

Research Abstracts: R-lipoic Acid

R-lipoic acid inhibits mammalian pyruvate dehydrogenase kinase

The four pyruvate dehydrogenase kinase (PDK) and two pyruvate dehydrogenase phosphatase (PDP) isoenzymes that are present in mammalian tissues regulate activity of the pyruvate dehydrogenase complex (PDC) by phosphorylation/dephosphorylation of its pyruvate dehydrogenase (E1) component. The effect of lipoic acids on the activity of PDKs and PDPs was investigated in purified proteins system. R-lipoic acid, S-lipoic acid and R-dihydrolipoic acid did not significantly affect activities of PDPs and at the same time inhibited PDKs to different extents (PDK1>PDK4 approximately PDK2>PDK3 for R-LA). Since lipoic acids inhibited PDKs activity both when reconstituted in PDC and in the presence of E1 alone, dissociation of PDK from the lipoyl domains of dihydrolipoamide acetyltransferase in the presence of lipoic acids is not a likely explanation for inhibition. The activity of PDK1 towards phosphorylation sites 1, 2 and 3 of E1 was decreased to the same extent in the presence of R-lipoic acid, thus excluding protection of the E1 active site by lipoic acid from phosphorylation. R-lipoic acid inhibited autophosphorylation of PDK2 indicating that it exerted its effect on PDKs directly. Inhibition of PDK1 by R-lipoic acid was not altered by ADP but was decreased in the presence of pyruvate which itself inhibits PDKs. An inhibitory effect of lipoic acid on PDKs would result in less phosphorylation of E1 and hence increased PDC activity. This finding provides a possible mechanism for a glucose (and lactate) lowering effect of R-lipoic acid in diabetic subjects. Korotchkina LG, Sidhu S, Patel MS. *Free Radic Res.* 2004 Oct;38(10):1083-92.

Engagement of the insulin-sensitive pathway in the stimulation of glucose transport by alpha-lipoic acid in 3T3-L1 adipocytes

AIMS/HYPOTHESIS: A natural cofactor of mitochondrial dehydrogenase complexes and a potent antioxidant, alpha-lipoic acid improves glucose metabolism in people with Type II (non-insulin-dependent) diabetes mellitus and in animal models of diabetes. In this study we investigated the cellular mechanism of action of alpha-lipoic acid in 3T3-L1 adipocytes. **METHODS:** We treated 3T3-L1 adipocytes with 2.5 mmol/l R (+) alpha-lipoic acid for 2 to 60 min, followed by assays of: 2-deoxyglucose uptake; glucose transporter 1 and 4 (GLUT1 and GLUT4) subcellular localization; tyrosine phosphorylation of the insulin receptor or of the insulin receptor substrate-1 in cell lysates; association of phosphatidylinositol 3-kinase activity with immunoprecipitates of proteins containing phosphotyrosine or of insulin receptor substrate-1 using a *in vitro* kinase assay; association of the p85 subunit of phosphatidylinositol 3-kinase with phosphotyrosine proteins or with insulin receptor substrate-1; and *in vitro* activity of immunoprecipitated Akt1. The effect of R (+) alpha-lipoic acid was also compared with that of S(-) alpha-lipoic acid. **RESULTS:** Short-term treatment of 3T3-L1 adipocytes with R (+) alpha-lipoic acid rapidly stimulated glucose uptake in a wortmannin-sensitive manner, induced a redistribution of GLUT1 and GLUT4 to the plasma membrane, caused tyrosine phosphorylation of insulin receptor substrate-1 and of the insulin receptor, increased the antiphosphotyrosine-associated and insulin receptor substrate-1 associated phosphatidylinositol 3-kinase activity and stimulated Akt activity. **CONCLUSION/INTERPRETATION:** These results indicate that R (+) alpha-lipoic acid directly activates lipid, tyrosine and serine/threonine kinases in target cells, which could lead to the stimulation of glucose uptake induced by this natural cofactor. These properties are unique among all agents currently used to lower glycaemia in animals and humans with diabetes. Yaworsky K, Somwar R, Ramlal T, Tritschler HJ, Klip A. *Diabetologia*, 2000 Mar;43(3):294-303.

Effect of R(+)-alpha-lipoic acid on pyruvate metabolism and fatty acid oxidation in rat hepatocytes

R-(+)-alpha-lipoic acid (R-LA) is the naturally occurring enantiomer of LA. It is a strong antioxidant and cofactor of key metabolic enzyme complexes catalyzing the decarboxylation of alpha-keto acids. Racemic LA (rac-LA) has shown promise in treating diabetic polyneuropathy, and some studies suggest that it improves glucose homeostasis in patients with type 2 diabetes. We examined the effects of R-LA on pyruvate metabolism and free fatty acid (FFA) oxidation in primary cultured hepatocytes isolated from 24-hour fasted rats. After overnight culture in serum-free medium, cells were pre-exposed to R-LA for 3 hours before assays. R-LA (25 to 200 micromol/L) significantly increased pyruvate oxidation (approximately 2-fold at the highest dose tested) measured as $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]\text{pyruvate}$ by the cells over 1 hour post-treatment. These effects correlated with proportional, significant increases in the activation state of the pyruvate dehydrogenase (PDH) complex. R-LA treatment inhibited glucose production from pyruvate by approximately 50% at 50 micromol/L R-LA and approximately 90% at 200 micromol/L. Palmitate oxidation was measured in hepatocytes cultured in the presence of albumin and physiological (0.1 mmol/L) or high (1.5 mmol/L) concentrations of FFA. The latter markedly enhanced FFA oxidation. R-LA treatment significantly inhibited FFA oxidation in both media, but was more effective in high FFA, where it reduced FFA oxidation by 48% to 82% at 25 to 200 micromol/L, respectively. Identical doses of R-LA did not affect FFA oxidation by L6 myotubes (a cell culture model for skeletal muscle) in either high or low FFA medium, but enhanced pyruvate oxidation. In conclusion, 3-hour exposure of primary cultured rat hepatocytes to R-LA at therapeutically relevant concentrations increased pyruvate oxidation, apparently by activation of the PDH complex, and decreased gluconeogenesis and FFA oxidation. These features may prove useful in the control of type 2 diabetes. Walgren JL, Amani Z, McMillan JM, Locher M, Buse MG. *Metabolism*. 2004 Feb;53(2):165-73.

Vascular endothelial dysfunction in aging: loss of Akt-dependent endothelial nitric oxide synthase phosphorylation and partial restoration by (R)-alpha-lipoic acid

Aging is the single largest risk factor for cardiovascular diseases, which in turn are the leading cause of death of individuals over the age of 65 years. In part, this risk is due to a profound loss of vasomotor function of the major conduit arteries, primarily because of lower levels of endothelial-derived nitric oxide. The mechanisms involved in vascular dysfunction are not entirely understood, but age-related alterations in eNOS (endothelial nitric oxide synthase) activity appear to be a likely source of the aging lesion. However, age-related changes in cell signalling that ultimately affect eNOS phosphorylation and its activity have not been explored. Results in our laboratory indicate that levels of eNOS phosphorylation in aortas from aged F344xBN rats (28-30 months old) are almost 50% lower than in aortas from young animals (3 months old). Lower eNOS phosphorylation is directly attributable to loss of constitutive Akt/protein kinase B activity. The decline in eNOS phosphorylation can be partially restored by treating old rats with (R)-alpha-lipoic acid. These results thus suggest that age-related changes in eNOS phosphorylation may be a significant factor in the overall loss of vasomotor function in the elderly. Smith AR, Hagen TM. *Biochem Soc Trans*. 2003 Dec;31(Pt 6):1447-9.

Interactions of exercise training and alpha-lipoic acid on insulin signaling in skeletal muscle of obese Zucker rats

We have shown previously (Saengsirisuwan V, Kinnick TR, Schmit MB, and Henriksen EJ. *J Appl Physiol* 91: 145-153, 2001) that the antioxidant R-(+)-alpha-lipoic acid (R-ALA), combined with endurance exercise training (ET), increases glucose transport in insulin-resistant skeletal muscle in an additive fashion. The purpose of the present study was to investigate possible cellular mechanisms

responsible for this interactive effect. We evaluated the effects of R-ALA alone, ET alone, or R-ALA and ET in combination on insulin-stimulated glucose transport, protein expression, and functionality of specific insulin-signaling factors in soleus muscle of obese Zucker (*fa/fa*) rats. Obese animals remained sedentary, received R-ALA (30 mg.kg body wt⁻¹.day⁻¹), performed ET (daily treadmill running for < or =60 min), or underwent both R-ALA treatment and ET for 15 days. R-ALA or ET individually increased ($P < 0.05$) insulin-mediated (5 mU/ml) glucose transport (2-deoxyglucose uptake) in soleus muscle by 45 and 68%, respectively, and this value was increased to the greatest extent (124%) in the combined treatment group. Soleus insulin receptor substrate (IRS)-1 protein was significantly increased by R-ALA alone (30%) or ET alone (31%), and a further enhancement (55%) was observed after the combination treatment in the obese animals. Enhanced levels of IRS-1 protein expression after individual or combined interventions were significantly correlated with insulin action on glucose transport activity ($r = 0.597$, $P = 0.0055$). Similarly, insulin-mediated IRS-1 associated with the p85 regulatory subunit of phosphatidylinositol 3-kinase was increased by R-ALA (317%) and ET (319%) and to the greatest extent (435%) (all $P < 0.05$) by the combination treatment. These results indicate that the improvements of insulin action in insulin-resistant skeletal muscle after R-ALA or ET, alone and in combination, were associated with increases in IRS-1 protein expression and IRS-1 associated with p85. Saengsirisuwan V, Perez FR, Sloniger JA, Maier T, Henriksen EJ. *Am J Physiol Endocrinol Metab.* 2004 Sep;287(3):E529-36.

(r)-, but not (s)-alpha lipoic acid stimulates deficient brain pyruvate dehydrogenase complex in vascular dementia, but not in Alzheimer dementia

In dementia of Alzheimer type (DAT), cerebral glucose metabolism is reduced in vivo, and enzymes involved in glucose breakdown are impaired in post-mortem brain tissue. Pyruvate dehydrogenase complex activity (PDHc) is one of the enzymes known to be reduced, while succinate dehydrogenase activity (SDH), another enzyme of oxidative glucose metabolism is unchanged. In dementia of vascular type (DVT), variable changes in glucose metabolism have been demonstrated in vivo, while changes of enzyme activities in post-mortem brain tissue are unknown. Here, PDHc and SDH activity were stimulated with each of the two stereoisomers of alpha lipoic acid in post-mortem parietal brain cortex of patients with DAT, DVT, and one case of Pick's disease and compared to stimulation effects in a control group, matched for age, sex, post-mortem delay, and storage time of brain tissue. PDHc in DAT and DVT, but not in Pick's disease was reduced. PDHc activity could be slightly stimulated by 10 micro M of the physiological stereoisomer (r)-alpha-lipoic acid, in controls and DVT (possibly also in Pick's disease), but not in DAT. In all groups investigated SDH was activated by 100 micro M and 1 mM of both isomers of alpha-lipoic acid, whereas 10 mM of both stereoisomers of alpha-lipoic acid caused an inhibition of both, PDHc and SDH activity. The loss of basal and of (r)-alpha-lipoic acid stimulated PDHc activity indicate that a functional or structural impairment of PDHc may exist in DAT and DVT which is not merely attributable to loss of mitochondria since basal and stimulated SDH activities are similar in controls, DVT and DAT, thus indicating selective vulnerability of PDHc. Frolich L, Gotz ME, Weinmuller M, et al. *J Neural Transm.* 2004 Mar;111(3):295-310.

(R)-alpha-lipoic acid reverses the age-related loss in GSH redox status in post-mitotic tissues: evidence for increased cysteine requirement for GSH synthesis

Age-related depletion of GSH levels and perturbations in its redox state may be especially deleterious to metabolically active tissues, such as the heart and brain. We examined the extent and the mechanisms underlying the potential age-related changes in cerebral and myocardial GSH status in young and old F344 rats and whether administration of (R)-alpha-lipoic acid (LA) can reverse these changes. Our results show that GSH/GSSG ratios in the aging heart and the brain declined by 58 and 66% relative to

young controls, respectively ($p < 0.001$). Despite a consistent loss in GSH redox status in both tissues, only cerebral GSH levels declined with age ($p < 0.001$). To discern the potential mechanisms underlying this differential loss, the levels and the activities of gamma-glutamylcysteine ligase (GCL) and cysteine availability were determined. There were no significant age-related changes in substrate or enzyme levels, or GCL activity when saturating amounts of substrates were provided. However, kinetic analysis of GCL in brains of old rats displayed a significant increase ($p < 0.05$) in the apparent [Km] for cysteine (Km cys) vs. young rats (84.3 \pm 25.4 vs. 179.0 \pm 49.0; young and old, respectively), resulting in a 40% loss in apparent catalytic turnover of the enzyme. Thus, the age-related decline in total GSH appears to be mediated, in part, by a general decrement in GCL catalytic efficiency. Treating old rats with LA (40 mg/kg body wt; by i.p.) markedly increased tissue cysteine levels by 54% 12 h following treatment and subsequently restored the cerebral GSH levels. Moreover, LA improved the age-related changes in the tissue GSH/GSSG ratios in both heart and the brain. These results demonstrate that LA is an effective agent to restore both the age-associated decline in thiol redox ratio as well as increase cerebral GSH levels that otherwise decline with age. Suh JH, Wang H, Liu RM, Liu J, Hagen TM. *Arch Biochem Biophys*. 2004 Mar 1;423(1):126-35.

Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid

Glutathione (GSH) significantly declines in the aging rat liver. Because GSH levels are partly a reflection of its synthetic capacity, we measured the levels and activity of gamma-glutamylcysteine ligase (GCL), the rate-controlling enzyme in GSH synthesis. With age, both the catalytic (GCLC) and modulatory (GCLM) subunits of GCL decreased by 47% and 52%, respectively ($P < 0.005$). Concomitant with lower subunit levels, GCL activity also declined by 53% ($P < 0.05$). Because nuclear factor erythroid2-related factor 2 (Nrf2) governs basal and inducible GCLC and GCLM expression by means of the antioxidant response element (ARE), we hypothesized that aging results in dysregulation of Nrf2-mediated GCL expression. We observed an approximately 50% age-related loss in total ($P < 0.001$) and nuclear ($P < 0.0001$) Nrf2 levels, which suggests attenuation in Nrf2-dependent gene transcription. By using gel-shift and supershift assays, a marked reduction in Nrf2/ARE binding in old vs. young rats was noted. To determine whether the constitutive loss of Nrf2 transcriptional activity also affects the inducible nature of Nrf2 nuclear translocation, old rats were treated with (R)-alpha-lipoic acid (LA; 40 mg/kg i.p. up to 48 h), a disulfide compound shown to induce Nrf2 activation in vitro and improve GSH levels in vivo. LA administration increased nuclear Nrf2 levels in old rats after 12 h. LA also induced Nrf2 binding to the ARE, and, consequently, higher GCLC levels and GCL activity were observed 24 h after LA injection. Thus, the age-related loss in GSH synthesis may be caused by dysregulation of ARE-mediated gene expression, but chemoprotective agents, like LA, can attenuate this loss. Suh JH, Shenvi SV, Dixon BM, et al. *Proc Natl Acad Sci U S A*. 2004 Mar 9;101(10):3381-6.

Dietary supplementation with (R)-alpha-lipoic acid reverses the age-related accumulation of iron and depletion of antioxidants in the rat cerebral cortex

Accumulation of divalent metal ions (e.g. iron and copper) has been proposed to contribute to heightened oxidative stress evident in aging and neurodegenerative disorders. To understand the extent of iron accumulation and its effect on antioxidant status, we monitored iron content in the cerebral cortex of F344 rats by inductively coupled plasma atomic emission spectrometry (ICP-AES) and found that the cerebral iron levels in 24-28-month-old rats were increased by 80% ($p < 0.01$) relative to 3-month-old rats. Iron accumulation correlated with a decline in glutathione (GSH) and the GSH/GSSG ratio, indicating that iron accumulation altered antioxidant capacity and thiol redox state in aged animals. Because (R)-alpha-Lipoic acid (LA) is a potent chelator of divalent metal ions in vitro and also regenerates other antioxidants, we monitored whether feeding LA (0.2% [w/w]; 2 weeks) could

lower cortical iron and improve antioxidant status. Results show that cerebral iron levels in old LA-fed animals were lower when compared to controls and were similar to levels seen in young rats. Antioxidant status and thiol redox state also improved markedly in old LA-fed rats versus controls. These results thus show that LA supplementation may be a means to modulate the age-related accumulation of cortical iron content, thereby lowering oxidative stress associated with aging. Suh JH, Moreau R, Heath SH, Hagen TM. *Redox Rep.* 2005;10(1):52-60.

Inhibition of L-homocysteic acid and buthionine sulphoximine-mediated neurotoxicity in rat embryonic neuronal cultures with alpha-lipoic acid enantiomers

In the present report, we have set out to investigate the potential capacity of both the oxidised and reduced forms of RS-alpha-lipoic acid, and its separate R-(+) and S-(-)enantiomers, to prevent cell death induced with L-homocysteic acid (L-HCA) and buthionine sulphoximine (BSO) in rat primary cortical and hippocampal neurons. L-HCA induced a concentration-dependent neurotoxic effect, estimated by cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) reduction, in primary neurons, but was significantly more toxic for hippocampal (EC(50)=197 microM) compared with cortical neurons (EC(50)=1016 microM) whereas D-HCA demonstrated only moderate (<20%) toxicity. On the other hand, cortical and hippocampal cultures were equally susceptible (341 and 326 microM, respectively) to the neurotoxic action of BSO. Antioxidants including butylated hydroxyanisole, propyl gallate and vitamin E protected cells against the neurotoxic effect of L-HCA and BSO. However, N-acetyl-cysteine and tert-butylphenyl nitron, although capable of abrogating L-HCA-mediated cell death showed no protective effect against BSO-mediated toxicity. RS-alpha-lipoic acid, RS-alpha-dihydrolipoic acid and the enantiomers R-alpha-lipoic acid and S-alpha-lipoic acid protected cells against L-HCA-mediated toxicity with EC(50) values between 3.1-8.3 microM in primary hippocampal neurons and 2.6-16.8 microM for cortical neurons. However, RS-alpha-lipoic acid, RS-alpha-dihydrolipoic acid, and S-alpha-lipoic acid failed to protect cells against the degeneration induced by prolonged exposure to BSO, whereas the natural form, R-alpha-lipoic, was partially active under the same conditions. The present results indicate a unique sensitivity of hippocampal neurons to the effect of L-HCA-mediated toxicity, and suggest that RS-alpha-lipoic acid, and in particular the R-alpha-enantiomeric form is capable of preventing oxidative stress-mediated neuronal cell death in primary cell culture. Lockhart B, Jones C, Cuisinier C, Villain N, Peyroulan D, Lestage P. *Brain Res* 2000 Feb 14;855(2):292-7.

Stereospecific effects of R-lipoic acid on buthionine sulfoximine-induced cataract formation in newborn rats

This study revealed a marked stereospecificity in the prevention of buthionine sulfoximine-induced cataract, and in the protection of lens antioxidants, in newborn rats by alpha-lipoate, R- and racemic alpha-lipoate decreased cataract formation from 100% (buthionine sulfoximine only) to 55% (buthionine sulfoximine + R-alpha-lipoic acid) and 40% (buthionine sulfoximine + rac-alpha-lipoic acid) (p<0.05 compared to buthionine sulfoximine only). S-alpha-lipoic acid had no effect on cataract formation induced by buthionine sulfoximine. The lens antioxidants glutathione, ascorbate, and vitamin E were depleted to 45, 62, and 23% of control levels, respectively, by buthionine sulfoximine treatment, but were maintained at 84-97% of control levels when R-alpha-lipoic acid or rac-alpha-lipoic acid were administered with buthionine sulfoximine; S-alpha-lipoic acid administration had no protective effect on lens antioxidants. When enantiomers of alpha-lipoic acid were administered to animals, R-alpha-lipoic acid was taken up by lens and reached concentrations 2- to 7-fold greater than those of S-alpha-lipoic acid, with rac-alpha-lipoic acid reaching levels midway between the R-isomer and racemic form.

Reduced lipoic acid, dihydrolipoic acid, reached the highest levels in lens of the rac-alpha-lipoic acid-treated animals and the lowest levels in S-alpha-lipoic acid-treated animals. These results indicate that the protective effects of alpha-lipoic acid against buthionine sulfoximine-induced cataract are probably due to its protective effects on lens antioxidants, and that the stereospecificity exhibited is due to selective uptake and reduction of R-alpha-lipoic acid by lens cells. Maitra I, Serbinova E, Tritschler HJ, Packer L. *Biochem Biophys Res Commun* 1996 Apr 16;221(2):422-9.

Reduction of lipoic acid by lipoamide dehydrogenase

Racemic lipoic acid is therapeutically applied in pathologies in which free radicals are involved. The in vivo reduction of lipoic acid may play an essential role in its antioxidant effect. It was found that mitochondrial lipoamide dehydrogenase (LipDH, EC 1.8.1.4.) reduces the R-enantiomer 28 times faster than the S-enantiomer of lipoic acid. Moreover, it was observed that the metabolites of lipoic acid, bisnor-, tetranor-, and beta-lipoic acid are poor substrates of LipDH. S-lipoic acid inhibits the reduction of the R enantiomer only at relatively high concentrations. The reduction of R-lipoic acid by mitochondria-rich tissues may proceed smoothly, even if the racemic mixture is applied. This is of importance in elucidating the molecular mechanism of the pharmacotherapeutic effect of lipoic acid. Biewenga GP, Dorstijn MA, Verhagen JV, Haenen GR, Bast A. *Biochem Pharmacol* 1996 Feb 9;51(3):233-8.

Cytosolic and mitochondrial systems for NADH- and NADPH-dependent reduction of alpha-lipoic acid

In cellular, tissue, and organismal systems, exogenously supplied alpha-lipoic acid (thioctic acid) has a variety of significant effects, including direct radical scavenging, redox modulation of cell metabolism, and potential to inhibit oxidatively-induced injury. Because reduction of lipoate to dihydrolipoate is a crucial step in many of these processes, we investigated mechanisms of its reduction. The mitochondrial NADH-dependent dihydrolipoamide dehydrogenase exhibits a marked preference for R(+)-lipoate, whereas NADPH-dependent glutathione reductase shows slightly greater activity toward the S(-)-lipoate stereoisomer. Rat liver mitochondria also reduced exogenous lipoic acid. The rate of reduction was stimulated by substrates which increased the NADH content of the mitochondria, and was inhibited by methoxyindole-2-carboxylic acid, a dihydrolipoamide dehydrogenase inhibitor. In rat liver cytosol, NADPH-dependent reduction was greater than NADH, and lipoate reduction was inhibited by glutathione disulfide. In rat heart, kidney, and brain whole cell-soluble fractions, NADH contributed more to reduction (70-90%) than NADPH, whereas with liver, NADH and NADPH were about equally active. An intact organ, the isolated perfused rat heart, reduced R-lipoate six to eight times more rapidly than S-lipoate, consistent with high mitochondrial dihydrolipoamide dehydrogenase activity and results with isolated cardiac mitochondria. On the other hand, erythrocytes, which lack mitochondria, somewhat more actively reduced S- than R-lipoate. These results demonstrate differing stereospecific reduction by intact cells and tissues. Thus, mechanisms of reduction of alpha-lipoate are highly tissue-specific and effects of exogenously supplied alpha-lipoate are determined by tissue glutathione reductase and dihydrolipoamide dehydrogenase activity. Haramaki N, Han D, Handelman GJ, Tritschler HJ, Packer L. *Free Radic Biol Med* 1997;22(3):535-42.

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