DUNEDIN STUDY CONCEPT PAPER FORM

Provisional Paper Title: Association between mitochondrial dysfunction, oxidative stress and accelerated biological ageing in midlife

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Please describe your proposal in 2-3 pages with sufficient detail for helpful review.

Objective of the study:

Mitochondrial dysfunction is proposed to be one of the fundamental hallmarks of ageing (1). Mitochondria have a critical role in energy production and supply essential metabolites for a wide range of cellular processes. These organelles control activities ranging from proliferation and differentiation through to the regulation of inflammation and cell death. It was eloquently stated that "no structure is so intimately and simultaneously connected to both the energy of youth and the decline of the old" (2). Mitochondrial quality declines during ageing (2), and stem cell exhaustion and cellular senescence is associated with defects in the ability to clear dysfunctional mitochondria through a process termed mitophagy (3). Interventions that protect mitochondria or promote mitophagy have significantly improved the functioning of stem cells (4-6) and slowed the ageing of fruit flies (7).

Oxidative stress has also been closely associated with ageing. The mitochondrial free radical theory of ageing (8) is based on observations that mitochondria are both a source of reactive oxidants and extremely sensitive to oxidative stress. Increased levels of oxidised proteins, lipids and DNA are detected in aged tissue (9), but it is not clear if this damage plays a fundamental role in the ageing process or is simply a late marker of age-related pathology. This relationship has become more complex in recent years, with growing evidence that oxidants play vital roles as signalling molecules. In some animal models, mild oxidative stress can trigger adaptive responses that increase lifespan and high-level antioxidant supplementation is reported to have detrimental effects (10).

Various markers of mitochondrial dysfunction and oxidative stress were measured in Dunedin Study participants at age 45. Protein carbonyls, allantoin and growth differentiation factor 15 (GDF-15) were measured in plasma. Carbonylation is an irreversible protein modification generated during a range of different oxidation processes (11), allantoin is an oxidation product of uric acid (12), and GDF-15 is a cytokine released systemically that is proposed to play a role in adaptive responses to mitochondrial stress (13,14). Plasma protein carbonyls and GDF-15 are reported to increase in older populations (14-17), while allantoin has not previously been assessed. The redox status of two members of the peroxiredoxin family, which are extremely sensitive to oxidation, was also measured in blood cells. Mitochondrial peroxiredoxin 3 oxidation was measured in platelets, and peroxiredoxin 2 oxidation was measured in red blood cells before and after challenge with hydrogen peroxide. These cellular measures of redox homeostasis may provide more sensitive markers of oxidative stress than end-point markers of oxidative damage.

Mitochondrial dysfunction and oxidative stress biomarkers at age 45 will provide baseline measures for each participant as they journey from middle age to old age. However, previous research with the Dunedin study cohort from age 26 to age 45 has revealed that some participants are ageing significantly faster than others. This was quantified from a composite score from 19 biomarkers measured at four chronological ages (18). An increased pace-of-ageing is associated with accelerated ageing of the brain (brainAGE) as determined structural MRI data (19), and a DNA methylation biomarker (DunedinPACE) (20). We hypothesize that the fastest-ageing individuals will show increased levels of mitochondrial dysfunction and oxidative stress.

Data analysis methods:

We will test the association of six markers of mitochondrial dysfunction and oxidative stress with accelerated ageing at midlife (assess by pace-of-ageing, facial ageing, BrainAGE and DunedinPACE).

First, we will test the associations between each mitochondrial dysfunction/oxidative stress marker and ageing measures using regression analyses. Next, we will test the association between all mitochondrial dysfunction/oxidative stress markers and ageing measures in a multiple regression model. To investigate if these associations are due to potentially confounding variables (smoking, BMI, urea, hematological measures), we will consecutively add these variables to the model as covariates.

We will then perform several sensitivity analyses: 1) to investigate if associations between mitochondrial dysfunction/oxidative stress markers with pace of ageing scores are driven by a particular biomarker, we will performs correlation analyses with mitochondrial dysfunction/oxidative stress markers and each of the 19 individual biomarkers that comprise the pace of ageing score, then systematically remove individual biomarkers from the pace of ageing score and reinvestigate associations with mitochondrial dysfunction and oxidative stress markers 2) we will remove study members with common age-related diseases (i.e., cancer, diabetes) and test if associations remain, 3) to test if the associations are driven by the long right-hand tail of the pace of ageing, we will repeat analyses after log-transforming and winsorizing pace of ageing scores, and 4) to assess if previously identified batch effects in the mitochondrial dysfunction and oxidative stress data (variation associated with batch processing

necessary for large-scale proteomic/metabolomic studies, such as inter-operator variability and storage time), we will reinvestigate associations between ageing measures and normalized values for mitochondrial dysfunction/oxidative stress markers, using the residuals, or variation not explained by batch effects.

All statistical analyses will be performed in R (Rstudio), with appropriate packages. Models will be adjusted for sex variables will be transformed and standardized as appropriate.

Variables needed at which ages:

Age 45:

Mitochondrial dysfunction and oxidative stress markers

- GDF-15, allantoin, protein carbonyls, Prx3, Prx2 basal and challenge

Ageing measures

- Pace-of-aging (including constituent biomarkers), Facial ageing, BrainAGE, DunedinPACE

Covariates

- Sex, BMI, smoking status, urea, haematological measures, disease status (age-related chronic conditions)

Significance of the Study (for theory, research methods or clinical practice):

Evidence for an association between the rate of ageing and mitochondrial dysfunction/oxidative stress does not indicate a causative role in ageing, but detection of an association prior to the onset of age-related disease would reveal these as early events in human ageing. In addition to providing insight into the fundamental processes associated with ageing, the mitochondrial dysfunction and oxidative stress biomarkers may prove useful as biomarkers of ageing that can be used to monitor the impact of therapies proposed to slow or reduce the detrimental effects of ageing.

References:

1. Lopez-Otin, C., M.A. Blasco, L. Partridge, M. Serrano, and G. Kroemer. The hallmarks of aging. Cell, 2013. 153:1194-217.

2. Sun, N., R.J. Youle, and T. Finkel. The mitochondrial basis of aging. Mol Cell, 2016. 61:654-66.

3. Yamamoto-Imoto, H., S. Minami, T. Shioda, Y. Yamashita, S. Sakai, S. Maeda, T. Yamamoto, S. Oki, M. Takashima, T. Yamamuro, K. Yanagawa, R. Edahiro, M. Iwatani, M. So, A. Tokumura, T. Abe, R. Imamura, N. Nonomura, Y. Okada, D.E. Ayer, H. Ogawa, E. Hara, Y. Takabatake, Y. Isaka, S. Nakamura, and T. Yoshimori. Age-associated decline of MondoA drives cellular senescence through impaired autophagy and mitochondrial homeostasis. Cell Rep, 2022. 38:110444.

4. Mansell, E., V. Sigurdsson, E. Deltcheva, J. Brown, C. James, K. Miharada, S. Soneji, J. Larsson, and T. Enver. Mitochondrial potentiation ameliorates age-related heterogeneity in hematopoietic stem cell

function. Cell Stem Cell, 2021. 28:241-56 e6.

5. Sun, X., B. Cao, M. Naval-Sanchez, T. Pham, Y.B.Y. Sun, B. Williams, S.Y. Heazlewood, N. Deshpande, J. Li, F. Kraus, J. Rae, Q. Nguyen, H. Yari, J. Schroder, C.K. Heazlewood, M. Fulton, J. Hatwell-Humble, K. Das Gupta, R. Kapetanovic, X. Chen, M.J. Sweet, R.G. Parton, M.T. Ryan, J.M. Polo, C.M. Nefzger, and S.K. Nilsson. Nicotinamide riboside attenuates age-associated metabolic and functional changes in hematopoietic stem cells. Nat Commun, 2021. 12:2665.

6. Vannini, N., V. Campos, M. Girotra, V. Trachsel, S. Rojas-Sutterlin, J. Tratwal, S. Ragusa, E. Stefanidis, D. Ryu, P.Y. Rainer, G. Nikitin, S. Giger, T.Y. Li, A. Semilietof, A. Oggier, Y. Yersin, L. Tauzin, E. Pirinen, W.C. Cheng, J. Ratajczak, C. Canto, M. Ehrbar, F. Sizzano, T.V. Petrova, D. Vanhecke, L. Zhang, P. Romero, A. Nahimana, S. Cherix, M.A. Duchosal, P.C. Ho, B. Deplancke, G. Coukos, J. Auwerx, M.P. Lutolf, and O. Naveiras. The NAD-booster nicotinamide riboside potently stimulates hematopoiesis through increased mitochondrial clearance. Cell Stem Cell, 2019. 24:405-18 e7.

7. Schmid, E.T., J.-H. Pyo, and D.W. Walker. Neuronal induction of BNIP3-mediated mitophagy slows systemic aging in Drosophila. Nat Aging, 2022. 2:494-507.

8. Harman, D. The biologic clock: the mitochondria? J Am Geriatr Soc, 1972. 20:145-7.

9. Bokov, A., A. Chaudhuri, and A. Richardson. The role of oxidative damage and stress in aging. Mech Ageing Dev, 2004. 125:811-26.

10. Shields, H.J., A. Traa, and J.M. Van Raamsdonk. Beneficial and detrimental effects of reactive oxygen species on lifespan: a comprehensive review of comparative and experimental studies. Front Cell Dev Biol, 2021. 9:628157.

11. Buss, I.H. and C.C. Winterbourn. Protein carbonyl measurement by ELISA. Methods Mol Biol, 2002. 186:123-8.

12. Kozlik, P., L. Hasikova, B. Stiburkova, J. Zavada, and K. Kalikova. Rapid and reliable HILIC-MS/MS method for monitoring allantoin as a biomarker of oxidative stress. Anal Biochem, 2020. 589:113509.

13. Hubens, W.H.G., A. Vallbona-Garcia, I.F.M. de Coo, F.H.J. van Tienen, C.A.B. Webers, H.J.M. Smeets, and T.G.M.F. Gorgels. Blood biomarkers for assessment of mitochondrial dysfunction: an expert review. Mitochondrion, 2022. 62:187-204.

14. Conte, M., C. Giuliani, A. Chiariello, V. Iannuzzi, C. Franceschi, and S. Salvioli. GDF15, an emerging key player in human aging. Ageing Res Rev, 2022. 75:101569.

15. Tanaka, T., A. Biancotto, R. Moaddel, A.Z. Moore, M. Gonzalez-Freire, M.A. Aon, J. Candia, P. Zhang, F. Cheung, G. Fantoni, C.H.I. consortium, R.D. Semba, and L. Ferrucci. Plasma proteomic signature of age in healthy humans. Aging Cell, 2018. 17:e12799.

16. Gil, L., W. Siems, B. Mazurek, J. Gross, P. Schroeder, P. Voss, and T. Grune. Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. Free Radic Res, 2006. 40:495-505.

17. Pandey, K.B., M.M. Mehdi, P.K. Maurya, and S.I. Rizvi. Plasma protein oxidation and its correlation with antioxidant potential during human aging. Dis Markers, 2010. 29:31-6.

18. Elliott, M.L., A. Caspi, R.M. Houts, A. Ambler, J.M. Broadbent, R.J. Hancox, H. Harrington, S. Hogan,

R. Keenan, A. Knodt, J.H. Leung, T.R. Melzer, S.C. Purdy, S. Ramrakha, L.S. Richmond-Rakerd, A. Righarts, K. Sugden, W.M. Thomson, P.R. Thorne, B.S. Williams, G. Wilson, A.R. Hariri, R. Poulton, and T.E. Moffitt. Disparities in the pace of biological aging among midlife adults of the same chronological age have implications for future frailty risk and policy. Nat Aging, 2021. 1:295-308.

19. Elliott, M.L., D.W. Belsky, A.R. Knodt, D. Ireland, T.R. Melzer, R. Poulton, S. Ramrakha, A. Caspi, T.E. Moffitt, and A.R. Hariri. Brain-age in midlife is associated with accelerated biological aging and cognitive decline in a longitudinal birth cohort. Mol Psychiatry, 2021. 26:3829-38.

20. Belsky, D.W., A. Caspi, D.L. Corcoran, K. Sugden, R. Poulton, L. Arseneault, A. Baccarelli, K. Chamarti, X. Gao, E. Hannon, H.L. Harrington, R. Houts, M. Kothari, D. Kwon, J. Mill, J. Schwartz, P. Vokonas, C. Wang, B.S. Williams, and T.E. Moffitt. DunedinPACE, a DNA methylation biomarker of the pace of aging. Elife, 2022. 11:e73420.