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Alpha-lipoic acid increases energy expenditure by enhancing AMPK-PGC-1α signalling in the skeletal muscle of aged mice

Yi Wang[1,2], **Xiaojie Li**[1,2], **Yuming Guo**[1], **Lawrence Chan**[3], and **Xinfu Guan**[2,3,*]

[1] State Key Laboratory of Animal Nutrition, College of Animal Science & Technology, China Agricultural University, Beijing, China

[2] USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX

[3] Division of Diabetes, Endocrinology & Metabolism, Department of Medicine, Baylor College of Medicine, Houston, TX

Abstract

Objectives—Skeletal muscle mitochondrial dysfunction is associated with aging and diabetes, which decreases respiratory capacity and increases reactive oxygen species. Lipoic acid (LA) possesses antioxidative and antidiabetic properties. Metabolic action of LA ismediated by activation of AMP-activated protein kinase (AMPK), a cellular energy sensor that can regulate PGC-1α, a master regulator of mitochondrial biogenesis. We hypothesized that LA improves energy metabolism and mitochondrial biogenesis by enhancing AMPK-PGC-1α signalling in the skeletal muscle of aged mice.

Methods—C57BL/6 mice (24-month old, male) were supplemented with or without α-LA (0.75% in drinking water) for one month. In addition, metabolic action and cellular signalling of LA were studied in cultured mouse myoblastoma C2C12 cells.

Results—LA supplementation improved body composition, glucose tolerance, and energy expenditure in the aged mice. LA increased skeletal muscle mitochondrial biogenesis with increased phosphorylation of AMPK and mRNA expression of PGC-1α and glucose transporter-4 (GLUT-4). Besides body fat mass, LA decreased lean mass and attenuated phosphorylation of mammalian target of rapamycin (mTOR) signalling in the skeletal muscle. In cultured C2C12 cells, LA increased glucose uptake and palmitate β-oxidation, but decreased protein synthesis, which was associated with increased phosphorylation of AMPK and expression of PGC-1α and GLUT-4, and attenuated phosphorylation of mTOR and p70S6 kinase.

Conclusions—We conclude that LA improves skeletal muscle energy metabolism in the aged mouse possibly through enhancing AMPK-PGC-1α-mediated mitochondrial biogenesis and function. Moreover, LA increases lean mass loss possibly by suppressing protein synthesis in the skeletal muscle by down-regulating the mTOR signalling pathway. Thus, LA may be a promising supplement for treatment of obesity and/or insulin resistance in older patients.

[*]whom correspondence should be addressed: Xinfu Guan, USDA/ARS, Children's Nutrition Research Centre, Department of Paediatrics, Baylor College of Medicine, 1100 Bates St., Houston, Texas 77030; Tel: 713-798-9322; Fax: 713-798-7100; xguan@bcm.edu.

USDA/ARS Children's Nutrition Research Center where the work was done.

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Keywords

Lipoic acid; Aging; Mitochondrial biogenesis; Protein synthesis; Energy metabolism

Introduction

Obesity and related metabolic syndrome continue to be a major public health problem in the developed world. Both obesity and insulin resistance increase with aging, which is associated with reduced mitochondrial mass and function, leading to a defective energy homeostasis [1–3]. A substantial decline in mitochondrial oxidative capacity in the skeletal muscle may contribute to the whole body aging process [4]. A reduction in respiration rate and mitochondria biogenesis accounts for a defective energy expenditure, which predisposes to obesity, type 2 diabetes, and other metabolic consequences [5]. Energy metabolism in the skeletal muscle is finely regulated in healthy subjects; however, such regulation may be impaired in aging and diabetes [6]. The mechanisms that regulate body composition and energy homeostasis are not fully understood.

Nutrient supplementation has been applied to slow down the aging process and improve the quality of life. Supplemented lipoic acid (LA)[4], an essential cofactor in mitochondrial dehydrogenase complexes, might protect against aging-related mitochondrial dysfunction [7, 8], and increases glucose utilization in type 2 diabetes mellitus *in vivo* [9, 10]. Recently, LA is shown to induce body weight loss by inhibiting hypothalamic AMPK activity, resulting from suppressed food intake and stimulated energy expenditure [11]. In addition, LA treatment combined with acetyl-carnitine increases ambulatory activity in aged rats [12], and improves mitochondrial function with attenuated oxidative damage [13]. Skeletal muscle is a key tissue and a major contributor to whole-body energy homeostasis in humans [14]. However, it is unknown whether LA supplementation increases mitochondrial biogenesis and energy metabolism in skeletal muscle of aged mice.

AMPK is a highly conserved, cellular energy sensor. It appears as an intracellular fuel gauge that is activated by a drop in the ATP/AMP ratio [15]. One mechanism by which activated AMPK stimulates glucose uptake, fatty acid oxidation, and mitochondrial biogenesis in skeletal muscle is by increasing GLUT-4 and PGC-1α expression [16–18]. Furthermore, metformin treatment in type 2 diabetes mellitus activates AMPK, leading to enhanced glucose disposal in skeletal muscle [19]. Interestingly, the increase in AMPK activity results in suppressed skeletal muscle protein synthesis. Lipoic acid has been reported to increase AMPK activity in skeletal muscles in diabetes-prone obese rats and in C2C12 myotubes, which is accompanied by improved glucose metabolism and fatty acid oxidation [20, 21]. Previous studies focused on LA-mediated anti-oxidative protective effects in aged mice [22, 23], though its metabolic effects in energy metabolism are also evident in obese and/or diabetic mice [24, 25]. Considering LA-mediated activation of AMPK, however, it is not known whether LA supplementation facilitates mitochondrial biogenesis, and/or inhibits protein loss in aged mice. Therefore, we hypothesized that α-LA improves energy metabolism and mitochondrial biogenesis by enhancing AMPK-PGC-1α signalling in the skeletal muscle of aged mice. Our objectives in the present study are to determine whether LA-stimulated energy expenditure and protein loss are mediated by activating the AMPK-PGC-1α signalling pathway in the skeletal muscle of aged mice.

[4]**Abbreviations used:** AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; DXA, dual-energy X-ray absorptiometry; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; GLUT-4, glucose transporter-4; GTT, glucose tolerance test; LA, lipoic acid; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; nDNA, nuclear DNA; PGC-1α, peroxisome proliferator–activated receptor-γ coactivator-1α

Methods

Experiment procedures

All experiments were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. The C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were fed *ad libitum* (standard rodent diet # 2920, Harlan Teklad) and given free access to water for 24 months. Individual male mice (at the age of 24 months) were provided with water supplemented with 0% (n=10, as control group) or 0.75% α-lipoic acid (Sigma-Aldrich, St. Louis, MO; n=10, as treatment group) for 1 month. Body weight was recorded weekly. During the 3rd week of treatment, food intake of mice was recorded daily for one week. After 1 month the mice were killed under isoflurane anaesthesia, and tissues were taken and frozen immediately in liquid nitrogen.

Body composition

During the 4th week of treatment, the mice in the fed status were anesthetized with isoflurane shortly, and then body composition was assessed *in vivo* by dual-energy X-ray absorptiometry (DXA; Lunar, Madison, WI). Body fat mass, lean mass and bone density were analyzed using GE Lunar PIXImus software version 1.45 (Lunar, Madison, WI).

Indirect calorimetry

During the 4th week of treatment, the mice were housed in individual chambers at 24 ± 1°C with free access to food and water with/without 0.75% α-LA. Oxygen consumption and CO_2 production were measured individually over 48 h using the Columbus Instrument Oxymax System (Columbus Instruments, Columbus, OH). Two time points were sampled per hour. Energy expenditure was calculated using the formula provided by the manufacturer: energy expenditure (kcal) = $(3.815 + 1.232VO_2/VCO_2)\cdot VO_2$.

Glucose tolerance test

Glucose tolerance test (GTT) was performed during the 4th week of treatment. The mice were fasted for 16 h and then were i.p. injected at 2.0 g D-glucose/kg BW. Blood samples were taken at 0, 30, 60 and 90 min post glucose administration. Blood glucose concentrations were measured by the glucose oxidase method using an Ultrasmart™ Glucose Meter (Life-Scan, Milpitas, Canada). The area under the curve was calculated by an integration method.

RNA extraction and quantitative RT-PCR

Total RNA was isolated from skeletal muscles (gastrocnemius muscles) and C2C12 cells using Trizol reagent (Invitrogen, Carlsbad, CA). RNA concentration was determined by NanoDrop 1000 (NanoDrop products, Wilmington, DE). Reverse transcription was performed with 1 μg of RNA as template using SuperScript-III-First-Strand-Synthesis-SuperMix kit (Invitrogen, Carlsbad, CA). An abundance of mRNA was quantified by real-time qRT-PCR. Primers and probes for PGC-1α were based on GenBank Accession No. NM_008904.1 as follows: Forward, 5′-AGAAGCGGGAGTCTGAAAGG-3′; Reverse, 5′-CAGTTCTGTCCGCGTTGTG-3′; Probe, 5′-FAM-AGAAAGCAATTGAAGAGCGCCGTGTG-TAMRA-3′. Primers for GLUT-4 were based on GenBank Accession No. NM_009204 as follows: Forward, 5′-ATGGCTGTCGCTGGTTTCTC-3′; Reverse, 5′-ACCCATGCCGACAATGAAGT-3′; Probe, Sybergreen (Bio-Rad, Hercules, CA). The housekeeping gene 18S ribosomal RNA was not altered, and thus used as an internal control in the study. Assays were performed in triplicate with an ABI Prism 7900 sequence detector (Applied Biosystems Inc., Foster City, CA). Data were normalized to 18S ribosomal RNA ($\Delta\Delta C_T$ analysis).

Mitochondrial biogenesis

Mitochondrial biogenesis was determined by the ratio of mitochondrial DNA (mtDNA) to nuclear DNA (nDNA) contents quantified by real-time qPCR as described [26] assuming that nuclear DNA remains constant. DNA was extracted from gastrocnemius muscles samples using UltraPure™ Phenol:Chloroform:Isoamyl Alcohol (25:24:1)(Invitrogen) followed by ethanol precipitation. For each DNA extract, the nuclear gene for ribosomal protein large p0 and the mitochondrial gene cytochrome c oxidase subunit I (CoxI) were quantified individually by real-time qPCR. The specific primers were used as follows: p0 forward, 5′-GCACTTTCGCTTTCTGGAGGGTGT-3′; p0 reverse, 5′-TGACTTGGTTGCTTTGGCGGGATT-3′; CoxI forward, 5′-TCTACTATTCGGAGCCTGAG-3′; and CoxI reverse, 5′-CTACTGATgCTCCTGCATGG-3′. Sybergreen was used as the probe. Data were normalized to the nuclear gene p0 DNA ($\Delta\Delta C_T$ analysis).

Protein extraction and western blotting

Proteins were extracted from mouse gastrocnemius muscles and C2C12 cells. Muscle samples were homogenized on ice in RIPA buffer (50 mM Tris-HCl at pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM sodium orthovanadate, 1mM sodium fluoride) and centrifuged at 10,000 g for 15 min at 4 °C. Protein concentration was determined using BCA protein assay kit (Pierce, Rockford, IL) using BSA as a standard. Samples were boiled at 100 °C for 10 min in 2× sample buffer. Total protein (100 μg per sample) was loaded per lane and electrophoresed in running buffer on a 7.5 ~ 12% Tris-glycine SDS-Polyacrylamide gel. Following the SDS PAGE, proteins were transferred to nitrocellulose membrane. After blocking, the membrane was incubated with primary antibodies. Primary antibodies were purchased from Cell Signalling Technology (Danvers, MA): phosphor-AMPK (Thr^{172}; 1:1000), phospho-mTOR (Ser^{2448}; 1:750), phospho-p70 S6K1 (Thr^{389}; 1:500), phospho-4E-BP1 ($Thr^{37/46}$; 1:500), total-AMPK (1:1000), total-mTOR (1:1000), total-p70 S6K1 (1:500), and total-4E-BP1 (1:500). After washing, the membrane was incubated with anti-rabbit IgG horseradish-peroxidase-conjugated secondary antibody (Bio-Rad, 1:3000), and reacted with ECL-Plus chemiluminescent detection HRP reagents (Amersham Biosciences, Piscataway, NJ). Western blot images were scanned and analyzed on a Storm 860 PhosphorImager (GE Healthcare). The densitometry of phosphorylatedprotein was normalized to its total protein.

Cell culture

The mouse myoblastoma C2C12 cells were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in six-well plates and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (Invitrogen), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were allowed to differentiate in DMEM with 2% horse serum, 100 U/ml penicillin, and 100 μg/ml streptomycin for 3 days before treatment. Each treatment was repeated at least three times. After treatment as indicated, cells were washed twice with PBS prior to protein extraction with the RIPA buffer, or to RNA extraction using Trizol reagent. The samples were then applied for western blotting or real-time qRT-PCR.

[^{3}H]-phenylalanine incorporation assay

Protein synthesis was determined by [^{3}H]-phenylalanine incorporation assay [27]. In brief, C2C12 myotubes were grown in 6-well plates and changed to serum-free DMEM for 1 h. C2C12 cells were then treated with α-LA at 1 mM for 0.5, 2, or 24 h. The stimulated cells were pulsed with 2 μCi/ml [^{3}H]-phenylalanine (Sigma-Aldrich) for additional 1 h prior to harvest.

After washed with ice-cold PBS, the cells were scraped in 0.5 ml PBS, lysed and precipited in 0.5 ml 20% trichloroacetic acid for 30 min on ice, and centrifuged at 10,000 g for 15 min at 4 °C. The pellets (precipited proteins) were washed twice with 10% trichloroacetic acid, and solubilised in 1 ml of 0.3 N NaOH for 1 h. An aliquot (400 μl) was taken to determine the incorporated radioactivity by liquid scintillation counter (Beckman LS 3801, Fullerton, CA). Incorporation rate of [^{3}H]-phenylalanine into total protein was expressed as DPM/μg protein mass and considered as protein synthesis.

[^{3}H]-2-deoxyglucose uptake

Glucose uptake was determined by [^{3}H]-2-deoxy-glucose uptake with modification [28]. In brief, the [^{3}H]-2-deoxy-glucose uptake assay was performed with six-well plates. C2C12 myotubes were washed twice with DMEM, starved for 1 h in serum-free DMEM at 37 °C, and then treated with α-LA at 0 ~ 2 mM. Cells were washed twice with KRH buffer (136 mM NaCl, 4.7 mM KCl, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$, 20 mM HEPES [pH 7.4]) without glucose, and then assayed for the glucose uptake for 20 min at 0.5 mM 2-deoxy-glucose containing 1 μCi/ml [^{3}H]-2-deoxy-glucose (Amersham Biosciences). Nonspecific uptake was determined with the presence or absence of 5 μM cytochalasin B. The cells were then solubilised in 1 ml of 0.3 N NaOH for 30 min before aliquots were taken for liquid scintillation counting. Measurements for glucose uptake were corrected for nonspecific uptake. Glucose uptake was expressed as DPM/μg protein mass.

Fatty acid β-oxidation

Fatty acid β-oxidation was determined by [14C]-palmitate assay with modification [29, 30]. C2C12 myotubes were grown in T-25 cm^2 flasks, starved for 1 h in serum-free DMEM, treated with α-LA at 1 mM for 6 h, and then incubated with 2% BSA and 0.4 mM palmitate (plus 0.25 μCi/ml [^{14}C]-palmitate (Amersham Biosciences)) for 1 h, and finally quenched by adding 2 ml of 6 M HCl. The release of $^{14}CO_2$ was trapped with 300 μl of 1 N NaOH. The trapped $^{14}CO_2$ were determined by liquid scintillation counting.

Statistical analysis

Data (body composition, metabolic action, relative abundance of PGC-1α and GLUT-4 mRNA, relative abundance of mtDNA, and phosphorylation of proteins (AMPK, mTOR, P70S6, and 4E-BP1)) were analyzed by ANOVA. Data (glucose concentration, O_2 consumption, CO_2 production, energy expenditure, and respiratory quotient) were analyzed by repeated measures ANOVA using the MIXED Procedure (SAS Version 9.1, SAS Institute Inc., Cary, NC), in which time of sampling was considered as the repeated effect. The relationship between α-LA dose and glucose uptake was fitted by an exponential regression model. Data were expressed as means ± SEM. *P* values < 0.05 or 0.01 were considered as statistical significance.

Results

Food intake and body composition

Daily intake of food was decreased ($P < 0.05$) by 18% in LA-treated mice (4.50 ± 0.30 g/d) compared with that (5.50 ± 0.30 g/d) in control mice. Over the period of four weeks, LA-treated mice lost ($P < 0.05$) body weight of 5.27 ± 0.62 g (an equivalent to 15.8% of initial body weight) while the control mice maintained their body weight. The LA-treated mice had less adipose as well as lean mass (Fig. 1A). Therefore, the LA-treated mice had lower percentage of fat mass (Fig. 1B), but higher percentage of lean and bone mass.

Energy expenditure

LA-treated mice had higher oxygen consumption (25.7 ± 0.35 mL/kg$^{0.75}$/min) and CO_2 production (23.9 ± 0.37 mL/kg$^{0.75}$/min) than control mice (20.2 ± 0.35 and 17.2 ± 0.37 for O_2 consumption and CO_2 production, respectively)(Fig. 2A and 2B). Note that the difference between two groups was significant ($P < 0.01$) at individual time points in Fig. 2. Calculated energy expenditure was higher (7.64 ± 0.10 kcal/kg$^{0.75}$/h) in the LA-treated mice than that (5.90 ± 0.10 kcal/kg$^{0.75}$/h) in the control mice (Fig. 2C). Calculated respiratory quotient (i.e., ratio of VCO_2 to VO_2) was higher (0.93 ± 0.01) in the LA-treated mice than that (0.85 ± 0.01) in the control mice. However, the difference for respiratory quotient was even more profound during the inactive (light) phase (Fig. 2D), indicating that LA stimulated more glucose utilization.

Glucose tolerance

After glucose challenge in overnight fasted mice, blood glucose concentrations were lower at 30 and 60 min ($P < 0.01$) during a GTT in the LA-treated group than those in the control group (Fig. 3A). Moreover, the area under curve was dramatically reduced by 47% ($P < 0.01$) in the LA-treated mice (Fig. 3B). These results suggest that LA not only stimulated glucose utilization, but also increased insulin sensitivity.

LA stimulates PGC-1α and GLUT-4 gene expression in skeletal muscle

The mRNA abundance for PGC-1α in the skeletal muscle was increased ($P < 0.05$) by 80.0% in the LA-treated mice compared to that in the control mice (Fig. 4A). Interestingly, the mRNA abundance for GLUT-4 in the skeletal muscle was increased ($P < 0.01$) by 105.0% in the LA-treated mice as well (Fig. 4A).

AMPK and mTOR signalling in the skeletal muscle

To determine whether AMPK accounts for LA-induced metabolic changes, we measured AMPK activation. AMPK phosphorylation at Thr^{172} increased ($P < 0.05$) by 107.5 % in response to LA treatment (Fig. 4B). In contrast to AMPK, mTOR phosphorylation at Ser^{2448} was decreased by 31.5 % and followed by a reduction of p70S6 kinase phosphorylation at Thr^{389} by 43.0 %. However, there was no difference ($P > 0.05$) in 4E-BP1 phosphorylation at Thr$^{37/46}$ between the two groups.

Mitochondrial biogenesis in the skeletal muscle

The relative abundance of mtDNA content (indicated by the mitochondrial gene cytochrome c oxidase subunit I) in the skeletal muscle was increased ($P < 0.05$) by 138% in the LA-treated mice compared to that in the control mice (Fig. 5).

Glucose uptake in C2C12 myotubes

We next evaluated LA-stimulated glucose uptake in C2C12 myotubes using [^{3}H]-deoxy-glucose assay (Fig. 6A). LA dose-dependently stimulated glucose uptake ($P < 0.01$). Significant stimulation was observed at 50 μM ($P < 0.01$), and a maximal stimulation was achieved at 1,000 μM ($P < 0.01$). The LA dosage was estimated at 512.4 μM for glucose uptake plateau.

Fatty acid β-oxidation in C2C12 myotubes

To further establish whether LA affects fatty acid β-oxidation, we quantified 14C-CO_2 released from [^{14}C]-palmitate oxidation upon LA stimulation in C2C12 myotubes. LA at 1000 μM for 6 h increased ($P < 0.05$) fatty acid β-oxidation by 33.2% compared with the control (Fig. 6B).

Protein synthesis in C2C12 myotubes

Lean mass was decreased in the LA-treated mice, which might be attributed to decreased protein synthesis and/or increased protein degradation. Therefore, we determined whether LA affected protein synthesis in C2C12 myotubes. The time course indicated that at least 24-h time of LA treatment (1 mM) was needed to suppress protein synthesis (data not shown). LA at 1,000 μM for 24 h significantly inhibited (by 24%, $P < 0.01$) [^{3}H]-phenylalanine incorporation into total protein in C2C12 myotubes (Fig. 6B).

Gene expression in C2C12 myotubes

The mRNA abundance for PGC-1α was increased ($P < 0.01$) by 2.1 folds in the LA-treated C2C12 myotubes compared to that in the control (Fig. 7). Furthermore, the mRNA abundance for GLUT-4 was increased ($P < 0.01$) by 13.7 folds in the LA-treated C2C12 myotubes as well (Fig. 7).

AMPK and mTOR signalling in C2C12 myotubes

To further investigate LA-mediated metabolic action and cellular signalling, we explored LA-mediated effects in C2C12 myotubes. LA treatment (100 ~ 2000 μM for 2 h; or 1,000 μM for 0.5, 2, and 24 h) stimulated phosphorylation of AMPKα at Thr^{172} in a dose- and time-dependent manner in C2C12 myotubes (Fig. 8). AMPK activation reached its maximum at 2 h post LA treatment. Phosphorylation of mTOR at Ser^{2448} and its downstream target P70S6 kinase at Thr^{389} was stimulated at 0.5 h post LA treatment, but was inhibited later on. This inhibited phosphorylation of mTOR and p70S6 kinase was correlated in a time-dependent manner with increased AMPK activity (Fig. 8). Prior to activation of AMPK, phosphorylation of mTOR and p70S6 kinase was increased. After activation of AMPK, however, time- and dose-dependent decreases in mTOR and p70S6 kinase phosphorylation were observed in the LA-treated C2C12 myotubes. However, 4E-BP1 phosphorylation was not altered by time or dosage.

Discussion

In the present study, we demonstrated that oral intake of α-LA improved body composition, glucose tolerance, and energy expenditure of the aged mice, which was associated with increased mitochondrial biogenesis in the skeletal muscle. It is well known that the aging in humans is associated with increased fat mass, declined lean mass [31, 32], and decreased basal metabolic rate [33]. Previous studies have demonstrated that dietary LA increases whole-body energy expenditure in adult mice [34]; however, our data are the first to show that LA had the same metabolic action in aged mice. Moreover, this LA-mediated effect on energy metabolism appears to be mediated partially by increased mitochondrial biogenesis in the skeletal muscle. As a result, oral intake of LA might decrease body fat accumulation and increase glucose utilization in the aging mice. However, it should be pointed out that oral intake of LA also increased body protein loss possibly by decreasing protein synthesis. Overall, LA might facilitate the whole-body energy catabolism and glucose utilization in the aged mice.

One of our major findings in the present study is that oral intake of LA increased mitochondrial biogenesis in the skeletal muscle. Brown adipose tissue in small mammals makes the largest contribution towards adaptive thermogenesis. Since humans have relatively little brown fat, however, this effect might thus have limited clinical relevance. In contrast, the skeletal muscle in large adult mammals is a major determinant of energy expenditure [35]. Thus, it is critical to establish whether LA has any beneficial outcomes in the skeletal muscle. In agreement with previous reports [36], our data showed that LA activated AMPK in the skeletal muscle. As a cellular energy sensor, activation of AMPK in peripheral tissues stimulates glucose uptake through initiated GLUT-4 translocation and enhanced GLUT-4 expression [37, 38]; and increases

fatty acid oxidation by inhibiting the activity of acetyl coenzyme A carboxylase [39, 40]. We note that peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) is not only a key transcriptional coactivator, but also a major regulator of energy metabolism [41, 42]. Forced over-expression of PGC-1α in the skeletal muscle dramatically increases mitochondrial respiration and elevates GLUT-4 transcript levels [43]. In contrast, PGC-1α deficient mice have diminished mitochondrial number and respiratory capacity in slow-twitch skeletal muscle [44]. Notably, PGC-1α expression is decreased in the skeletal muscle of aged mice [45, 46]. In the present study, we show that LA increases mRNA expression of PGC-1α and GLUT-4 in the skeletal muscle of aged mice, which might account for improved glucose homeostasis. Moreover, LA increases the skeletal muscle mitochondrial respiration in aged rats [47]. We note that caloric restriction has shown to increase longevity and mitochondrial biogenesis [48]. Thus, increased biogenesis of mitochondria could be attributed to LA-specific metabolic action and decreased intake of energy as well. In addition to suppression of AMPK activity in the hypothalamus, however, LA-increased energy expenditure is partially through augmentation of AMPK activity in the skeletal muscle, which increases energy fuel (substrate) oxidation and results in reduced whole-body adiposity. Our data on cultured C2C12 cells not only support this notion, but also agree with those published previously [49]. Most recently, AMPK has been shown to stimulate mitochondrial biogenesis through up-regulating PGC-1α [50]. Therefore, it appears that LA-mediated metabolic phenomenon is mediated partially through activation of AMPK-PGC-1α signalling in the skeletal muscle.

Lipoic acid-mediated loss of body weight and improvement in glucose tolerance might be related to suppression of food intake. The evidence that LA stimulated energy expenditure even under suppression of food intake, however, indicates LA-mediated metabolic effects probably independent of reduced food intake in the present study. It should be pointed out that LA-mediated metabolic effects might be dose dependent. As demonstrated previously [51], daily intake of dietary LA (0.5~1%, equivalently to ~500 mg/kg $BW^{0.75}$) not only inhibits food intake, but also stimulates energy expenditure in pair-fed, three-week treatments. In the present study, daily intake of water was estimated at 5.5 mL/d (=$BW^{0.667}$ × 60 mL/kg $BW^{0.667}$/d)[52]. Thus, the daily intake of LA through 0.75% in drinking water would be approximate at 550 mg/kg $BW^{0.75}$. AMPK, as a metabolic “fuel gauge” that oscillates between anabolic and catabolic metabolism, integrates nutritional and hormonal signals to regulate food intake and energy metabolism. Dominant negative AMPK expression in the hypothalamus is sufficient to reduce food intake and body weight, whereas constitutively active AMPK increases both [53]. Importantly, energy expenditure is increased not only by stimulation of AMPK activity in peripheral tissues but also suppression of it in the hypothalamus. Note that the hypothalamus coordinates signals from peripheral tissues to control energy homeostasis. It seems that LA-mediated increase in the whole-body energy expenditure might be mainly due to its modulation of the hypothalamic function. In addition to direct effects on the muscle, LA inhibited the hypothalamic activity of APMK to promote negative energy balance [54]. However, further studies are warranted to define LA-mediated central vs. peripheral actions using tissue-specific AMPK deficiency mouse models. Therefore, LA-mediated metabolic effects in the present study might not only result from suppression of food intake, but also stimulation of energy expenditure, which is regulated by LA-mediated AMPK signaling in the hypothalamus and peripheral tissues (such as muscle, adipose tissue, and liver). Notably, there was a reduction in lean mass in LA-treated mice. Due to a greater loss in fat mass, however, lean mass expressed as the percentage of body composition actually increased in the LA-treated mice. It is unclear whether LA-mediated loss of lean mass resulted from increased rates of protein degradation and/or decreased rates of protein synthesis. The mammalian target of rapamycin (mTOR, a nutrient sensor) regulates cell growth and survival and controls protein synthesis. Phosphorylation of both mTOR and p70S6 kinase decreased in the skeletal muscle of the LA-treated mice, probably indicating decreased protein synthesis. In consistent with this, protein synthesis was suppressed in LA-treated C2C12 cells. Importantly, activation of AMPK by

AICAR appears to suppress protein synthesis in rat skeletal muscle through down-regulating mTOR signalling [55, 56]. Thus, LA might suppress protein synthesis in the skeletal muscle, probably resulting from activated AMPK-mediated suppression of the mTOR signalling pathway. Further studies are warranted to establish how LA down-regulates the mTOR signalling involved in protein and energy metabolism to optimise a LA-based treatment for the management of the metabolic syndrome, which might offer the maximum benefit on energy expenditure and glucose metabolism with the minimum loss of lean body mass.

Body fat mass (especially visceral fat) tends to increase with increasing age, while physical activity energy expenditure tends to decrease. Reduced levels of physical activity energy expenditure might be attributed to obesity in the older persons. LA improved glucose tolerance and energy expenditure (possibly through increasing mitochondrial biogenesis in the skeletal muscle), suggesting that oral intake of LA be able to restore reduced oxidative phosphorylation in the older persons. Thus, the results here may provide further insight into LA-mediated metabolic benefits and potential treatment in older patients who suffer from obesity and/or insulin resistance. However, it would be better to compare LA-mediated beneficial, metabolic effects on aged animals with those on young ones. Thus, a lack of this comparison is a pitfall in the present study. A complete factorial design, i.e., 2 ages (young vs. old) × 2 treatments (vehicle vs. LA) should be employed in future studies to address LA-mediated age-specific beneficial effects if any.

In summary, our present study has demonstrated that LA increased energy expenditure in the skeletal muscle of aged mice partially through enhancing AMPK-PGC-1α-mediated mitochondrial biogenesis and function. Moreover, LA increases lean mass loss possibly by suppressing protein synthesis in the skeletal muscle, which might be attributed to LA-activated AMPK-PGC-1α-mediated down-regulation of the mTOR signalling pathway. With beneficial metabolic actions, LA may be a promising supplement for treatment of obesity and/or insulin resistance in older patients.

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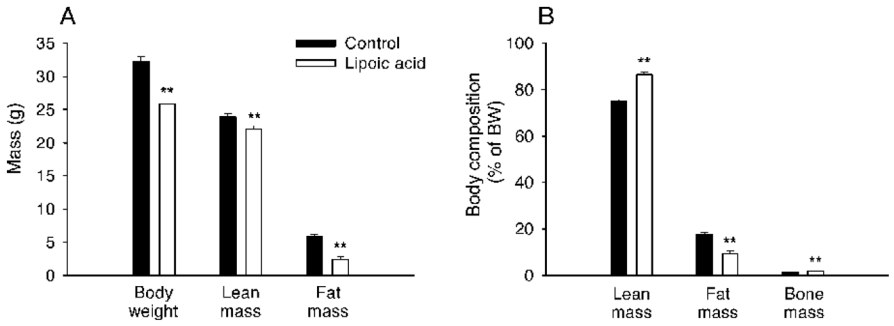

FIG. 1.

Lipoic acid improved body composition in the aged mice. A: Lipoic acid-treated mice had significantly lower final body weight, fat mass, and lean mass than the control mice. B: lipoic acid decreased body proportion of fat mass, but increased those of lean mass and bone mass. The data were analyzed by ANOVA and expressed as mean ± SEM (n = 10 per group). *P < 0.05; **P < 0.01.

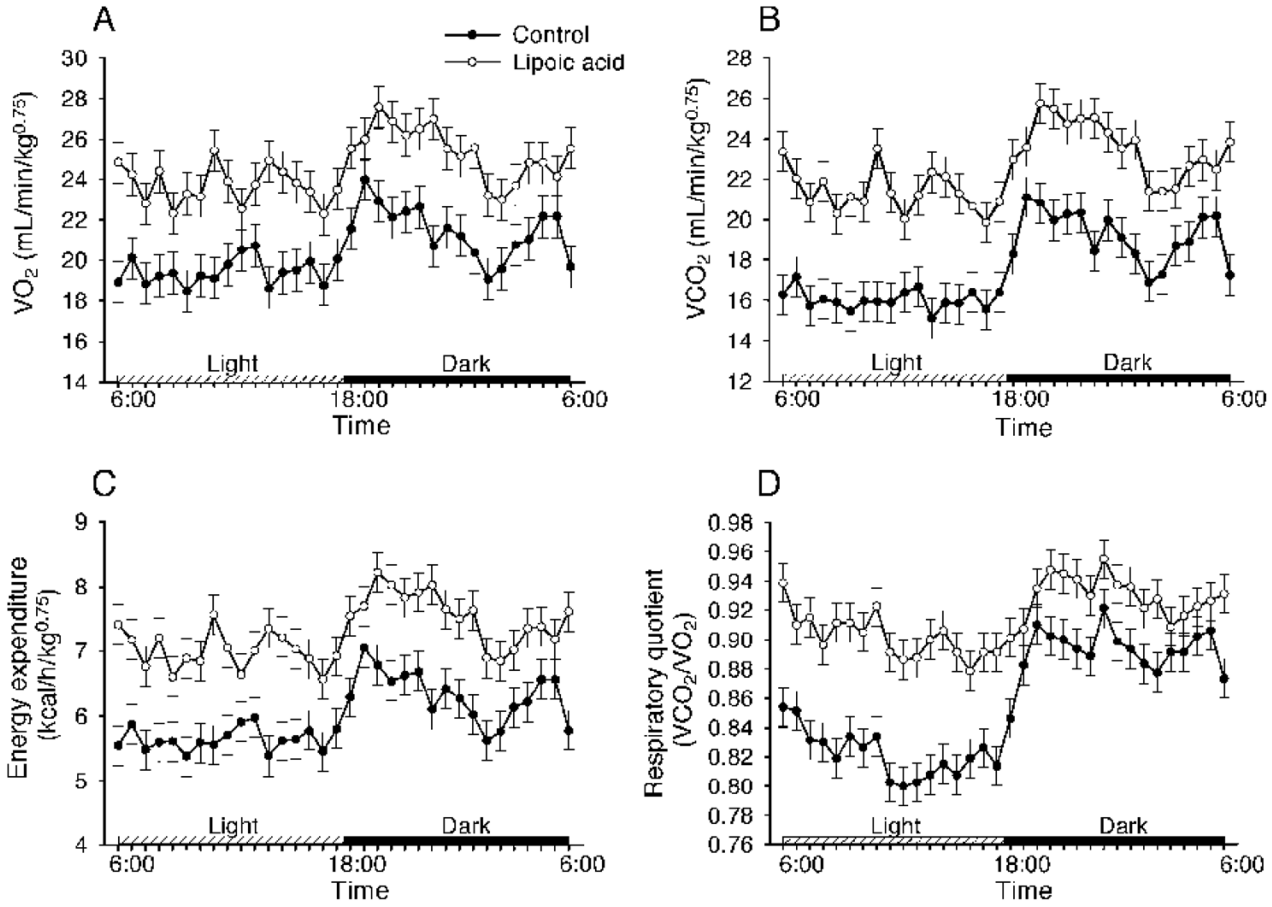

FIG. 2.

Lipoic acid increased energy expenditure in the aged mice. Energy expenditure was measured with a 30-min interval for 48 h during the 4th week of lipoic acid treatment. A: Lipoic acid treatment increased O_2 consumption compared with the control. B: Lipoic acid treatment increased CO_2 production compared with the control. C and D: Energy expenditure and respiratory quotient were significantly higher in the lipoic acid-treated mice than those in the control mice during the light and dark cycles and at each sampling time point. Data were analyzed by repeated measures ANOVA using the MIXED Procedure, and expressed as means ± SEM (n = 10 per group). Note that significance symbols (** for $P < 0.01$) are not shown.

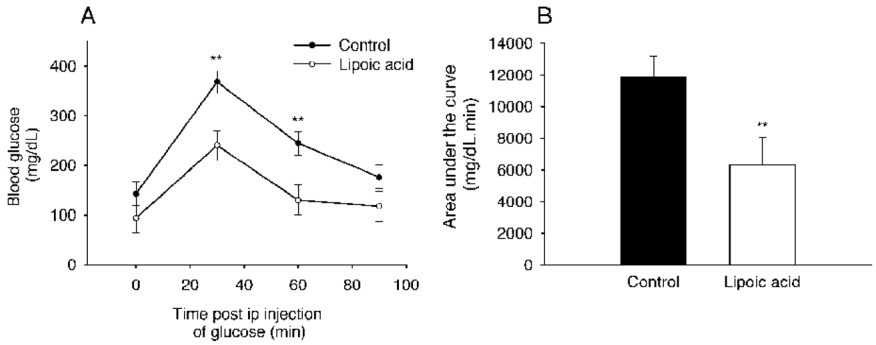

FIG. 3.

Lipoic acid increased glucose tolerance in the aged mice. Glucose tolerance test was performed in the aged mice fasted for 16 h. D-glucose was administered intraperitoneally at 2 g/kg BW. A: After glucose load, the lipoic acid-treated mice had faster clearance than the control mice. However, no significant difference in basal levels of blood glucose was observed between the groups. B: There was a significant reduction in area under the curve in the lipoic acid-treated mice compared with that in the control mice. Data are presented as mean ± SEM (n = 6 per group). *P < 0.05; **P < 0.01.

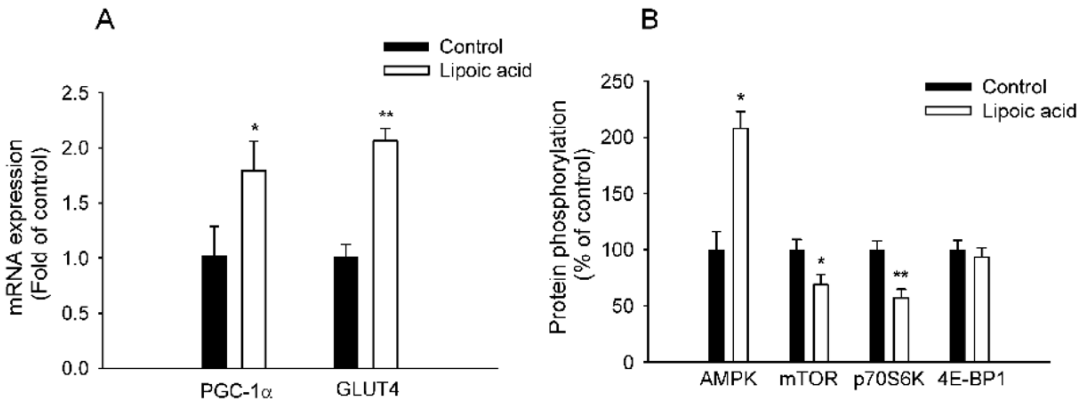

FIG. 4.

Lipoic acid altered gene and protein expression in the skeletal muscle. A: Gene expression was measured by real-time qRT-PCR and expressed in terms of mRNA levels relative to 18S rRNA. The lipoic acid-treated mice had significantly elevated mRNA levels of PGC-1α and GLUT-4 in the skeletal muscle. B: Lipoic acid increased AMPK activation with decreased phosphorylation of mTOR and p70S6K; however, it did not significantly alter 4E-BP1 phosphorylation. Data are presented as mean ± SEM (n = 6 per group). *P < 0.05; **P < 0.01.

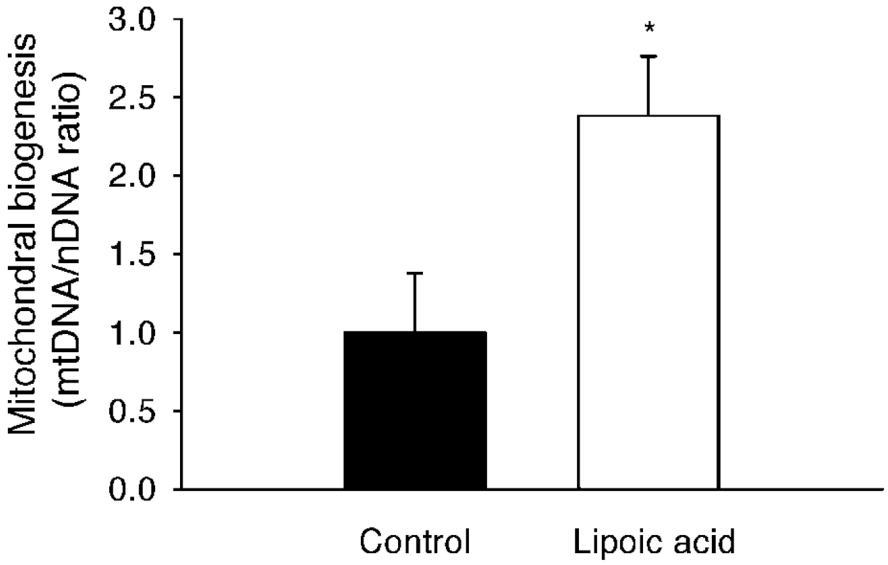

FIG. 5.

Lipoic acid improved mitochondrial biogenesis in the skeletal muscle. Mitochondrial biogenesis was determined by the ratio between mitochondrial and nuclear DNA contents. The abundance of mitochondrial gene cytochrome c oxidase subunit I (CoxI) was quantified by real-time qPCR and expressed in relative to that of the nuclear gene ribosomal protein large p0. Data are presented as mean ± SEM (n = 6 per group). *$P < 0.05$.

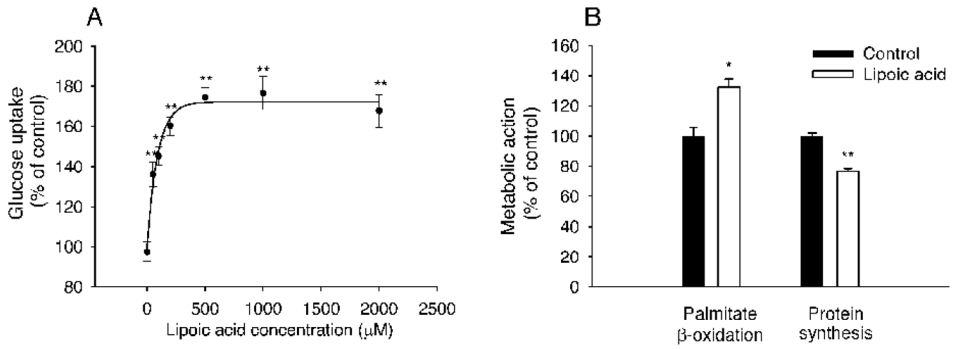

FIG. 6.

Lipoic acid altered energy metabolism and protein synthesis in differentiated C2C12 cells. A: Lipoic acid dose-dependently increased glucose uptake compared with the control. B: Lipoic acid at 1000 μM increased fatty acid oxidation and decreased protein synthesis compared with the control. Each treatment was repeated at least three times. Data are presented as mean ± SEM. **P* < 0.05; ***P* < 0.01.

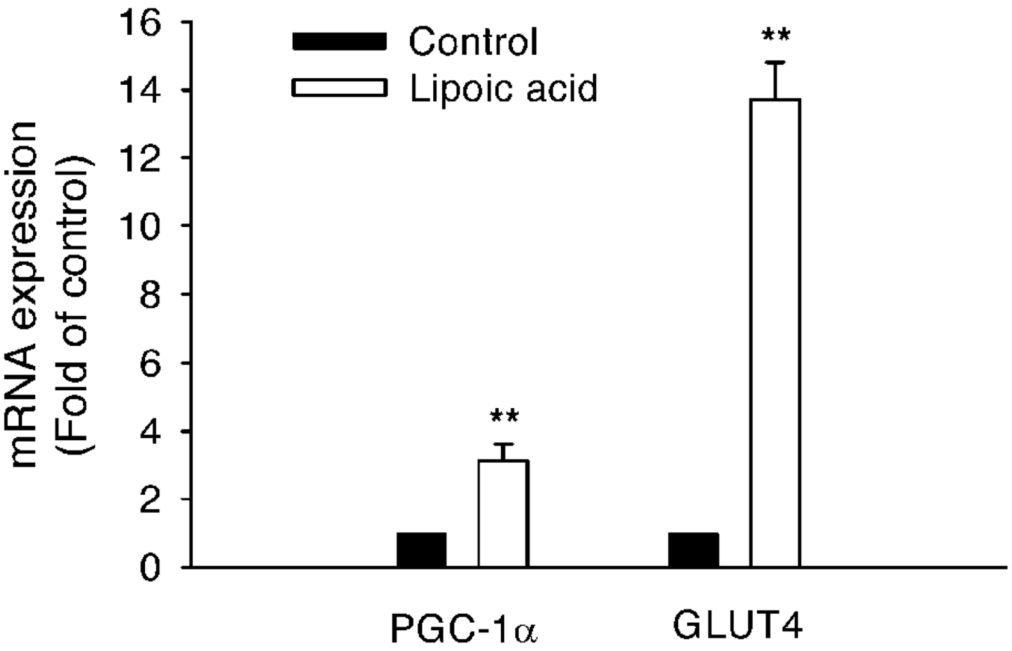

FIG. 7.

Lipoic acid altered gene expression in differentiated C2C12 cells. Lipoic acid at 1000 μM for 16 h induced gene expressions in differentiated C2C12 cells. Gene expression was measured by real-time qRT-PCR and expressed in terms of mRNA levels relative to 18S rRNA. Lipoic acid stimulation had significantly elevated mRNA levels of PGC-1α and GLUT-4. Each treatment was repeated at least three times. Data are presented as mean ± SEM. $^{**}P < 0.01$.

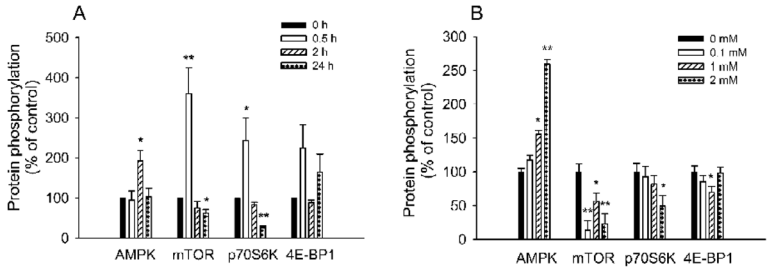

FIG. 8.

Lipoic acid altered protein phosphorylation in a time- and dose-dependent manner in differentiated C2C12 cells. A: Lipoic acid at 1000 μM increased AMPK activation after 2 h treatment; increased phosphorylation of mTOR and p70S6K at 0.5 h; but decreased them after 24-h treatment. However, it did not significantly alter 4E-BP1 phosphorylation. B: Lipoic acid dose-dependently increased AMPK activation with decreased phosphorylation of mTOR and p70S6K; however, it did not significantly alter 4E-BP1 phosphorylation. Each treatment was repeated at least three times. Data are presented as mean ± SEM. *P < 0.05; **P < 0.01.