(*R*)-α-Lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate

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ABSTRACT **A diet supplemented with (*R*)-lipoic acid, a mitochondrial coenzyme, was fed to old rats to determine its efficacy in reversing the decline in metabolism seen with age. Young (3 to 5 months) and old (24 to 26 months) rats were fed an AIN-93M diet with or without (*R*)-lipoic acid (0.5% w/w) for 2 wk, killed, and their liver parenchymal cells were isolated. Hepatocytes from untreated old rats vs. young controls had significantly lower oxygen consumption ($P<0.03$) and mitochondrial membrane potential. (*R*)-Lipoic acid supplementation reversed the age-related decline in O_2 consumption and increased ($P<0.03$) mitochondrial membrane potential. Ambulatory activity, a measure of general metabolic activity, was almost threefold lower in untreated old rats vs. controls, but this decline was reversed ($P<0.005$) in old rats fed (*R*)-lipoic acid. The increase of oxidants with age, as measured by the fluorescence produced on oxidizing 2′,7′-dichlorofluorescin, was significantly lowered in (*R*)-lipoic acid supplemented old rats ($P<0.01$). Malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were increased fivefold with age in cells from unsupplemented rats. Feeding rats the (*R*)-lipoic acid diet reduced MDA levels markedly ($P<0.01$). Both glutathione and ascorbic acid levels declined in hepatocytes with age, but their loss was completely reversed with (*R*)-lipoic acid supplementation. Thus, (*R*)-lipoic acid supplementation improves indices of metabolic activity as well as lowers oxidative stress and damage evident in aging.—Hagen, T. M., Ingersoll, R. T., Lykkesfeldt, J., Liu, J., Wehr, C. M., Vinarsky, V., Bartholomew, J. C., Ames, B. N. (*R*)-α-Lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *FASEB J.* 13, 411–418 (1999)**

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AGING IS A MULTIFACTORIAL PROCESS that leads to loss of function and the inability to adequately respond to external stress. Mitochondrial dysfunction appears to contribute to some of the loss of function accompanying aging (1, 2). Mitochondria from aged tissue use oxygen inefficiently, which impairs ATP synthesis and results in increased oxidant production (3, 4). The high flux of oxidants not only damages mitochondria, but other important cell biomolecules as well. Antioxidant defenses also decline with age (5, 6), making mitochondria even more susceptible to oxidative injury (1). The resultant mitochondrial decay may eventually cause inadequate energy production and/or the loss of calcium homeostasis. Such changes could result in unwarranted cellular apoptosis and also lead to the general metabolic decline evident in aging.

Lipoic acid is a disulfide compound found naturally in mitochondria as the coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. It has been used as therapy for many diseases associated with impaired energy utilization, such as type II diabetes (7) and diabetic polyneuropathies (8, 9). Dietary supplementation also increases unbound lipoic acid, which can act as a potent antioxidant and ameliorate oxidative stress both *in vitro* and *in vivo* (10–15). To a degree, aging results in the same type(s) of metabolic impairment and increased oxidative stress as shown in these conditions.

Though its ability to improve energy metabolism (16) and lower oxidative stress (11–15) for certain disease states has been described, it is not known whether lipoic acid supplementation may also re-

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[4] Abbreviations: ALCAR, acetyl-L-carnitine; GSH: glutathione; HPLC, high-performance liquid chromatography; DCFH: 2′,7′-dichlorofluorescin diacetate; MDA: malondialdehyde; R123, rhodamine 123.

verse energy-linked metabolic deficits or reduce the increased oxidative stress seen in aging. The purpose of this study was twofold: to *1*) determine whether (*R*)-lipoic acid supplementation increased cellular and general metabolic activity in old rats, and *2*) examine whether this supplementation affected hepatocellular antioxidant status, oxidant production, and oxidative damage.

We show that supplementing old rats with 0.5% (w/w) lipoic acid for 2 wk partially reverses the age-associated loss of mitochondrial function, an increase in oxidative stress, and the damage and decline in general metabolic activity.

MATERIALS AND METHODS

The following chemicals were used: EGTA (ethylene glycol-bis (β-aminoethyl ether) N,N, N′,N′-tetraacetic acid), 2,4-dinitrofluorobenzene, Trypan blue, heparin (sodium salt), rhodamine 123 (R123),[4] glutathione, reduced form (GSH), and dithiothreitol (Sigma, St. Louis, Mo.); 2′,7′-dichlorofluoroscin diacetate (DCFH) (Molecular Probes, Eugene, Oreg.); collagenase (type D) (Boehringer Mannheim, Indianapolis, Ind.); L-ascorbic acid, and *meta*-phosphoric acid (Fluka, Ronkonkoma, N.Y.). All other reagents were reagent grade or better. Double-distilled/deionized water was used throughout.

Animals

Rats (Fisher 344, virgin male, outbred albino), both young (3–5 months; Simonsen, Gilroy, Calif.) and old (24–26 months; National Institute of Aging animal colonies), were acclimatized in the Berkeley animal facilities for at least 1 wk prior to experimentation. The AIN-93M standard diet or one supplemented with 0.5% (w/w) (*R*)-lipoic acid, and water ad libitum was given throughout.

Cell isolation

Liver tissue was dispersed into single cells by collagenase perfusion (17). Cell number was assessed using a hemocytometer, and viability (typically greater than 90% in both age groups) was determined by Trypan blue exclusion.

Mitochondrial membrane potential

The average mitochondrial membrane potential in intact hepatocytes was measured by flow cytometry using R123 as the fluorescent probe (4). Hepatocytes (2.0×10^6 cells) were incubated with R123 (0.01 mg/ml) for 30 min at 37°C, then subjected to flow cytometry using an instrument constructed according to the design of Steinkamp et al. (18). Nonspecific light scatter was subtracted and cells showing a particular fluorescence were quantified.

Oxygen consumption studies

Hepatocellular oxygen consumption was analyzed using a YSI 5300 oxygen electrode and monitor (Yellow Springs, Ohio).

DCFH measurement

Formation of oxidants in cells were determined by assaying the fluorescence of 2′,7′-dichlorofluorescein, the oxidation product of DCFH (19). Quadruplicate samples were routinely analyzed. Fluorescence was monitored using a Cytofluor 2350 fluorescent measurement system (Millipore, Bedford, Mass.) using standard fluorescein filters and Cytocalc software. Oxygen consumption was measured and data were expressed as the fluorescence per μM O_2 consumed/10^6 cells.

GSH analysis

Reduced GSH was measured by high-performance liquid chromatography (HPLC) as described by Reed et al. (20). Briefly, cells were mixed with 5-sulfosalicylic acid [7.5% (w/v), final concentration] and the samples were spun for 1 min at 13,000 RPM in a microcentrifuge to remove denatured debris. An aliquot of the supernatant was added to 100 μl of 1M Trizma Base buffer (pH 8), followed by addition of 100 μl of 40 mM fresh aqueous iodoacetic acid (4 μmol). The reaction mixture was brought to pH 8 with $NaHCO_3$ and dinitrophenyl derivatives were made by addition of 500 μl of 2,4-dinitrofluorobenzene [1.5% (v/v) in absolute ethanol] and 100 to 200 μl of K_2CO_3. The resultant derivatives were separated on a 10 μm Ultrasphere-amine column (4.6 mm×25 cm) using a Waters HPLC system and solvents, as described (20). GSH was quantified relative to standards.

Ascorbic acid analysis

Total ascorbic acid quantification was performed after reduction with dithiothreitol, as described (21). The samples were placed in a chilled (2°C) auto sampler for analysis. The system used for separation was reversed-phase HPLC (Hewlett-Packard, Mountain View, Calif.) with coulometric detection (ESA Inc., Bedford, Mass.). The peak area corresponding to ascorbic acid was integrated using HP ChemStation software (Hewlett-Packard).

Malondialdehyde analysis

Lipid peroxidation was assayed using a recently developed sensitive and specific gas chromatography-mass spectrometry method for malondialdehyde (MDA) (22, 23). Briefly, the hepatocytes were lysed with phosphate-buffered saline containing 2.8 mM butylated hydroxytoluene and 1% sodium dodecyl sulfate, pH 7.4. The protein-bound MDA was hydrolyzed with H_2SO_4. MDA was converted into a stable derivative, using pentafluorophenyl hydrazine at room temperature, and the derivative was detected with a Hewlett Packard 5890 Series II gas chromatograph interfaced to a 5989 mass spectrometry system equipped with a J & W Scientific DBWAX capillary column (15 m×0.25 mm i. d., 0.25 μm film thickness) in the negative chemical ionization mode. The results were indexed with protein, which was measured with a modified Lowry method.

Ambulatory activity tests

Each night rats were moved from group housing to individual cages (48 cm l×25 w×20 h) at least 4 h prior to the quantification of ambulatory parameters. The room was on a 12 h light/dark cycle (lights on 6 AM to 6 PM). At 8 PM, a very low light illuminated the test subjects for video tracking. Quantification began at 9 PM and continued for 4 h. One hour later the low light was turned off and the room

remained in total darkness until 6 AM, when the standard light/dark cycle began. A video signal from a camera suspended directly above the individual cages was fed directly into a Videomex-V (Columbus Instruments, Columbus, Ohio) computer system running the Multiple Objects Multiple Zones software. The system quantified ambulatory activity parameters and was calibrated to report distance traveled in centimeters. In addition to total distance traveled, the time each subject spent in ambulatory (locomotor), stereotypic (grooming), and resting (nonmovement) activity was recorded by an IBM computer. No additional modifications (such as fur dying) were needed to continuously track the subjects. At 9 AM animals were removed from individual housing and returned to group housing. Results are shown as the mean centimeters traveled per hour ±SEM.

The ambulatory activity of each rat was recorded before lipoic acid supplementation and for two consecutive nights. After lipoic acid supplementation and for two consecutive nights, the same spontaneous locomotor parameters were determined. With this design, each rat acted as its own control. After measurement of ambulatory activity, some lipoic acid-supplemented animals were placed on an AIN-93M diet for three additional weeks and activity was again measured.

Statistical analysis

Statistical significance was determined using the paired Student's *t* test or one way analysis of variance. Results are expressed as the mean ±SEM. A *P* value of less than 0.05 was considered significant.

RESULTS

Effect of lipoic acid supplementation on metabolic activity

Hepatocellular oxygen consumption was monitored to assess age-related changes in mitochondrial activity and whether (*R*)-lipoic acid supplementation affected cellular metabolic rate. Oxygen consumption in hepatocytes isolated from old rats was only 59% of that of hepatocytes from young animals (**Table 1**). This significant decline ($P<0.03$) was completely reversed after a 2 wk feeding regimen of (*R*)-lipoic acid (Table 1). (*R*)-Lipoic acid supplementation had no significant effect on oxygen consumption in cells isolated from young rats.

TABLE 1. *Lipoic acid supplementation increases hepatocellular oxygen consumption in old rats*[a]

	O_2 Consumption (μmol O_2/min per 10^7 cells)	
	Unsupplemented	(*R*)-Lipoic acid supplemented
Young	480 ± 60 (5)	500 ± 48 (8)
Old	281 ± 27 (5)	533 ± 28 (8)

[a] Numbers are expressed as the mean ± SEM. N values are indicated in parentheses.

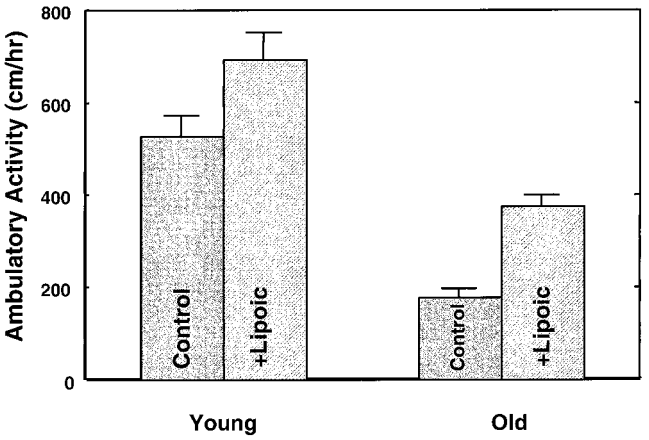

Figure 1. (*R*)-Lipoic acid supplementation increases ambulatory activity in old rats. Ambulatory activity was measured as distance traveled in young and old rats, fed with or without 0.5% (w/w) lipoic acid. Each bar represents the mean distance traveled (cm/h) ±SEM from 8 h of quantification as described in Materials and Methods. Distance was determined from the same young ($n=5$) and old ($n=5$) rats before and after (*R*)-lipoic acid treatment. Comparing old animals before and after lipoic acid supplementation: $P = 0.0005$. Comparing lipoic acid supplemented to unsupplemented young rats: $P = 0.06$.

The mitochondrial membrane potential in hepatocytes was measured using R123 fluorescence in order to test whether the lipoic acid-induced increase in O_2 consumption in hepatocytes from old rats was attributable to enhanced mitochondrial function. The average mitochondrial membrane potential in the majority of hepatocytes from old rats has previously been shown to be approximately 40% that of hepatocytes from young rats, a significant loss ($P<0.02$; N=8) of the driving force for ATP production (4). (*R*)-Lipoic acid supplementation caused the mitochondrial membrane potential to increase by 50.0% ±7.9 (N=4) over that of unsupplemented old rats, a marked improvement ($P<0.03$), but still significantly lower ($P<0.04$) when compared to cells from young untreated rats. Thus, (*R*)-lipoic acid supplementation partially improves mitochondrial function in old rats and may alleviate some loss of metabolic activity associated with aging.

To determine whether (*R*)-lipoic acid improved metabolic activity on a physiological basis, we quantified ambulatory activity in rats with and without lipoic acid treatment. Ambulatory activity declined almost threefold with age (**Fig. 1**). This significant decline was partially reversed by (*R*)-lipoic acid supplementation, which increased ambulatory activity by twofold over untreated old animals ($P<0.0005$). Activity in treated old rats compared to untreated young rats was lower, but not significantly so ($P<0.06$). Feeding (*R*)-lipoic acid to young rats also increased ambulatory activity, but this increase was not significant.

To confirm the effect of (*R*)-lipoic acid on meta-

bolic activity, a three-staged feeding regimen using the same aged rats was designed. Ambulatory activity was monitored *1*) after feeding an AIN-93M diet for 2 wk, *2*) after feeding (*R*)-lipoic acid supplemented AIN-93M diet for 2 wk, and *3*) after feeding an AIN-93M diet (without lipoic acid) for three additional weeks. The results of this experiment showed that during the lipoic acid supplementation period, ambulatory activity again was significantly higher ($P<0.03$; **Fig. 2**). Removal of (*R*)-lipoic acid from the diet reversed this improvement (Fig. 2). Control experiments where the AIN-93M was given to old rats throughout the study, but otherwise treated similarly to the experimental group, showed no change in ambulatory activity (data not shown). Thus, (*R*)-lipoic acid significantly increases overall physiological activity among old rats.

Effect of lipoic acid supplementation on oxidant stress

(*R*)-Lipoic acid acts as a cofactor in several mitochondrial enzyme complexes, but is also a powerful antioxidant and increases levels of other endogenous antioxidants when given as a supplement. The effect of (*R*)-lipoic acid supplementation on antioxidant status, oxidant production, and levels of oxidative damage in hepatocytes from old rats was examined.

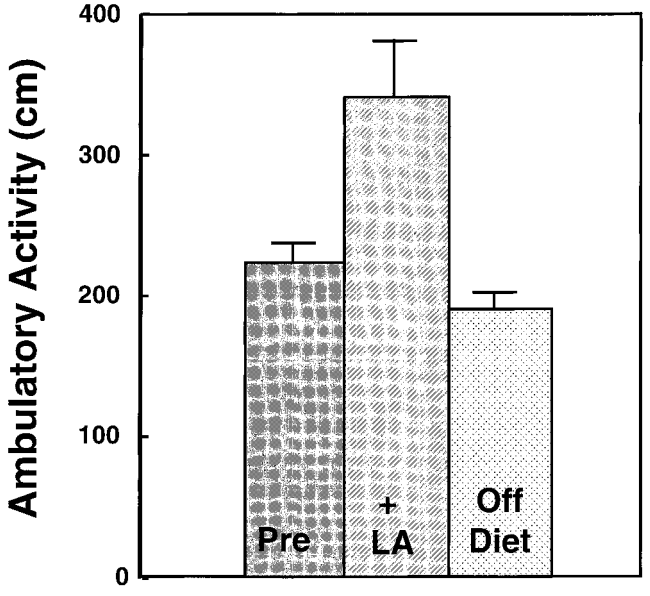

Figure 2. Removal of (*R*)-lipoic acid from the diet reverses the improvement in ambulatory activity. Ambulatory activity (distance traveled in cm/h) was measured in old rats after 2 wk on the AIN-93M diet ('Pre'), after 2 wk of the (*R*)-lipoic acid supplemented diet ('+LA'), and finally 3 wk after removal of (*R*)-lipoic acid from the diet ('off diet'). (*R*)-Lipoic acid increased ambulatory activity ($P<0.03$) whereas its removal resulted in a complete reversal of the observed improvement. Control experiments where AIN-93M was given throughout resulted in no change in ambulatory activity (data not shown).

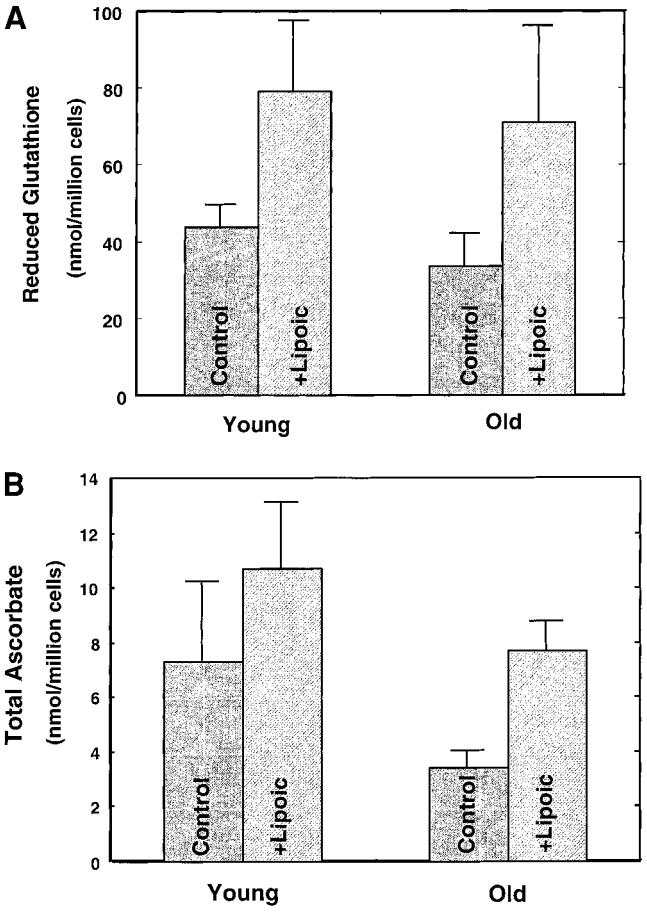

Figure 3. Hepatocellular glutathione (GSH) and ascorbate levels increase after (*R*)-lipoic acid supplementation. GSH and ascorbate levels in hepatocytes from rats either on control diet (AIN-93M) or a diet supplemented with (*R*)-lipoic acid were measured as described. *A*) GSH levels in young and old rats fed either with or without 0.5% (w/w) (*R*)-lipoic acid. (*R*)-Lipoic acid supplementation significantly ($P<0.03$) increased GSH levels in both young and old rat hepatocytes. *B*) Hepatocellular ascorbate levels in young and old rats supplemented with or without 0.5% (w/w) lipoic acid. Lipoic acid treatment reversed the age-associated decline in cellular ascorbate levels. Results are the mean ±SEM for at least 5 experiments.

Hepatocellular GSH and ascorbic acid concentrations were measured to determine whether these low molecular weight antioxidants declined with age. Both GSH and ascorbic acid levels were significantly lower ($P<0.05$) in hepatocytes from old compared to young rats, with declines of 23% and 50%, respectively (**Fig. 3***A, B*). Supplementation of (*R*)-lipoic acid for 2 wk prior to cell isolation restored the level of antioxidants to that of young animals. In both young and old rats, hepatocellular GSH levels were significantly higher vs. their corresponding controls ($P<0.03$; Fig. 3*A*); GSH levels were more than twofold higher in old rats than in unsupplemented animals. Lipoic acid supplementation also restored

the cellular ascorbic acid levels to that of young rats (Fig. 3*B*). Thus, (*R*)-lipoic acid reverses the age-associated decline in endogenous low molecular weight antioxidants, and therefore may lower the increased risk for oxidative damage that occurs during aging.

We previously showed that hepatocytes from old rats have a higher rate of oxidant production per oxygen consumed, as measured by the fluorescence formed on oxidizing DCFH (4). To determine whether dietary (*R*)-lipoic acid could lower the increased rate of oxidant production seen in aging, we measured the fluorescence in supplemented animals and their corresponding controls. Cells from old rats had significantly higher oxidant production ($P<0.005$), nearly twofold more than that in young rats (**Fig. 4**). In contrast, oxidant production was lowered in cells from lipoic acid-treated old rats to a level not significantly different from those of untreated young rats (Fig. 4).

To gauge whether the lipoic acid-induced decline in oxidant production translated into lower levels of oxidative damage, MDA was measured as an indicator of cellular lipid peroxidation (22, 23). Hepatocytes from unsupplemented old rats had fivefold more MDA than the cells from young rats ($P<0.01$; **Fig. 5**). (*R*)-Lipoic acid supplementation reduced MDA levels markedly in old rats ($P<0.01$) (Fig. 5). Although this decline in lipid peroxidation was substantial, the levels observed in the lipoic acid-supplemented old rats were still significantly higher than those found in cells from unsupplemented young rats ($P=0.05$). MDA levels were higher in lipoic acid-supplemented young animals, but this increase was not significant. Thus, (*R*)-lipoic acid supplementation markedly lowers oxidant production and the attendant increase in oxidative damage associated with aging.

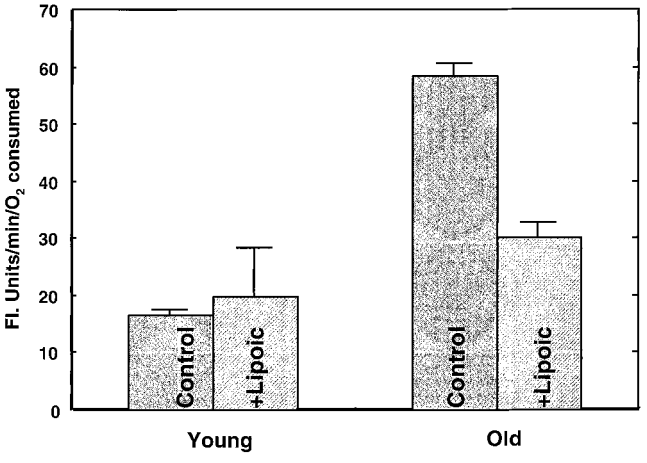

Figure 4. (*R*)-Lipoic acid treatment lowers the age-associated increase in oxidant production per μM O_2 used as measured by the fluorescence formed on oxidizing DCFH. Hepatocytes from young and old rats that had been supplemented with or without (*R*)-lipoic acid were incubated with DCFH, as described, and the increase in mean fluorescence was monitored to determine the rate of oxidant production. Results show that cells isolated from old untreated rats had a significantly higher rate of oxidant appearance. Lipoic acid treatment reversed this increase. Results are the mean ±SEM for 7 experiments.

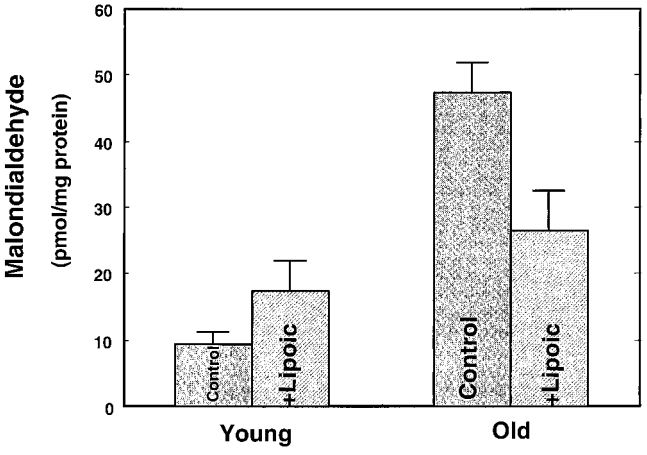

Figure 5. (*R*)-Lipoic acid supplementation lowers the age-related increase in hepatocellular lipid peroxidation. Lipid peroxidation, as measured by hepatocellular MDA levels, was measured in cells isolated from rats fed with or without (*R*)-lipoic acid. The MDA levels increased significantly with age ($P<0.01$). 2 wk of (*R*)-lipoic acid supplementation significantly lowered MDA levels in old rats ($P<0.01$). Similar supplementation to young rats resulted in no significant change in MDA levels ($P=0.05$). Results are expressed as the mean ±SEM for 5 experiments.

DISCUSSION

The (*R*)-form of lipoic acid used in this study is the naturally occurring enantiomer in mammalian cells (24). Only the (*R*)-form is used by mitochondrial α-keto acid dehydrogenases and specifically reduced to dihydrolipoic acid, a powerful antioxidant, via mitochondrial lipoamide dehydrogenase. There is evidence that (*R*)-lipoic acid supplementation may be more potent than either the racemic mixture (the form sold commercially as α-lipoic acid) or (*S*)-enantiomer, and thus a more relevant supplement for this study. Addition of (*R*)-lipoic acid increases ATP synthesis and aortic blood flow during reoxygenation after hypoxia in a working heart model (25). The (*S*)-enantiomer had no effect on ATP synthesis and improved blood flow at only 10-fold the effective dose of (*R*)-lipoic acid. Packer and colleagues (26) also showed that (*R*)-lipoic acid significantly reduced buthionine-S,R-sulfoximine-induced cataract formation, but (*S*)-lipoic acid had little effect at the same concentration. (*R*)-Lipoic acid increased glucose uptake and the number of glucose transporters in muscle tissue much more effectively

than (*S*)-lipoic acid (27). The (*R*)-enantiomer more effectively chelated copper and prevented copper-induced lipid peroxidation (28).

We did not measure hepatic tissue concentrations of (*R*)-lipoic acid or dihydrolipoic acid after oral supplementation. However, the characteristics of its uptake and tissue distribution in the rat have previously been examined, although not on an age-related basis. Lipoic acid is rapidly absorbed in the gastrointestinal tract but is subject to considerable presystemic elimination (29, 30). Between 27 to 34% of orally administered lipoic acid is available for tissue uptake, and the liver is one of the major organs of clearance (31). Studies where radiolabeled lipoic acid was infused into rats revealed that the liver has a high capacity for both uptake and accumulation of lipoic acid (32). Thus, dietary supplementation of lipoic acid would be expected to elevate hepatocellular lipoic acid concentrations considerably in both young and old rats, though its release from the liver may also be rapid.

A pharmacological dose of (*R*)-lipoic acid was given to maximize the possibility of observing whether it could affect metabolic activity and lower the increased oxidative stress evident in old rats. Even though the supplemental dose given was relatively high, it was considerably lower than the reported LD_{50} concentration for (*R*)- or (*R,S*)-lipoic acid for old rats (24). Rats fed the lipoic acid-supplemented diet for 2 wk exhibited no adverse side effects other than a small amount of weight loss, which we attribute to increased general metabolic activity. We are currently determining whether lower levels of (*R*)-lipoic acid in the diet would be equally effective in partially restoring metabolic function and decreasing oxidative stress in old rats.

We demonstrate that lipoic acid supplementation of old rats markedly improves the average mitochondrial membrane potential and restores the cellular oxygen consumption (Table 1) in hepatocytes to that of young rats. Rats on this feeding regimen were significantly more active, which further shows that (*R*)-lipoic acid acts physiologically to increase general metabolic activity. While the underlying causes for this increased energy metabolism were not explored, it is plausible that lipoic acid improves mitochondrial function through a number of mechanisms. Administration of lipoic acid stimulates insulin-dependent and independent glucose uptake into cells (33) and also enhances nonoxidative and oxidative glucose metabolism. Reduced (*R*)-lipoic acid has also been shown to increase ATP synthase activity (16), which in combination with increased glucose utilization would be expected to enhance overall cellular metabolism. Finally, as a potent antioxidant, dihydrolipoic acid may also maintain critical thiol groups in a reduced state and allow mitochondrial protein carriers to function more effectively (16).

We also show that feeding (*R*)-lipoic acid significantly attenuates the age-related increase in hepatocellular oxidant production as well as lipid peroxidation. This reduction in oxidative stress may be directly attributable to increased unbound dihydrolipoic acid or indirectly due to higher levels of other antioxidants. Lipoic acid raises GSH values by increasing cysteine availability (12), which is the rate-limiting factor in its biosynthesis. Lipoic acid decreases levels of GSH protein-mixed disulfides (34). Lipoic acid also causes faster ascorbic acid recycling (13). This may be important because ascorbic acid recycling in times of oxidative insult is markedly impaired in cells from old rats (14) and (*R*)-lipoic acid supplementation reverses this decline (14). Thus, feeding lipoic acid generally improves cellular antioxidant status, which declines with age.

α-Lipoic acid has been used as a therapeutic agent in humans, especially for diabetes (7, 9, 35) as well as certain toxicological and pathological conditions of the liver (24, 36, 37). However, little is known about whether (*R*)-lipoic acid may be an effective anti-aging supplement or therapy for certain diseases in humans. Our present findings using rats would suggest that (*R*)-lipoic acid supplementation may be a safe and effective means to improve general metabolic activity and increase antioxidant status, affording increased protection against external oxidative and xenobiotic insults with age.

Other critical metabolites that become limiting due to age-associated metabolic changes may also be beneficial as dietary supplements. A number of studies report that administration of acetyl-L-carnitine (ALCAR), a derivative of carnitine involved in fatty acid transport into mitochondria, enhanced mitochondrial function in aged tissue (38–42). We previously found (43) that ALCAR fed to old rats restores decayed mitochondria for cardiolipin content, membrane potential, and oxygen consumption and restores ambulatory activity of the rats. However, ALCAR supplementation also increased the rate of oxidant production, oxidative damage, and decreased cellular antioxidant levels (43). This indicated that ALCAR supplementation improved mitochondrial electron flux but did not reverse the increased inefficiency of electron transport. In a separate study (T. M. Hagen et al., unpublished results), we find that feeding ALCAR in combination with lipoic acid to old rats effectively increases mitochondrial metabolism without an increase in oxidative stress. Long-term feeding studies are warranted to determine whether these observed changes in mitochondria will significantly diminish decline in energy metabolism and the increased oxidative stress evident in aging. FJ

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