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Postmortem mitochondrial membrane permeability transition assessment of apoptotic cell death in brain and liver of diabetic, insulin-resistant, ovariectomised rats

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Mitochondria perform a constitutional role in normal brain and liverfunctions, and in their disease processes. The adverse alterations in mitochondrial functionscan affect neuronal function negatively, as they play a crucial role in neuronal plasticity anddeath. Direct measurements of mitochondrial activity, including membrane potential andATP production, are not easily achieved in post-mortem brain and liver samples becausemost organ functions cease to work after death; in fact, with increasing postmortemintervals (PMI), the brain and liver tissues deteriorate rapidly. In the mitochondria, estrogen receptors play a pivotal role in regulating energy expenditures and protection from oxidative stress. Mitochondrial actions provide a model for assessing the relationship among menopause, estrogen exposures, Alzheimer's disease and estrogen effects on mitochondrial function. Therefore, this study sought to investigate the effects of PMI on mitochondrial membrane permeability transition (MMPT) pore status of control and insulin-resistant, ovariectomised rats; if (a) intact mitochondria can be isolated from brain and liver of these rats after storage in animal body (*in situ*) at -20°C for 7 days (168 hours, post-mortem), (b) these mitochondria can still take up exogenous Ca^{2+} and (c) they can still undergo osmotically induced swelling in Mannitol: Sucrose: HEPES (MSH) buffer

Materials and Methods: Rats were randomly divided into five groups: group 1 served as the control non-ovariectomised group fed normal pellet diet; group 2: control non-ovariectomised group fed high fat diet; group 3: an ovariectomised group fed normal diet; group 4: an ovariectomised group fed high fat diet; group 5: an ovariectomised, β- estradiol-treated group fed high fat diet. Standard procedures for mitochondria isolation (Johnson and Lardy,1967, Sims, 1990), protein determination expressed as BSA equivalent (Lowry et al., 1951)and spectrophotometric assessment of pore opening at 540 nm (Lapidus and Sokolove,1994) were employed. The results revealed that (a) intact mitochondria can be isolated from rat brain andliver of after storage in animal body (*in situ*) at -20 °C for 7 days (168 hours, post-mortem), (b) these mitochondria can still take up exogenous Ca^{2+} and (c) they can stillundergo osmotically-induced low amplitude swelling in a suitable buffer.Furthermore, using ovariectomized middle-aged rats to mimic the post-menopausal pathophysiological changes in women, we have demonstrated that the *in situ* post-morterm brain and liver may not be ample sources of mitochondria for functional assays at a PMI after 168 hours. Also, our results show that high-fat diet (lifestyle), ovarectomy (menopause), and a combination of both tend to enhance MMPT pore opening in 168–hour post-mortem brain. The other groups showed that mitochondrial pore opening wastime-dependent and consistent up to 5 minutes only, beyond which the amplitude swelling was notconsistent. The liver showed post-mortem deterioration in the normal, non-ovariectomisedgroup. Observation for the other groups show that 168–hour post-mortem liver mitochodria were still able to take up Ca^{2+} in a consistent time-dependent manner, though the degree of intactness in the absence of exogenous Ca^{2+} were low. β–estradiol treated group did not show this, meaningthat β–estradiol may not be able to restore the liver mitochondria to the pre-menopausal status.Mitochondrial dysfunction disrupts calcium homeostasis and increases oxidative stress within apoptotic subpopulations of neurones, as proposed to underlie neurodegenerative pathologies and associated cognitive decline estrogen-inducible neuroprotective mechanisms converge onto mitochondria, which are pivotal to sustaining calcium homeostasis and cell survival. Interestingly, estrogen-inducible neuroprotective mechanisms converge onto mitochondria, which are pivotal to sustaining calcium homeostasis and cell survival (Irwin et al., 2008; Grimm, 2016). Estrogen-activated cellular signaling cascade promotes enhanced mitochondrial function, leading to increased calcium load tolerance, enhanced electron transport chain efficiency, and promotion of antioxidant defense mechanisms (Morrison et al., 2006). The need for mitochondrial purity, structural integrity and abundance forfunctional studies are common hindrances that can encumber mitochondrial research. Therefore, this study is significant to have shown that PMI up to 7 days does not diminishMMPT pore status in normal and diabetic, ovariectomised rats. This can be relevant forforensic data mining.

Keywords: Post-mortem intervals; mitochondrial membrane permeability transition; diabetes ovariectomy.

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