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## The “rejuvenatory” impact of lipoic acid on mitochondrial function in aging rats may reflect induction and activation of PPAR- $\gamma$ coactivator-1 $\alpha$

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## SUMMARY

In aging rats, lipoic acid exerts a “rejuvenative” impact on mitochondria in various tissues, boosting mitochondrial membrane potential and oxygen consumption, while decreasing mitochondrial production of oxidants. A likely explanation for this phenomenon is that the mitochondria in aging rodents are structurally and functionally impaired by excessive oxidant stress – and that lipoic acid reverses this damage by amplifying key antioxidant mechanisms that protect mitochondria. A likely mediator of this effect is PPAR $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), which recently has been shown to promote transcription of the manganese-dependent superoxide dismutase, uncoupling protein-2, and an array of other proteins which provide antioxidant protection to mitochondria. Lipoic acid has been reported to activate both p38 MAP kinase and AMP-activated kinase (AMPK); p38 MAP kinase can boost the transcription, half-life, and coactivational activity of PGC-1 $\alpha$ , and AMPK is known to promote its transcription in skeletal muscle and endothelial cells. Thus, it is intriguing to speculate that the remarkable antioxidant effects of lipoic acid therapy reflect not only induction of phase 2 antioxidants (e.g. glutathione and heme oxygenase-1), but also induction of various proteins that function expressly to protect mitochondria from self-generated oxidant stress. Further research is required to evaluate this model.

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### Lipoic acid rejuvenates mitochondrial function of aging rats

Ames and colleagues have demonstrated that ample dietary intakes of lipoic acid, with or without concurrent acetyl-L-carnitine supplementation, have a remarkable rejuvenatory impact on the function of hepatic mitochondria in of aging rats [1,2]. As compared to hepatocytes obtained from young rats, those obtained from aging rats showed a marked reduction in *ex vivo* oxygen consumption and in mitochondrial membrane potential – whereas oxidant production was severalfold higher. Pre-administration of a diet enriched in (*R*)-lipoic acid (0.5% w/w), while it had little impact on these parameters in young rats, virtually restored these parameters to “youthful” levels in the aged rats. This effect was associated with a substantial increase in the diminished ambulatory activity of aging rats. The authors suggested that the impairment of mitochondrial function observed in the aging rats may have reflected structural damage secondary to increased mitochondrial oxidant stress; the rejuvenatory effects of lipoic acid may thus have been attributable to suppression of this elevated oxidant stress. The minimal impact of lipoic acid on the young rats could then be attributed to the fact that baseline oxidant stress was low in these animals.

In subsequent studies, these researchers examined the age-dependent impact of lipoic acid feeding on mitochondrial ultrastructure and oxidant stress in the brain of rats [3,4]. They confirmed that this nutrient exerted an antioxidant effect in aging rat brain that was associated with a reversal of age-related ultrastructural mitochondrial decay and a normalization of carnitine acetyltransferase binding affinity. Moreover, supplemental lipoic acid was found to improve the performance of aging rats in tests assessing spatial and temporal memory.

Indian researchers have provided confirmatory evidence, examining the impact of DL-lipoic acid supplementation on oxidant stress and the activities of various mitochondrial enzymes (from the citric acid cycle and respiratory chain) in the liver and kidney of aging rats. They found that lipoic acid corrected the age-related increase in oxidative stress as well as the age-related decline in mitochondrial enzyme activities [5,6]. Subsequent studies, employing co-administration of DL-lipoic acid and L-carnitine, demonstrated comparable effects in the hearts of aging rats, and also showed a normalization of mitochondrial membrane potential and in mitochondrial membrane cardiolipin content [7–9] (other researchers have likewise shown that supplemental lipoic acid reverses an age-related increase of oxidative stress in cardiac myocytes of aging rats [10]). Their most recent study examined the function of mitochondria isolated from the skeletal muscle of young and aging rats [11]. Aging was associated with a decline in

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state 3 respiration and the respiratory control ratio, whereas state 4 respiration (reflecting proton leak) increased. Pre-feeding lipoic acid and carnitine for 30 days restored muscle mitochondria to more youthful function in these regards.

Of related interest is a report that glucose feeding, leading to insulin resistance, was associated with increased superoxide production by mitochondria isolated from rat hearts; co-administration of lipoic acid prevented this glucose-induced increase in mitochondrial superoxide production [12].

A parsimonious explanation for all of these observations – as suggested by Ames and colleagues – is that mitochondrial generation of oxidants increases during the aging process, leading to impairments of mitochondrial structure that compromise the efficiency of coupled mitochondrial respiration. Lipoic acid, by somehow suppressing mitochondrial generation of oxidants – or by amplifying mitochondrial mechanisms which scavenge these oxidants – helps to restore the “youthful” structure and function of mitochondria.

### Does PPAR $\gamma$ coactivator-1 $\alpha$ mediate these effects?

PPAR $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) can serve as a coactivator for a wide range of transcription factors [13]. It has drawn particular attention because it has been shown to play a prominent role in mitochondrial biogenesis [14,15]. Increased expression of PGC-1 $\alpha$  has been found to promote transcription and synthesis of the nuclear respiratory factors NRF-1 and NRF-2, transcription factors which promote the transcription of numerous mitochondrial proteins and enzymes [14,16,17]. PGC-1 $\alpha$  is a crucial coactivator for NRF-1/2 function in this regard, and in particular these factors work together to promote transcription of mitochondrial transcription factor A (mtTFA) as well as the more recently characterized mitochondrial transcription specificity factors (TFB1M/TFB2M), required for the efficient transcription and replication of mitochondrial DNA [14,18]. When the PGC-1 $\alpha$  gene is transfected into cultured myotubes, a marked increase in cellular respiration is noted, in conjunction with an increase in mitochondrial DNA content and the levels of many mitochondrial proteins [14]. Increased expression of PGC-1 $\alpha$ , mediated by activation of p38 MAP kinase, as well as an increase in free intracellular calcium that induces increased activity of the ATF2 and MEF2 transcription factors via calcium/calmodulin-dependent protein kinase IV and calcineurin, appears to be largely responsible for the increase in muscle mitochondrial mass and aerobic power that is induced by exercise training [13,16,19,20].

There is very recent evidence that PGC-1 $\alpha$  likewise enhances the expression of a wide range of proteins that contribute importantly to the antioxidant protection of mitochondria. Valle and colleagues have shown that overexpression of PGC-1 $\alpha$  in various types of endothelial cells induces markedly increases expression of the manganese-dependent superoxide dismutase (SOD2), UCP-2, peroxiredoxins 3 and 5, mitochondrial thioredoxin, mitochondrial thioredoxin reductase, and catalase – all of which contribute importantly to mitochondrial antioxidant defense [21]. Such transfection also increases mitochondrial membrane potential in these cells, suggestive of improved mitochondrial function. In particular, these transfected cells experienced considerably less oxidant stress, and the increase in oxidant stress associated with exposure to elevated glucose was substantially mitigated.

The ability of PGC-1 $\alpha$  to decrease mitochondrial oxidant stress – despite an increase in mitochondrial membrane potential – is reminiscent of the effects of lipoic acid supplementation in various tissues. Could lipoic acid be increasing the expression and/or activity of PGC-1 $\alpha$ ?

There are several reasons to believe that lipoic acid might indeed have such an effect. For one thing, there are two reports that

lipoic acid can promote activation of p38 MAP kinase (in myotubes and monocytes) [22,23]. Activation of this kinase is known to increase both the expression and activity of PGC-1 $\alpha$  [20,24,25]. Phosphorylation of PGC-1 $\alpha$  by p38 MAP kinase has at least two key effects – this inhibits the binding of p160 myb binding protein, a ubiquitously expressed protein which functions to inhibit the coactivational activity of PGC-1 $\alpha$ ; furthermore, this phosphorylation prolongs the half-life of PGC-1 $\alpha$  by suppressing its proteosomal degradation [24,25]. Thus, p38 MAP kinase activity enhances both the level and the coactivational activity of PGC-1 $\alpha$ . Furthermore, this increase in PGC-1 $\alpha$ 's activity can be expected to increase the transcription of the PGC-1 $\alpha$  gene in skeletal muscle, as PGC-1 $\alpha$  functions as a coactivator for myocyte enhancer factor 2, which binds to the PGC-1 $\alpha$  promoter to activate transcription [26]. Since PGC-1 $\alpha$  appears to promote transcription of SOD2, it is of interest to note a report that arachidonate's ability to trigger SOD2 induction in hepatocytes is mediated by activation of p38 MAP kinase [27].

Lipoic acid is also reported to activate AMP-activated kinase (AMPK) in skeletal muscle, vascular endothelium, and hepatocytes [28–30]. For unclear reasons, it appears to *inhibit* activity of this kinase in the hypothalamus – an effect associated with increased satiety [31]. These discordant effects of lipoic acid on AMPK activity are precisely parallel to those of leptin – for which reason it has been suggested that lipoic acid may somehow activate the signaling pathway downstream from leptin [31]. One of the well documented effects of AMPK is to activate p38 MAP kinase – an effect which mediates its impact on glucose transport [32–36]. Whether this might be the sole basis of lipoic acid's ability to activate p38 is unclear.

Other activators of AMPK (such as metformin, AICAR, and the creatine antagonist  $\beta$ -guanidinopropionic acid – GPA) have been shown to increase the expression of PGC-1 $\alpha$  mRNA in endothelial cells and in rat skeletal muscle; transfection with dominant negative forms of AMPK abolished these effects [37–41] (AMPK does not appear to mediate the impact of exercise on PGC-1 $\alpha$  expression, however [42]). In cultured endothelial cells, the increased expression of PGC-1 $\alpha$  associated with metformin or AICAR exposure was accompanied by increased expression of the manganese-dependent mitochondrial superoxide dismutase and a substantial abrogation of hyperglycemia-induced mitochondrial oxidant production [38]. Other researchers have confirmed that metformin can inhibit mitochondrial oxidant generation in endothelial cells [43]. In skeletal muscle *in vivo*, GPA-mediated activation of AMPK was associated with a twofold increase in mitochondrial DNA, suggestive of mitochondrial biogenesis [41].

How AMPK might increase PGC-1 $\alpha$  expression has not been studied. The impact of AMPK on p38 MAP kinase may play a role in this regard in skeletal muscle – while also boosting the coactivational activity of PGC-1 $\alpha$ . It has also been suggested that an induced increase in expression of calcium/calmodulin-dependent protein kinase IV, as observed in GPA-treated muscle, might mediate an increase in the transcription of the PGC-1 $\alpha$  gene, as this kinase mediates the stimulatory impact of intracellular calcium on PGC-1 $\alpha$  transcription [19,41]. It should be noted that AMPK has the potential to *inhibit* transcription of PGC-1 $\alpha$  in hepatocytes, since AMPK phosphorylates and thereby excludes from the nucleus TORC2, a transcriptional coactivator for CREB that promotes hepatic expression of PGC-1 $\alpha$ ; this mechanism may contribute to the inhibitory impact of metformin on hepatic gluconeogenesis [44].

Recently, Unger and colleagues have reported that 4 days of high-dose lipoic acid feeding markedly enhanced activation of AMPK and increased the expression of PGC-1 $\alpha$  in the liver of rats; these effects lost statistical significance after 42 days [30]. Another recent report describes a reduction of the expression and activity of SOD2 in the retina of diabetic rats; long-term lipoic acid treatment

reversed this decrease [45] (it will be recalled that this form of superoxide dismutase is a target of PGC-1 $\alpha$  activity). This study did not clarify whether lipoic acid promoted SOD2 induction directly, or instead alleviated an inhibitory impact of hyperglycemia on this induction.

Intriguingly, partial deficiency of either SOD2 or peroxiredoxin 3 – mitochondrial antioxidant enzymes induced by PGC-1 $\alpha$  in endothelial cells – leads to a reduction in mitochondrial membrane potential, associated with a reduction in state 3, but an increase in state 4, respiration [46,47]. Note that administration of lipoic acid to aging rats is reported to have precisely the opposite impact on mitochondrial function [1,11].

### Possible inconsistencies

At least a couple of observations appear inconsistent with the hypothesis that lipoic acid boosts the expression and/or activity of PGC-1 $\alpha$ . For one, Ames et al. did not observe an impact of lipoic acid feeding on the oxygen uptake of hepatocytes from young rats [1]. Since PGC-1 $\alpha$  would be expected to increase mitochondrial mass in these cells, a failure to increase oxygen uptake may seem paradoxical. However, it might be argued that oxygen consumption in these cells is determined primarily by ATP demand, and that youthful mitochondria are quite proficient at meeting that demand; thus, an increase in mitochondrial mass, if it does not notably influence ATP demand, might not be expected to increase oxygen uptake – or only to the extent that there is an increase in uncoupled oxygen uptake (there was indeed a modest but statistically insignificant increase in oxygen uptake in the hepatocytes from lipote-supplemented young rats). On the other hand, the functionally impaired hepatic mitochondria of aged rats might be incapable of maintaining an ATP level that could maximize hepatocyte ATP utilization; the functional “rejuvenation” associated with lipoic acid supplementation might then boost ATP utilization by increasing the ATP level – an effect that would be associated with increased oxygen consumption, as was observed.

Another seemingly inconsistent observation is that Kumaran et al. observed a reduction in state 4 (uncoupled) respiration in mitochondria derived from the skeletal muscle of lipoate-fed aged rats [11] – whereas transfection of PGC-1 $\alpha$  into myotubes was found to *enhance* the oxygen consumption observed after oligomycin treatment [14] (oligomycin inhibits the mitochondrial ATPase, so that only uncoupled respiration is observed in its presence). Conceivably, this disparity could reflect differences in the function of myotubes and mature skeletal muscle.

Of course, it is conceivable that lipoic acid influences the expression or activity of mitochondrial antioxidant enzymes for reasons unrelated to PGC-1 $\alpha$  modulation. At this point, little is known about the transcription factors primarily responsible for expression of these enzymes, or about the mechanisms that regulate the half-lives of these enzymes. However, with respect to SOD2, forkhead transcription factors have been shown to promote its transcription [48], and PGC-1 $\alpha$  can act as a coactivator for FOXO1 [49] – so perhaps an interaction of PGC-1 $\alpha$  and forkhead factors plays a role in the induction of this enzyme. In any case, no relationship between lipoic acid and forkhead activity has been established. If indeed forkheads and PGC-1 $\alpha$  collaborate in the induction of SOD2, then measures which blunt Akt activation (caloric restriction or other strategies that down-regulate insulin/IGF-1, for example [50]) or diminish p66Shc expression or activation, used in conjunction with AMPK activators (metformin and lipoic acid), might be expected to have a particularly strong impact on expression of this key antioxidant enzyme. Whether forkheads participate in the induction of other mitochondrial-specific antioxidant enzymes is not yet known, but the key role of forkhead activ-

ity in longevity promotion suggests that this may be a likely possibility.

### Lipoic acid – an innocuous oxidant that evokes hormesis

It is clear that high-dose supplemental lipoic acid has great potential for alleviating the complications of diabetes [51–56]. This benefit is presumed to reflect antioxidant activity, and mitochondria are a prominent source of the excess superoxide generated when glucose-permeable cells are exposed to elevated glucose levels [57,58]. Lipoic acid is now known to have a phase 2 inductive effect [23,59,60], presumably because lipoic acid can act as a reactive electrophile for one or more key cysteine residues of Keap1, the protein that in its native form sequesters the Nrf2 transcription factor in the cytoplasm, preventing it from promoting expression of phase 2 proteins [61] (*note*: Nrf2 and NRF-2 are completely different!). Phase 2 inducers support antioxidant defenses, most notably by boosting glutathione synthesis and inducing heme oxygenase-1 [23,60,62]. It seems unlikely, however, that these effects would be sufficient to explain the ability of lipoic acid to inhibit oxidant production by aging mitochondria. Nor is the direct antioxidant activity of dihydrolipoic acid likely to explain this effect, in light of the fact that only a tiny percentage of the lipoic acid pool occurs in reduced form in cells [63]. Thus, it would be of great interest to examine the impact of lipoic acid treatment on the expression of a range of mitochondrial antioxidant enzymes, and to determine whether increased expression or activity of PGC-1 $\alpha$  mediates any effects observed in this regard.

Although lipoic acid is generally presumed to function as an antioxidant when administered in therapeutic doses, lipoic acid per se (as opposed to its derivative dihydrolipoic acid) is an oxidant, capable of reacting covalently with free cysteine groups. Conceivably, the main therapeutic effects of lipoic acid reflect its ability to serve as an innocuous oxidant that, most fortunately, has a high affinity for certain signaling proteins that function to detect oxidative stress or reactive electrophiles [63]; activation of these signaling proteins then triggers various adaptive responses which protect cells from stressors. This model is concordant with lipoic acid's phase 2 inductive activity; not unlikely, lipoic acid's impact on p38 MAP kinase, AMPK – and possibly PGC-1 $\alpha$  – will ultimately be shown to fit this model as well. The ability of mild stressors to evoke long lasting protection against subsequent assault by greater stressors is often referred to as “hormesis”; arguably, lipoic acid is an outstanding clinical tool for evoking hormesis. Its remarkable antioxidant activity will be adequately explained if future research establishes that it can induce not only phase 2 antioxidants, but also the diverse array of antioxidant enzymes that provide protection to mitochondria.

### A role in promoting longevity?

Recent evidence suggests that induction of PGC-1 $\alpha$  may be a key mechanism whereby caloric restriction enhances lifespan in rodents [13,64]. This induction likely reflects a prominent role for forkhead transcription factors in transcription of the PGC-1 $\alpha$  gene [13,65]; growth factors such as insulin or IGF-I inhibit forkhead function via Akt [66,67]. The increased mitochondrial biogenesis consequent to PGC-1 $\alpha$  expression implies that electron flux per mitochondrion decreases, leading to a reduction in mitochondrial membrane potential and a consequent decrease in mitochondrial superoxide production [64]. This can be expected to decrease the rate at which mitochondrial DNA accumulates mutations. There is growing evidence that mutagenesis of mitochondrial DNA plays a pacesetter role in the aging process – a view known as “the mitochondrial theory of aging” [68–73]. If indeed lipoic acid can

promote PGC-1 $\alpha$  expression/activity via p38 MAP kinase, it will be of interest to determine whether high-dose lipoic acid can prolong longevity in rodents, and possibly potentiate the utility of caloric restriction in this regard. Although a recent study failed to observe an impact of lipoic acid feeding, commencing in middle age, on longevity in mice, the dose used in this study, 0.06% of diet, was barely a tenth as high as the dose (0.5%) shown to modulate mitochondrial function in rats [74].

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