

**ORIGINS AND CONSEQUENCES OF MITOCHONDRIAL
DECLINE IN NUCLEATED ERYTHROCYTES**

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Erythrocytes have many roles in ion regulation and respiratory gas exchange, processes that depend upon energy provided by mitochondrial metabolism. Within a few months after red cells are released into the circulation, they exhibit changes in structure and function. Aging is accompanied by reduced membrane fluidity, losses in organelles and a reduction in gene expression and protein synthesis. The impact of cellular aging on the responsibilities of erythrocytes has only recently been studied. We have previously shown that erythrocytes defend ion and gas exchange properties as they age (1). Conversely, aging has a profound effect on the ability of cells to mount heat shock responses (2). Recent work has focused on the impact of age-dependent changes in mitochondria and bioenergetics. Aging erythrocytes experience dramatic losses in mitochondria. These changes could reduce the capacity of the older erythrocytes to perform energy dependent functions. Age-induced changes in mitochondria could also increase the vulnerability of aged cells to mitochondria-initiated programmed cell death, or apoptosis.

We mounted a series of studies to assess the origins and consequences of mitochondrial losses (3). What mechanisms are responsible for causing the changes in bioenergetics during aging? Do these mitochondrial changes alter energetics? Do the changes increase the propensity for apoptosis? In addition to investigating the effects of aging on erythrocyte energetics (discussed below), we assessed the relationship between cell aging periodicity and seasonal remodelling of tissue energetics. It is well known that Winter acclimation causes significant changes in muscle bioenergetics (4). Because fish experience a round of erythropoiesis in Spring, they enter Winter with aged cells. We examined the hypothesis that muscle remodelling was related to the age of the erythrocyte

population. Although older cells retain their capacity for anti-oxidant protection (3), it is possible that the combination of cold and cell age alters the rigidity of erythrocyte membranes, altering oxygen delivery/antioxidant protection. Specifically, we tested the hypothesis that seasonal changes in fish muscle energetics were imposed through these effects on the ability of erythrocyte to penetrate the vasculature.

Trout erythrocytes were separated into age classes using inherent differences in buoyant density. Based upon enzyme activities, the oldest cells demonstrate had lost about half of their mitochondria. Studies with isolated mitochondria showed there were no changes in mitochondrial enzymes stoichiometries, effects that are often seen in models of organismal aging. The oldest cells showed a profound decline in mtDNA transcripts, due to reductions in both transcription and mtDNA copy number but we saw no evidence for qualitative changes mitochondrial gene expression. Collectively, these data suggest that mitochondrial losses arise through controlled reductions in synthesis of mitochondrial precursors, rather than an accumulation of spontaneous defects.

Age also appeared to have pronounced effects on energy metabolism. Older cells had lower basal respiration than young cells. Despite the reduced rates of respiration, there was no effect of age on tissue ATP levels. In addition, the maximal respiratory rates were similar in old and young cells. Thus, old cells retain a capacity for elevating respiration, even if basal respiratory rates are lower than in young cells. The reduction in metabolic rate probably reflects the lower costs of other metabolic processes.

Mammalian erythrocytes lack nuclei and are unable to induce apoptosis. It was not known if nucleated erythrocytes of fish retain the capacity to induce apoptosis. Apoptosis could not be induced in either whole blood, young or old erythrocytes by pro-oxidants, mitochondrial inhibitors or the mitochondrial permeability transition agonist staurosporine. Surprisingly, treatment with cyclosporin A caused caspase 3 activation, DNA laddering and LDH leakage, but only in young cells. Fluorescence microscopy, caspase inhibitor studies suggest that mitochondria are not involved in erythrocyte apoptosis. However, the ability of erythrocytes to undergo apoptosis has important ramifications to the use of erythrocytes as biomarkers.

Collectively, these studies suggest that the mitochondrial changes with aging do not compromise cellular function, although trout erythrocytes can initiate apoptosis by non-mitochondrial pathways.

References

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