Marissa J. Schafer,[1,2] Thomas A. White,[1] Glenda Evans,[1] Jason M. Tonne,[3] Grace C. Verzosa,[4] Michael B. Stout,[1,5] Daniel L. Mazula,[1] Allyson K. Palmer,[1] Darren J. Baker,[1,6] Michael D. Jensen,[7] Michael S. Torbenson,[8] Jordan D. Miller,[1,4] Yasuhiro Ikeda,[3] Tamara Tchkonia,[1] Jan M. van Deursen,[1,9] James L. Kirkland,[1,5] and Nathan K. LeBrasseur[1,2]

Exercise Prevents Diet-Induced Cellular Senescence in Adipose Tissue

Diabetes 2016;65:1606–1615 | DOI: 10.2337/db15-0291

OBESITY STUDIES

Considerable evidence implicates cellular senescence in the biology of aging and chronic disease. Diet and exercise are determinants of healthy aging; however, the extent to which they affect the behavior and accretion of senescent cells within distinct tissues is not clear. Here we tested the hypothesis that exercise prevents premature senescent cell accumulation and systemic metabolic dysfunction induced by a fast-food diet (FFD). Using transgenic mice that express EGFP in response to activation of the senescence-associated $p16^{INK4a}$ promoter, we demonstrate that FFD consumption causes deleterious changes in body weight and composition as well as in measures of physical, cardiac, and metabolic health. The harmful effects of the FFD were associated with dramatic increases in several markers of senescence, including p16, EGFP, senescence-associated β-galactosidase, and the senescence-associated secretory phenotype (SASP) specifically in visceral adipose tissue. We show that exercise prevents the accumulation of senescent cells and the expression of the SASP while nullifying the damaging effects of the FFD on parameters of health. We also demonstrate that exercise initiated after long-term FFD feeding reduces senescent phenotype markers in visceral adipose tissue while attenuating physical impairments, suggesting that exercise may provide restorative benefit by mitigating accrued senescent burden. These findings highlight a novel mechanism by which exercise mediates its beneficial effects and reinforces the effect of modifiable lifestyle choices on health span.

Unhealthy diets and sedentary lifestyles are factors fueling the obesity epidemic, wherein ~35% of middle-aged Americans are obese (1). Heavily implicated in this public health issue is routine consumption of calorie-dense, nutrient-poor fast foods and sugar-sweetened beverages, akin to a fast-food diet (FFD) (2). Nutrient excess leading to metabolic dysfunction increases the risk for and accelerates the onset of numerous age-related conditions, including diabetes, cardiovascular disease, Alzheimer disease, and cancer (3,4). Fat mass distribution further influences chronic disease risk, with visceral adiposity serving as a stronger predictor of all-cause mortality relative to subcutaneous adiposity (5). In contrast, exercise positively affects body composition, enhances physical fitness, and is protective against numerous age-related diseases (6). Despite the widely recognized effects of diet and exercise on health span, the fundamental mechanisms by which they influence the biology of aging and chronic disease remain elusive.

Cellular senescence is a state of stable growth arrest triggered by telomere erosion, DNA lesions, reactive oxygen species, and other mitogenic and metabolic stressors. It is mediated by the inhibition of cell cycle progression through $p16^{INK4a}$/retinoblastoma protein and/or the activation of cell cycle arrest through p53/p21. Characteristic gene expression signature and morphological shifts define the transition into a senescence state, but the functional role of senescent cells within a given tissue milieu is highly dependent on cell type, concentration, and context (7). Multiple

[1]Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN
[2]Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN
[3]Department of Molecular Medicine, Mayo Clinic, Rochester, MN
[4]Department of Surgery, Mayo Clinic, Rochester, MN
[5]Department of Internal Medicine, Mayo Clinic, Rochester, MN
[6]Department of Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN
[7]Division of Endocrinology, Department of Medicine, Mayo Clinic, Rochester, MN
[8]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN
[9]Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN

Corresponding author: Nathan K. LeBrasseur, lebrasseur.nathan@mayo.edu.

Received 4 March 2015 and accepted 29 February 2016.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db15-0291/-/DC1.

lines of evidence implicate cellular senescence in the biology of aging and the genesis of age-related conditions (8,9). In particular, biomarkers of senescent cells, including p16 and senescence-associated β-galactosidase (SA-β-gal) levels, increase in multiple tissues with advancing age and in the context of chronic disease (10).

Senescent cells actively secrete a broad repertoire of cytokines, chemokines, matrix-remodeling proteases, and growth factors, collectively referred to as the senescence-associated secretory phenotype (SASP) (11). Despite their cell-autonomous role in the prevention of malignant transformation, through the SASP, senescent cells damage neighboring cells, paradoxically fuel the aberrant growth and invasion of malignant cells, and promote inflammation (7,8). Senescent cells and the SASP are thus believed to drive degenerative, hyperproliferative, and inflammatory conditions of aging (12). This premise is further supported by studies demonstrating that targeted deletion of senescent cells expressing $p16^{INK4a}$ delays the onset of several age-related phenotypes, including thymic involution (13), and, in a mouse model of accelerated aging, cataracts, lordokyphosis, and diminished exercise capacity (14). More recently, senolytics, the term given to pharmacological agents selected for their ability to kill senescent cells or inhibit the SASP, have shown therapeutic benefit on parameters of physical health and function when administered to chronologically aged, progeroid, and/or irradiated mice (15,16).

Whether and how lifestyle choices in middle age influence the premature genesis of proaging senescent phenotypes in distinct tissues remains unclear. Accordingly, we sought to determine the extent to which nutrient excess and exercise affect the onset and progression of cellular senescence and the SASP using adult transgenic mice that express a construct harboring EGFP in response to the senescence-sensitive promoter, $p16^{INK4a}$.

RESEARCH DESIGN AND METHODS

Mice and Experimental Interventions

Mice harboring the $p16^{INK4a}$-EGFP transgenic construct (14) were generated on a genetically heterogeneous background (four-strain cross, as previously detailed [17]). For the prevention study, 8-month-old male mice were divided into four groups of comparable mean body weights. The groups were randomly assigned to one of the following 16-week interventions: normal diet (ND) (13% energy as fat; PicoLab Rodent Diet 20 [5053]; LabDiet, St. Louis, MO), FFD (40% energy as fat [milk fat, 12% saturated] with 0.2% cholesterol; Western Diet [5342]; TestDiet, St. Louis, MO), and high-fructose corn syrup in the drinking water (42 g/L; see [18]), ND plus exercise, or FFD plus exercise.

For the treatment study, 5- to 6-month-old male mice were provided ND or FFD for 16 weeks. FFD mice were then randomized to sedentary or exercise groups based on body weight, for a total of three groups, which were monitored for an additional 14 weeks. Thus, all mice in the prevention and treatment studies were ~1 year old at necropsy. All mice were individually housed in ventilated cages and provided food and water ad libitum. Exercised mice were provided wireless running wheels, and exercise behavior was monitored using Wheel Manager Data Acquisition Software (Med Associates, St. Albans, VT). Experiments were performed under protocols approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Body Composition and Health Span Measures

Body weight and food intake were measured weekly. Body composition (total body lean and fat mass) was assessed monthly in unanesthetized mice by quantitative MRI (EchoMRI-100; Houston, TX), as previously described (19). At the end of the study, subcutaneous and visceral fat in the lumbar region was quantified in anesthetized mice using microcomputed tomography (vivaCT 40; Scanco Medical, Wayne, PA). As a measure of physical function, exercise capacity was determined on a motorized treadmill (Columbus Instruments, Columbus, OH), as previously described (20). Cardiac function in mice under light isoflurane anesthesia was assessed by echocardiography using the Vevo 2100 system (FUJIFILM VisualSonics, Inc., Toronto, Ontario, Canada), as recently described (21). For metabolic function, glucose and insulin concentrations and glucose tolerance after a 6-h fast were assessed, as previously described (22).

Tissue Assessments

Individual tissues were harvested, weighed, and processed for downstream analyses. Portions of individual adipose tissue depots were fixed in PBS containing 2.0% formaldehyde and 0.2% glutaraldehyde for cell size determination and SA-β-gal activity. The sizes of adipocytes in fat tissue were determined using Metamorph software (Molecular Devices, Sunnyvale, CA). Liver tissue was fixed in 10% formalin, dehydrated, and embedded in paraffin. Liver sections were stained with hematoxylin and eosin for overall morphology. A pathologist who was not aware of treatment assignments gave grades of 0, 1, 2, 3, and 4 to sections in which 0, 1–4, 5–30, 31–60, and 61–100% of hepatocytes, respectively, had lipid macrovesicles. Grades of 0, 1, 2, and 3 were assigned to liver sections with 0, 1–30, 31–60, and 61–100% of hepatocytes containing lipid microvesicles. Liver ceramides were quantified using ultraperformance liquid chromatography/tandem mass spectrometry, as recently described (23). Pancreata were embedded and frozen in Optimal Cutting Temperature Compound (Sakura Finetek USA, Inc., Torrance, CA). Immunostaining of cryosections and quantification of insulin-positive mass was performed, as previously described (24).

Markers of Cellular Senescence and the SASP

For transcriptional analysis, TRIzol-based extraction was used to isolate RNA from whole mouse tissues, which were subjected to nanodrop concentration and purity analysis before cDNA synthesis. TaqMan quantitative PCR (qPCR) assays (Life Technologies, Carlsbad, CA) were used for detection of p16 (Mm00494449_m1), monocyte

chemoattractant protein 1 (Mcp1; Mm00441243_g1), and insulin-like growth factor 1 (Igf1; Mm00439561_m1). PrimeTime 5′ nuclease qPCR assays (Integrated DNA Technologies, Coralville, IA) were used for detection of p21 (Mm.PT.56a.17125846), p53 (Mm.PT.56a.44013092), interleukin 6 (Il6; Mm.PT.56a.10005566), plasminogen activator 1 (Pai1; Mm.PT.58.6413525), matrix metalloprotease 3 (Mmp3; Mm.PT.58.9719290), CD68 (Mm.PT.58.32698807), and Tbp (Mm.PT.39a.22214839). A SYBR qPCR assay (Integrated DNA Technologies) was used for detection of EGFP (forward 5′-CAA CTA CAA CAG CCA CAA CG-3′; reverse 5′-GGT CAC GAA CTC CAG CAG-3′). Adipose tissue depots were stained for SA-β-gal activity, as previously described (25).

Statistical Analysis

Significant differences between groups for the dependent variables of diet (ND and FFD) and behavior (sedentary and exercise) were tested using one- or two-way ANOVA. The Tukey multiple comparisons test was used for post hoc analyses for between-group comparisons. Analyses were conducted using GraphPad Prism Statistical Software Version 6.0 (GraphPad Software, Inc., San Diego, CA).

RESULTS

Exercise Prevents Multiple Indices of Diet-Induced Metabolic Dysfunction

To investigate the potential role of cellular senescence in diet-induced dyshomeostasis, which may be attenuated by exercise, we provided 8-month-old male mice harboring an EGFP transgene driven by the $p16^{INK4a}$ promoter with an ND or a high-fat diet enriched with saturated fat, cholesterol, and high fructose corn syrup, equivalent to an FFD, for 4 months. Subsets of ND- and FFD-fed mice were provided with running wheels. Mice provided with the FFD consumed more total calories within the first weeks of the study, but within 1 month, total calorie intake was not different among any of the groups (Supplementary Fig. 1*A*). Increased energy intake in exercising FFD mice corresponded to elevated average daily running distances, albeit nonstatistically significant, within the first 2 months of the study (Supplementary Fig. 1*B*).

We assessed whether clinically relevant health indices, including body weight, adipose mass, physical activity, circulating insulin and glucose concentrations, cardiac function, and liver health were altered by diet and exercise. At study onset, average body weight and fat mass were equivalent among ND- and FFD-fed mice, but after 4 months, FFD-fed mice weighed 31% more and accumulated twice the total fat of ND-fed mice (Fig. 1*A* and *B*). Differences between ND- and FFD-fed mice in subcutaneous and visceral fat were evident by weight (Supplementary Fig. 2*A*) and in microcomputed tomography scans of the lumbar region (Fig. 1*C*). The visceral fat of FFD-fed mice was composed of significantly larger adipocytes and a greater percentage of large adipocytes than that of ND-fed mice (Supplementary Fig. 2*B*). Exercise blunted the ND- and, more dramatically, the FFD-induced accretion of body weight and fat mass (Fig. 1*A*–*C* and Supplementary

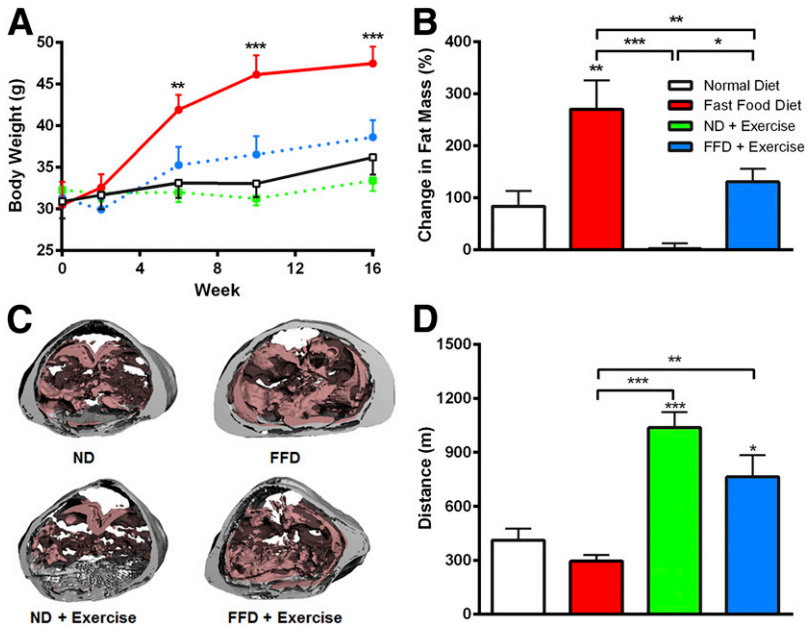

Figure 1—Nutrient excess and exercise exert opposing effects on body composition and physical endurance. Compared with mice fed an ND, mice fed an FFD for 16 weeks exhibited marked gains in body weight (*A*) and fat mass as determined by quantitative MRI (*B*). *C*: FFD-induced obesity was further evident in volumes of visceral (pink) and subcutaneous (gray) fat in the lumbar region of mice as assessed by computed tomography. *D*: Exercised mice fed the ND or FFD exhibited significantly greater distances run to exhaustion on a treadmill than sedentary mice fed either diet. For all analyses, n = 6–7 mice/group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Fig. 2*A*). In fact, the visceral and subcutaneous fat weights of exercised FFD-fed mice were not statistically different from those of ND-fed mice (Supplementary Fig. 2*A*). Exercise also prevented the FFD-induced hypertrophy of adipocytes (Supplementary Fig. 2*B*).

Assessment of physical function using a treadmill test revealed that the FFD diet alone caused a modest but nonsignificant decrease in physical performance, whereas exercised mice fed the ND and FFD both ran a significantly greater distance to exhaustion than sedentary peers (Fig. 1*D*). Exercised mice also had positive cardiac adaptations relative to sedentary peers, including increased heart weight–to–body weight ratios, indicative of physiological hypertrophy (Supplementary Fig. 3*A*), and improved ejection fractions measured by echocardiography (Supplementary Fig. 3*B*). Sedentary mice fed the FFD exhibited poorer values for both of these parameters of cardiac health (Supplementary Fig. 3*A* and *B*) and a deleterious increase in the left ventricular end diastolic dimension relative to mice fed the ND and exercised FFD-fed peers (Supplementary Fig. 3*C*).

With respect to metabolic function, no differences in fasting glucose were observed between groups (data not shown); however, sedentary mice fed the FFD had dramatically increased insulin concentrations. This hallmark of diet-induced insulin resistance was robustly reduced by exercise (Fig. 2*A*). Correspondingly, we observed grossly enlarged β-cell masses and insulin-positive areas in the pancreata of sedentary but not exercised FFD-fed mice (Fig. 2*B* and *C*). Further evidence of preserved insulin action in exercised mice fed the FFD was apparent in a glucose tolerance test, in which they were indistinguishable from mice fed the ND (Fig. 2*D*). In contrast, sedentary mice fed the FFD had more pronounced excursions and impaired clearance of circulating glucose.

Nutrient excess can lead to adipocyte dysfunction, reflected in impaired triglyceride deposition, increased lipolysis and lipotoxicity, or the accumulation of lipids in peripheral tissues (26). As expected, liver weights of sedentary FFD-fed mice were significantly greater than ND-fed mice (Supplementary Fig. 4*A*). Also significantly elevated in the livers of sedentary mice were longer chain ceramides, C16 and C24:1, and liver triglycerides, which are associated with insulin resistance (27) (Supplementary Fig. 4*B* and *C*). These markers of hepatic lipotoxicity were prevented by exercise (Supplementary Fig. 4*B* and *C*), as

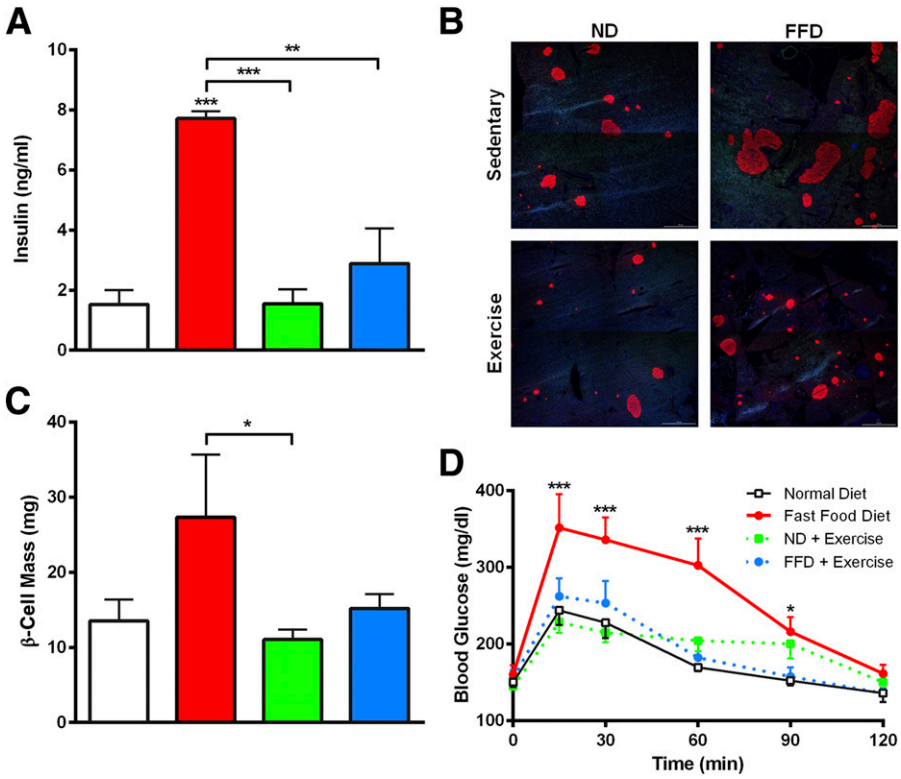

Figure 2—Diet-induced deterioration of metabolic health is attenuated by exercise. *A*: Compared with sedentary and exercised mice fed the ND, sedentary FFD-fed mice exhibited significantly higher circulating insulin concentrations. *B* and *C*: Correspondingly, cross sections of the pancreata of sedentary FFD-fed mice exhibited markedly greater β-cell masses and insulin-positive areas (representative images, *B*). *A–C*: Remarkably, these features of insulin resistance in FFD-fed mice were abrogated by exercise. *D*: Compromised and improved insulin actions in sedentary and exercised FFD-fed groups, respectively, were apparent in a glucose tolerance test. Namely, the higher peak and greater excursions in glucose concentrations observed in sedentary FFD-fed mice relative to ND-fed mice were erased by exercise. For all analyses, n = 6–7 mice/group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

were select FFD-induced gross morphological changes (Supplementary Fig. 4*D*). Compared with ND-fed mice, livers of sedentary and exercised FFD-fed mice demonstrated increases in the percentage of hepatocytes with lipid macrovesicles in their cytoplasm, an early event in the pathogenesis of steatosis (Supplementary Fig. 4*E*). Most of the hepatocytes in the livers of sedentary mice fed the FFD also had highly abundant lipid microvesicles, a feature associated with mitochondrial injury or dysfunction (28). This consequence of the FFD was abrogated by exercise (Supplementary Fig. 4*F*). Collectively, these data underscore the salutary influence of exercise on several parameters of health and its ability to prevent multiple harmful effects of nutrient excess.

The Detrimental Effects of Nutrient Excess on Senescent Cell Burden and the SASP in Visceral Fat Are Prevented by Exercise

Although accumulation of senescent cells occurs with advancing age, prematurely elevated senescent cell burden may be both a cause and consequence of metabolic dysfunction (9,29). We hypothesized that routine FFD consumption in middle age promotes accretion of senescent cells, and accordingly, we probed expression of senescent biomarkers p16, p21, and p53 within discrete tissues. Compared with ND-fed mice, the visceral fat of sedentary FFD-fed mice contained significantly higher mRNA levels of p16 (Fig. 3*A*) and p53 (Fig. 3*B*) as well as p21 (Fig. 3*C*), its downstream target, indicating pronounced activation of senescence effectors. Exercise completely blocked FFD-induced increases in p53 and p21 (Fig. 3*B* and *C*). The expression of p16 in subcutaneous adipose tissue, liver, skeletal muscle, pancreas, kidney, heart (left ventricle), and aorta was not altered in response to diet or exercise (Fig. 3*A*). Similar outcomes were observed for the expression of p53, which, in addition, was higher in the subcutaneous fat of FFD-fed mice relative to that of exercised ND-fed mice (Fig. 3*B*). Expression of p21 was significantly elevated in subcutaneous fat and liver of sedentary FFD-fed mice, an effect that was prevented by exercise (Fig. 3*C*). These findings suggest that nutrient excess in middle age activates expression of prosenescence markers in visceral adipose tissue and that this effect is robustly attenuated by exercise. To a lesser degree, senescent signaling may also occur in tissues other than visceral fat and may be prevented by exercise. However, this likely involves mechanisms other than p16, such as the p53/p21 pathway.

Elevated expression of senescence markers distinctly within visceral adipose led us to further investigate the influence of nutrient excess and exercise on additional indicators of cellular senescence and the SASP in this tissue. Quantification of EGFP expression confirmed that FFD-feeding greatly enhanced $p16^{INK4a}$ promoter activity, which was strongly prevented by exercise (Fig. 4*A*). To validate expression-based data, we stained visceral adipose tissue for the classic biomarker of senescence, SA-β-gal. In sedentary and exercised mice fed the ND, ~2% of cells stained were positive for SA-β-gal (Fig. 4*B* and *C*). In comparison, more than 12% of cells in sedentary mice fed the FFD stained positively. Strikingly, exercise nullified this effect of the FFD, and as a result, the percentage of cells positive for SA-β-gal in exercised FFD-fed mice was identical to that of ND-fed middle-aged mice (Fig. 4*B* and *C*).

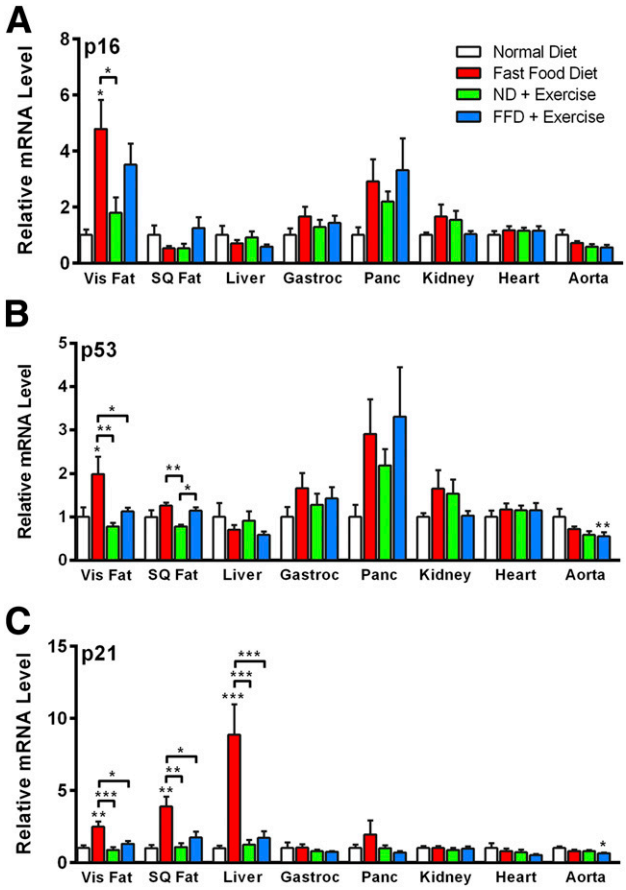

Figure 3—The effects of diet and exercise on senescence markers in multiple tissues. To determine the extent to which nutrient excess and exercise affected cellular senescence in middle-aged mice, we compared the expression of p16 (*A*), p53 (*B*), and p21 (*C*) by qPCR in multiple tissues, including visceral (Vis) fat, subcutaneous (SQ) fat, liver, gastrocnemius (gastroc), pancreas (panc), kidney, heart, and aorta. For all analyses, n = 6–7 mice/group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Senescent cells partly disrupt a tissue's structure and function and affect the systemic environment through the factors they secrete. Indeed, FFD-fed mice demonstrated significant increases in the expression of proinflammatory SASP markers, including Il6, Pai1, and Mcp1 (Fig. 3*D*). We also observed significantly increased expression of Mmp3, a matrix remodeling protein and SASP component, within visceral fat after FFD-feeding. No significant differences were found in Igf1 (Fig. 4*D*). With the exception of Pai1, exercise prevented the induction of the SASP by the FFD. SASP signaling may instigate senescence in a paracrine manner while recruiting inflammatory cells (30), ultimately

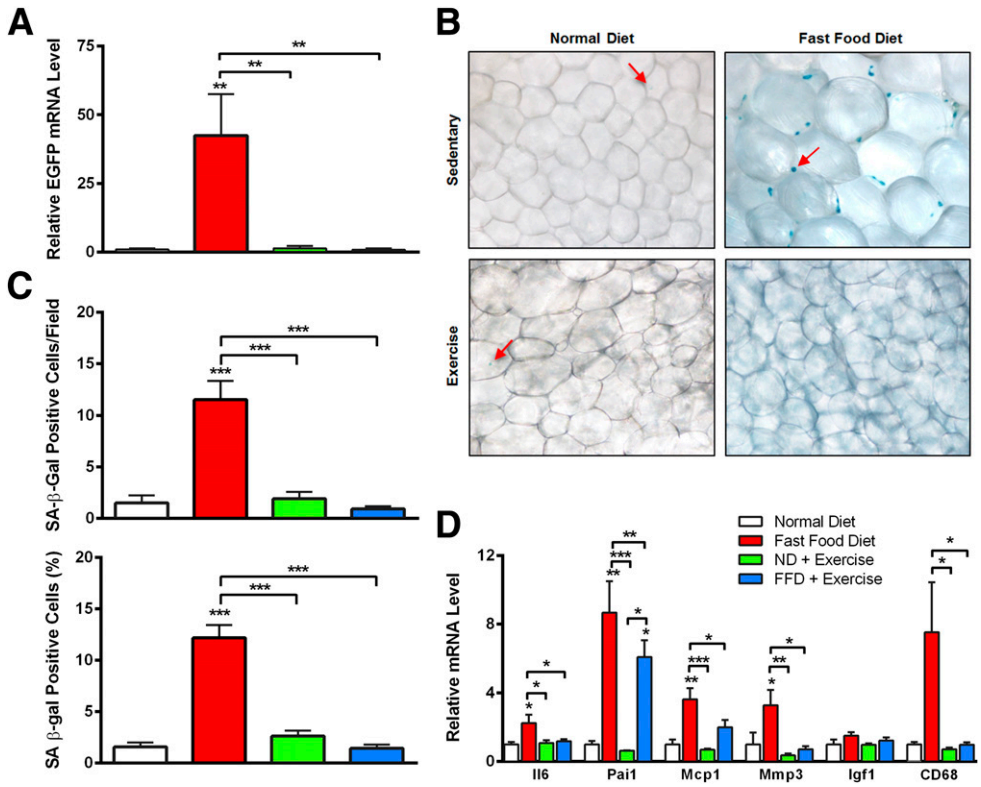

Figure 4—Exercise prevents diet-induced cellular senescence and the SASP within visceral adipose tissue. *A*: Compared with the ND, the FFD caused a marked increase in the activity of the senescence-associated $p16^{INK4a}$ promoter, as measured by EGFP expression. *B*: Representative images show the abundance of cells positive for SA-β-gal (arrow) in harvested visceral adipose tissue further validated the pro- and antisenescent effects of nutrient excess and exercise, respectively (summary data, *C*). *D*: The expression of SASP and inflammatory factors was also increased in response to FFD, and these increases were attenuated by exercise. For all analyses, n = 6–7 mice/group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

propagating sterile inflammation (31). Indeed, we observed a 7.5-fold increase in the expression of the pan-macrophage marker, CD68, in visceral adipose (Fig. 4*D*). Exercise completely abrogated CD68 induction (Fig. 4*D*). These findings indicate that diet and exercise are potent mediators of cellular senescence and the SASP, which may be a central mediator of obesity-induced inflammation in visceral adipose tissue.

Exercise Reduces Markers of Cellular Senescence and the SASP in Diet-Induced Obese Mice

Given the efficacy of exercise at preventing the molecular phenotype of senescence in the visceral fat of FFD-fed mice, we next tested whether exercise may be leveraged to revert these consequences. Accordingly, we provided mice with the ND or FFD for 16 weeks, during which the FFD led to gains in total body weight (Fig. 5*A*) and fat mass as measured by quantitative MRI (Fig. 5*B*). The FFD mice were then randomized to voluntary wheel running or sedentary groups. Over the subsequent 14 weeks, body weight (Fig. 5*A*) and fat mass (Fig. 5*B*) declined in exercising mice. Microcomputed tomography scans further showed that exercise-mediated fat mass reductions occurred in visceral and subcutaneous adipose depots (Fig. 5*C*). Exercise adaptations were corroborated by a forced-run treadmill test to exhaustion, during which exercised mice ran 126% farther than sedentary FFD-fed peers (Fig. 5*D*).

To explore whether exercise mitigates diet-induced cellular senescence, we conducted gene expression profiling on visceral adipose extracts. Similar to the prevention study, exposure to the FFD for 30 weeks resulted in a significant fourfold increase in p16 expression in visceral fat of sedentary mice (Fig. 5*E*). Despite continued consumption of the FFD, the treatment of obese mice with exercise for 14 weeks reverted this effect, as evidenced by reduced expression of p16 to levels that were not different from ND-fed control mice. Expression of p53 and p21 did not significantly differ between ND- and FFD-fed mice (Fig. 5*E*), suggesting that p16 rather than p53/p21 signaling may drive long-term senescence maintenance. Consistent with induction of the senescence biomarker p16, we observed significant increases in the SASP component, Pai1, in sedentary FFD-fed mice. Increases in Mcp1 were not significant (Fig. 5*F*). CD68 transcriptional levels were also higher in sedentary FFD-fed mice than in ND-fed peers (Fig. 5*F*). Treatment of FFD-fed mice with exercise attenuated elevated Pai1 and

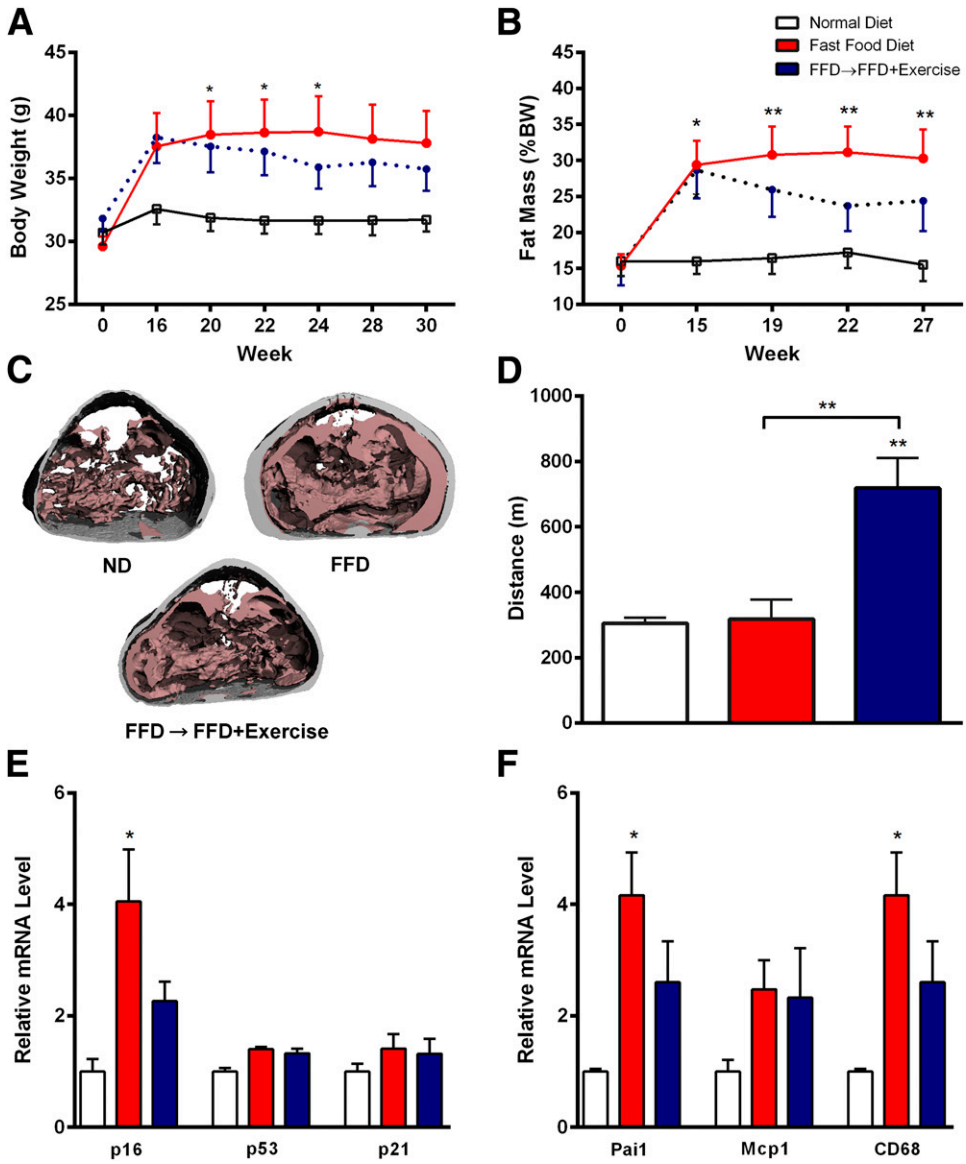

Figure 5—Exercise initiated after long-term FFD feeding improves physical parameters and attenuates markers of visceral adipose senescence. After 16 weeks of FFD feeding and sedentary lifestyle, exercise "treatment" in the form of voluntary running wheels for a subsequent 14 weeks (FFD → FFD + Exercise) led to reductions in total body weight (*A*) and fat mass (*B*). *C*: Computed tomography assessment of the lumbar region at end point revealed that exercise-mediated reductions corresponded to both visceral (pink) and subcutaneous (gray) adipose depots. *D*: Exercise capacity determined by treadmill testing dramatically increased in FFD-fed mice switched from sedentary to running wheel exercise, relative to both ND- and FFD-sedentary groups. qPCR was used to compare expression of senescence induction factors p16, p53, and p21 (*E*) and SASP and inflammatory factors Pai1, Mcp1, and CD68 (*F*). For all analyses, $n = 5–7$ mice/group. $^*P < 0.05$, $^{**}P < 0.01$.

CD68 levels (Fig. 5*F*). Cumulatively, these results point to diminution of a diet-induced senescent phenotype in visceral adipose tissue as a means by which exercise provides restorative benefit to abate chronic disease after long-term sedentary behavior and nutrient excess.

DISCUSSION

The current study demonstrates the robust effects of modifiable lifestyle factors on the accumulation of senescent cells and the expression of the SASP in middle age. Our data highlight the harmful consequences of nutrient excess and the remarkably protective influence of exercise on this biological process and, in turn, measures of physical, cardiovascular, and metabolic function. In the face of population aging, an obesity epidemic, and global reductions in physical activity, these findings have significant implications for human health.

Obesity is, fundamentally, a condition of energy imbalance caused by the consumption of more energy than is expended. The increased storage demands placed on adipocytes in the face of nutrient excess ultimately compromise their function, reflected in impaired storage of lipids and augmented release of free fatty acids and inflammatory mediators (26). Our data support the premise

that visceral adipose tissue dysfunction and its sequelae are partly mediated through cellular senescence (12). The stromal vascular fraction of adipose tissue is rich in progenitor cells, or preadipocytes, that are prone to senescence and exhibit a proinflammatory profile (12,16,29). We show that nutrient excess markedly increased the expression of p16 and other markers of senescence, including p53 and p21 and the activity of SA-β-gal. These changes were associated with induction of proinflammatory cytokines, chemokines, and matrix remodeling proteins (e.g., Il6, Mcp1, Pai1, and Mmp3, respectively), collectively referred to as the SASP.

SASP factors mechanistically contribute to metabolic disease. Knockout of Pai1 abrogates insulin resistance and obesity brought on by high-fat feeding (32). Similarly, blockade of adipose Mcp1 signaling exerts anti-inflammatory effects (33), and ablation of the Mcp1 receptor (C-C motif chemokine receptor 2 [$Ccr2^{-/-}$]) increases adiponectin levels and improves glucose homeostasis after high-fat feeding relative to $Ccr2^{+/+}$ controls matched for adiposity (34). SASP signaling has been further implicated as a means of senescence transmission to neighboring cells (30), suggesting that the SASP may be responsible for induction and amplification of inflammation arising from nutrient excess. We demonstrate that FFD feeding strongly induces visceral adipose expression of Pai1, Mcp1, and CD68 coincident with increases in p16 expression and cells positive for SA-β-gal, which is prevented by exercise. Similarly, we show that treatment of obese mice with voluntary exercise is able to revert aspects of this molecular phenotype. Our data and prior evidence support the premise that senescent cells may be a primary source of obesity-associated inflammation, which is central to the pathogenesis of type 2 diabetes and its complications (9) and highlights the potential of exercise as an effective intervention.

In agreement with our findings, Minamino et al. (35) reported that senescent cells accumulate in the adipose tissue of younger mice with ectopic expression of agouti peptide, which leads to excessive nutrient intake, obesity, and diabetes. However, a more recent study failed to show that high-fat feeding accelerates age-related $p16^{INK4a}$ expression as quantified by whole-body luciferase imaging or mRNA abundance in isolated livers or spleens (36). It is plausible that imaging was not adequately sensitive to detect diet-induced changes in the abundance of $p16^{INK4a}$-positive senescent cells in vivo. Furthermore, nutrient excess may more potently induce the accumulation of $p16^{INK4a}$ and/or p53-positive senescent cells in adipose tissue compared with other organs. Our results show that a senescence phenotype is readily activated specifically within visceral fat and, to a lesser degree within subcutaneous fat, in middle age by the FFD. Because visceral adipose is a stronger driver of metabolic-induced morbidity, relative to other depots (5), senescent signaling may mediate this organ's unique role in sensing and negatively affecting the body in response to nutrient excess, particularly regarding the inflammatory component of obesity-induced dyshomeostasis. The causal role of senescent cells and the SASP in the genesis of obesity-associated conditions and the therapeutic efficacy of their removal, therefore, requires further examination.

The tissue specificity and temporal induction of cellular senescence warrants further consideration. Other groups have demonstrated increased senescence markers in variable tissues in response to nutrient excess, including elevated aortic p16 levels after 20 weeks of high-fat feeding in 4-week-old C57BL/6 J mice (37), elevated hepatic p16 and p21 levels after 13 weeks of high-fat feeding in 5-week-old rats (38), and elevated pancreatic p38 and SA-β-gal levels after 12 months of high-fat feeding in 4-week-old C57BL/6 J mice (39). We conducted analyses in 12-month-old mice administered dietary and/or exercise intervention for the previous 16 or 30 weeks, and our experiments used a diet that was high in sugar and fat. We observed robust induction of p16 and p53 in visceral adipose and induction of p21 in visceral and subcutaneous adipose and liver. Expression of these markers also appeared to increase in other tissues, including the pancreas, but did not reach statistical significance. Given the differences in age, genetic background, and diet composition between our study and the noted reports, that others have identified senescence signatures in tissues that were not prominent in our exploration is not surprising. However, the composite results unanimously show that nutrient excess leads to induction of senescence in multiple tissues responsible for coordinating metabolic health and cumulatively highlight the need for additional work to tease out the time course of this progression in uniform contexts.

The beneficial effects of exercise on health span are irrefutable; however, the biological mechanisms through which they act are not completely understood. Our findings confirm that exercise can positively affect multiple parameters of physical, cardiac, and metabolic health in middle-aged mice and, importantly, overcome the damaging effects of nutrient excess. Moreover, using established biomarkers, we show for the first time that exercise prevents and reduces indicators of cellular senescence in visceral adipose tissue induced by an FFD. This is significant given the considerable evidence implicating senescent cells and the SASP in the biology of chronic diseases (8) and the beneficial effects of their removal on several parameters of health, at least in a model of accelerated aging (14). There are three possibilities by which exercise prevented the diet-induced accumulation of senescent cells:

First, the increased energy demands and use of dietary macronutrients during bouts of exercise may have limited the metabolic and replicative stresses experienced by cells in adipose tissue and, consequently, their transition into a senescent state. Smaller adipocytes and fat depots as well as lower liver weights and abundance of liver ceramides in exercised FFD-fed mice, compared with their sedentary peers, may reflect this.

Second, it is plausible that exercise may have augmented the clearance of senescent cells. Their fate is highly variable. In benign melanocytic nevi, senescent cells can persist for

decades (40), whereas senescent liver carcinoma cells acutely activate the innate immune system to mediate their clearance and limit tumor growth (41). This possibility is supported by our data showing exercise reduces p16 expression in mice even after obesity and its consequences have been established.

Third, exercise could have invoked protective responses against triggers of cellular senescence in the context of nutrient excess, because exercise counters DNA damage (42), telomere erosion (43), oxidative stress (44), protein aggregation (45), and mitochondrial dysfunction (46) in multiple cell types. We speculate that exercise may have induced such defense systems to prevent adipose tissue cells from senescing. Indeed, additional work is needed to better understand how and when to leverage exercise to affect the accumulation, behavior, and persistence of senescent cells in the context of nutrient excess.

Adipose tissue is a critically important tissue in organismal health and aging. In contrast to the associations between obesity and phenotypes suggestive of accelerated aging, reductions in fat mass through calorie restriction (47), surgery (48), mutations in the insulin-signaling pathway (49), or exercise, as shown here, enhance health span in various organisms. Our findings lend support to the concept that the SASP is a major determinant of the secretory profile, or endocrine function, of adipose tissue. In aging and obesity, adipose tissue is a primary source of inflammatory mediators implicated in the genesis of diabetes and other chronic diseases (12). We previously demonstrated that the expression of components of the SASP, including Pai1 and Il6, were distinctly higher in $p16^{INK4a}$-positive senescent cells than nonsenescent cells residing in adipose tissue (9). In the current study, our results show that exercise prevents the SASP within visceral adipose tissue, and remarkably, reduces aspects of the SASP when initiated after its accumulation. We propose this is an unappreciated mechanism through which physical activity interventions may affect health span, particularly in those who are overweight or obese. Of note, the association between obesity-associated subclinical inflammation and the genesis of type 2 diabetes has been reported to be stronger in women than in men (50). Additional work is needed to determine the extent to which senescent cell burden and the SASP may account for this sex difference and whether exercise is as protective in females as we observed in males. Therapeutic approaches to suppress the SASP, such as exercise, may offer a means to negate the deleterious systemic effects of senescent cells in the genesis of obesity- and age-related chronic diseases.

In sum, our data highlight a novel and significant mechanism by which exercise positively affects organismal health. Given the considerable evidence that cellular senescence is a fundamental mechanism of aging and the genesis of chronic diseases, our findings reinforce the notion that lifestyle choices are powerful determinants of health span. Additional studies are necessary to determine the mechanisms by which exercise prevents and reverses cellular senescence and the SASP and at what ages and in what disease states it is most effective.

Acknowledgments. The authors greatly appreciate the technical expertise and support of Tamara Pirtskhalava, Kurt Johnson, Nathan W. Werneburg, Carolyn M. Roos, Anthony J. Croatt, Xuan-Mai Persson, and all of the Mayo Clinic.

Funding. This work was supported by the Glenn Foundation for Medical Research (D.J.B., J.M.v.D., J.L.K., N.K.L.), National Institutes of Health, National Institute on Aging grant AG-041122 (J.M.v.D., J.L.K., N.K.L.), the Pritzker Foundation (N.K.L.), a generous gift from Robert and Arlene Kogod, and by the Metabolic Studies Core of the Minnesota Obesity Center (DK-50456).

Duality of Interest. Mayo Clinic, A.K.P., D.J.B., T.T., J.M.v.D., J.L.K., and N.K.L. have a financial interest related to this research with intellectual property licensed to a commercial entity. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. M.J.S. helped collect and analyze data, designed and implemented follow-up experiments, and drafted and revised the manuscript. T.A.W. helped design the study, collected and analyzed data, and drafted the manuscript. G.E., J.M.T., G.C.V., M.B.S., D.L.M., A.K.P., M.S.T., and Y.I. collected and analyzed data. D.J.B. and J.M.v.D. helped design the study and provided study resources. M.D.J., J.D.M., and T.T. helped design the study and collected and analyzed data. J.L.K. helped design study, interpreted data, and drafted the manuscript. N.K.L. designed the study, interpreted data, and drafted and revised the manuscript. N.K.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 2014;311:806–814

2. Pereira MA, Kartashov AI, Ebbeling CB, et al. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet 2005;365:36–42

3. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA 1999;282:1523–1529

4. Whitmer RA, Gunderson EP, Barrett-Connor E, Quesenberry CP Jr, Yaffe K. Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study. BMJ 2005;330:1360

5. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 2007;116:39–48

6. Blair SN, Kohl HW 3rd, Barlow CE, Paffenbarger RS Jr, Gibbons LW, Macera CA. Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. JAMA 1995;273:1093–1098

7. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 2005;120:513–522

8. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 2013;123:966–972

9. Palmer AK, Tchkonia T, LeBrasseur NK, Chini EN, Xu M, Kirkland JL. Cellular senescence in type 2 diabetes: a therapeutic opportunity. Diabetes 2015;64:2289–2298

10. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell 2007;130:223–233

11. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 2010;5:99–118

12. Tchkonia T, Morbeck DE, Von Zglinicki T, et al. Fat tissue, aging, and cellular senescence. Aging Cell 2010;9:667–684

13. Liu Y, Johnson SM, Fedoriw Y, et al. Expression of p16(INK4a) prevents cancer and promotes aging in lymphocytes. Blood 2011;117:3257–3267
14. Baker DJ, Wijshake T, Tchkonia T, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 2011;479:232–236
15. Zhu Y, Tchkonia T, Pirtskhalava T, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 2015;14:644–658
16. Xu M, Tchkonia T, Ding H, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. Proc Natl Acad Sci U S A 2015;112:E6301–E6310
17. Miller RA, Austad S, Burke D, et al. Exotic mice as models for aging research: polemic and prospectus. Neurobiol Aging 1999;20:217–231
18. Charlton M, Krishnan A, Viker K, et al. Fast food diet mouse: novel small animal model of NASH with ballooning, progressive fibrosis, and high physiological fidelity to the human condition. Am J Physiol Gastrointest Liver Physiol 2011;301:G825–G834
19. Akasaki Y, Ouchi N, Izumiya Y, Bernardo BL, Lebrasseur NK, Walsh K. Glycolytic fast-twitch muscle fiber restoration counters adverse age-related changes in body composition and metabolism. Aging Cell 2014; 13:80–91
20. LeBrasseur NK, Schelhorn TM, Bernardo BL, Cosgrove PG, Loria PM, Brown TA. Myostatin inhibition enhances the effects of exercise on performance and metabolic outcomes in aged mice. J Gerontol A Biol Sci Med Sci 2009;64:940–948
21. Roos CM, Hagler M, Zhang B, Oehler EA, Arghami A, Miller JD. Transcriptional and phenotypic changes in aorta and aortic valve with aging and MnSOD deficiency in mice. Am J Physiol Heart Circ Physiol 2013;305:H1428–H1439
22. Bernardo BL, Wachtmann TS, Cosgrove PG, et al. Postnatal PPARdelta activation and myostatin inhibition exert distinct yet complimentary effects on the metabolic profile of obese insulin-resistant mice. PLoS One 2010;5:e11307
23. Chow LS, Mashek DG, Austin E, et al. Training status diverges muscle diacylglycerol accumulation during free fatty acid elevation. Am J Physiol Endocrinol Metab 2014;307:E124–E131
24. Tonne JM, Sakuma T, Deeds MC, et al. Global gene expression profiling of pancreatic islets in mice during streptozotocin-induced β-cell damage and pancreatic Glp-1 gene therapy. Dis Model Mech 2013;6:1236–1245
25. Villaret A, Galitzky J, Decaunes P, et al. Adipose tissue endothelial cells from obese human subjects: differences among depots in angiogenic, metabolic, and inflammatory gene expression and cellular senescence. Diabetes 2010;59:2755–2763
26. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 2008;9:367–377
27. Haus JM, Kashyap SR, Kasumov T, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. Diabetes 2009;58:337–343
28. Fromenty B, Pessayre D. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. Pharmacol Ther 1995;67:101–154
29. Escande C, Nin V, Pirtskhalava T, et al. Deleted in Breast Cancer 1 regulates cellular senescence during obesity. Aging Cell 2014;13:951–953
30. Acosta JC, Banito A, Wuestefeld T, et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol 2013;15:978–990
31. Freund A, Orjalo AV, Desprez PY, Campisi J. Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med 2010;16:238–246
32. Ma LJ, Mao SL, Taylor KL, et al. Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. Diabetes 2004;53:336–346
33. Yu R, Kim CS, Kwon BS, Kawada T. Mesenteric adipose tissue-derived monocyte chemoattractant protein-1 plays a crucial role in adipose tissue macrophage migration and activation in obese mice. Obesity (Silver Spring) 2006; 14:1353–1362
34. Weisberg SP, Hunter D, Huber R, et al. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J Clin Invest 2006;116:115–124
35. Minamino T, Orimo M, Shimizu I, et al. A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat Med 2009;15:1082–1087
36. Sorrentino JA, Krishnamurthy J, Tilley S, Alb JG Jr, Burd CE, Sharpless NE. p16INK4a reporter mice reveal age-promoting effects of environmental toxicants. J Clin Invest 2014;124:169–173
37. Wang CY, Kim HH, Hiroi Y, et al. Obesity increases vascular senescence and susceptibility to ischemic injury through chronic activation of Akt and mTOR. Sci Signal 2009;2:ra11
38. Zhang X, Zhou D, Strakovsky R, Zhang Y, Pan YX. Hepatic cellular senescence pathway genes are induced through histone modifications in a diet-induced obese rat model. Am J Physiol Gastrointest Liver Physiol 2012;302: G558–G564
39. Sone H, Kagawa Y. Pancreatic beta cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. Diabetologia 2005;48:58–67
40. Michaloglou C, Vredeveld LC, Soengas MS, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature 2005;436:720–724
41. Xue W, Zender L, Miething C, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 2007;445:656–660
42. Radák Z, Naito H, Kaneko T, et al. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. Pflugers Arch 2002;445:273–278
43. Werner C, Hanhoun M, Widmann T, et al. Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. J Am Coll Cardiol 2008;52:470–482
44. Ji LL. Exercise-induced modulation of antioxidant defense. Ann N Y Acad Sci 2002;959:82–92
45. He C, Bassik MC, Moresi V, et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature 2012;481:511–515
46. Safdar A, Bourgeois JM, Ogborn DI, et al. Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. Proc Natl Acad Sci U S A 2011;108:4135–4140
47. Masoro EJ. Caloric restriction and aging: an update. Exp Gerontol 2000;35: 299–305
48. Muzumdar R, Allison DB, Huffman DM, et al. Visceral adipose tissue modulates mammalian longevity. Aging Cell 2008;7:438–440
49. Blüher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. Science 2003;299:572–574
50. Thorand B, Baumert J, Kolb H, et al. Sex differences in the prediction of type 2 diabetes by inflammatory markers: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. Diabetes Care 2007;30:854–860