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# Cardiolipin as an oxidative target in cardiac mitochondria in the aged rat

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ABSTRACT

The aged heart sustains greater injury during ischemia (ISC) and reperfusion (REP) compared to the adult heart. In the Fischer 344 (F344) rat, aging decreases oxidative phosphorylation and complex III activity increasing the production of reactive oxygen species in interfibrillar mitochondria (IFM) located among the myofibrils. In the isolated, perfused 24 month old elderly F344 rat heart 25 min of stop–flow ISC causes additional damage to complex III, further decreasing the rate of oxidative phosphorylation. We did not observe further progressive mitochondrial damage during REP. We next asked if ISC or REP increased oxidative damage within mitochondria of the aged heart. Cardiolipin (CL) is a phospholipid unique to mitochondria consisting predominantly of four linoleic acid residues (C18:2). Following ISC and REP in the aged heart, there is a new CL species containing three oxygen atoms added to one linoleic residue. ISC alone was sufficient to generate this new oxidized molecular species of CL. Based upon oxidative damage to CL, complex III activity, and oxidative phosphorylation, mitochondrial damage thus occurs in the aged heart mainly during ISC, rather than during REP. Mitochondrial damage during ischemia sets the stage for mitochondrial-driven cardiomyocyte injury during reperfusion in the aged heart.



## 1. Introduction

Despite timely and successful reperfusion elderly patients sustain greater mortality and cardiac injury with acute myocardial infarction [1]. The Fischer 344 rat model of aging (F344) was used to investigate mechanisms of the enhanced susceptibility to myocardial damage. Isolated, buffer-perfused hearts from 24 mo. F344 rats (aged) exhibit decreased hemodynamic recovery and greater myocardial cell death following 25 min of 37 °C global stop–flow ischemia and 30 min reperfusion compared to hearts from 6 mo. adult controls [2], providing an appropriate cardiac model for study. Other laboratories also have observed enhanced cardiac damage in the aged Fischer 344 rat heart [3–5] as well as age-related increases in other rat strains [6] and other species [7,8]. Ischemic preconditioning, where antecedent periods of brief ischemia limit injury from a subsequent longer period of ischemia [5,9,10], is ineffective in the aged rat [5,11,12] and human hearts [13,14]. Thus, additional understanding of the mechanisms that underlie the age-enhanced susceptibility to ischemic damage are needed in order to develop strategies to protect the elderly heart during ischemia and reperfusion.

Mitochondrial dysfunction contributes to aging in the heart by increasing the production of reactive oxygen species (ROS) [15] and by favoring the release of cytochrome *c* to activate cell death pathways [16]. Mitochondrial defects predispose to an increase in cardiomyocyte death that leads to age-related decreases in cardiomyocyte number and an increase in areas of fibrosis [17]. Cardiac mitochondria exist in two functionally distinct populations within the myocyte. Subsarcolemmal mitochondria (SSM) are located underneath the plasma membrane and interfibrillar mitochondria (IFM) are situated among the myofibrils [18]. The content of IFM is decreased in the aged heart [19]. The rate of oxidative phosphorylation (OXPHOS) is decreased in IFM from hearts in 24 mo aged F334 rats, whereas SSM remain unaffected [19]. OXPHOS is tightly coupled in IFM from aged rats [19]. Dinitrophenol-uncoupled respiration is decreased, localizing the defect to the electron transport chain (ETC) [19]. IFM exhibit a decrease in OXPHOS with age using TMPD-ascorbate, an electron donor to complex IV, as a substrate [19]. Complex IV enzyme activity decreases with aging and is reversed by the addition of exogenous phospholipid liposomes [19,20], localizing the defect to the lipid environment of the inner mitochondrial membrane, rather than to the peptide subunits of complex IV [19–21].

Aging decreases the maximally expressed activity of complex III measured in detergent-solubilized mitochondria in IFM from aged hearts, but SSM are unaffected [22]. Complex III catalyzes electron transfer from ubiquinol to cytochrome *c* coupled to proton translocation

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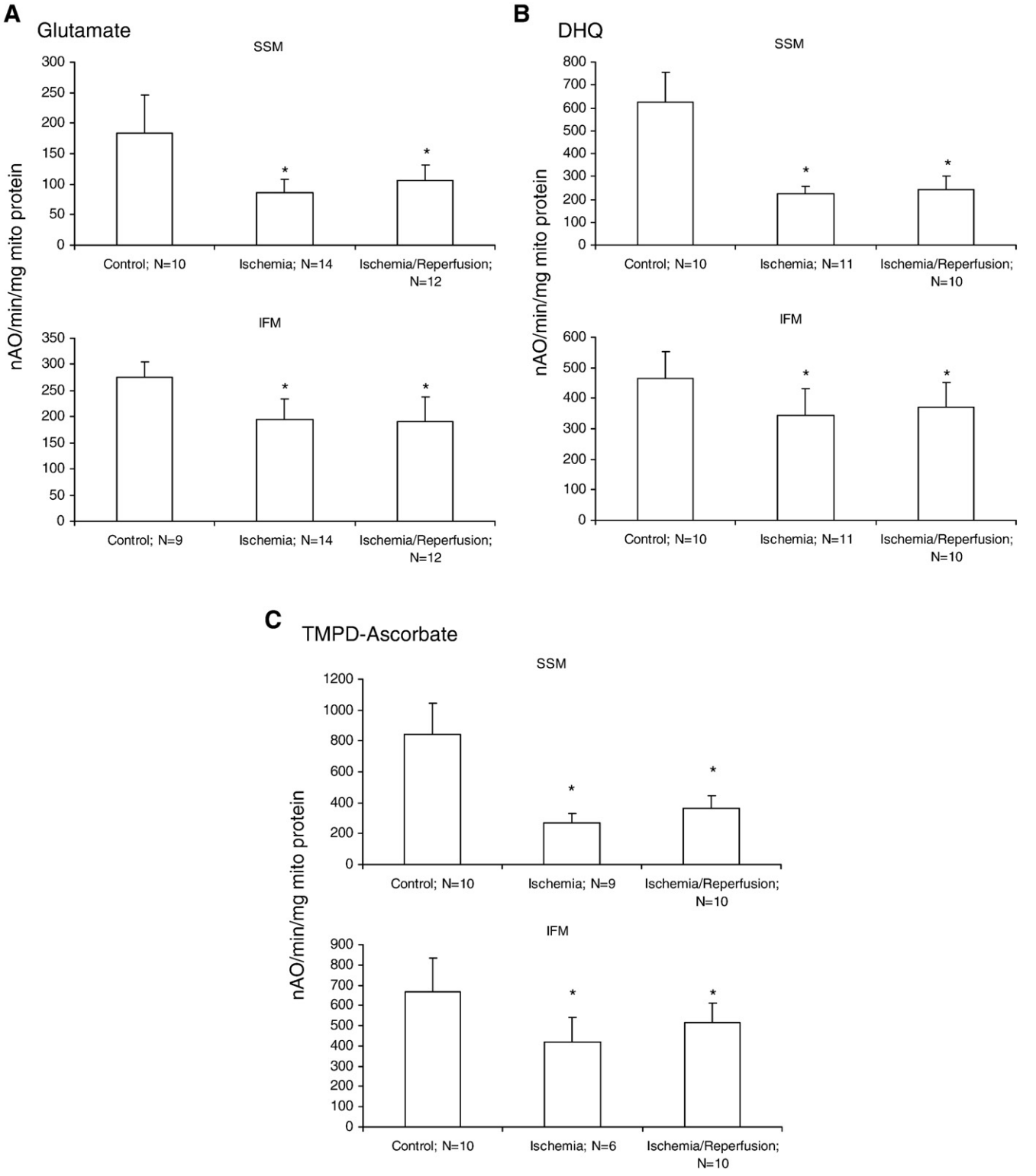


**Fig. 1.** Ischemia markedly decreases the maximal ADP-stimulated rate of oxidative phosphorylation in both subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria obtained from the 24 month old Fischer 344 rat heart. Reperfusion does not result in additional decreases in the rate of oxidative phosphorylation. Decreases are observed with glutamate (A), duroquinol, DHQ (B), and TMPD-ascorbate (C) as substrates. The decrease in the rate of respiration is similar in the mitochondria isolated both following 25' ischemia and following 25' ischemia plus 30' reperfusion. (Mean ± SD; $*p<0.05$; $p$ = NS Ischemia vs. Ischemia–Reperfusion.)

[23–25]. The complex is composed of two 11 subunit monomers. Each monomer contains three subunits, cytochrome *b*, cytochrome $c_1$, and the iron–sulfur protein (ISP) that participate in electron transfer [23,24,26,27]. The content of subunit peptides is not altered by age [22]. Functional studies using partial reactions within complex III localized the aging defect to the ubiquinone oxidation site of cytochrome *b* in complex III (Qo site) in IFM whereas SSM were unaffected [28]. The aging defect in the Qo site of complex III in IFM increases ROS production [28]. The net release of $H_2O_2$ was increased in IFM isolated from aged Fischer 344 rat hearts compared to adult controls, whereas SSM, without