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Research Article

Cellular Senescence Biomarker p16^{INK4a}+ Cell Burden in Thigh Adipose is Associated With Poor Physical Function in Older Women

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Abstract

Background: Ample evidence implicates cellular senescence as a contributor to frailty and functional decline in rodents, but considerable effort remains to translate these findings to human aging.

Methods: We quantified senescence biomarker p16^{INK4a}-expressing cells in thigh adipose tissue obtained from older women previously enrolled in a 5-month resistance training intervention, with or without caloric restriction (RT ± CR, n = 11 baseline, 8 pre–post-intervention pairs). Women in this subsample were older (72.9 ± 3.4 y) and overweight/obese (body mass index: 30.6 ± 2.4 kg/m^2). p16^{INK4a}+ cells were identified from 12 to 20 random visual fields/sample at 20× magnification (immunohistochemical, nuclear staining) and were present in all adipose samples.

Results: Cross-sectional associations were observed between p16^{INK4a}+ cell burden and physical function, including grip strength (r = –0.74), 400-m walk time (r = 0.74), 4-m gait speed (r = –0.73), and self-perceived mobility (r = –0.78) ($p \leq .05$). These relationships remained significant after independent adjustments for age and adiposity ($p \leq .05$). p16^{INK4a}+ cell abundance was lower following the intervention (pre: 5.47 ± 3.4%, post: 2.17 ± 1.1% count p16^{INK4a}+ cells, $p \leq .05$).

Conclusions: These results provide proof-of-concept that p16^{INK4a}+ cells in thigh adipose are associated with physical function, and may be sensitive to change with RT ± CR in overweight/obese older women.

Keywords: Exercise, Caloric restriction, Mobility, Biology of aging

A key strategy advanced by the interdisciplinary field of *geroscience* is to therapeutically target the biologic aging process to prevent or reverse declines in health and function (1,2). One such biological process, cellular senescence, has emerged as a promising target for drug development based on a growing body of animal literature (3,4). Cellular senescence entails cell cycle arrest triggered by DNA damage, oxidative stress, or metabolic stressors (5,6). This molecular damage can engage cellular signaling cascades and activate p53 and/or p16^{INK4a} cellular senescence programs leading to a senescent phenotype demonstrated to have deleterious consequences on health and function in animal models of aging (3,6–8).

Senescent cells can accumulate within mitotic tissues such as adipose leading to detrimental local and systemic effects that can compromise function (3,4,8,9). For example, up to 30-fold greater senescent cell accumulation is observed in visceral adipose tissue from obese compared with non-obese adults (9), possibly owing to a combination of replicative, cytokine-induced, and metabolic

stresses coupled with reduced apoptosis (4,9,10). Elevated adipose tissue senescent cell burden is hypothesized to contribute to local and systemic dysfunction as adipose is frequently the largest tissue in the human body with important immune and endocrine functions (10,11). In rodents, elevated adipose senescent cell burden induced by aging or "fast-food" westernized-diet accompanies systemic inflammation and physical dysfunction (4,11), but even modest reductions in the number of senescent cells can positively impact health and function. For example, adipose senescent cell abundance in middle-aged mice is attenuated from 12% in sedentary to ~2% in exercised animals exposed to a fast-food diet, which is attended by robust improvements in function and physical endurance (4). However, the physical function and clinical health consequences of cellular senescence in human aging have yet to be determined.

Determining the abundance and functional consequences of cellular senescence in humans is necessary to evaluate the translational potential of therapeutically targeting senescent cells (12–14). We hypothesized that elevated burden of senescent cell biomarker cyclin-dependent kinase inhibitor $p16^{INK4a}$ in adipose tissue is uniquely associated with poorer physical function in older persons. We further hypothesized that $p16^{INK4a}$+ senescent cell burden can be quantified serially as a biologic outcome for clinical trials in humans, and may be sensitive to change with intervention. To gain initial insight, we leveraged previously collected cryopreserved thigh adipose tissue (intermuscular or perimuscular) from well-characterized overweight/obese older women previously enrolled in a clinical study (15). We sought to demonstrate proof-of-concept that: (1) senescent cells could be detected by immunohistochemical (IHC) staining of $p16^{INK4a}$+ cells in intermuscular/perimuscular thigh adipose of older women, (2) $p16^{INK4a}$ senescent cell abundance would be associated with poor physical function, and (3) adipose $p16^{INK4a}$+ cell burden may be sensitive to change following a 5-month resistance training with or without caloric restriction intervention (RT ± CR).

Materials and Methods

Participants and Study Design

This study took advantage of cryopreserved adipose samples collected coincident to biopsy of the *vastus lateralis* during a previously conducted randomized clinical trial of resistance exercise (3 d/wk) either with –600 kcal/day caloric restriction or without caloric restriction in overweight/obese (body mass index [BMI] ≥ 28–35 kg/m^2) older adults (65–79 y) (Improving Muscle for Functional Independence Trial, I'M FIT; clinicaltrials.gov NCT01049698) (15). The study was approved by the Wake Forest School of Medicine Institutional Review Board and all participants provided written informed consent.

Detailed description parent study design, participants, and measures of body composition and adiposity are provided in Supplementary Methods and previous publication (15). Main outcome measures in the parent I'M FIT study that are relevant to the present investigation are: (a) body composition (height, weight, body mass index, dual-energy X-ray [DEXA]), (b) thigh intermuscular adipose tissue volume (IMAT, by computed tomography [CT scan]), (c) physical function (grip strength, 400-m walk performance, 4-m usual gait speed, and self-perceived mobility), and (d) biopsy of thigh intermuscular or perimuscular adipose tissue coincident to harvest of *vastus lateralis*; when adipose tissue (deep to fascia) was harvested with the skeletal muscle sample, it was immediately dissected and stored at –80°C (n = 56 total, n = 40 with ≥20 mg adipose sample needed for analysis). A subset of these cryopreserved thigh adipose samples obtained at baseline (n = 11) and follow-up (n = 8 pre-post intervention pairs with n = 4 RT and n = 4 RT + CR) was analyzed, using selection criteria to minimize impact on biorepository resources, and to ensure heterogeneity in physical function (See Supplementary Methods for detail).

Physical Function

Physical function was measured as grip strength, time to complete 400-m walk at a brisk pace, 4-m usual gait speed and self-perceived mobility (Mobility Assessment Tool short form, MAT-sf). Grip strength was measured twice to the nearest kilogram by using an isometric hydraulic hand dynamometer (Jamar); the maximal value by the right hand was used in analyses. Performance on 400-m walk was determined as time to complete the distance (10 laps on an indoor 20-m long course) as quickly as possible without running. Gait speed was determined as the usual walking speed on a 4-m course. The MAT-sf consisted of a 10-item computer-based assessment of mobility using animated video clips and covers a broad range of functioning, with score range 0–60 (60 best).

Quantification of Adipose $p16^{INK4a}$

Biopsy of *vastus lateralis* and coincident adipose was performed in the morning following an overnight fast following standard procedures (Supplementary Methods). Briefly, a modified Bergstrom needle was used to harvest skeletal muscle and surrounding tissue, including intermuscular and/or perimuscular (subfascial) adipose. The residual adipose was manually teased apart, flash frozen, and stored at –80°C. Cryopreserved adipose samples were thawed, paraffin embedded, and IHC nuclear staining for $p16^{INK4a}$ as detailed in Supplemental Methods. Cells expressing $p16^{INK4a}$ were quantified by an investigator blinded to function and pre-post status via manual visual inspection of 12–20 visual fields at 20× mag (representative images Supplementary Figure 1), with indices reported as the average across serial sections. Abundance of $p16^{INK4a}$+ cells was quantified as: (a) percent of the total fields analyzed containing $p16^{INK4a}$+ cells, (b) average number of $p16^{INK4a}$+ cells counted per visual field, and (c) percent of the total cells counted identified as $p16^{INK4a}$+. $p16^{INK4a}$+ cells were quantified by investigators blind to subject physical function *via* manual visual inspection at 20× magnification (positive stained nuclei confirmed at 40× magnification); total nuclei and $p16^{INK4a}$+ cells were counted in each visual field (Figure 1). In each section, 12–20 visual fields were analyzed and 3–4 serial sections were analyzed per subject sample. Indices of $p16^{INK4a}$ abundance are reported as the average across serial sections for each subject sample (Figure 1C and D). To assess the intra-examiner reliability, the visual analysis and quantification of $p16^{INK4a}$+ cell abundance was repeated in randomized order by the same reader blinded to original values separated by ~6–8 weeks.

Statistical Analyses

All statistical analyses were performed with SPSS software (version 23.0, IBM); $\alpha = 0.05$ was used to assess significance. Descriptive characteristics are reported as means ± SD and ranges. Intra-examiner reliability was determined by intraclass correlation coefficient using a one-way random effects model, and inter-item Pearson correlations across analyses were examined. Pearson correlations coefficients were calculated to assess cross-sectional relations at baseline and post-intervention between $p16^{INK4a}$ abundance (percent fields

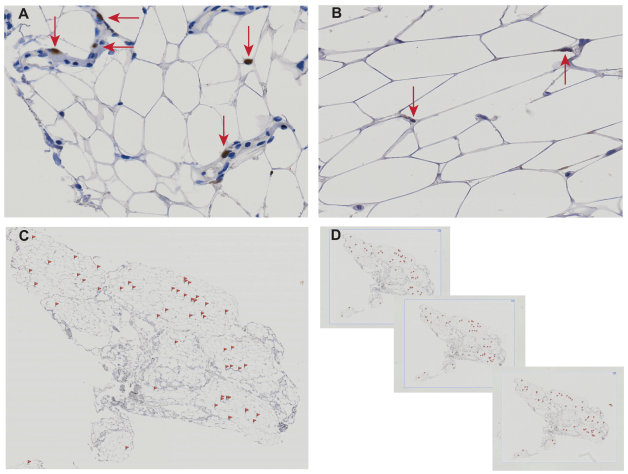

Figure 1. Representative images. p16^{INK4a}+ nuclear cell staining (IHC) of representative adipose tissue sample obtained from single subject. All thigh adipose samples were obtained during biopsy of the *vastus lateralis,* and were harvested deep to the fascia and likely of intermuscular or perimuscular origin. Upper panels (**A**, **B**) 20 × magnification single visual fields with p16^{INK4a}+ cells (red arrows); bottom panels p16^{INK4a}+ cell (red flags) distribution across sample section (2×mag, **C**), and three sections from one sample (1.2× mag, **D**).

with p16^{INK4a}-positive cells, average p16^{INK4a}-positive cells per field, and percent of total cells that were p16^{INK4a}-positive) and (a) age, (b) body composition (body weight, BMI, waist circumference, total fat mass, total lean mass, thigh IMAT volume), and (c) physical function (grip strength, 400-m walk time, 4-m usual gait speed, MAT-sf score, knee extensor power, five repetition chair-rise time, and SPPB). To examine potential confounders of the observed significant relationships between p16^{INK4a}+ cell abundance with the physical function measures (grip strength, 400-m walk time, 4-m gait speed, MAT-sf score), partial correlations were calculated (individually adjusted for age, BMI, waist circumference, total fat mass, total lean mass, or thigh IMAT volume). To determine change in indices of p16^{INK4a}+ cell abundance, body composition and physical function over time and estimates of intervention effects, the statistical differences within subjects were calculated using repeated measures ANOVA (RT and RT + CR intervention arms collapsed). Finally, to gain exploratory insight into possible relations between change in p16^{INK4a}+ cell abundance and change in physical function and body composition, Pearson correlations between the absolute change (post-RT ± CR–pre-RT ± CR) in each variable were calculated.

Results

Subject Characteristics and Senescent Cell Abundance in Thigh Adipose Samples

Subject characteristics at baseline are summarized in Table 1, including age, body composition, and adiposity (total body fat, fat mass, and lean mass by DEXA, intermuscular adipose tissue volume, IMAT by CT scan), and measures of physical function and self-perceived mobility. Descriptive characteristics for primary indices of p16^{INK4a}+ abundance are shown in Table 1. The intraclass correlation coefficients between repeat analyses ranged from 0.87 to 0.92, indicating strong reliability in p16^{INK4a}+ cell identification and quantification (Table 2).

Table 1. Subject characteristics (age, body composition/adiposity, and physical function) and indices of thigh adipose p16^{INK4a}+ cell abundance

	Mean ± SD	Range
n (women)	11	
Age (y)	72.9 ± 3.4	67–77
Body composition and adiposity		
Body weight (kg)	79.5 ± 7.2	64.9–90.0
BMI (kg/m^2)	30.6 ± 2.4	27.0–34.7
Waist circumference (cm)	88.4 ± 5.0	78.9–98.9
Total body fat (%)	45.5 ± 2.6	40.3–48.8
Fat mass (kg)	36.8 ± 4.7	26.1–44.6
Lean mass (kg)	43.8 ± 3.6	37.1–47.2
IMAT (cm^3)	24.5 ± 16.6	9.9–69.3
Physical function		
Grip strength (kg)	23.9 ± 6.0	11–32
400-m walk time (s)	332 ± 46	271–421
4-m usual gait speed (m/s)	1.06 ± 0.16	0.81–1.39
MAT-sf (score)	62.8 ± 4.0	54.7–67.6
Leg muscle power (W)	103 ± 47	44–193
rep chair rise time (s)	11.5 ± 4.0	8.1–21.9
SPPB (score)	11.2 ± 1.2	9.0–12.0
Adipose p16^{INK4a}+ cell abundance		
Fields w p16^{INK4a}+ cell (%)	75.7 ± 24.6	20–100
Avg. p16^{INK4a}+ cell count/field	2.2 ± 1.1	0.3–4.3
Total cell count	771 ± 220	358–1122
Cell count p16^{INK4a}+ (%)	5.6 ± 2.9	0.6–11.1

Note: Fat mass as whole body fat from DEXA. IMAT, thigh intermuscular adipose volume from CT scan. MAT-sf, Mobility Assessment Tool short form (higher score indicative of greater mobility). For indices of p16^{INK4a}+ cell abundance, 3–4 sections per sample were mounted, stained for p16^{INK4a} (immunohistochemistry), and manually visually analyzed; averages across the sample sections are shown.

Associations Among Thigh Adipose p16^{INK4a}+ Cell Burden and Age, Body Composition, and Physical Function

Overall, at baseline, cross-sectional correlations indicate that elevated p16^{INK4a}+ cell burden is associated with poorer physical function, even with adjustment for age and body composition. Significant negative correlations exist between p16^{INK4a}+ abundance with grip strength, usual gait speed on 4-m course, and MAT-sf, and though not statistically significant, moderate correlations were also observed for leg power and SPPB score (Table 3, Supplementary Figure 1). Likewise, 400-m walk time was positively correlated with percent total cell count p16^{INK4a}+ and a moderate (but non-significant) correlation was observed with five-repetition chair rise time, such that higher p16^{INK4a}+ cell burden was associated with poorer endurance walk and chair rise performance (Table 3, Supplementary Figure 1). p16^{INK4a}+ cell burden was significantly associated with waist circumference, but was only modestly associated with age and other indices of body composition, such that elevated p16^{INK4a}+ cell burden was either unrelated or moderately related to older age, lower lean body mass, and greater adiposity (Table 3). Partial correlations between physical function measures with significant correlations with p16^{INK4a}+ burden are shown in Supplementary Table 1 (independent adjustments for age, BMI, total fat mass, total lean mass, and IMAT); adjusting for age, adiposity, and lean mass did not affect the magnitude or direction of the associations.

Table 2. Intra-examiner reliability (intraclass correlation coefficient) and inter-item correlation matrix for primary indices of adipose $p16^{INK4a}$+ cell abundance across repeat analyses

		Initial Analyses		
	ICC	Fields w $p16^{INK4a}$+ Cells (%)	Avg. $p16^{INK4a}$+ Cells/Field	Cell Count $p16^{INK4a}$+ (%)
Fields with $p16^{INK4a}$+ cells (%)	0.87*	0.91*	0.78*	0.81*
Avg. $p16^{INK4a}$+ cells/field	0.87*	0.84*	0.88*	0.85*
Cell count $p16^{INK4a}$+ cells (%)	0.92*	0.85*	0.90*	0.97*

Note: ICC, single measure intraclass correlation coefficient. Pearson (r) correlations; *$p < .05$ ($n = 19$). $p16^{INK4a}$+ cell quantification was repeated 6–8 wk following initial analyses under same procedures, by investigator blind to physical function status and cell quantifications from initial analyses.

Table 3. Associations between indices of thigh adipose $p16^{INK4a}$+ cells with age and body composition, and physical function measures

	Fields with $p16^{INK4a}$+ Cells (%)	Avg. $p16^{INK4a}$+ Cells/Field	Cell couNt $p16^{INK4a}$+ (%)
Age	0.37	0.34	0.31
Body weight (kg)	0.29	0.39	0.24
BMI (kg/m^2)	0.40	0.49	0.55
Waist circumference (cm)	0.54	0.74*	0.63*
Total body fat (%)	0.54	0.38	0.38
Fat mass (kg)	0.49	0.49	0.38
Lean mass (kg)	–0.02	0.17	0.04
IMAT (cm^3)	0.07	–0.06	0.02
Grip strength (kg)	–0.45	–0.65*	–0.74*
400-m walk time (s)	0.50	0.53	0.74*
4-m gait speed (m/s)	–0.73*	–0.57	–0.54
MAT-sf (score)	–0.55	–0.66*	–0.78*
Leg power (W)	–0.36	–0.46	–0.53
5-rep chair rise time (s)	0.34	0.43	0.45
SPPB (score)	–0.41	–0.36	–0.57

Note: Pearson correlations (r); *$p < .05$ ($n = 11$). Whole body fat and lean mass from DEXA. IMAT, thigh intermuscular adipose volume from CT scan. 400-m walk time (s) performed at brisk pace; 4-m Gait Speed performed at usual walking speed. MAT-sf, Mobility Assessment Tool, short form. Leg muscle power (W) of right leg from Biodex. SPPB, short physical performance battery.

At intervention follow-up, despite the smaller sample size, overall moderate correlations were observed between $p16^{INK4a}$+ cell abundance and indices of adiposity, and physical function (Supplementary Table 2). The relationships between $p16^{INK4a}$+ burden and grip strength, and chair-rise performance remained statistically significant ($p < .05$), and though not significant, moderate relations with 400-m walk speed and MAT-sf, were observed ($r > 0.50$), but not with 4-m usual gait speed, leg power, and SPPB (Supplementary Table 2).

Thigh Adipose $p16^{INK4a}$+ Cell Burden Before and After RT ± CR

Eight subjects with thigh adipose tissue $p16^{INK4a}$+ cells quantified at baseline had samples obtained at intervention follow-up for paired pre–post intervention $p16^{INK4a}$+ cell analyses. Overall, $p16^{INK4a}$+ cell abundance was lower following the 5-months RT ± CR intervention, with attenuated burden in 6 of the 8 sample pairs (individual data shown in Figure 2, effect estimates in Supplementary Table 4). In this study subset, the 5-month intervention elicited 3 ± 4% total weight loss and reduced adiposity (baseline vs 5-mo RT ± CR, $p < .05$ all), but lean mass, physical function and self-perceived mobility were unchanged (Supplementary Table 4). Bivariate correlations were assessed between absolute changes in $p16^{INK4a}$+ cell abundance and changes in body composition (Supplementary Table 5), with moderate correlations observed with change in indices of adiposity including body weight, total fat mass, and percent body fat ($p \leq .05$), but not physical function, though modest and inconclusive given the small sample size.

Discussion

This study provides proof-of-concept that $p16^{INK4a}$+ cell abundance from thigh adipose is associated with worse physical function in community-dwelling older overweight/obese women. Moreover, these relations were not mediated by age, adipose quantity, or lean mass, suggesting that $p16^{INK4a}$+ cell burden derived from thigh adipose tissue is uniquely related to physical function. We demonstrate that $p16^{INK4a}$+ cell abundance can be quantified serially and may be sensitive to change with an intervention. Additionally, though our data suggest a possible association between change in $p16^{INK4a}$+ expression and reduction in body weight and total fat with intervention—but not with change in physical function—the evidence is not conclusive and must be replicated in a larger sample with a control group for reference. These preliminary observations require replication by a larger prospective study with supplementary senescence biomarkers and senescence-associated secretory products, however, we do provide the first evidence for translational utility of an *in vivo* cellular senescence biomarker and its associations with physical function and adiposity in older humans.

We found a robust cross-sectional association between thigh adipose tissue $p16^{INK4a}$+ cell abundance and several subdomains of physical function, including grip strength, 400-m walk performance, gait speed, and self-perceived mobility that predict higher rates of disability, institutionalization, and mortality (16–18). Preclinical investigations in rodents suggest depot specificity, with elevated senescent cells accumulation in visceral adipose tissue compared

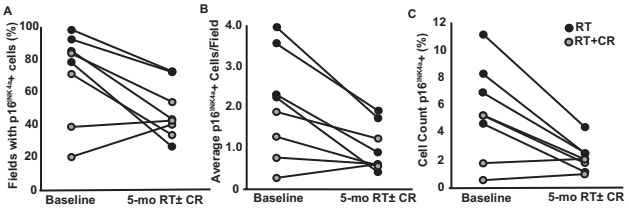

Figure 2. p16^{INK4a}+ cell burden before and after 5-mo RT ± CR intervention in older overweight/obese women. p16^{INK4a}+ cell burden, measured as percent of visual fields with p16^{INK4a}+ cells (**A**), average p16^{INK4a}+ cells per field (**B**), and percent cell count p16^{INK4a}+ (**C**), from thigh adipose tissue samples collected before and after a 5-month RT (3 d/wk on weight-stack resistance machines), with a CR intervention designed to elicit moderate weight-loss (–600 kcal/d) or without CR. Black circles: RT only; gray: RT + CR.

with subcutaneous adipose (4,10). While we do not have access to visceral adipose tissue in this study, our findings do suggest that p16^{INK4a} expressing cells do accumulate in thigh adipose harvested deep to fascia (not subcutaneous), and this depot may serve as a source of senescent cells that are relatively easier to access than visceral depots. Excess adipose tissue is associated with worse physical function in older adults (19), but thigh intermuscular fat in particular is more closely associated with physical dysfunction and annual gait speed declines in older persons, such that every 5.75 cm^2 increase in intermuscular fat is associated with a 0.01 ± 0.0 m/s annual decline in gait speed, and this decline is exacerbated with intermuscular adipose gain over time in older persons (20). That robust cross-sectional associations were observed between physical function and p16^{INK4a}+ cells harvested from thigh intermuscular or perimuscular adipose, an adipose depot known to independently predict gait speed and functional decline, is intriguing and warrants further investigation to determine depot-specificity and possible systemic mediators.

We were able to detect p16^{INK4a}+ cells in adipose tissue biopsies performed before and after a clinical intervention, which suggests the potential of thigh adipose tissue p16^{INK4a}-positive cell abundance as *in vivo* senescence biomarker for clinical trials in humans. Though senescent cells are known to accumulate in aging rodents, the relative abundance and longitudinal quantification in humans by histochemical detection methods has been limited by the rarity of these cells, potential non-specificity, and other practical issues (21). Our findings also suggest potential modulation in p16^{INK4a} senescent cell abundance with an RT ± CR intervention, which is in accord with preclinical evidence from rodents (4,22) though conclusions from the present study are dampened by absence of a control group. Nevertheless, we illustrate that p16^{INK4a} can be measured serially in biopsies in the context of a clinical trial in aging, and provide a conceptual foundation for future work to determine the appropriateness of p16^{INK4a}+ cells as a surrogate marker of senescence with interventions (3). This is especially important with discovery of senolytic drugs that reduce senescent cell burden and alleviate age- and disease-related phenotypes, including frailty and declines in physical endurance in mice (12–14), and could potentially be translated to humans.

Though mechanisms have yet to be defined, we provide direct evidence linking p16^{INK4a}+ cell abundance in thigh adipose tissue to physical function in humans for the first time, and our findings are substantiated by foundational evidence in rodents and association studies in humans. p16^{INK4a} acts to establish retinoblastoma protein (pRB)-regulated growth arrest and is commonly used as an *in vivo* senescence marker owing largely to its function as a tumor suppressor and highly dynamic expression (21,23). p16^{INK4a} is expressed by many (though not all) senescent cells, and ample evidence demonstrates elevated p16^{INK4a} expression in mammals with advanced chronologic age across multiple tissue types (24,25). The physiologic effect of p16^{INK4a}-expressing cells has been examined in a transgenic mouse model (INK-ATTAC), in which p16^{INK4a}-expressing cell apoptosis can be induced (26,27). p16^{INK4a}-expressing cell ablation partially rescues vascular reactivity and several age-related phenotypes and disorders in these mice and may extend median, but not maximum lifespan, though the effects on lifespan are not definitive (4,13,26,27). Clearance of p16^{INK4a}-expressing cells beginning at mid-life in naturally-aged rodents attenuated age-dependent adipose tissue and stem cell dysfunction, and delays in the trajectory of age-related declines in spontaneous and exploratory behavior were noted (28,29). This is further supported by the discovery that senolytic drugs reduce p16^{INK4a}-expressing cells in tandem with declines in other markers of cellular senescence, and affects on multiple age- and senescence-related phenotypes parallel those of genetic clearance from INK-ATTAC mice (4,12,13). p16^{INK4a}+ cells isolated from adipose tissue exhibit elevated expression of pro-inflammatory senescence-associated secretory phenotype (SASP) products compared to cells from the same tissues not expressing p16^{INK4a} (3,11,26,28). However, the relation between p16^{INK4a} and the SASP is nuanced and by p16^{INK4a} overexpression may not consistently activate a SASP (30,31). Whether driven through SASP-dependent mechanisms or not, collectively these data support a model in which p16^{INK4a}+ cells shorten healthy lifespan and negatively impact function with age in rodents.

The negative health consequences of increased p16^{INK4a}+ cell burden may extend to humans. For example, peripheral blood T-lymphocyte expression of p16^{INK4a}, but not other INK4/ARF transcripts, was found to be uniquely related to chronologic age and associated with health behaviors such as tobacco use and physical inactivity (24). Unbiased human association studies demonstrate that the genotype of single nucleotide polymorphisms near the INK/ARF locus coding for p16^{INK4a} are associated with chronic age-related diseases (32–34). A strong association between a common inherited variant of the p16^{INK4a} genetic region and reduced limitation in physical function was found in a heterogeneous older adult population (35). Collectively, these works and our present findings implicate p16^{INK4a} senescent cells in maintenance of health and physical function in mammalian species from rodents to humans.

We acknowledge several limitations. Important next steps should confirm whether these p16^{INK4a}+ cells are indeed senescent and express SASP factors and determine cell types through secondary staining. In future work, adipose samples should be harvested from different depots and tissues, as p16^{INK4a}+ cells may be differentially-expressed and could vary in their systemic effects and functional consequences on age-related adipose dysfunction, physical function declines, and frailty (8,10,36). The quantification of p16^{INK4a}+ cell burden from adipose tissue biopsies should also be coupled with circulating SASP products, senescence biomarkers, and T-cell p16^{INK4}expression, as blood-based biomarkers could have particular utility in clinical trials (3,24). Finally, p16^{INK4a}+ cell burden should be assessed longitudinally in both women and men not undergoing an intervention to determine p16^{INK4a} accumulation with normal human aging. A non-treatment reference group (which was not part of the parent trial) is critically important to determine relations between change in p16^{INK4a}+ senescent cell burden and change in functional outcomes over time. Thus, longitudinal assessment without intervention will enable calculation of effect size that will be critical for future clinical trials with senolytics and other interventions designed to directly or indirectly alleviate senescent cell burden.

In conclusion, this study provides the first direct evidence that the senescence biomarker $p16^{INK4a}$ is associated with physical dysfunction that could increase risk of disability, loss of independence, and mortality in older adults, and these relations are independent of age and adiposity or lean mass. Additionally, $p16^{INK4a}$+ cell burden in biopsied thigh intermuscular or perimuscular adipose tissue can be quantified serially in the context of a clinical trial in older persons, and is potentially modifiable with intervention. These results are preliminary and require replication and validation, including determination of $p16^{INK4a}$+ cell type, confirmation of permanent cell-cycle arrest, and correlation with additional senescence and SASP markers. Though additional data are needed to identify the mechanisms of action, this study provides novel evidence that thigh adipose tissue $p16^{INK4a}$+ cell burden may have functional consequences in community-dwelling older women, and opens a fertile area for future research seeking to translate groundbreaking insights on cellular senescence from the lab to the clinic.

Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

The authors declare no competing interests.

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