References

1. Ben-Shlomo Y, Cooper R, Kuh D. The last two decades of life course epidemiology, and its relevance for research on ageing. *Int J Epidemiol* 2016;**45**:973–88.
2. Fontana L, Partridge L, Longo VD. Extending Healthy Life Span—From Yeast to Humans. *Science* 2010;**328**:321–26.
3. Justice JN, Carter CS, Beck HJ *et al*. Battery of behavioral tests in mice that models age-associated changes in human motor function. *Age (Dordr)* 2013;**36**:583–95.
4. Sanders J, Boudreau R, Newman A. Understanding the Ageing Process using Epidemiologic Approaches. In: Newman AB, Cauley JA (eds). *The Epidemiology of Ageing*. Rotterdam, The Netherlands: Springer Netherlands, 2012.
5. Newman AB, Arnold AM, Sachs MC *et al*. long-term function in an older cohort - the cardiovascular health study all stars study. *J Am Geriatr Soc* 2009;**57**:432–40.
6. Lara J, Cooper R, Nissan J *et al*. A proposed panel of biomarkers of healthy ageing. *BMC Med* 2015;**13**:1–8.
7. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in Utero and Early-Life Conditions on Adult Health and Disease. *N Engl J Med* 2008; **59**:61–73.
8. Broer L, Codd V, Nyholt Dr *et al*. Meta-analysis of telomere length in 19[thinsp]713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *Eur J Hum Genet* 2013;**21**:1163–68.
9. Keogh M, Chinnery Patrick F. Hereditary mtDNA Heteroplasmy: A Baseline for Ageing? *Cell Metab* 2013;**18**: 463–64.
10. Fleg JL, Morrell CH, Bos AG *et al*. Accelerated Longitudinal Decline of Aerobic Capacity in Healthy Older Adults. *Circulation* 2005;**112**:674–82.
11. Cauley JA, Greendale GA, Ruppert K *et al*. Serum 25 Hydroxyvitamin D, Bone Mineral Density and Fracture Risk Across the Menopause. *J Clin Endocrinol Metab* 2015;1002046–54.
12. Metter EJ, Conwit R, Tobin J, Fozard JL. Age-Associated Loss of Power and Strength in the Upper Extremities in Women and Men. *J Gerontol A Bio Sci Med Sci* 1997;**52a**:b267–76.
13. Arnold AM, Newman AB, Cushman M, Ding J, Kritchevsky S. Body Weight Dynamics and Their Association with Physical Function and Mortality in Older Adults: The Cardiovascular Health Study. *J Gerontol A Bio Sci Med Sci* 2010;**65a**:63–70.
14. Whitehead BR, Bergeman CS. Ups and Downs of Daily Life: Age Effects on the Impact of Daily Appraisal Variability on Depressive Symptoms. *J Gerontol B Psychol Sci Soc Sci* 2014;**69**:387–96.
15. Levine Me. Modeling the Rate of Senescence: Can Estimated Biological Age Predict Mortality More Accurately Than Chronological Age? *J Gerontol A Bio Sci Med Sci* 2013;**68**: 667–74.
16. Holford TR, Levy DT, Mckay LA *et al*. Patterns of Birth Cohort–Specific Smoking Histories, 1965–2009. *Am J Prev Med* 2014;**46**:e31–7.

International Journal of Epidemiology, 2016, 1015–1019
doi: 10.1093/ije/dyw104

Commentary: Life course epidemiology embraces geroscience

Luigi Ferrucci

Intramural Research Program, National Institute on Aging – NIH, 251 Bayview Boulevard, Baltimore, MD, 21224 USA. E-mail: Ferruccilu@mail.nih.gov

Accepted 3 March 2016

In this issue of the *Journal*, Yoav Ben Shlomo, Rachel Cooper and Diana Kuh present a comprehensive and state-of-the-art review of life course epidemiology; they describe their own unique scientific contributions and offer a much needed update on conceptual and methodological advancements that have occurred in this field over the past few years.[1] Of relevance for this commentary, Ben Shlomo *et al*. observe that the focus of life course epidemiology has broadened from simply identifying early life predictors of disease development to depicting physiological and functional trajectories over the entire lifespan and seeking explanatory models for heterogeneous pathways. A corollary to this expanded focus is the need to distinguish between chronological and biological ageing, which represents a radical departure from traditional epidemiology and opens a window of opportunity to the study of ageing as a dynamic process that, perhaps, one day may be modulated through specific interventions.[2]

Right from the first day of training, epidemiologists learn that ageing is a powerful risk factor for chronic disease, disability and mortality. The effect of ageing on health is so strong that the only possible way of dealing with it … is by ignoring it or, more precisely, by dissecting it out from the analyses. 'Adjusting for age and sex' remains a mantram today, a *sine qua non* of any study that aims to connect specific risk factors to a disease outcome and pass the peer review process. There are, of course, good reasons for this approach. Up to very recently, the

mechanisms of ageing completely escaped our understanding and there was little hope that the ageing process and its phenotypic consequences could be modified. However, something has changed in recent years. We have witnessed extraordinary progress in ageing research that challenges some of the most rooted assumptions.[3] In particular, research in several laboratory species points to specific biological mechanisms that drive many of the phenotypic manifestations of ageing.[4] In other words, the idea of slowing down ageing is no longer considered the delirium of creative novelists, such as Dorian Grey for Oscar Wilde, but rather a hypothesis that could be scientifically tested.[5]

Research has shown that the longevity of yeast, worms and even small mammals can be extended through genetic manipulation, dietary restriction or pharmacological intervention.[6–9] Recently, López-Otín and colleagues conducted an extensive review of this literature and outlined the major mechanisms hypothesized to drive ageing and likely to be the targets of these intervention. These mechanisms include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication.[10] Methodologies are available or in advanced stages of development that permit evaluation of some of these mechanisms in humans. Telomere length, epigenetic DNA methylation changes, mitochondrial function and inflammation already have been assessed in large epidemiological studies[11–14] and genomic instability and DNA damage repair capacity can be effectively assessed in blood mononuclear cells using high-throughput sequencing.[15,16]

Although detection of senescent cells in humans is still problematic, $P16^{4ink}$, a specific biomarker of senescence, can be easily measured in circulating lymphocytes and has been found to be associated with ageing and negative health outcomes.[17] Chaperone and other proteins involved in proteostasis can be assessed by liquid chromatography/mass spectrometry (LC-MS), but whether their quantification has biological and clinical meaning is still under investigation.[18] Problems with many of these assays remain: they are expensive, time consuming, mostly limited to blood cells and have unknown sensitivity to changes over time and with environmental stresses. History tells us that changes in technology will help overcome these limitations, for example by substantially reducing the cost of sequencing, extensive use of robotics and developing micromethods that can assess multiple biomarkers from small quantities of biological material. Introducing these assays in epidemiological studies should facilitate testing the hypothesis that change in biomarkers of ageing track the basic ageing phenotypes, such as muscle strength decline or loss of aerobic fitness, above and beyond chronological ageing, and may also help stimulate new hypotheses about the biological nature of ageing in humans and its relationship with disease and the ageing phenotype.

Understanding the mechanisms underpinning the ageing process opens the door to research aimed at modulating this process and preventing its phenotypic manifestations. Theoretically, slowing down ageing would reduce age-specific incidence and prevalence of major chronic diseases and delay age-associated cognitive and physical decline and perhaps even push the debilitating consequences much closer to the end of life. Thus, slowing the rate of ageing could substantially improve the health of the global population and reduce health care needs.[19]

There is still a long journey before we can translate basic research findings to clinical applications. To reconcile research findings on basic biological mechanisms of ageing with what we are learning from epidemiological studies will require the development of new methodology tailored to this new purpose. In this regard, integrating geroscience in the design of life course epidemiological studies offers a particularly promising perspective. Indeed, geroscience aims at understanding the molecular and cellular mechanisms through which ageing becomes the major risk factor for chronic disease. Most of what we know about human ageing comes from either cross-sectional studies that compare individuals of different ages or from longitudinal studies that assess multiple phenotypes in ageing individuals with serial evaluations over limited time periods. Attempts to bridge this gap exist.[17] For example, considerable resources have been dedicated to identifying biological biomarkers that correlate with chronological ageing and at the same time track and predict the rate of deterioration with ageing of some phenotypic parameters. To illustrate, large studies of whole blood gene expression consistently have found that transcripts in the pathways of 'innate and adaptive immunity' and 'cytokines and chemokines signalling' appear to be upregulated whereas those related to 'oxidative phosphorylation' or 'mitochondrial function' are downregulated with ageing.[20]

Consistent with these findings, it has been demonstrated that high levels of pro-inflammatory cytokines predict multiple chronic diseases as well as sarcopenia and mobility loss.[21] Similarly, mitochondrial dysfunction has been linked to many age-related chronic diseases, such as diabetes and sarcopenia.[22,23] More recently, epigenetic clocks developed using methylation levels at 71 and 353 CpG sites, respectively, accurately estimate chronological age in humans and, independent of age, predict mortality and other health outcomes.[13,24,25] Strong evidence exists for the predictive validity of these exemplary biomarkers for multiple adverse health outcomes, but the mechanisms that alter their expression and may explain their predictive

qualities, remain unknown. For instance, we don't know why blood levels of pro-inflammatory markers increase, mitochondrial function declines or specific patterns of methylation track ageing. Given the dynamic nature of the ageing process, it is reasonable to hypothesize that these biological changes are adaptive in nature, although at later stages towards the end of life they may contribute to loss of functional integrity with ageing.[26]

It is intrinsically difficult to interpret the biological role of 'accelerated ageing' biomarkers without information on the mechanisms that lead to them. For example, a pro-inflammatory state may emerge in response to a challenge and therefore serve to dampen the effect of that challenge on health. That is, the predictive validity of the pro-inflammatory state of ageing could be a function of an unknown condition that stimulated production of pro-inflammatory cytokines in the first place, rather than the cytokines themselves. Thus, without further information, we cannot exclude that reducing inflammation may remove the adaptive compensation and, ultimately, have negative effects on health. To further illustrate, the consistent finding of an inverse association between blood levels of pro-inflammatory markers and brain health and cognition[27–29] has been interpreted to indicate that inflammation is a risk factor as well as a protective agent. Similarly, some investigators have proposed that insulin resistance protects cells from being overloaded by fuel that cannot be processed and, therefore, that removing insulin resistance could be more deleterious than useful.[30] Finally, there is some evidence that age-related epigenetic changes may represent evolutionary selected adaptive strategies aimed at re-adjusting cell physiology to compensate for changes in metabolism due to ageing. Importantly, these examples demonstrate why we need to be extremely cautious in initiating clinical trials that target a particular biomarker based on correlative data only, without understanding the mechanisms underlying its alteration.

Traditional cross-sectional and short-term longitudinal studies cannot differentiate adaptive from causative biomarkers because they do not have the length of time to capture both the precipitating biological process(es) that alter the biomarker and the cascade of response and/or consequences. New science emerging from life course epidemiology has begun to reveal the limits of these 'snapshot' approaches by demonstrating that the processes that lead to disease and loss of function evolve over many decades and are characterized by recognizable stages.[1] For example, we can hypothesize that increased visceral fat, or the accumulation of senescent cells, is followed by a pro-inflammatory state, that in turn increases risk of several diseases and adverse phenotypes, including sarcopenia. But what happens when people accumulate visceral fat but do not develop inflammation? Traditional time-limited epidemiological studies cannot adequately address this question because they do not have sufficient data to compare the long-term consequences of developing or not developing a hypothetical adaptive strategy.

The ideal setting for conducting these type of studies is a life course design where a birth cohort is identified (ideally with information on parents and gestation environment) and followed to exhaustion. Incidentally, this perspective is consistent with the description in Ben Shlomo's paper of predictive adaptive response (PAR) that occurs in early life to maximize the chances of survival and reproduction, but may be maladaptive in the long term. The biological basis of this version of 'antagonistic pleiotropy' probably relies on epigenetic, metabolic, hormonal, neurological, immunological and energetic adaptations that are then interpreted later in life as disease risk biomarkers. According to the literature, diseases develop when one of these PARs becomes maladaptive.[31,32] However, an alternative interpretation is that deployment of different sorts of pre-packaged PARs over the lifespan is necessary to respond to challenges at different ages, at least during the reproductive stage. This view is consistent with the idea that some ageing phenotypes derive from damage accumulation due to incomplete or inadequate stress response, in organisms that are losing resilience.

Generating hypotheses about the biological nature of PARs that develop with ageing, and developing measures to assess these PARs and follow up their long term consequences in terms of their impact on trajectories of physiological and functional variables across the life span, including peak values and rate of age-related change, would tremendously enrich future life course epidemiological projects and provide a critical tool to test some of the hypotheses generated by geroscience. It is a win-win situation: neither geroscience nor life course epidemiology can be fully exploited without the another.

Although a few milestones for this transformation have already been achieved, many design elements are still missing and need further development. To make real progress, future life course epidemiological studies will require the following:

i. long-term financial commitments that span over decades. Although this element is somewhat outside the scope of this commentary, it is nonetheless critical because it represents a straight departure from the funding mechanisms of the past. Without some certainty of funding continuity, the initial effort required to establish a new birth cohort may be considered unjustified;

ii. a study design with a core set of measures maintained relatively stable over time and with pre-defined strategies to offset unavoidable changes in assessment technology;
iii. explicit strategies to minimize the burden to participants, encourage their participation and minimize attrition. These may include motivation through provision of feedback, administration of questionnaires through the INTERNET and use of wearable biosensors to collect data while minimizing the burden on participants;
iv. mechanisms to ensure that knowledge about the study is conveyed across generations of investigators;
v. a clear operational definition of the physiological and functional systems to be targeted, together with some understanding about how frequently they should be assessed to adequately capture change trajectories and avoid missing important departures, and methods to minimize floor and ceiling effects;
vi. pre-specification of a limited number of hypotheses concerning the biology of ageing that are supposed to drive, directly or indirectly, the main ageing phenotypes of interest.

Points (i)-(ii) are self-explanatory and (v) was extensively considered by Ben Shlomo and colleagues, but point (vi) requires some more explanation. If we consider that physiological and functional trajectories of ageing phenotypes are driven by the ageing process itself, then a starting point would be to consider measuring the biological mechanisms proposed for ageing in model organisms. [9] As mentioned above, measures of the 'hallmarks of ageing' outlined by López-Otín and colleagues may serve as reasonable starting points. However, analogous to many physiological variables, static measures of these mechanisms would not adequately track the ageing process, especially in early life which is characterized by a preponderance of developmental processes and great functional reserve. For this scope, challenge tests and measures of resilience would probably work better. For example, measuring mitochondrial respiration at rest would not be very useful, whereas obtaining the same parameter before and after a bout of exercise may be more useful. Analogously, measures of DNA repair capacity could be developed in blood cells in which a standard amount of DNA damage was introduced; epigenetic changes that follow and acute intervention could be studied, or the accumulation of senescent cells in the skin could be quantified.[33] Pursuing this approach requires considerable pilot work and validation. Hopefully in the future, the technology of 'omics' will allow assessments of these mechanisms with methodologies that are both feasible in living subjects and affordable. However, at this stage there are no such easy shortcuts.

Life course epidemiology and geroscience are natural partners; as in any partnership, we may see in the future some incomprehension and conflicting findings, but their integration is key to success in the study of human ageing. There is no doubt that Ben Shlomo and collaborators opened the door to this development. It is to be hoped that funding agencies and researchers from multiple disciplines, and especially gerontologists, will follow their lead.

Funding

This research was supported entirely by the Intramural Research Program of the National Institutes of Health, National Institute on Aging.

Acknowledgments

The author would like to thank Felipe Sierra of the National Institute of Aging for comments and suggestions and Eleanor Simonsick for reviewing the final manuscript.

Conflict of interest: None declared.

References

1. Ben-Shlomo Y, Cooper R, Kuh D. The last two decades of life course epidemiology, and its relevance for research on ageing. *Int J Epidemiol* 2016;**45**:973–88.
2. Rockwood K, Mitnitski A. Frailty in relation to the accumulation of deficits. *J Gerontol A Biol Sci Med Sci* 2007;**62**:722–27.
3. Kennedy BK, Berger SL, Brunet A *et al*. Geroscience: linking aging to chronic disease. *Cell* 2014;**159**: 709–13.
4. Burch JB, Augustine AD,, Frieden LA *et al*. Advances in geroscience: impact on healthspan and chronic disease. *J Gerontol A Biol Sci Med Sci* 2014;**69**(**Suppl 1**):S1–3.
5. Franceschi C, Valensin S, Bonafè M *et al*. The network and the remodeling theories of aging: historical background and new perspectives. *Exp Gerontol* 2000;**35**: 879–96.
6. Harrison DE, Strong R, Sharp ZD *et al*. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 2009;**460**: 392-95.
7. Mitchell SJ, Scheibye-Knudsen M, Longo DL, de Cabo R. Animal models of aging research: implications for human aging and age-related diseases. *Annu Rev Anim Biosci* 2015;**3**:283–303.
8. Liao C-Y, Kennedy BK. Mouse models and aging: longevity and progeria. *Curr Top Dev Biol* 2014;**109**: 249–85.
9. Kennedy BK, Pennypacker JK. Drugs that modulate aging: the promising yet difficult path ahead. *Transl Res* 2014;**163**: 456–65.
10. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;**153**: 1194–217.
11. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science* 2015;**350**:1193–98.
12. Coen PM, Jubrias SA, Distefano G *et al*. Skeletal muscle mitochondrial energetics are associated with maximal aerobic

capacity and walking speed in older adults. *J Gerontol A Biol Sci Med Sci* 2013;**68**:447–55.
13. Marioni RE, Shah S, McRae AF *et al*. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol* 2015;**16**:25.
14. Ferrucci L, Harris TB, Guralnik JM *et al*. Serum IL-6 level and the development of disability in older persons. *J Am Geriatr Soc* 1999;**47**:639–46.
15. Maslov AY, Quispe-Tintaya W, Gorbacheva T, White RR, Vijg J. High-throughput sequencing in mutation detection: A new generation of genotoxicity tests? *Mutat Res* 2015;**776**:136–43.
16. Nagel ZD, Chaim IA, Samson LD. Inter-individual variation in DNA repair capacity: a need for multi-pathway functional assays to promote translational DNA repair research. *DNA Repair (Amst)* 2014;**19**:199–213.
17. Newgard CB, Sharpless NE. Coming of age: molecular drivers of aging and therapeutic opportunities. *J Clin Invest* 2013;**123**: 946–50.
18. Kaushik S, Cuervo AM. Proteostasis and aging. *Nat Med* 2015;**21**: 1406–15.
19. Blagosklonny MV. Prospective treatment of age-related diseases by slowing down aging. *Am J Pathol* 2012;**181**:1142–46.
20. Peters MJ, Joehanes R, Pilling LC *et al*. The transcriptional landscape of age in human peripheral blood. *Nat Commun* 2015;**6**: 8570.
21. Fabbri E, An Y, Zoli M *et al*. Aging and the burden of multimorbidity: associations with inflammatory and anabolic hormonal biomarkers. *J Gerontol A Biol Sci Med Sci* 2015;**70**:63–70.
22. Gonzalez-Freire M, de Cabo R, Bernier M *et al*. Reconsidering the role of mitochondria in aging. *J Gerontol A Biol Sci Med Sci* 2015;**70**:1334–42.
23. Remmen H. Oxidative damage to mitochondria and aging. *Exp Gerontol* 2001;**36**:957–68.
24. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol* 2013;**14**: R115.
25. Hannum G, Guinney J, Zhao L *et al*. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 2013;**49**:359–67.
26. Blagosklonny MV. Revisiting the antagonistic pleiotropy theory of aging: TOR-driven program and quasi-program. *Cell Cycle* 2014;**9**:3171–76.
27. Thambisetty M, Ferrucci L. Soluble interleukin-6 receptor levels and risk of dementia: one more signpost on a long road ahead. *J Am Geriatr Soc* 2014;**62**:772–74.
28. McCarrey A, Pacheco J, Carlson O *et al*. Interleukin-6 (IL-6) is associated with longitudinal rates of cortical thinning. *Alzheimer's Dement* 2013;**9**:307.
29. Schram MT, Euser SM, De Craen AJM *et al*. Systemic markers of inflammation and cognitive decline in old age. *J Am Geriatr Soc* 2007;**55**:708–16.
30. Barzilai N, Ferrucci L. Insulin resistance and aging: a cause or a protective response? *J Gerontol A Biol Sci Med Sci* 2012;**67**: 1329–31.
31. Gluckman PD, Hanson MA, Beedle AS. Early life events and their consequences for later disease: A life history and evolutionary perspective. *Am J Hum Biol* 2007;**19**:1–19.
32. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev* 2014;**94**:1027–76.
33. Rodier F, Campisi J. Four faces of cellular senescence. *J Cell Biol* 2011;**192**:547–56.