Effects of Aging on Metabolic Characteristics of Human B Cells

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Abstract: Metabolic changes represent the most common sign of aging and lead to increased risk of developing diseases typical of old age. Age-associated metabolic changes, such as decreased insulin sensitivity, decreased mitochondrial function, and dysregulated nutrient uptake, fuel the low-grade chronic systemic inflammation, known as inflammaging, a leading cause of morbidity and mortality, linked to the development of several diseases of old age. How aging affects the metabolic phenotype of immune cells, and B cells in particular, is not well known and is under intensive investigation by several groups. In this study, we summarized the few published results linking intrinsic B-cell metabolism and B-cell function in different groups of young and elderly individuals: healthy, with type-2 diabetes mellitus, or with HIV infection. Although preliminary, these results suggest the intriguing possibility that metabolic pathways can represent potential novel therapeutic targets to reduce inflammaging and improve humoral immunity.

Key Words: aging, inflammation, metabolism, humoral immunity

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AGING IS ASSOCIATED WITH INCREASED SYSTEMIC INFLAMMATION

Aging is associated with low-grade chronic systemic inflammation, a status called inflammaging.[1] While acute inflammation is a beneficial protective response to harmful conditions and is fundamental for survival,[2] inflammaging is considered a leading cause of morbidity and mortality because it is involved in initiation and establishment of several age-driven health conditions and diseases.[3–5] These include cardiovascular diseases, cancer, type-2 diabetes mellitus (T2DM), chronic kidney disease, nonalcoholic fatty liver disease, osteoporosis, sarcopenia, autoimmune diseases, and neurodegenerative conditions.[6] To evaluate the link between inflammaging and disease risk, a multiomics study has performed high-throughput molecular profiling of adult individuals followed up longitudinally, measuring the transcriptome (whole blood gene expression), the immunome (whole blood expression of cytokines and chemokines in immune cells), and the frequencies of circulating immune cells (T/B/NK cells). Results have led to the construction of a high-dimensional trajectory of immune aging, called IMM-AGE, which described the immune status of the individuals better than chronological age and predicted all-cause mortality.[7] A more recent study has applied artificial intelligence in systems and computational immunology of aging to identify an inflammatory clock of aging. This clock, called iAGE, has been shown to be able to track with multimorbidities, immunosenescence, frailty and cardiovascular aging, and also predict extreme life span in centenarians.[8] Using iAGE, it has also been possible to identify crucial contributors to iAGE and inflammaging, which can be blocked to silence at least in vitro aging phenotypes of mouse and human cells.

The quality of life in childhood, especially the presence of psychological stressors, such as poverty and malnutrition, is significantly associated with the risk of developing inflammatory conditions and diseases, and to die, later in life.[9] These effects persist throughout life span.[10] From an evolutionary point of view, inflammaging is induced by the ability of the body to adapt to and to counteract all stressors encountered during life, but this process causes the accumulation of cellular and molecular scars.[11] Other factors able to regulate inflammaging include polymorphisms in the promoter regions of proinflammatory and anti-inflammatory genes, chronic stimulation of immune cells with viruses such as HIV and cytomegalovirus, cellular senescence, increased deposition of fat in internal organs, increased permeability of the gut, changes in the composition of the gut microbiome, increased production of altered molecules generated by damaged or dead cells and organelles, and exposure to xenobiotics (air pollutants, hazardous waste products, industrial chemicals, and tobacco smoking).[11,12] All these factors, alone and in combination, induce defects in cells of the innate and adaptive immune system and increase the frequency and severity of infectious diseases in the elderly individuals. It has been proposed that the balance between inflammaging and anti-inflammaging determines the rate of aging, the onset of age-associated diseases and their severity and the individual's ability to reach extreme longevity.[2] Therefore, the identification of pathways inducing inflammaging and anti-inflammaging across multiple systems is needed to design

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strategic therapeutic interventions aimed to increase health span of elderly individuals.

INFLAMMAGING DECREASES HUMORAL RESPONSES

Acute inflammation is crucial for the protective, biological, and immunological response to infections and is therefore crucial for survival. However, if the acute inflammatory reaction cannot be properly turned out, the inflammatory response may progress to the chronic stage, inflammaging, which is negatively associated with protective responses to infections and vaccines such as the influenza,[13] hepatitis B,[14,15] and yellow fever[16] vaccines, supporting the concept that the inflammatory status of an individual before vaccination determines their capacity to respond to the vaccine. Therefore, efforts to reduce background inflammation might be a promising strategy of intervention to improve vaccine responses not only in the elderly individuals but also in individuals with inflammatory conditions and diseases such as obesity, autoimmunity, and chronic viral infections. This may be particularly important for individuals developing severe inflammation after SARS-Cov-2 where reducing inflammation may boost vaccine efficacy.

Mechanistically, inflammaging induces intrinsic inflammation and chronic immune activation (IA) in B cells, both negatively associated with decreased immune responses against infections and vaccines,[17–19] but positively associated with increased autoimmune pathogenic responses. Higher levels of inflammaging (circulating TNF-α) induce higher intrinsic TNF-α in B cells from old mice[20] and humans.[18] This higher inflammatory phenotype makes B cells from old mice and humans refractory to further stimulation and reduces their capacity to make protective antibodies in response to infections or vaccination.[18,20] Mechanistically, serum TNF-α upregulates the expression of its receptors on B cells and induces NF-kB activation and secretion of TNF-α and other proinflammatory cytokines and chemokines.[21] Of importance, blocking TNF-α with specific antibodies has been shown to increase B-cell function, at least in vitro, in mice[20] and humans.[18]

Similar to B cells, elevated TNF-α levels are negatively associated with T-cell function because TNF-α directly downregulates the transcription of the *CD28* gene leading to reduced membrane expression of *CD28*.[17] Experiments in vitro have clearly shown that the incubation of $CD28^{+}$ T-cell lines with TNF-α induces the appearance and the expansion of $CD28^{null}$ clones. $CD28^{null}$ clones also expand in the blood of elderly individuals, and their presence is associated with a low response to the influenza vaccine.[22]

METABOLIC DYSFUNCTION IS A HALLMARK OF AGING

Inflammaging is involved in the establishment of insulin resistance (IR),[23,24] as shown in mouse studies conducted by Hotamisligil who showed that blocking inflammaging through neutralization of TNF-α improves IR.[25,26] Other major metabolic changes observed in older adults in addition to increased IR include changes in body composition and increased fat mass[27,28] and decreased mitochondrial function.[29,30] All these factors are interconnected and in turn fuel inflammaging leading to increased risks of developing diseases typical of old age. Metabolic dysfunction due to either hypernutrition or sedentary lifestyle has been shown to be associated with each of the 9 hallmarks of aging (genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication).[31]

Increased IR with age is not only a primary risk factor for the development of T2DM,[32] but it is also associated with several cardiometabolic changes that include dyslipidemia and increased hypertension and thrombogenesis and is therefore a significant risk factor of cardiovascular disease and all-cause mortality. Increased physical activity is an effective lifestyle change that has a robust impact on health outcomes in elderly individuals.[33] Exercise training has indeed been shown to significantly decrease IR and enhance insulin-stimulated glucose uptake in the whole muscle and individual muscle fibers.[34–36]

Aging induces changes in the distribution of fat mass. A recently published work conducted in mice has shown that visceral adipose tissue (AT) is the most affected tissue by aging, suggesting that the AT is crucial for the organism's adaptation and response to aging.[37] Indeed, age-associated changes in the AT accelerate the onset of age-associated disease.[38,39] Computational tomography scans have shown that subcutaneous AT mass decreases while visceral AT mass increases with age,[40] with the latter being more inflammatory. An age-associated increase in the accumulation of triglycerides outside fat depots (liver, muscle, heart, pancreas, and kidney)[41–45] and in blood vessels[46] has also been reported. Changes in the AT with age include abundance, distribution, and cellular composition. Studies in mice have shown that obesity, similar to aging, leads to reduced lifespan,[47] whereas caloric restriction increases lifespan due to reduction in the amount of visceral adipose tissue depots.[48]

Aging increases the secretion of inflammatory mediators (IL-1β, IL-6, TNF-α, COX-2, and phospholipids) by adipocytes.[49,50] Ceramides are particularly interesting molecules because they link the excess of lipids with proinflammatory cytokines and IR.[49] Aging also increases the accumulation of senescent cells in the AT and the secretion of multiple factors that constitute the senescence-associated secretory phenotype (SASP),[51] such as proinflammatory factors (cytokines, chemokines, and micro-RNAs), soluble receptors (TNF receptors), nonprotein soluble factors (nitric oxide), growth factors (epidermal growth factor, vascular endothelial growth factor, and nerve growth factor), and extracellular matrix macromolecules (fibronectin, collagens, and laminin).[52] The age-dependent accumulation of senescent cells represents a favorable environment for the development of inflammatory-based age-associated diseases. Secretion of SASP products by adipose cells has been shown to inhibit adipogenesis, recruit immune cells, and induce IR.

One of the major theories of aging supports mitochondrial dysfunction as one of the primary causes of cellular

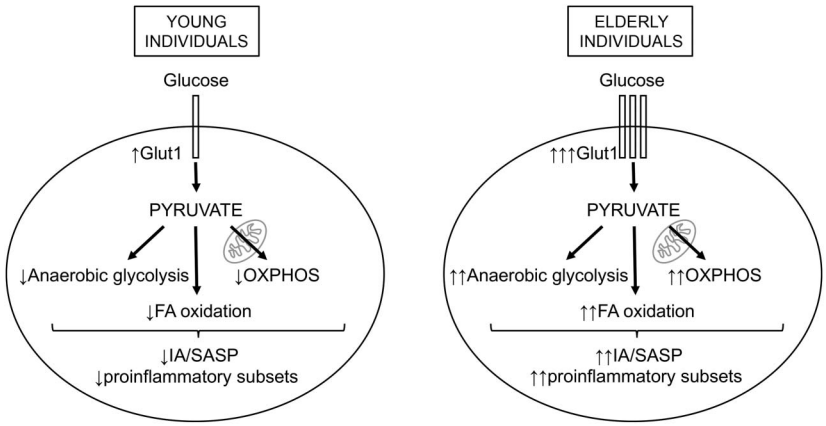

FIGURE 1. Effects of aging on the metabolic phenotype of peripheral blood B cells. Unstimulated B cells from elderly individuals, when compared with those from younger controls, are hypermetabolic. They upregulate the membrane expression of Glut1 and enroll in anaerobic glycolysis (in which glucose is incompletely oxidized in the cytosol, producing lactate as final product), OXPHOS, and FA oxidation (in which carbon substrates such as glucose-derived pyruvate or FAs are oxidized in the mitochondria to generate ATP, respectively). This hypermetabolic phenotype is associated with higher frequencies of proinflammatory subsets and IA and with higher secretion of inflammatory and metabolic mediators.

senescence, mainly but not exclusively due to the release of reactive oxygen species (ROS) causing oxidative damage to macromolecules. Studies conducted in mutant mice have clearly indicated a significant association between impaired mitochondrial DNA repair, accelerated aging, and sarcopenia.[53] An association between IR, glucose tolerance, and mitochondrial dysfunction has also been reported.[54,55] Mitochondrial DNA (mtDNA) can be released in the extracellular space and can function as a damage-associated molecular pattern signaling molecule, suggesting a role for mtDNA in the maintenance of the low-grade chronic inflammation observed in elderly individuals.[56]

AGING CHANGES THE METABOLIC PROFILE OF HUMAN B CELLS

Cell function is strictly determined by its metabolic status and its ability to regulate metabolic pathways. As opposed to T cells,[57–59] B-cell metabolic characteristics have not been thoroughly elucidated. Resting T cells require a minimum amount of energy, and they rely on oxidative phosphorylation (OXPHOS) fueled by glucose-derived pyruvate, exogenous fatty acids (FAs), and glutamine for adenosine triphosphate (ATP) production. After stimulation, T cells enter the cell cycle, rapidly divide, and also enroll in anaerobic glycolysis. Activated T cells are characterized by their enhanced metabolic flexibility because they can use multiple nutrients for energy production and biomolecule synthesis. Nutrient uptake supports their increased metabolic activity. The transition of T cells from OXPHOS to glycolysis and vice versa regulates cell survival and the expansion of antigen-specific T-cell clones.

Similar to T cells, B cells perform a metabolic reprogramming to meet the bioenergetic and biosynthetic demands associated with their function, and they rely on both anaerobic glycolysis and OXPHOS. One crucial pathway to upregulate anaerobic glycolysis and OXPHOS is through increased glucose uptake through Glut1, which is highly expressed in B cells, as evaluated by both mRNA and protein expression.[60]

As to the effects of aging on T-cell metabolism, it has been shown that after stimulation, $CD4^+$ T cells from young and elderly individuals equally increase glucose uptake and OXPHOS, with both measures being higher in T cells from elderly individuals when compared with those from younger controls. Increased OXPHOS was found to be associated with increased mitochondrial ROS and ATP production and secretion.[61]

How aging affects metabolic programs of human B cells is under intense investigation by several groups, including ours. However, only a few studies have evaluated so far the metabolic phenotype of unstimulated B cells from young and elderly individuals and how this associates with B-cell activation and differentiation. We have shown that the peripheral blood of elderly individuals when compared with that of younger controls is enriched in proinflammatory B cells that express RNA for multiple inflammatory mediators (cytokines, chemokines, and micro-RNAs). B cells from elderly individuals upregulate Glut1 expression and increase glucose uptake. Glucose uptake is associated with a higher metabolic phenotype, measured by increased OXPHOS, anaerobic glycolysis, and FA oxidation. This phenotype is necessary to support their secretory phenotype and increased IA. Figure 1 summarizes our recently published results[62] showing that aging changes the metabolic phenotype of peripheral blood B cells.

One of the first published studies has shown the link between lower antibody responses to the trivalent inactivated influenza vaccine in elderly individuals and the expression of metabolic markers in B cells.[63] Results have shown that antibody secreting cells in the blood of elderly individuals have higher mitochondrial mass and mitochondrial ROS but express lower levels of Sirtuin1 (SIRT1). SIRT1 has been shown to play crucial roles in determining life span of different species through the deacetylation of heterochomatin regions of the genome and of telomere regions, thus maintaining telomere stability and preventing DNA damage responses; in the regulation of responses to conditions such as fasting, caloric restriction, and exercise; in protection from oxidative stress-related cellular events; in inflammatory conditions being anti-inflammatory; and in the amelioration of neurodegenerative conditions, metabolic dysfunction, and cancer.[64,65] Levels of SIRT1 were higher in antibody secreting cells from individuals who had higher levels of H1N1-specific and H3N2-specific IgG antibody secretions, both young and elderly individuals. When naive B cells were

sorted from the blood of young and elderly individuals and analyzed by Seahorse to measure OXPHOS by OCR and glycolysis by ECAR, unstimulated B cells from young individuals were found to be higher in both measures when compared with B cells from elderly individuals. After culture with polyclonal stimuli, both OXPHOS and glycolysis increased when compared with unstimulated B cells, and significant defects in OXPHOS and mild defects in glycolysis were observed in B cells from elderly versus young individuals.

In a recently published article, we have evaluated the metabolic requirements of B-cell antibody responses in elderly patients with T2DM (E_{T2DM}) taking metformin or not, and compared with those in healthy elderly (E_H) and healthy young (Y_H) individuals.[62] Results have clearly showed that metformin increases in vivo B-cell function, measured by trivalent inactivated influenza vaccine–specific serum antibodies, in E_{T2DM} to the levels observed in E_H and Y_H individuals. Metformin also decreases the frequencies of proinflammatory B-cell subsets and intrinsic inflammation and metabolic requirements of peripheral B cells from E_{T2DM}. This hypermetabolic phenotype of B cells from E_{T2DM} is needed to support intrinsic inflammation, measured by the expression of transcripts for markers of the SASP, and the secretion of autoimmune antibodies. Of importance, B-cell function in E_{T2DM} taking metformin is not only increased when compared with that in E_{T2DM} not taking metformin, but is comparable with B-cell function measured in Y_H individuals. Although not evaluated in the abovementioned study, the effects of metformin on B cells from E_{T2DM} may have also been due to inhibitory effects on insulin/insulin-like growth factor signaling, activation of AMP-activated kinase and consequent inhibition of mTOR signaling, inhibition of mitochondrial complex 1 in the electron transport chain, and reduced production of ROS.[66] These results altogether highlight the relationship between metabolism and intrinsic inflammation and provide examples of how metabolic pathways can represent potential novel therapeutic targets, supporting the importance of clinical trials to improve health span with metformin.

AGING, HIV, AND IMMUNOMETABOLISM

HIV selectively infects highly metabolic $CD4^+$ T cells, characterized by high anaerobic glycolysis and OXPHOS,[67,68] and the susceptibility to HIV infection correlates with levels of expression of Glut1 expression and metabolic enzymes and with a hypermetabolic phenotype of both $CD4^+$ T cells[69] and monocytes.[70] There are no published findings on the effects of aging on the metabolic phenotype of T cells in HIV+ individuals.

We have recently shown that B cells from young and elderly HIV+ individuals, when compared with healthy controls, are enriched in proinflammatory B-cell subsets, express higher levels of RNA for proinflammatory markers and are hypermetabolic, and more in elderly HIV+ than in young HIV+ individuals.[71] Similar to what we have observed in healthy elderly individuals,[62] this higher metabolic phenotype of B cells from HIV+ individuals is needed to support IA, suggesting the intriguing possibility that metabolic pathways in B cells from HIV+ individuals may be targeted to limit inflammaging and IA and improve B-cell function and antibody responses in this vulnerable population.

CONCLUSIONS

Immune cell function and metabolism over the life course are closely related. A better understanding of the mechanisms by which metabolism influences B-cell function will help to identify novel targets to improve or normalize humoral immunity. We have shown that proinflammatory B cells, which express RNA for multiple inflammatory mediators, are increased in the blood of elderly when compared with young individuals. B cells from elderly individuals upregulate Glut1 expression, increase glucose uptake, and show increased OXPHOS, anaerobic glycolysis, and FA oxidation. This phenotype is necessary to support their secretory phenotype and increased IA. HIV also induces proinflammatory B cells that are hypermetabolic and more in elderly HIV+ than in young HIV+ individuals. Although more studies are needed to better clarify the link between B-cell metabolism and B-cell function, results so far obtained have suggested that it is possible to target the metabolic phenotype of B cells to reduce inflammaging and IA and improve B-cell function and antibody responses.

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REFERENCES

1. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* 2000;908:244–254.
2. Franceschi C, Capri M, Monti D, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev.* 2007;128:92–105.
3. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. *Nat Med.* 2019;25:1822–1832.
4. Netea MG, Balkwill F, Chonchol M, et al. A guiding map for inflammation. *Nat Immunol.* 2017;18:826–831.
5. Slavich GM. Understanding inflammation, its regulation, and relevance for health: a top scientific and public priority. *Brain Behav Immun.* 2015; 45:13–14.
6. Collaborators GBDCoD. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* 2018;392:1736–1788.
7. Alpert A, Pickman Y, Leipold M, et al. A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring. *Nat Med.* 2019;25:487–495.
8. Sayed N, Huang Y, Nguyen K, et al. An inflammatory aging clock (iAge) based on deep learning tracks multimorbidity, immunosenescence, frailty and cardiovascular aging. *Nat Aging.* 2021;1:598–615.
9. Miller GE, Chen E, Parker KJ. Psychological stress in childhood and susceptibility to the chronic diseases of aging: moving toward a model of behavioral and biological mechanisms. *Psychol Bull.* 2011;137:959–997.

10. Renz H, Holt PG, Inouye M, et al. An exposome perspective: early-life events and immune development in a changing world. *J Allergy Clin Immunol.* 2017;140:24–40.
11. Franceschi C, Garagnani P, Parini P, et al. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol.* 2018; 14:576–590.
12. Frasca D, Diaz A, Romero M, et al. B cell immunosenescence. *Annu Rev Cell Dev Biol.* 2020;36:551–574.
13. Nakaya HI, Hagan T, Duraisingham SS, et al. Systems analysis of immunity to influenza vaccination across multiple years and in diverse populations reveals shared molecular signatures. *Immunity*. 2015;43: 1186–1198.
14. Bartholomeus E, De Neuter N, Meysman P, et al. Transcriptome profiling in blood before and after hepatitis B vaccination shows significant differences in gene expression between responders and non-responders. *Vaccine*. 2018;36:6282–6289.
15. Fourati S, Cristescu R, Loboda A, et al. Pre-vaccination inflammation and B-cell signalling predict age-related hyporesponse to hepatitis B vaccination. *Nat Commun.* 2016;7:10369.
16. Muyanja E, Ssemaganda A, Ngauv P, et al. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. *J Clin Invest.* 2014;124:3147–3158.
17. Bryl E, Vallejo AN, Weyand CM, et al. Down-regulation of CD28 expression by TNF-alpha. *J Immunol.* 2001;167:3231–3238.
18. Frasca D, Diaz A, Romero M, et al. High TNF-alpha levels in resting B cells negatively correlate with their response. *Exp Gerontol.* 2014;54: 116–122.
19. Parish ST, Wu JE, Effros RB. Modulation of T lymphocyte replicative senescence via TNF-{alpha} inhibition: role of caspase-3. *J Immunol.* 2009;182:4237–4243.
20. Frasca D, Romero M, Diaz A, et al. A molecular mechanism for TNF-alpha-mediated downregulation of B cell responses. *J Immunol.* 2012; 188:279–286.
21. Miscia S, Marchisio M, Grilli A, et al. Tumor necrosis factor alpha (TNF-alpha) activates Jak1/Stat3-Stat5B signaling through TNFR-1 in human B cells. *Cell Growth Differ.* 2002;13:13–18.
22. Saurwein-Teissl M, Lung TL, Marx F, et al. Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. *J Immunol.* 2002;168:5893–5899.
23. Fink RI, Kolterman OG, Griffin J, et al. Mechanisms of insulin resistance in aging. *J Clin Invest.* 1983;71:1523–1535.
24. Ryan AS. Insulin resistance with aging: effects of diet and exercise. *Sports Med.* 2000;30:327–346.
25. Hotamisligil GS, Johnson RS, Distel RJ, et al. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. *Science*. 1996;274:1377–1379.
26. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259:87–91.
27. Bosy-Westphal A, Geisler C, Onur S, et al. Value of body fat mass vs anthropometric obesity indices in the assessment of metabolic risk factors. *Int J Obes (Lond).* 2006;30:475–483.
28. Zeng Q, Dong SY, Sun XN, et al. Percent body fat is a better predictor of cardiovascular risk factors than body mass index. *Braz J Med Biol Res.* 2012;45:591–600.
29. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. *Proc Natl Acad Sci U S A.* 1994;91: 10771–10778.
30. Sun N, Youle RJ, Finkel T. The mitochondrial basis of aging. *Mol Cell.* 2016;61:654–666.
31. Lopez-Otin C, Blasco MA, Partridge L, et al. The hallmarks of aging. *Cell*. 2013;153:1194–1217.
32. Wild S, Roglic G, Green A, et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047–1053.
33. Houmard JA, Tanner CJ, Slentz CA, et al. Effect of the volume and intensity of exercise training on insulin sensitivity. *J Appl Physiol (1985).* 2004;96:101–106.
34. Cartee GD, Arias EB, Yu CS, et al. Novel single skeletal muscle fiber analysis reveals a fiber type-selective effect of acute exercise on glucose uptake. *Am J Physiol Endocrinol Metab.* 2016;311:E818–E824.
35. Richter EA, Garetto LP, Goodman MN, et al. Muscle glucose metabolism following exercise in the rat: increased sensitivity to insulin. *J Clin Invest.* 1982;69:785–793.
36. Wojtaszewski JF, Hansen BF, Gade S, et al. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. *Diabetes.* 2000;49: 325–331.
37. Yu Q, Xiao H, Jedrychowski MP, et al. Sample multiplexing for targeted pathway proteomics in aging mice. *Proc Natl Acad Sci U S A.* 2020;117: 9723–9732.
38. Guo SS, Zeller C, Chumlea WC, et al. Aging, body composition, and lifestyle: the fels longitudinal study. *Am J Clin Nutr.* 1999;70:405–411.
39. Lutz W, Sanderson W, Scherbov S. The coming acceleration of global population ageing. *Nature*. 2008;451:716–719.
40. Zamboni M, Rossi AP, Fantin F, et al. Adipose tissue, diet and aging. *Mech Ageing Dev.* 2014;136–137:129–137.
41. Foster MC, Hwang SJ, Porter SA, et al. Fatty kidney, hypertension, and chronic kidney disease: the Framingham Heart Study. *Hypertension*. 2011;58:784–790.
42. Machann J, Thamer C, Schnoedt B, et al. Age and gender related effects on adipose tissue compartments of subjects with increased risk for type 2 diabetes: a whole body MRI/MRS study. *MAGMA*. 2005;18:128–137.
43. Ryan AS, Nicklas BJ. Age-related changes in fat deposition in mid-thigh muscle in women: relationships with metabolic cardiovascular disease risk factors. *Int J Obes Relat Metab Disord.* 1999;23:126–132.
44. Saisho Y, Butler AE, Meier JJ, et al. Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. *Clin Anat.* 2007;20:933–942.
45. Silaghi A, Piercecchi-Marti MD, Grino M, et al. Epicardial adipose tissue extent: relationship with age, body fat distribution, and coronaropathy. *Obesity (Silver Spring)*. 2008;16:2424–2430.
46. Robert L. Aging of the vascular-wall and atherosclerosis. *Exp Gerontol.* 1999;34:491–501.
47. Ahima RS. Connecting obesity, aging and diabetes. *Nat Med.* 2009;15: 996–997.
48. Barzilai N, Gupta G. Revisiting the role of fat mass in the life extension induced by caloric restriction. *J Gerontol A Biol Sci Med Sci.* 1999;54: B89–B96; discussion B97–8.
49. Summers SA. Ceramides in insulin resistance and lipotoxicity. *Prog Lipid Res.* 2006;45:42–72.
50. Wu D, Ren Z, Pae M, et al. Aging up-regulates expression of inflammatory mediators in mouse adipose tissue. *J Immunol.* 2007;179: 4829–4839.
51. Tchkonia T, Morbeck DE, Von Zglinicki T, et al. Fat tissue, aging, and cellular senescence. *Aging Cell.* 2010;9:667–684.
52. Campisi J. Cellular senescence: putting the paradoxes in perspective. *Curr Opin Genet Dev.* 2011;21:107–112.
53. Alway SE, Mohamed JS, Myers MJ. Mitochondria initiate and regulate sarcopenia. *Exerc Sport Sci Rev.* 2017;45:58–69.
54. Jeong EM, Chung J, Liu H, et al. Role of mitochondrial oxidative stress in glucose tolerance, insulin resistance, and cardiac diastolic dysfunction. *J Am Heart Assoc.* 2016;5:e003046.
55. Montgomery MK, Turner N. Mitochondrial dysfunction and insulin resistance: an update. *Endocr Connect.* 2015;4:R1–R15.
56. Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010;464:104–107.
57. Rathmell JC. Metabolism and autophagy in the immune system: immunometabolism comes of age. *Immunol Rev.* 2012;249:5–13.
58. Ron-Harel N, Sharpe AH, Haigis MC. Mitochondrial metabolism in T cell activation and senescence: a mini-review. *Gerontology*. 2015;61: 131–138.
59. Wu B, Goronzy JJ, Weyand CM. Metabolic fitness of T cells in autoimmune disease. *Immunometabolism*. 2020;2:e200017.
60. Caro-Maldonado A, Wang R, Nichols AG, et al. Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. *J Immunol.* 2014;192: 3626–3636.
61. Yanes RE, Zhang H, Shen Y, et al. Metabolic reprogramming in memory CD4 T cell responses of old adults. *Clin Immunol.* 2019;207:58–67.
62. Frasca D, Diaz A, Romero M, et al. Metformin enhances B cell function and antibody responses of elderly individuals with type-2 diabetes mellitus. *Front Aging*. 2021. doi: 10.3389/fragi.2021.715981.

63. Kurupati RK, Haut LH, Schmader KE, et al. Age-related changes in B cell metabolism. *Aging (Albany NY)*. 2019;11:4367–4381.
64. Guarente L. Sirtuins, aging, and metabolism. *Cold Spring Harb Symp Quant Biol.* 2011;76:81–90.
65. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J.* 2007;404:1–13.
66. Barzilai N, Crandall JP, Kritchevsky SB, et al. Metformin as a tool to target aging. *Cell Metab.* 2016;23:1060–1065.
67. Butterfield TR, Landay AL, Anzinger JJ. Dysfunctional immunometabolism in HIV infection: contributing factors and implications for age-related comorbid diseases. *Curr HIV/AIDS Rep.* 2020;17:125–137.
68. Valle-Casuso JC, Angin M, Volant S, et al. Cellular metabolism is a major determinant of HIV-1 reservoir seeding in CD4(+) T cells and offers an opportunity to tackle infection. *Cell Metab.* 2019;29:611–626.e5.
69. Clerc I, Moussa DA, Vahlas Z, et al. Entry of glucose- and glutamine-derived carbons into the citric acid cycle supports early steps of HIV-1 infection in CD4 T cells. *Nat Metab.* 2019;1:717–730.
70. Butterfield TR, Hanna DB, Kaplan RC, et al. Increased glucose transporter-1 expression on intermediate monocytes from HIV-infected women with subclinical cardiovascular disease. *AIDS*. 2017;31:199–205.
71. Frasca D, Pallikkuth S, Pahwa S. Metabolic phenotype of B cells from young and elderly HIV individuals. *Immun Ageing*. 2021;18:35.