Review

Lipoic acid: energy metabolism and redox regulation of transcription and cell signaling

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The role of R-α-lipoic acid as a cofactor (lipoyllysine) in mitochondrial energy metabolism is well established. Lipoic acid non-covalently bound and exogenously administered to cells or supplemented in the diet is a potent modulator of the cell's redox status. The diversity of beneficial effects of lipoic acid in a variety of tissues can be mechanistically viewed in terms of thiol/disulfide exchange reactions that modulate the environment's redox and energy status. Lipoic acid-driven thiol/disulfide exchange reactions appear critical for the modulation of proteins involved in cell signaling and transcription factors. This review emphasizes the effects of lipoic acid on PI3K and AMPK signaling and related transcriptional pathways that are integrated by PGC-1α, a critical regulator of energy homoestasis. The effects of lipoic acid on the neuronal energy-redox axis are largely reviewed in terms of their outcomes for aging and age-related neurodegenerative diseases.

Key Words:　lipoic acid, dihydrolipoic acid, energy, redox, AMPK, insulin, mitochondria, PGC1α

Lipoic acid (1,2-dithiolane-3-pentanoic acid)—first isolated and chemically identified in 1951 by Lester Reed and colleagues[1]—occurs in the R- and S-enantiomeric structures, only the R-form being essential in biological systems. The discovery of lipoic acid led to an unprecedented interest in basic research because of its role as a coenzyme in energy metabolism and the non-covalently bound form as a modulator of the cell's redox status. The biochemistry, physiology, and pharmacokinetics of lipoic acid as well as its effects on several disease states have been extensively reviewed (see Ref. 2, 3); the diversity of effects of lipoic acid in a variety of tissues can be viewed within the realm of antioxidant activity, metal chelation, transcriptional responses—related to inflammation and induction of phase II enzymes—, and cell signaling responses, especially in terms of cardiovascular function and glucose metabolism.[2,3] These effects of lipoic acid can be mechanistically accounted for in terms of thiol/disulfide exchange reactions that modulate the environment's redox and energy status (Fig. 1). The energy and redox components are integrated into an *energy–redox axis*; hence, on a mechanistic basis, lipoic acid co-regulates both components in the several subcellular compartments. R-Lipoic acid—as a micronutrient and

a therapeutic agent—stimulated interest in clinical research because of its therapeutic implications for the metabolic syndrome,[4] diabetic polyneuropathies,[5] and neurodegenerative diseases (with emphasis on Alzheimer's disease).[6]

The Cell's Redox Status

The redox environment of a linked set of redox couples—as found in biological fluids, organelles, cells, or tissues—is defined as the summation of the products of the reduction potential and reducing capacity of the linked redox couples.[7] Quantification of thioredoxin, glutathione/glutathione disulfide (GSH/GSSG), and cysteine/cystine redox couples—termed redox control nodes[8]—brings new dimensions to redox systems biology; assessment of these major cellular thiol/disulfide systems in different cellular compartments indicated that individual signaling and control events occur through discrete redox pathways, thereby leading to a new definition of oxidative stress as a disruption of redox signaling and control.[9]

Lipoic acid—either as a dietary supplement or a therapeutic agent—modulates distinct redox circuits because of its ability to equilibrate between different subcellular compartments as well as extracellularly. As such, lipoic acid is a critical component of the antioxidant network because of its ability to regenerate other antioxidants, such as vitamins E and C, increase intracellular GSH levels, and provide redox regulation of proteins and transcription factors.[10]

The extracellular thiol/disulfide redox environment (determined by the cysteine/cystine couple) has been reported to modulate cell proliferation, apoptosis, cell adhesion molecules, and pro-inflammatory signaling.[11] Lipoic acid may modulate the extracellular redox state inasmuch as dihydrolipoic acid is involved in the reduction of cystine to cysteine, thus facilitating rapid uptake of the latter into the cell through the ASC transport system and, consequently, its availability to stimulate GSH synthesis (Fig. 2).[12,13] Cellular transport of lipoic acid occurs probably by several systems, such as the medium chain fatty acid transporter,[14] a Na$^+$-dependent vitamin[15] transport system,[16] and a H$^+$-linked monocarboxylate transporter for intestinal uptake.[17] The cellular reduction of lipoic acid to dihydrolipoic acid is
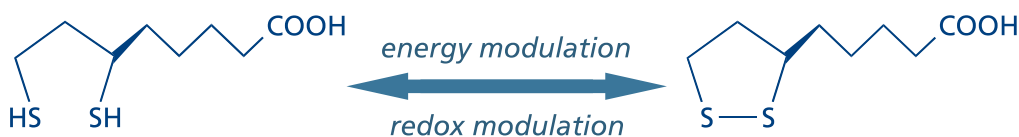

Fig. 1.　Thiol/disulfide exchange by lipoic acid is the basis for its modulation of the cell's energy and redox status.

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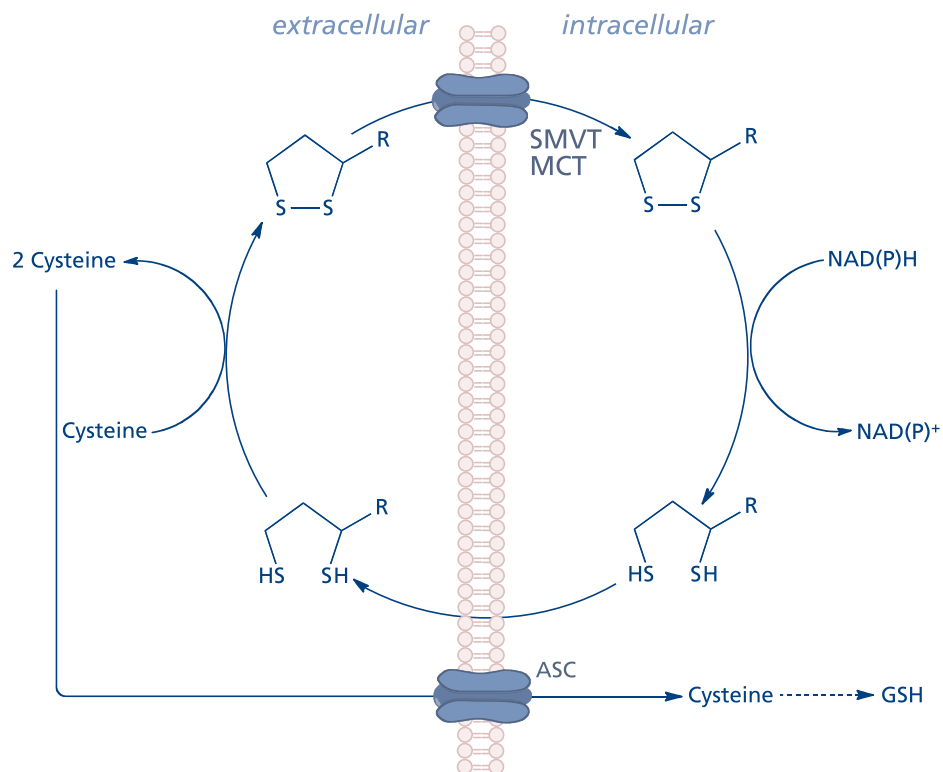E-mail: packer@usc.edu

Fig. 2. Cellular uptake and release of lipoic acid and modification of the extracellular redox state.

accomplished by NAD(P)H-driven enzymes, thioredoxin reductase, lipoamide dehydrogenase, and glutathione reductase. Erythrocytes take up and reduce lipoic acid by glucose metabolism; subsequently, dihydrolipoic acid is released to the extracellular milieu, thus reflecting the activity of disulfide reductases.[14] This phenomenon was observed in several cell types;[18] 3T3-L1 adipocytes, however, possess a low capacity to reduce lipoic acid and most of the intracellular effects in these cells are due to its pro-oxidant function.[19,20]

R-(+)-lipoic acid (as lipoyllisine) is present in both plant and animal tissues only in small amounts, thus its bioavailability is low;[21] however, lipoic acid is now available as a nutritional supplement: the human plasma pharmacokinetics of R-(+)-lipoic acid (administered as a sodium salt to healthy individuals) revealed that R-(+)-lipoic acid displayed high plasma maximum concentration and area under the concentration versus time curve values; the study reported negligible unbound R-(+)-lipoic acid at the highest achievable plasma concentrations.[22]

The intracellular redox status is usually determined by the GSH/GSSG, thioredoxin$_{reduced}$/thioredoxin$_{oxidized}$, and cysteine/cystine couples[8] and their ability to reversibly modulate cysteine- and methionine moieties in proteins. The participation of R-lipoic acid in thiol/disulfide exchange is the basis for its redox modulation of cell signaling and transcription: NFκB-, MAPK-, and PI3K/Akt signaling as well as transcription factors.[23–29]

Lipoic acid and redox control of glucose uptake and metabolism. Extensive evidence suggests that lipoic acid has potential therapeutic value in lowering glucose levels in diabetic conditions and that the intracellular redox status plays a role in the modulation of insulin action (insulin resistance). Mechanistic studies on the effects of lipoic acid on the redox status of insulin-responsive cells revealed that lipoic acid stimulated glucose uptake by affecting components of the insulin signaling pathway. The signaling networks of insulin receptors entail binding of

insulin to the receptor followed by autophosphorylation of the intracellular tyrosine kinase domain of the β-subunits and activation of signaling pathways that may be considered in three sequential nodes[30] encompassing the insulin receptor substrate (IRS1/2/3/4), PI3K, and Akt (also known as PKB). PI3K/Akt activity was shown to be necessary for the translocation of glucose transporter-4 (GLUT4) from an intracellular pool to the plasma membrane.[31–33] In a comprehensive series of studies it was found that lipoic acid augmented tyrosine phosphorylation and the activity of components of insulin signaling: insulin receptor, insulin receptor substrate-1, PI3K (type I), Akt1, and p38[34,35] (Fig. 4). The authors concluded that lipoic acid stimulated glucose uptake upon translocation and regulation of the intrinsic activity of GLUT4, an effect that might be mediated by p38 MAPK.[34] (Akt phosphorylates 160 kDa AS160, facilitating its dissociation from the GLUT4 storage vesicle and preventing inactivation of Rab-GTP). R-α-lipoic acid and oxidized isoforms are effective in stimulating glucose transport in differentiated 3T3-L1 adipocytes by a mechanism entailing changes in the intracellular redox status (but not changes in the GSH levels); lipoic acid also facilitated the autophosphorylation of the insulin receptor by a mechanism that may involve oxidation of the cysteine residues in the α- and β-subunits.[19] These effects of lipoic acid are in agreement with an alteration of the thiol reactivity of redox components of the insulin pathway caused by a thiol/disulfide exchange mechanism (Fig. 3). The inhibition of protein tyrosine phosphatase 1B activity by lipoic acid was also associated with a decrease in thiol reactivity of the enzyme.[20] Lipoic acid inhibits differentiation of 3T3-L1 pre-adipocytes by activation of JNK and ERK pathways and, in turn, transcription factors,[24] a different mechanism by which lipoic acid increases glucose uptake (i.e., activation of the insulin receptor/Akt pathway).

The stress-activated MAPK, JNK, plays a central role in the progression of insulin resistance and diabetic neuropathies;[36,37]
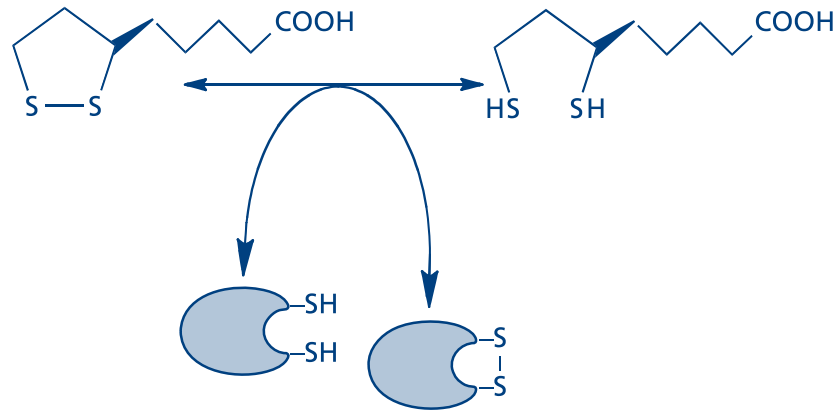
Fig. 3. Thiol/disulfide exchange as the basis for the activation/inhibition of cell signaling and transcription.
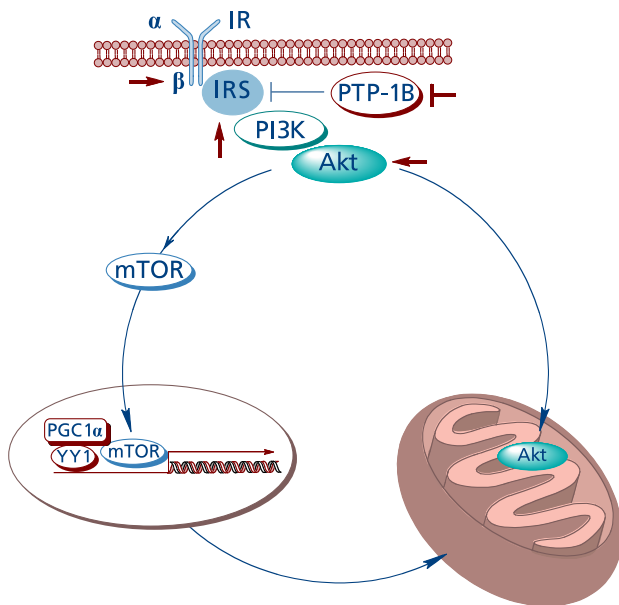
Fig. 4. The insulin-like effect of lipoic acid and Akt-dependent stimulation of mitochondrial function.

a likely mechanism entails the phosphorylation of the insulin receptor substrate-1 serine 307 and, as a consequence, inhibition of the insulin-promoted tyrosine phosphorylation of IRS-1.[38] Lipoic acid was shown to inhibit the JNK pathway and IRS-1 serine phosphorylation, thereby improving insulin sensitivity. Although the exact mechanism by which lipoic acid inhibits the JNK pathway remains unclear, these effects place lipoic acid at the cross-road of insulin- and JNK signaling favoring glucose uptake and metabolism, thus ameliorating insulin resistance. A plausible mechanism suggests that lipoic acid-mediated induction of heat shock proteins and the subsequent inhibition of JNK and IKKβ.[39] In L6 muscle cells, lipoic acid prevented the activation of JNK triggered by either anisomycin or TNF-α.[39]

In hepatocytes, active Akt (Akt phosphorylated at Ser[473]), decreases as a function of age, whereas basal Akt phosphorylated at Thr[308] remained unchanged; lipoic acid partially recovered Akt activation[40] and, as observed also in 3T3-L1 adipocytes, lipoic acid inhibited the phosphatase activities of PTEN and PP2A.

Full activation of Akt is a complex process entailing different pathways;[41] Akt activation affects mitochondrial bioenergetics by

at least two pathways (Fig. 4).

First, it was shown that Akt translocates to the mitochondrion of several cell types upon stimulation with insulin, insulin-like growth factor-1, or heat stress, where the phosphorylation targets identified were the β-subunit of ATPase and GSK3β; phosphorylation of the latter at a serine residue leads to its inactivation;[42] in unstimulated cells, heat shock protein-90 is responsible for Akt accumulation in the mitochondrion.[43] Mitochondrion-targeted Akt also protected neuroblastoma cells from apoptosis.[44] Whether or not the insulin-like effects of lipoic acid facilitate the translocation of Akt to mitochondria remains to be investigated.

Second, Akt is a positive regulator of the mammalian target of rapamycin (mTOR)[45] by mechanisms entailing the Akt-mediated phosphorylation and inhibition of TSC1 or the Akt-mediated inhibition of AMPK.[46] mTOR regulates the transcription of several genes and regulates mitochondrial activity, i.e., controls mitochondrial gene expression by modulation of YY1-PGC-1α.[47]

The Cell's Energy Status

Lipoic acid is an essential cofactor for the E2 component of α-ketoacid dehydrogenase complexes, exclusively located in mitochondria, e.g., the pyruvate dehydrogenase (PDH)-, α-ketoglutarate dehydrogenase (KGDH)-, and branched chain α-ketoacid dehydrogenase (BCKDH) complexes. The former catalyzes the oxidative carboxylation of pyruvate and plays a fundamental role in carbohydrate metabolism and bioenergetics (Fig. 5), for PDH bridges anaerobic and aerobic energy metabolism, and it is the entry point of carbohydrates into the tricarboxylic acid cycle as acetyl-CoA. The latter is a regulatory control point in the tricarboxylic acid cycle; the activities of both PDH and KGDH is substantially decreased during aging and in neurodegenerative disorders.[48–50] Lipoic acid is reduced to dihydrolipoic acid by dihydrolipoamide dehydrogenase, the E3 component of PDH and KGDH. PDH activity is regulated by products, nucleotides, and reversible phosphorylation; lipoic acid supplementation increases PDH activity in hepatocyte mitochondria and it inhibits the pyruvate dehydrogenase kinase (PDK),[51,52] hence leading to a lower phosphorylation (and inactivation) of PDH. The mechanism of PDK inactivation by lipoic acid is not known yet.[51] 4-Hydroxynonenal (HNE) inhibited rather specifically KGDH, which accounted for the inhibitory effects of HNE on mitochondrial respiration;[53,54] the inactivation of KGDH (and PDH) was ascribed to the electrophilic attack of HNE on the reduced lipoyl moiety covalently bound to E2 component of the complex). Lipoic acid at the E2 of KGDH is glutathionylated upon treatment of mitochondria with H2O2; glutathionylation of the lipoyl moiety is reversible and appears to serve as a transient protection against

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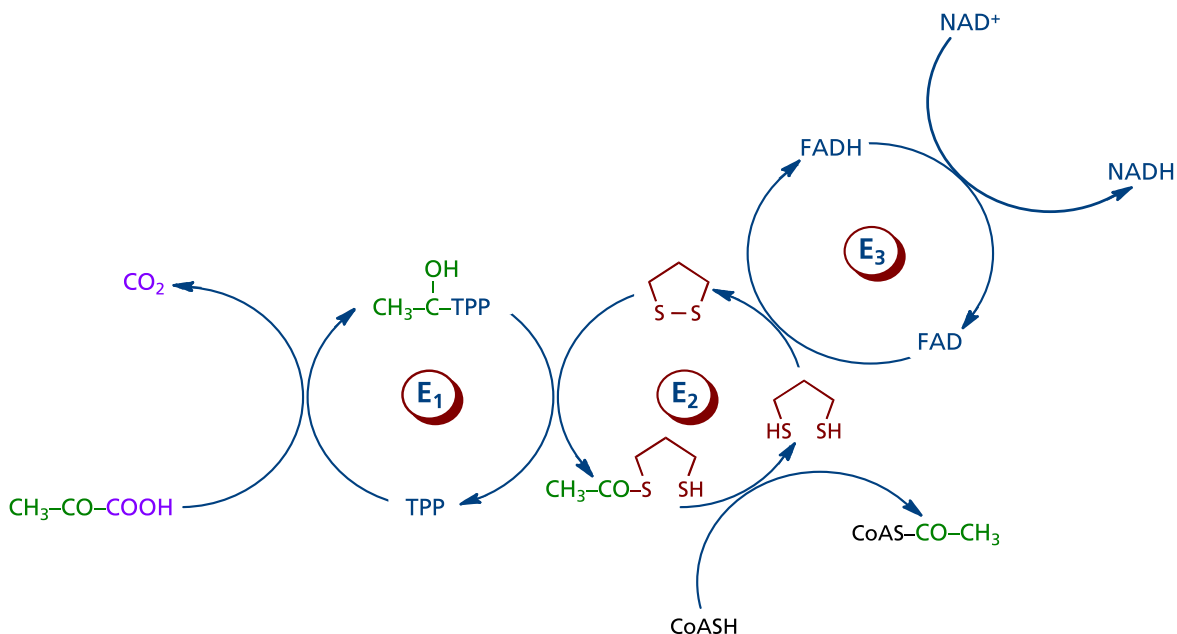
Fig. 5. Pyruvate dehydrogenase-catalyzed oxidative decarboxylation of pyruvate. The lipoyl moiety is shown in red. E_1, a-ketoacid decarboxylase; E_2, dihydrolipoyl transcetilase; E_3, dihydrolipoyl dehydrogenase.
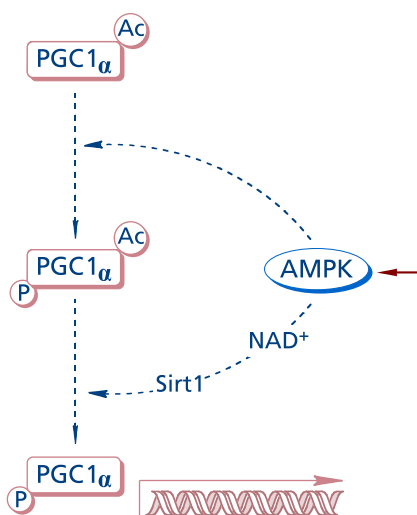
Fig. 6. AMPK-dependent transcriptional pathway for PGC-1α activation.

electrophilic attack by HNE.[55]

AMP-activated protein kinase (AMPK) is a sensitive cellular energy sensor[56] that supports ATP-generating catabolic pathways and decreases ATP-consuming anabolic processes by post-translational modifications and modulation of gene transcription (Fig. 6). AMPK consists of a catalytic (α) and two regulatory (β and γ) subunits, the γ subunit being the center of allosteric regulation (stimulated by AMP). Enzyme activation requires phosphorylation of a threonine residue by LKB1 or elevation of intracellular Ca^{++} via CaMKK. The effects of lipoic acid on AMPK differ depending on whether its action is on peripheral tissues or the hypothalamus[4] (AMPK in hypothalamic neurons integrates signals related to body's energy metabolism). The different roles of AMPK in neurons have been critically reviewed:[57] depending on the experimental model, AMPK may

function in a neuroprotective role or be harmful for neuronal survival or act as an autophagy mediator. AMPK is involved in transcriptional pathways that control mitochondrial function through PGC-1α.[58] The phosphorylation of PGC-1α protein[59] by AMPK at Thr^{177} and Ser^{538} appears to be a requirement for the induction of the PGC-1α promoter. Also, activation of AMPK was shown to enhance NAD^+ levels in muscle cells and induce Sirt1-mediated PGC-1α deacetylation; apparently, PGC1α phosphorylation by AMPK facilitates the subsequent deacetylation by Sirt1.[60] The energy status of the cell is also related to activity of sirtuins,[61] which—among others—can deacetylase PGC-1α, by means of which Sirt1 controls mitochondrial biogenesis and function.

PGC-1α is a transcriptional regulator of mitochondrial function and biogenesis and, as such, a critical regulator of energy homeostasis and integrates several transcriptional pathways driven by mTOR (Fig. 4), AMPK, and Sirt1 (Fig. 6).[62] Lipoic acid was reported to increase energy metabolism and mitochondrial biogenesis in the skeletal muscle of aged mice by increasing the phosphorylation of AMPK at Thr^{172} and expression of PGC-1α.[63] In this report, lipoic acid also increased the expression of GLUT4 (as observed in other cell types), but it decreased the phosphorylation of mTOR at Ser^{2448}.[63] As in the case of Akt-driven signaling, AMPK phosphorylates AS160, thereby facilitating its dissociation from the glucose transporter vesicle and preventing the inactivation of Rab-GTP. It remains to be investigated whether or not AMPK is a preferential pathway of lipoic acid for the transcriptional activation of PGC-1α (opposite to mTOR) in tissues other than skeletal muscle.

It is well established that aging is associated with a loss of mitochondrial function and insulin resistance. In brain, there is an increased activation of JNK (bisphosphorylation) with age as well as its translocation to mitochondria, thereby blunting the activity of pyruvate dehydrogenase.[48,49] In muscle, AMPK activity—of significance in the regulation of energy metabolism and maintenance of energy homeostasis through PGC1α—is also reduced as a function of age;[64] whether its activity also decreases with age in neurons remains to be determined.

Lipoic Acid and the Neuronal Redox-Energy Axis

Pharmacokinetic studies on the distribution of orally administered R-, and S-lipoic acid from the systemic circulation into rat brain tissues (with consideration of the transport efficiency across the brain blood barrier) showed blood endogenous levels of 0.05–0.27 μM and brain levels of 0–0.024 μM after either a single or chronic oral dosing at 50 mg/kg;[65] the authors concluded that lipoic acid does not readily cross the blood-brain barrier, thereby questioning a direct effect of lipoic acid in the central nervous system.[65] Despite this, there is a myriad of reports on the effects of lipoic acid on improving mitochondrial function in aging and neurodegenerative disorders. However, few studies have investigated the mechanistic implications of lipoic acid in terms of PI3K/Akt- and MAPK-driven signaling and transcription (as in peripheral tissues referred to above).

It is widely accepted that loss of mitochondrial function may be an underlying event in brain aging and neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's diseases. Loss of mitochondrial function entails a reduction of the energy-transducing systems partly due to oxidative/nitrative damage. From this perspective, exogenously administered lipoic acid has been considered a mitochondrial nutrient. Mitochondrial dysfunction inherent in the pathogenesis of neurodegenerative diseases is aggravated by downregulation of cytosolic glutaredoxin-1 (which helps maintain mitochondrial integrity in terms of VDAC redox status) and is recovered by lipoic acid.[66]

The properties of lipoic acid that help improve age-associated loss of cognitive function are elevation of cofactors of defective mitochondrial enzymes, such as PDH and KGDH, protection of enzymes against oxidative stress, and enhancement of antioxidant defense systems through the activation of phase II enzymes and an increase in mitochondrial biogenesis.[67] The amount and activity of PDH and KGDH are decreased in Alzheimer's disease,[68] a finding that might be partly explained by the higher susceptibility of mitochondria in Alzheimer's disease to autophagy.[69] PDH activity is decreased in post-mortem tissues from Alzheimer's dementia and vascular dementia, but R-lipoic acid (not S-lipoic acid) appears to stimulate PDH activity only in vascular dementia.[70]

In aged rats, spatial and temporary memory loss was associated with loss of brain mitochondrial function as well as RNA/DNA oxidation in hippocampus; these effects were partially reversed upon feeding animals with a combination of lipoic acid and acetyl-L-carnitine.[67] Age-related changes in synaptic function (in terms of impairment of long-term potentiation (LTP) and glutamate release) were reversed by dietary supplementation with lipoic acid (entailing restoration of IL-1β and tocopherol levels to values of young rats).[71]

Chronic administration of lipoic acid partially restored the age-associated loss of mitochondrial function to the level of young rats (in terms of activity of complex I, IV, and V) and improved oxidative stress markers.[72] A combination of lipoic acid and acetyl-L-carnitine was suggested to delay the loss of mitochondrial function associated with aging, restored mitochondrial ultrastructural changes, and increase mitochondrial biogenesis in the hippocampus.[73] Chronic administration of lipoic acid decreased biomarkers of oxidative stress in young and old control mice and a transgenic mouse model overexpressing the amyloid-β protein precursor without having an impact on end-point amyloid-β load; however, this reduction in oxidative stress was not correlated with an improvement on cognitive behavior (Y-maze performance).[74] Conversely, a combination of acetyl-L-carnitine and lipoic acid partially improved spatial and temporal memory in an ApoE4 transgenic mouse model.[75] Dietary supplementation with a combination of several micronutrients—among them lipoic acid—improved cognitive performance in ApoE-deficient mice.[76] Lipoic acid also improved survival in two transgenic mouse models of Huntington's disease.[77] Interestingly, dichloro-acetate—an inhibitor of pyruvate dehydrogenase kinase, which phosphorylates and inactivates PDH—also increased survival in these two transgenic models of Huntington's disease showing improved motor function and decreased striatal neuron atrophy.[78] Hence, the protective effect of lipoic acid on these models of Huntington's disease could be partly due to its inhibition of pyruvate dehydrogenase kinase (as reported in[52]). A recent study concluded that short-term supplementation with lipoic acid and acetyl-L-carnitine is insufficient to improve cognition in aged dogs, and that the beneficial effects of the full spectrum diet arose from either the cellular antioxidants alone or their interaction with lipoic acid and acetyl-L-carnitine.[79]

Lipoic acid protected cortical neurons against amyloid-β or H_2O_2-induced cytotoxicity and also induced the expression of Akt (and the downstream Akt signaling pathway).[26] Pretreatment of cortical neurons with lipoic acid (and in combination with acetyl-L-carnitine) results in the activation of the PI3K and ERK1/2 pathways and the inherent neuronal survival;[80] lipoic acid also protected against 4-hydroxy-2-nonenal (HNE)-mediated oxidative modifications in cortical neurons.[80]

Concluding Remarks

R-α-Lipoic acid, a cofactor for four enzyme complexes exclusively located in mitochondria, is essential for energy production and the regulation of carbohydrate and protein metabolism. Lipoic acid is synthesized *in vivo* and it is almost entirely covalently bound to the E_2 component of three α-ketodehydrogenase complexes and the glycine cleavage system. Hence, it would be expected that only trace amounts are available from dietary sources. However, when lipoic acid is supplemented in the diet, it is readily absorbed and present in all cell compartments and extracellular fluids where it acts as a redox modulator and antioxidant *par excellence*.

Although known for more than 60 years to have potent effects in biological systems, lipoic acid studies have been hampered by the inability to detect accurately its presence in tissue samples. A study of the plasma pharmacokinetics of R-(+)-lipoic acid revealed that maximum concentrations were reached within ~30 min of administration and had a short half life when administered as sodium R-(+)-lipoate to healthy human subjects. Therefore, due to its very rapid metabolism, precise sampling times are required to establish an association of lipoic acid concentration with function in cells and tissues. Nevertheless, the redox modulating action of lipoic acid on signaling and transcription exhibits remarkable promise. Future studies are warranted to elucidate the therapeutic effects of exogenous lipoic acid during aging and age-related diseases, with emphasis on Alzheimer's disease, of special interest to the co-authors of this review.

Acknowledgments

Abbreviations

AMPK	AMP-activated protein kinase
ASC	alanine, serine, cysteine transporter
CaMKK	calcium/calmodulin-dependent protein
AS160	Akt substrate of 160 kDa
HNE	4-hydroxy-2-nonenal
IRS	insulin receptor substrate
KGDH	α-ketoglutarate dehydrogenase
LKB1	liver kinase B1
mTOR	mammalian target of rapamycin
PDH	pyruvate dehydrogenase

PDK   pyruvate dehydrogenase kinase
PGC-1α   Peroxisome proliferator-activated receptor γ (PPARγ)
          coactivator-1α

PI3K   phosphotidylinositide 3-kinase
PP2A   protein phosphatase 2A
PTEN   phosphatase and tensin homologue

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