. Once activated, AMPK “switches on” glycolysis and other catabolic pathways that generate ATP (Sambandam and Lopaschuk, 2003). It is hypothesized that AMPK also plays a crucial role in muscle glycolysis, and the incidence of PSE meat may be prevented by inhibiting AMPK.

α-Lipoic acid (ALA) is a strong antioxidant that exists in foods (Packer et al., 1995). A recent study showed that dietary ALA decreased hypothalamic AMPK activity while increasing its activity in skeletal muscle (Kim et al., 2004). Thus, it is quite possible that dietary ALA also alters AMPK activity in postmortem muscle, which may slow glycolysis and prevent the incidence of PSE meat. Dietary ALA also has anti-obesity effects (Kim
et al., 2004), and supplementing ALA may decrease fat accumulation and influence growth performance of animals. Therefore, the objective of this study was to assess the effect of dietary ALA on growth, fat accumulation, AMPK activation, and postmortem glycolysis in mice.

Materials and Methods

Animals and Diets

Thirty, 2-mo-old, male, C57BL/6J mice (Charles River Laboratories, Wilmington, MA) were individually caged, and cages (10 per treatment) were assigned to one of three dietary treatments, where a commercial rodent diet (Laboratory Rodent Diet 5001; LabDiet, Richmond, IN), containing 23.4% CP, 5.5% fat, 43.1% carbohydrate, and 6.9% ash on DM basis, was supplemented with 0, 0.5 or 1.0% ALA on DM basis (MTC Industries, Inc., Great Neck, NY). To ensure that diets were isocaloric, 1.0, 0.5, and 0% palmitic acid was added to the 0, 0.5, and 1.0% ALA diets, respectively. Mice were maintained on a 14-h light:10-h dark cycle and were isograde fed a commercial diet (Laboratory Rodent Diet 5001; LabDiet, Richmond, IN) for 3 wk feeding period, mice were anesthetized with sodium pentobarbital, and killed by cervical dislocation. Heads, pelts, and carcasses were homogenized using a polytron homogenizer (top speed for 10 s; IKA Works, Inc., Wilmington, NC) in 500 μL of buffer (precooled in ice) containing 20 mM Tris-HCl (pH 7.4 at 4°C), 2% SDS, 5 mM EDTA, 5 mM EGTA, 1 mM DTT, 100 mM NaF, 2 mM sodium vanadate, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, and 10 μL/mL pepstatin (Raser et al., 1995; Veiseth et al., 2001). Each muscle homogenate was mixed with an equal amount of 2 × standard SDS sample loading buffer and boiled for 3 min.

For SDS-PAGE preparation, a separation gel containing 10% (wt/vol) acrylamide/bisacrylamide (29:1), 375 mM Tris-HCl (pH 8.8), 0.1% SDS, 0.04% ammonium persulfate, and 0.028% N,N,N',N’-tetramethyl-ethylendiamine (TEMED) was prepared; the stacking gel contained 4% (wt/vol) acrylamide/bisacrylamide (29:1), 330 mM Tris-HCl (pH 6.8), 0.04% ammonium persulfate, and 0.028% TEMED. Following electrophoresis, proteins on gels were transferred to a nitrocellulose membrane in a transfer buffer containing 20 mM Tris-base, 192 mM glycine, 0.1% SDS, and 20% methanol.

Membranes were incubated in a blocking solution consisting of 5% nonfat dry milk in TBS/T (0.1% Tween-20, 50 mM Tris-HCl [pH 7.6]), and 150 mM NaCl) for 1 h. Then, membranes were incubated overnight with a primary antibody (antiphospho-AMPKα [Thr172] or antiAMPKα; Cell Signaling Technology, Beverly, MA) to rabbit IgG (1:1,000; Amersham Bioscience, Piscataway, NJ) for 1 h in TBS/T with gentle agitation. After three 10-min washes, membranes were visualized using ECL Western blotting reagents (Amersham Bioscience) and exposed to film (MR; Kodak, Rochester, NY). Density of bands was quantified by using an Image Scanner II and ImageQuant TL software (Du et al., 2004). Glycolytic Potential

Frozen vastus lateralis muscle (0.1 g) was homogenized using a Polytron homogenizer (top speed for 10 s) in 0.5 mL of distilled water plus 0.5 mM phenylmethylsulfonyl fluoride, 10 μg of leupeptin/mL, and 10 μL of pepstatin/mL. A 100-μL homogenate was incubated with 0.5 mL of 1 mg/mL amylglucosidase in 0.2 M acetate buffer (pH 4.8) for 2 h at 37°C. Samples were cooled on ice and centrifuged at 12,000 × g at 4°C for 2 min. A 50-μL aliquot of resulting extract was incubated with 1 mL of assay buffer (1 mM ATP, 1 mM NADP, 3 U of glucose-6-phosphate dehydrogenase/mL, 3 U of hexokinase/mL, and 0.1 M Tris-HCl; pH 7.4) for 1 h at 25°C. Total concentrations of glycogen, glucose, and glucose-6-phosphate were determined simultaneously by the change in absorbance at 340 nm (Miller et al., 2000a). Lactic acid content was measured using 200 μL
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Figure 1. Body weight of mice receiving different levels of supplemental α-lipoic acid (ALA). Within a specific time on feed, points that do not have common letters differ, \( P < 0.05 \).

Figure 2. Daily feed consumption (DM basis) of mice receiving different levels of supplemental α-lipoic acid (ALA). Within a specific time on feed, points that do not have common letters differ, \( P < 0.05 \).

e of perchloric acid extract and a commercially available lactate analysis kit (Trinity Biotech, St. Louis, MO). Glycolytic potential was calculated using the formula of Monin and Sellier (1985), where glycolytic potential = \( 2 \times ([\text{glycogen}] + [\text{glucose}] + [\text{glucose-6-phosphate}]) + [\text{lactate}] \).

Statistical Analyses

The effect of dietary ALA on the growth and feed consumption of mice, chemical composition of carcasses, AMPK phosphorylation, and muscle glycolytic potential were analyzed as a completely randomized design using the GLM procedure of the SAS (SAS Inst., Inc., Cary, NC), with individual mouse as the experimental unit. The growth and intake measurements were collected over time and analyzed as a split-plot design with each time point as a subplot. The interaction between dietary treatment and feeding time was tested. The Student-Newman-Keul’s multiple range test was used to compare differences among dietary treatments when the \( F \)-test was significant (\( P < 0.05 \)).

Results

Dietary ALA decreased (\( P < 0.05 \)) weight gain in mice during the second and third weeks of the feeding trial compared with mice fed 0% ALA (dietary treatment \( \times \) feeding time; \( P < 0.05 \); Figure 1). Mice receiving the 1.0% ALA treatment had a net weight loss of 3.3 g, whereas mice receiving 0% ALA gained about 3.6 g and 0.5% ALA gained approximately 0.8 g during the entire 3-wk trial (Figure 1). During the first week on feed, mice fed 1.0% ALA had lower (\( P < 0.05 \)) ADFI than mice fed 0% ALA, whereas during the second week, mice fed 1.0% ALA had lower (\( P < 0.05 \)) ADFI than mice fed either 0 or 0.5% ALA (dietary treatment \( \times \) feeding time; \( P < 0.05 \); Figure 2). At the conclusion of the 3-wk feeding trial, daily feed consumption was 4.4, 3.8, and 3.3 g for mice receiving diets supplemented with 0, 0.5, and 1.0% ALA, respectively.

Average carcass weight decreased 1.7 and 3.5 g for mice receiving 0.5 and 1.0% ALA treatments, respectively, compared with the 0% ALA treatment (Table 1). No difference (\( P = 0.33 \)) in carcass weight:BW was observed, however, indicating that ALA supplementation did not affect the dressing percent. Supplementation of 1.0% ALA decreased (\( P < 0.05 \)) the gonadal fat weight in mice by 85% compared with mice fed 0% ALA. Gonadal fat:BW was greatest (\( P < 0.05 \)) for mice fed 0% ALA and least (\( P < 0.05 \)) for mice fed 1.0% ALA; thus, the decrease in gonadal fat weight was not merely the response to decreased BW but a result of ALA supplementation. Feeding mice 0.5 and 1.0% ALA decreased (\( P < 0.05 \)) vastus lateralis weight by 11 and 30%, respectively; however, muscle weight:BW were similar (\( P = 0.54 \)) among dietary treatments, indicating that muscle weight was a response to the decreased BW of mice receiving supplemental ALA.

Feeding 1.0% ALA decreased (\( P < 0.05 \)) carcass fat percent by 80% compared with control (0% ALA) mice; however, carcass moisture percent was increased (\( P < 0.05 \)) by feeding 1.0% ALA (Table 2). Carcass protein and ash content were unchanged (\( P = 0.07 \)) by dietary ALA supplementation. When expressed as a change in composition per day, carcass moisture, protein, ash, and fat contents were decreased (\( P < 0.05 \)) substantially by feeding 1.0% ALA; the greatest magnitude of change was observed in carcass fat content (Table 2). However, feeding 0.5% ALA increased (\( P < 0.05 \)) carcass moisture and protein and ash content, but it dramatically decreased (\( P < 0.05 \)) fat content (Table 2).

At 20 min postmortem, LM pH was greater (\( P < 0.05 \)) for mice receiving 1.0% ALA treatment than for mice receiving 0% ALA (Figure 3). Additionally, ultimate (24
Table 1. Carcass, gonadal fat, and vastus lateralis weights of mice receiving diets with different levels of α-lipoic acid (ALA) supplementation on a DM basis

<table>
<thead>
<tr>
<th>ALA</th>
<th>Item</th>
<th>0%</th>
<th>0.5%</th>
<th>1.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Carcass weight, g</td>
<td></td>
<td>11.55 ± 0.75x</td>
<td>9.93 ± 0.44vy</td>
<td>8.07 ± 0.36y</td>
</tr>
<tr>
<td>Carcass weight:BW</td>
<td></td>
<td>0.417 ± 0.027</td>
<td>0.430 ± 0.019</td>
<td>0.404 ± 0.018</td>
</tr>
<tr>
<td>Gonadal fat weight, g</td>
<td></td>
<td>0.61 ± 0.06x</td>
<td>0.31 ± 0.03yv</td>
<td>0.09 ± 0.01y</td>
</tr>
<tr>
<td>Gonadal fat:BW</td>
<td></td>
<td>0.022 ± 0.006x</td>
<td>0.013 ± 0.004y</td>
<td>0.005 ± 0.002y2</td>
</tr>
<tr>
<td>Vastus lateralis (VL) weight, g</td>
<td></td>
<td>0.101 ± 0.0022</td>
<td>0.090 ± 0.005y</td>
<td>0.071 ± 0.0052</td>
</tr>
<tr>
<td>VT:BW</td>
<td></td>
<td>0.0049 ± 0.0001</td>
<td>0.0050 ± 0.0003</td>
<td>0.0050 ± 0.0005</td>
</tr>
</tbody>
</table>

x,y,z Within a row, least squares means ± SE that do not have a common superscript letter differ, P < 0.05.

Table 2. Chemical composition and calculated net change of mice receiving different levels of α-lipoic acid (ALA) supplement on a DM basis

<table>
<thead>
<tr>
<th>ALA</th>
<th>Item</th>
<th>0.5%</th>
<th>0%</th>
<th>1.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Chemical composition of carcasses</td>
<td></td>
<td>Moisture, %</td>
<td>66.49 ± 0.59x</td>
<td>69.38 ± 0.42vy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protein, %</td>
<td>17.52 ± 0.39</td>
<td>18.43 ± 0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat, %</td>
<td>9.09 ± 0.60x</td>
<td>5.20 ± 0.34yv</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ash, %</td>
<td>5.23 ± 0.14</td>
<td>5.26 ± 0.23</td>
</tr>
<tr>
<td>Calculated change per day, mg</td>
<td></td>
<td>Water</td>
<td>118 ± 11x</td>
<td>60 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protein</td>
<td>33 ± 5x</td>
<td>19 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat</td>
<td>19 ± 9x</td>
<td>−40 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ash</td>
<td>11 ± 3x</td>
<td>6 ± 3</td>
</tr>
</tbody>
</table>

x,y,z Within a row, least squares means ± SE that do not have a common superscript letter differ, P < 0.05.

h) LM pH was greatest (P < 0.01) for mice fed 1.0% ALA and least (P < 0.01) for mice fed 0% ALA, with pH values of 5.83, 6.05, and 6.27 in the LM of mice fed 0, 0.5, and 1.0% ALA, respectively (Figure 3).

In this study, supplementation of ALA caused dramatic loss in body fat. The loss in body fat should be related to decreased food consumption as well as enhanced β-oxidation of fatty acid (Kim et al., 2004). In agreement with the results of Kim et al. (2004), dietary ALA supplementation decreased food consumption in mice as a response to the inhibition of AMPK activity in the hypothalamus. Several reports showed that inhibition of hypothalamic AMPK activity has an anorexigenic effect (Andersson et al., 2004; Minokoshi et al., 2004). The decrease in food consumption following ALA supplementation cannot be attributed to taste aversion to diets because an intraperitoneal injection of ALA caused a similar decrease in food consumption (unpublished results).

Supplementing mice diets with 1.0% ALA caused a net loss not only in fat, but also in protein, and it had detrimental effects on mouse performance. Nonetheless, 0.5% ALA supplement only induced a loss in fat; thus, it might be a good level of supplement to decrease fat accumulation. In addition to a decrease in food consumption, ALA supplements promote β-oxidation of fatty acids (Kim et al., 2004), which partially explains the dramatic fat loss in mice supplemented with ALA.

Discussion

α-Lipoic acid is a strong antioxidant in foods (Packer et al., 1995). Very recently, it was reported that ALA decreased the AMPK activity in the hypothalamus and caused profound weight loss in rats by decreasing food intake and enhancing energy expenditure (Kim et al., 2004). It is quite possible that ALA also alters AMPK activity in skeletal muscle and thereby affects the postmortem glycolysis and fat accumulation.
Lipoic acid and postmortem muscle

An important finding of this study was that dietary ALA increased the ultimate pH value of postmortem muscle, which is consistent with the elevation in pH at 45 min in the LM of pigs fed supplemental ALA (Berg et al., 2003). The lack of effect of ALA on pH of postmortem muscle at other time points could be due to very low levels of ALA supplementation used in that study, which might limit the effect of ALA on postmortem glycolysis (Berg et al., 2003). Fast glycolysis plus elevated temperature in postmortem muscle causes PSE pork, turkey, and chicken, and excessive glycolysis results in red, soft, and exudative meat in Hampshire pigs carrying the Rendement Napole (RN) gene (Solomon et al., 1998; van Laack and Kauffman, 1999; Miller et al., 2000b). The high incidence of PSE in pork, turkey, and chicken causes significant losses to the meat industry (Woelfel et al., 2002; Josell et al., 2003). Results of the current study indicate that supplementing diets with ALA before slaughter can downregulate postmortem glycolysis; thus, dietary ALA might be a possible method to prevent the occurrence of PSE meat.

Adenosine monophosphate-activated protein kinase is a heterotrimeric enzyme with α, β, and γ subunits. The α subunit is the catalytic unit, the β subunit has a regulatory function, and the γ unit provides anchorage sites for α and γ (Sambandam and Lopaschuk, 2003). Adenosine monophosphate-activated protein kinase is “switched on” by an increase in the AMP:ATP in muscle cells, which induces a phosphorylation at Thr172 in the α subunit (Hardie, 2004). Once activated, AMPK switches on glycolysis and other catabolic pathways that generate ATP. Current biomedical studies have shown that AMPK initiates glycolysis through two possible mechanisms. First, activated AMPK can phosphorylate 6-phosphofructo-2-kinase (Marsin et al., 2002). Phosphorylated 6-phosphofructo-2-kinase promotes the synthesis of fructose 2, 6-bisphosphate, a potent allosteric stimulator of the 6-phosphofructo-1-kinase (a key glycolysis-controlling enzyme). Second, AMPK can inhibit the activity of glycogen synthase and activate phosphorylase kinase by phosphorylation, which can further activate glycogen phosphorylase (Carling and Hardie, 1989; Young et al., 1996; Bergeron et al., 1999). Studies to test these mechanisms in postmortem muscle have not been conducted.

Results of this study demonstrated that feeding ALA for 3 wk before slaughter increased postmortem muscle pH and decreased phosphorylation of AMPK at Thr172.
of its α subunit in mice. Because the activity of AMPK is controlled through phosphorylation at Thr\textsuperscript{172} of α subunit, the lack of phosphorylation in postmortem muscle from mice fed ALA resulted in less activated AMPK, which might inhibit the initiation and progress of glycolysis. Reasons for the lower AMPK activation in postmortem muscle of mice receiving supplemental ALA were unclear, but could be due to feedback inhibition. Actually, Kim et al. (2004) reported that dietary ALA increased AMPK activity slightly in skeletal muscle in vivo; thus, evidence is available supporting the theory that antemortem AMPK activation inhibits postmortem AMPK activation. Therefore, the postmortem activation of AMPK was less for mice that received ALA treatment (Shen and Du, 2005).

Although there was no difference in glycolytic potential between muscles of mice fed 0 and 0.5% ALA, ultimate pH was greater in the LM from mice fed 0.5% ALA. The pH of postmortem muscle is a result of both glycogen content and glycolytic enzyme activity. When there is sufficient glycogen available, the glycolytic enzyme activity determines the rate of pH decline and establishment of ultimate pH. In beef, there seemed to be a glycolytic potential threshold at approximately 100 mM/g, below which lower glycolytic potential was associated with higher ultimate pH, and above which glycolytic potential had no effect on ultimate pH (Wulf et al., 2002). At slaughter, glycogen content poorly explains the variability in ultimate pH (El Rammouz et al., 2004) and supports the role of glycolytic enzymes in determining ultimate pH. Because AMPK has a controlling role in glycolytic enzyme activity, lower AMPK activation results in lower activities of glycolytic enzymes and promotes a greater ultimate pH.

It is unclear why the glycolytic potential was lower in muscle of mice fed 1.0% ALA than in muscle of those fed the 0 or 0.5% ALA. Because activation of AMPK in vivo promotes the accumulation of glycogen in muscle, it was expected that vastus lateralis glycolytic potential from mice fed 1.0% ALA would have been greater than control. For RN pigs, the high activity of AMPK, because of a mutation, enhances glycogen accumulation (Andersson, 2003); however, in rats, starvation decreases the glycogen content in skeletal muscle (Calder and Geddes, 1992). Thus, the severe decrease in feed consumption associated with feeding 1.0% ALA might have decreased the level of gluconeogenesis and/or glycogen synthesis in muscle.

**Implications**

Results of this study indicate that supplementing mice diets with α-lipoic acid can inhibit the activation of AMP-activated protein kinase in postmortem muscle and increase pH values of postmortem muscle. Therefore, dietary α-lipoic acid supplementation may be a practical way of preventing the development of pale, soft, and exudative meat. Furthermore, α-lipoic acid supplementation dramatically decreased carcass fat ac-

**Literature Cited**


Lipoic acid and postmortem muscle


