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Initial Assessment of Lactate as Mediator of Exercise-Induced Retinal Protection

Jana T Sellers¹, Micah A Chrenek¹, Preston E Girardot¹, John M Nickerson¹, Machel T Pardue^{2,3}, Jeffrey H Boatright^{4,5,6}

¹Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA, USA.

²Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA.

³Atlanta VA Center for Visual and Neurocognitive Rehabilitation, Decatur, GA, USA.

⁴Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA, USA

⁵Atlanta VA Center for Visual and Neurocognitive Rehabilitation, Decatur, GA, USA

⁶Atlanta VA Medical Center, Research Service (Oph151), Decatur, GA, USA

Abstract

Physical exercise is protective in rodent models of retinal injury and disease. Data suggest that this is in part mediated by brain-derived neurotrophic factor (BDNF) signal transduction. It has been hypothesized that exercised-induced neuroprotection may be mediated by increases in circulating lactate that in turn alter BDNF secretion. We therefore tested whether mice undergoing a treadmill running regimen previously shown to be protective in a mouse model of retinal degeneration (RD) have increased serum levels of lactate. Lactate levels in exercised and non-exercised mice were statistically indistinguishable. A role for circulating lactate in exercise-induced retinal protection is unsupported.

Keywords

Exercise; Retinal degeneration; Neuroprotection; Lactate; Brain-derived neurotrophic factor; BDNF; Mouse

74.1 Introduction

Exercise may protect vision and the retina in humans and animal models. Retrospective studies with retinal degeneration (RD) patients suggest that increased physical activity, including moderate exercise (walking) in people over age 75, is associated with reduced risk of age-related macular degeneration (AMD) development and progression, whereas decreased physical activity is associated with AMD precursors (reviewed in (Ong et al.)). We find that increased physical activity is associated with greater self-reported visual function in RP patients (Levinson et al.). Animal studies indicate that exercise has direct benefits for the retina and vision. An enriched environment paradigm that includes wheel

running protects against RD in the B6.CXB1-*Pde6b*^{rd10}/J mouse model of RP (the “rd10” mouse) (Barone et al.). Swimming protects mouse retinal ganglion cells following ischemic insult (Chrysostomou et al. ; Chrysostomou et al.). We (Allen et al.) and others (Kim et al.) find that treadmill exercise also protects retinas in streptozotocin-injected rats, a model of diabetic retinopathy. We find that modest running exercise alone protects the retinas and visual function of mice undergoing light-induced retinal degeneration (Lawson et al. ; Chrenek et al.) in the I307N Rho mouse (Zhang et al.), and in the rd10 mouse (Hanif et al.).

There are several potential mechanisms of action that may mediate exercise-induced retinal protection. We find that exercise increases circulating and retinal levels of BDNF, whereas treatment of mice with a BDNF TrkB receptor antagonist, ANA-12, prevents the protective effects of exercise, suggesting that BDNF signal transduction in part mediates the neuroprotective effects of exercise (Lawson et al. ; Hanif et al.). These data are similar to those from numerous studies in human and animals reporting that beneficial effects of exercise on various brain regions are mediated by increased levels of circulating and tissue-specific BDNF (Zoladz and Pilc).

Other studies examining the beneficial effects of exercise on the central nervous system (CNS) suggest a role for elevated levels of circulating lactate. For instance, mice exercised by running on treadmills at rates and durations that elevate circulating levels of lactate, but not circulating levels of BDNF, exhibit enhanced hippocampal biogenesis and increased VEGF expression, effects that are mimicked by exogenous local or systemic lactate infusion (Berthet et al. ; Bergersen ; Proia et al. ; Pensel et al.) or direct application of lactate to hippocampal slices (Billat et al. ; E et al.). Although the exercise regimens that we find are protective in models of retinal degeneration and diabetic retinopathy are likely to be below that needed to significantly increase circulating levels of lactate (cf. (Lawson et al. ; Hanif et al. ; Chrenek et al. ; Allen et al.) with (Billat et al.)), recent data from Hurley and colleagues suggest that retinal energy metabolism could be sensitive to changes in circulating lactate (Hurley et al. ; Kanow et al.), while other groups hypothesize that exercise-induced effects on CNS targets, including retina, may be mediated by changes in circulating lactate that in turn alter BDNF secretion (Bergersen ; Kolko et al. ; Proia et al. ; Pensel et al. ; Vohra et al.). We therefore tested whether serum lactate levels increase in mice following treadmill running at rates and durations at and beyond those that we have previously found to protect against retinal degeneration.

74.2 Materials and Methods

74.2.1 Animals

All mouse handling procedures and care were approved by the Emory Institutional Animal Care and Use Committee and followed ARVO guidelines of animal care. Adult (3–4 months old) BALB/cAnNCrl male mice were obtained from Charles River Laboratory, housed under a 12g12 hour (h) light-dark cycle, and had access to standard LabDiet 5001 mouse chow ad libitum.

74.2.2 Treadmill Exercise

Mice were exercised on a rodent treadmill with shock detection (Exer-3/6, Columbus Instruments) identical to protocols as previously reported in exercise-induced retinal protection experiments (Lawson et al. ; Chrenek et al.) with exceptions. Briefly, cohorts of mice were exercised 5 d for 60 min each day at a rate of 10 m/min (Lawson et al. ; Chrenek et al.) with the exception of a subgroup that was exercised at 16 m/min on the fifth day. In this same time, other mice were placed on a static acclimation platform made from the same materials and design as the treadmills (functionally, a “run rate” of 0 m/min).

74.2.3 Blood Collection

Immediately after the last exercise session, mice were sacrificed by CO₂ inhalation and eyes enucleated. Blood was collected from the enucleation site (~0.6 ml) directly into tubes specifically designed for serum separation (Sarstedt 1.1 ml Z-Gel microtubes, cat. #41.1378.005). Blood samples were kept at room temperature until centrifuged. Blood samples were centrifuged 30 min after the last blood sample was collected. Blood samples were centrifuged at 20 °C, 10,000 RCF for 5 min. Serum was transferred to fresh microfuge tubes and stored at –80 °C immediately after separation by centrifugation.

74.2.4 Lactate Assay

Lactate was measured from serum samples by colorimetric enzymatic reaction per manufacturer’s protocol (Abcam ab65331). Briefly, a standard curve was made using dilutions of lactate standard in lactate assay buffer. Serum samples were diluted 1g100 in lactate assay buffer. 50 µl of serum from diluted experimental samples and standard curve dilutions were distributed into a 96 well plate. Reaction mix was prepared by mixing 46 µl lactate assay buffer, 2 µl lactate substrate mix, and 2 µl lactate enzyme mix per sample and standard. 50 µl of reaction mix was added to each well and the plate placed in the plate reader (BioTek Synergy H1MF). Plates were incubated at room temperature for 30 min with orbital shaking at 80 rpm. Absorbance was measured at OD450 nm. Concentration of lactate in experimental samples was calculated from absorbance measures against the standard curve. Group means were statistically compared by ordinary one-way ANOVA with Tukey’s multiple comparisons test. Results were considered statistically significant if $p < 0.05$. Data of Fig. 74.1 are displayed as mean $\pm$ standard deviation (SD).

74.3 Results

Treadmill exercise of mice at a rate (10 m/min) and duration (1 h per day) previously shown to protect against retinal degeneration (Lawson et al. ; Chrenek et al.) did not increase the concentration of lactate in serum. As shown in Fig. 74.1, the concentration of serum lactate from unexercised mice (running rate = 0 m/min) is about 10 mM, similar to serum lactate concentrations of unexercised mice measured by this commercially available assay and reported elsewhere (Guglielmetti et al. ; Deng et al.). Even a treadmill running rate of 16 m/min, a running rate greater than that known to be needed to protect against retinal degeneration, did not result in increased serum concentrations of lactate (Fig. 74.1, far right bar). Indeed, though not statistically significant, concentrations trended downward with increasing rate.

74.4 Discussion

We report here that mice that underwent the same exercise regimen previously shown to be protective of photoreceptor function and morphology and visual function (Lawson et al. ; Chrenek et al.) did not increase the concentration of lactate in serum. It appears, to this first approximation, that elevation of circulating lactate may not be necessary for exercise-induced retinal neuroprotection.

There are caveats to this conclusion based on this initial work; it is clear that lactate local to the retina is critical for retinal function and health (Hurley et al. ; Kolko et al. ; Kanow et al. ; Vohra et al.). Future studies should examine the effects of exercise on lactate shuttles between photoreceptors and retinal pigment epithelial cells (Hurley et al. ; Kanow et al.) and between retinal neurons and inflammatory response cells such as Müller glia, astrocytes, and activated microglia. For instance, lactate released locally may protect adjacent retinal neurons and photoreceptor cells by acting as fuel for maintaining bioenergetic needs and as signal mediators, possibly by binding receptors and initiating second messenger cascades (Kolko et al. ; Mason ; Vohra et al.). Intriguingly, our pilot studies suggest that exercise alters Müller and microglia activation (data not shown).

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Fig. 74.1.

Modest treadmill exercise does not alter serum lactate levels. Mice were treadmill exercised at 0, 10, or 16 m/min $\times$ 1 h/day $\times$ 5 days. Blood was collected within 15 min of exercise cessation. Serum was assayed for lactate concentration by colorimetric enzyme reaction per manufacturer's instructions (Abcam ab65331). Data are means $\pm$ SD; sampling sizes for 0, 10, and 16 m/min groups were 6, 4, and 2, respectively. Means are statistically indistinguishable