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Occupational Years of Service and Leukocyte Epigenetic Aging

Relationships in United States Firefighters

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Objective: The aim of the study is to examine associations between years of firefighting service and eight chronological age-adjusted measures of blood leukocyte epigenetic age acceleration: Horvath, Hannum, SkinBloodClock, Intrinsic, Extrinsic, PhenoAge, GrimAge, and DNAm telomere length. **Methods:** The study used a repeated measures analysis of data from 379 incumbent firefighters from eight career departments and 100 recruit firefighters from two of the departments, across the United States. **Results:** Incumbent firefighters had on average greater epigenetic age acceleration compared with recruit firefighters, potentially due to the cumulative effect of occupational exposures. However, among incumbent firefighters, additional years of service were associated with epigenetic age deceleration, particularly for GrimAge, a strong predictor of mortality. **Conclusions:** Long-term studies with more specific occupational exposure classification are needed to better understand the relationship between years of service and aging biomarkers.

Keywords: epigenetic age, DNA methylation, healthy worker effect, EMT, EMS

LEARNING OUTCOMES

- Describe the concept of epigenetic age acceleration and recall key differences between the eight epigenetic age markers used in the present study.
- Summarize the observed relationships between years of occupational firefighter exposure and epigenetic age acceleration in incumbent firefighters while identifying differences in these observed relationships in incumbent versus recruit firefighters.

Firefighting is characterized by work in a variety of environments and is associated with multiple hazardous exposures including chemical toxicants, heat, shift work, and noise.[1] The International Agency for Research on Cancer has classified firefighting as "carcinogenic to humans" (group 1),[2] and there is further evidence linking firefighting to respiratory, cardiovascular, and mental illness.[3–5] DNA methylation, an epigenetic mechanism that influences gene expression and is influenced by lifestyle and environmental factors,[6] is one biological marker that has been used to better understand these health relationships. Existing studies have identified genes that are differentially methylated when comparing firefighters to nonfirefighting controls and incumbent firefighters to recruits. Many of these differentially methylated genes, including *FOXK2* and *DUSP22*, have known relationships with carcinogenesis.[7–9] To the best of our knowledge, only one study has examined relationships of firefighting with epigenetic age,[10] a DNA methylation-based biomarker of biological aging that surpasses single methylation loci in its prediction of healthspan and lifespan.[11–13] This study reported significant, positive associations of perfluorooctanoate, the sum of branched isomers of perfluorooctane sulfonate, and perfluorohexane sulfonate with several epigenetic aging biomarkers in municipal firefighters, suggesting important health risks from this group of chemical compounds.[10]

In the present study, we add to the knowledge of firefighting and epigenetic age by examining the relationships of occupational years of service with epigenetic aging using data from career firefighters across the United States. Furthermore, we explore whether epigenetic aging may differ between recruit and incumbent firefighters. We tested relationships with eight robust epigenetic age measures that provide different

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Ethical Considerations and Disclosures: This study was reviewed and approved by the University of Arizona Institutional Review Board (IRB) and the University of Miami IRB. All study participants provided their written informed consent.

Author Contributions: JB, AC, ACM, CG, JG, and JNE contributed to the conception and design of the study. JB, ACM, CG, JG, SB, SL, DU, JG, JH, JG, and DW contributed to the data collection and laboratory analyses. JNE, AC, MAF, and JB were major contributors to the analysis and interpretation of the data and writing of the manuscript. AJ, PC, SB, and SL also contributed to the data interpretation and writing of the manuscript. All authors read and approved of the final manuscript.

Data Availability Statement: The data generated or analyzed during the current study are not publicly available because of restrictions based on the consent forms and IRB application for this study, but deidentified data are available from the authors upon reasonable request. Data requests can be made through the Fire Fighter Cancer Cohort Study at the email address COPH-FFCCS@arizona.edu

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information on DNA methylation-based biological aging. The Hannum,[14] Horvath,[11] and the SkinBloodClock[15] epigenetic biomarkers are predictors of chronological age but also have reported relationships with environmental exposures and disease. For instance, the Horvath epigenetic clock is associated with increased exposure to fine particle air pollution[16] and chronic obstructive pulmonary disease.[17] The PhenoAge clock[12] is a more sensitive biomarker of morbidity, while the GrimAge clock[13] is an epigenetic predictor of mortality risk. Extrinsic (EEAA) and intrinsic (IEAA) epigenetic age acceleration are derived from the Hannum and Horvath measures, respectively.[18] Extrinsic epigenetic age acceleration measures immune system aging by upweighting age-dependent changes in leukocytes (naïve cytotoxic T cells, exhausted cytotoxic T cells, and plasmablasts). The IEAA measure reports age acceleration independent of leukocyte proportions and can be viewed as a metric of the intrinsic aging of cells. The DNAm telomere length (DNAmTL) biomarker is correlated with directly measured leukocyte TL and may reflect cell replication.[19]

Given the well-documented occupational hazards that firefighters experience, we hypothesized that occupational years of service would be positively associated with an increased cumulative burden of adverse health exposures and subsequently with accelerated epigenetic aging. Likewise, given that recruits are typically younger in chronological age and have experienced a lower burden of occupational exposures, we performed a sensitivity analysis comparing recruits to incumbent firefighters. Here, we hypothesized that recruits would be epigenetically younger, independent of their chronological age, compared with incumbent firefighters.

METHODS

Study Sample

Participants were drawn from two studies assessing disease and cancer risk factors among career firefighters in the United States (US). Study recruitment methods have been reported previously.[10,20] All study protocols and materials were reviewed and approved by institutional review boards (IRBs), and all participants provided written informed consent. The first study was conducted in partnership with the Tucson Fire Department. The second study, the Fire Fighter Cancer Cohort Study (FFCCS) (https://www.ffccs.org/), is an ongoing study involving academic and governmental institutions as well as fire departments across the US. At time of study enrollment (2015–2018 for Tucson Fire Department study and 2018–2021 for FFCCS), participants completed an online survey that collected demographic information including age, race, Hispanic ethnicity, height and weight measurements that were used to calculate body mass index (BMI, in kilograms per meter squared), years working as a firefighter, and fire department. Blood samples were also collected from participants at time of enrollment. For participants in the Tucson Fire Department study, an additional blood sample was collected at follow-up 20 to 37 months after enrollment. For a subset of participants in the FFCCS, a second blood sample was collected approximately 9 months after initial enrollment. The final sample set is composed of 479 firefighters (379 incumbents and 100 recruits) from eight fire departments across the US. New recruits were from two fire departments. Of the study participants, 144 had a follow-up collection resulting in 623 total observations.

DNA Methylation Processing

Blood samples were primarily collected in one 6-mL dipotassium ethylenediaminetetraacetic acid tube (Becton, Dickinson and Company, Franklin Lakes, NJ) for DNA methylation analyses, with eight samples collected in cell preparation tubes (Becton, Dickinson and Company). The ethylenediaminetetraacetic acid tubes were processed in one of two ways: 2.5 mL of whole blood was aliquoted, and the remaining blood centrifugated at 1300*g* for 15 minutes and plasma separated from the packed cell pellet, or the whole 6.0-mL blood sample was centrifuged at 1300*g* for 15 minutes and plasma separated from the packed cell pellet. Samples processed off site were temporarily stored at −20°C until transfer to the University of Arizona for long-term storage at −80°C. Cell preparation tubes were processed according to the product guidelines and packed cell pellets stored at −80°C.

DNA was isolated from blood leukocytes or packed cell pellets and quantified using a QuantiFluor dsDNA System (Promega, WI) or a Qubit Fluorometer (Thermo Fisher Scientific, MA). Approximately 500 ng per sample underwent bisulfite conversion using Zymo EZ DNA Methylation Kits (Zymo Research Corp, Irvine, CA) according to manufacturer recommendations for downstream Infinium array analysis. DNA methylation was quantified at greater than 850,000 CpG sites throughout the genome using the Infinium MethylationEPIC array (Illumina, CA).[21] Laboratory and data processing methods have been previously described.[10] Briefly, after completion of bisulfite conversion, samples were randomized across chips, hybridized, and scanned in batches at the University of Utah DNA Sequencing and Genomics Core Facility (Salt Lake City, UT) and/or the University of Michigan Advanced Genomics Core. Raw image files were read through the R package minfi, and quality control was performed in minfi and Enmix.[22] Samples failing at least one quality control measure (including sex match, low average intensities, poor bisulfite conversion efficiency, and/or mismatching SNP data for repeat samples from the same participant) were excluded.

Epigenetic Age Biomarker Calculation

We generated estimates of both cell type proportions and epigenetic age ("epigenetic clocks") using widely used algorithms as previously described.[10] Briefly, we uploaded EPIC data for all samples passing quality control to the New Methylation Age Calculator Web site (https://dnamage.genetics.ucla.edu/new).[11] Epigenetic age acceleration (EAA) measures are provided directly by the online calculator and represent the difference between the ages predicted by various epigenetic clocks and an individual's chronological age. Epigenetic age acceleration measures are derived from residuals of models that regress each epigenetic clock on chronological age. Cell type proportions of $CD4^+$ T cells, natural killer cells, B cells, monocytes, and granulocytes were estimated using an established algorithm[23] along with relative abundance of three additional blood cell types—plasma blasts, CD8 + CD28-CD45RA-T cells, and naive $CD8^+$ T cells.[11] Among epigenetic aging biomarkers generated by this calculator, we selected the Horvath, Hannum, and SkinBlood clocks along with composite clocks PhenoAge and GrimAge and a DNA methylation-based estimator of telomere length (DNAmTL) for downstream analysis. These six markers were regressed on chronological age and the residuals used in downstream statistical analysis. We also estimated intrinsic epigenetic age acceleration (IEAA) and EEAA in our analyses.[24]

Statistical Analysis

We used Pearson correlations between chronological age and epigenetic aging biomarkers to test the performance of the epigenetic clocks. For all measures except DNAmTL, we computed the median absolute error (MAE) in years (defined as the median absolute deviation between age predicted by each epigenetic clock and chronological age) to further evaluate accuracy.

We ran a 3-phase analysis. The first phase of our analysis focused on incumbent firefighters from all eight fire departments. In linear unadjusted models, we modeled the first visit relationships of occupational years of service with each of the eight epigenetic clocks. Next, we used linear mixed effects models to model first and second visit (longitudinal) relationships of occupational years of service with each of the eight epigenetic clocks in the firefighters. The longitudinal models included a random intercept for participants to account for repeated measures. We then reran the longitudinal models adjusted for race, sex, body mass index (BMI), and fire department. The second

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TABLE 1. Participant Characteristics for Incumbent and New Recruit Firefighters ($N = 479$)

	Incumbent ($n = 379$, 79%), Mean (SD)	Recruit ($n = 100$, 21%), Mean (SD)
Age variables		
Baseline chronological age, y	41.7 (9.4)	29.1 (5.7)
Hannum DNAmAge, y	33.5 (9.2)	22.5 (6.2)
Horvath DNAmAge, y	43.7 (9.5)	31.1 (7.8)
SkinBloodClock, y	38.5 (10.4)	25.0 (7.4)
DNAm PhenoAge, y	31.3 (10.4)	21.1 (8.1)
DNAm GrimAge, y	41.5 (8.6)	30.7 (6.6)
DNAmTL, kb	7.1 (0.3)	7.4 (0.2)
Demographic and occupational variables	*n* (%)	*n* (%)
BMI category		
Normal	80 (21)	41 (41)
Overweight	221 (58)	49 (49)
Obese	78 (21)	10 (10)
Race		
Black or African American	14 (4)	10 (10)
White	307 (81)	76 (76)
Other	58 (15)	14 (14)
Sex		
Female	42 (11)	14 (14)
Male	337 (89)	86 (86)
No. visits		
1	285 (75)	50 (50)
2	94 (25)	50 (50)
Years as firefighter, mean (SD)	14.5 (8.8)	0.3 (0.6)

phase of our analysis evaluated years of service and epigenetic aging relationships using first visit unadjusted, longitudinal unadjusted, and longitudinal adjusted models for recruits alone. Again, recruits came from two of the eight fire departments. In the third phase of our analysis, we focused on the two fire departments with recruits and examined if being an incumbent versus recruit firefighter was significantly associated with the epigenetic clocks using first visit unadjusted, longitudinal unadjusted, and longitudinal adjusted models. These adjusted models contain covariates for occupational years of service, race, sex, BMI, and fire department. Given evidence that weight/adiposity/BMI could also be a potential mediator of the adverse health effects of environmental and occupational exposures including air pollutants,[25,26] we performed a sensitivity analysis of adjusted models excluding BMI as a covariate. All

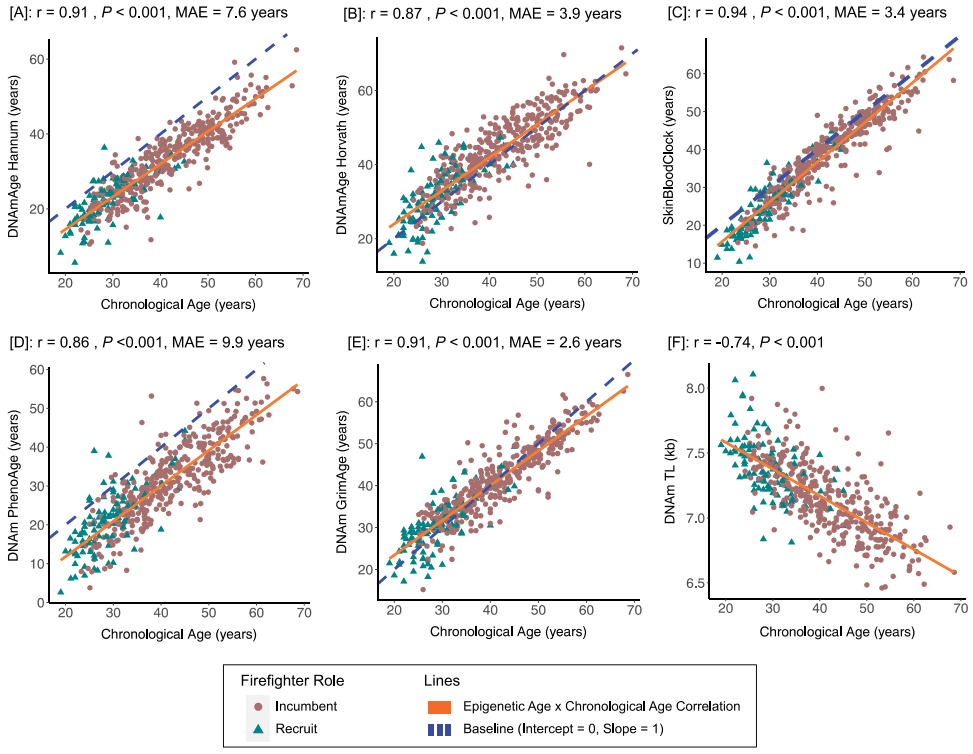

FIGURE 1. Epigenetic age and chronological age correlations. Pearson correlation coefficients (*r*) and MAEs for baseline (first visit) epigenetic aging biomarkers estimated in the study sample and chronological age.

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TABLE 2. Relationship between Years of Service and EAA in Incumbent Firefighters

Aging Biomarker Models	Baseline Unadjusted (Obs = 379)		Longitudinal Unadjusted (Obs = 473)		Longitudinal Adjusted (Obs = 473)		Sensitivity Longitudinal Adjusted w/o BMI (Obs = 473)	
	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*
EAA Hannum units: years	−0.01 (−0.05 to 0.04)	0.80	−0.003 (−0.05 to 0.04)	0.88	−0.02 (−0.06 to 0.03)	0.50	−0.01 (−0.05 to 0.04)	0.78
EAA Horvath units: years	−0.03 (−0.09 to 0.03)	0.27	−0.04 (−0.10 to 0.02)	0.16	−0.08 (−0.13 to −0.02)	0.01	−0.06 (−0.12 to −0.01)	0.02
EAA SkinBloodClock units: years	−0.02 (−0.07 to 0.02)	0.29	−0.02 (−0.06 to 0.03)	0.41	−0.04 (−0.08 to 0.01)	0.14	−0.02 (−0.07 to 0.02)	0.30
Intrinsic EAA (IEAA) units: years	−0.02 (−0.07 to 0.03)	0.46	−0.03 (−0.08 to 0.02)	0.30	−0.04 (−0.10 to 0.01)	0.15	−0.02 (−0.08 to 0.02)	0.31
Extrinsic EAA (EEAA) units: years	0.005 (−0.05 to 0.06)	0.87	0.01 (−0.05 to 0.06)	0.80	−0.01 (−0.07 to 0.05)	0.80	0.004 (−0.05 to 0.06)	0.89
EAA PhenoAge units: years	0.02 (−0.04 to 0.08)	0.51	0.02 (−0.04 to 0.08)	0.48	0.01 (−0.05 to 0.08)	0.66	0.04 (−0.02 to 0.10)	0.22
EAA GrimAge units: years	−0.02 (−0.06 to 0.02)	0.34	−0.02 (−0.07 to 0.02)	0.23	−0.05 (−0.09 to −0.01)	0.01	−0.05 (−0.08 to −0.01)	0.01
DNAm TL age adjusted units: kb	−0.001 (−0.003 to 0.002)	0.60	−0.001 (−0.003 to 0.001)	0.54	0.001 (−0.002 to 0.003)	0.58	0.0003 (−0.002 to 0.003)	0.76

Adjusted models contain covariates for race, sex, BMI, and fire department. The baseline model consists of first visits only. The longitudinal models include first and second study visits. These models include data from eight fire departments. The table depicts β values for each year of occupational service. For instance, for EAA Horvath in longitudinal adjusted models, each year of service is associated with a 0.08-year younger epigenetic age.

BMI, body mass index; CI, confidence interval.

statistical analyses were performed using R Version 4.1.0 (R Core Team, Vienna, Austria).

RESULTS

Study Sample Characteristics

Our study sample characteristics are presented in Table 1. The sample includes 479 firefighters (379 incumbent firefighters [79%] and 100 recruits [21%]). Four of the departments were municipal fire departments and four were airport fire departments; the four municipal fire departments accounted for 80% of the total participants and all the recruits. Baseline mean (SD) chronological ages for incumbent firefighters and recruits were 41.7 (9.4) and 29.1 (5.7) years, respectively. The study sample was majority male (88%) and White (80%). Most participants were overweight or obese according to BMI (75%). Most incumbents (75%) had one study visit; however, half of the recruits (50%) had two study visits. The mean (SD) years working as a firefighter in incumbents was 14.5 (8.8) years. The baseline mean (SD) years working as a firefighter in recruits was 0.3 (0.6) years.

Performance of Epigenetic Clocks

Epigenetic ages were higher in incumbents compared with recruits (Table 1). For instance, mean (SD) Horvath DNAmAge was 43.7 (9.5) years and 31.1 (7.8) years for incumbents and recruits respectively. DNAmTL estimates were comparable between the two groups. Mean (SD) DNAmTL was 7.1 (0.3) kilobases (kb) for incumbents and 7.4 (0.2) kb for recruits. All epigenetic clocks performed well and were significantly correlated with chronological age (Fig. 1). Positive correlations with chronological age were strongest for the SkinBloodClock ($r = 0.94$, $P < 0.001$; MAE = 3.4 years), Hannum DNAmAge ($r = 0.91$, $P < 0.001$; MAE = 7.6 years), and DNAm GrimAge ($r = 0.91$, $P < 0.001$; MAE = 2.6 years). As expected, DNAmTL was the only metric that was negatively correlated with chronological age ($r = -0.74$, $P < 0.001$). Notably, Hannum and PhenoAge estimates were generally younger than subjects' chronological ages and had the largest MAEs.

Associations of Years of Service and EAA

Table 2 presents relationships of years of service and EAA solely in incumbent firefighters from the eight fire departments. In longitudinal adjusted models, years of service was inversely associated with EAA Horvath and EAA GrimAge. Specifically, both EAA Horvath (β = −0.08 years; 95% CI, −0.13 to −0.02, $P = 0.01$) and EAA GrimAge (β = −0.05 years; 95% CI, −0.09 to −0.01, $P = 0.01$) were inversely associated with years of service in longitudinal models adjusting for race, sex, BMI, and fire department. Unadjusted baseline and longitudinal estimates were not statistically significant.

Table 3 presents relationships of years of service and EAA solely in recruit firefighters from two fire departments. In adjusted longitudinal models, years of service was inversely associated with EAA Hannum (β = −0.58 years; 95% CI, −1.15 to −0.02, $P = 0.04$), SkinBloodClock (β = −0.65 years; 95% CI, −1.09 to −0.21, $P = 0.005$), PhenoAge (β = −1.14 years; 95% CI, −1.87 to −0.41, $P = 0.003$), and EEAA (β = −0.98 years; 95% CI, −1.84 to −0.11, $P = 0.03$). Years of service was positively associated with EAA Horvath (β = 0.61 years; 95% CI, 0.02 to 1.20, $P = 0.04$). The observed relationships were of similar magnitude and statistical significance in the sensitivity analysis where BMI was removed as a covariate (Tables 2, 3).

Epigenetic Age Relationships Comparing Recruits With Incumbent Firefighters

Table 4 presents the results of models comparing EAA between recruits and incumbents. A significant association was only observed for EAA SkinBloodClock. In longitudinal age-adjusted models, recruits had a 1.16-year younger EAA SkinBloodClock when compared with incumbents (95% CI, −2.28 to −0.03, $P = 0.04$). This relationship was of similar magnitude but not statistically significant in the

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TABLE 3. Relationship between Years of Service and EAA in Recruit Firefighters

Aging Biomarker Models	Baseline Unadjusted (Obs = 100)		Longitudinal Unadjusted (Obs = 150)		Longitudinal Adjusted (Obs = 150)		Sensitivity Longitudinal Adjusted w/o BMI (Obs = 150)	
	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*
EAA Hannum units: years	−1.15 (−2.45, 0.14)	0.08	−0.76 (−1.26 to −0.27)	0.003	−0.58 (−1.15 to −0.02)	0.04	−0.58 (−1.14 to −0.03)	0.04
EAA Horvath units: years	−1.68 (−3.38 to 0.02)	0.05	−0.13 (−0.72 to 0.46)	0.66	0.61 (0.02 to 1.20)	0.04	0.62 (0.04 to 1.21)	0.04
EAA SkinBloodClock units: years	0.04 (−0.99 to 1.07)	0.94	−0.60 (−1.00 to −0.21)	0.003	−0.65 (−1.09 to −0.21)	0.005	−0.67 (−1.11 to −0.23)	0.004
Intrinsic EAA (IEAA) units: years	−0.75 (−2.18 to 0.69)	0.30	−0.13 (−0.58 to 0.33)	0.57	0.11 (−0.38 to 0.60)	0.66	0.12 (−0.37 to 0.60)	0.63
Extrinsic EAA (EEAA) units: years	−2.40 (−4.37 to −0.43)	0.02	−1.06 (−1.79 to −0.32)	0.006	−0.98 (−1.84 to −0.11)	0.03	−0.99 (−1.84 to −0.14)	0.02
EAA PhenoAge units: years	−1.47 (−3.24 to 0.29)	0.10	−0.70 (−1.34 to −0.06)	0.03	−1.14 (−1.87 to −0.41)	0.003	−1.14 (−1.86 to −0.42)	0.003
EAA GrimAge units: years	−3.03 (−4.33 to −1.73)	<0.001	−0.78 (−1.23 to −0.33)	0.001	−0.44 (−0.90 to 0.02)	0.06	−0.42 (−0.88 to 0.04)	0.07
DNAm TL age adjusted units: kb	−0.02 (−0.08 to 0.05)	0.61	0.001 (−0.02 to 0.02)	0.90	0.02 (−0.01 to 0.04)	0.18	0.01 (−0.01 to 0.04)	0.20

Adjusted models contain covariates for race, sex, BMI, and fire department. The baseline model consists of first visits only. The longitudinal models include first and second study visits. These models include data from two fire departments. The table depicts beta values for each year of occupational service. For instance, for EAA Hannum in longitudinal adjusted models, each year of service is associated with a 0.58-year younger epigenetic age.

BMI, body mass index; CI, confidence interval.

sensitivity analysis where BMI was removed as a covariate (β = −0.92 years; 95% CI, −2.05 to 0.20, *P* = 0.11).

DISCUSSION

In this study of US firefighters, we explored the relationships of occupational years of service with epigenetic age. Our results demonstrate that for the SkinBloodClock, incumbents were epigenetically older than new firefighters (recruits), even after accounting for chronological age. However, for incumbent firefighters, we observed that each occupational year of service was associated with lower EAA by the Horvath and GrimAge biomarkers, although the magnitude of this relationship was relatively small. Despite short follow-up time, in recruit firefighters, overall, each occupational year of service was also associated with lower epigenetic age and these effect sizes were larger than those observed in incumbents.

Existing studies demonstrate that firefighters have increased risks for certain cancers,[27] heart disease,[28,29] adverse reproductive

TABLE 4. Epigenetic Age Acceleration Relationships of Recruits Compared With Incumbent Firefighters

Aging Biomarker Models	Baseline Unadjusted (Obs = 300)		Longitudinal Unadjusted (Obs = 435)		Longitudinal Adjusted (Obs = 435)		Sensitivity Longitudinal Adjusted w/o BMI (Obs = 435)	
	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*	Difference in DNA Methylation Biomarker (95% CI)	*P*
EAA Hannum units: years	0.07 (−0.92 to 1.06)	0.89	−0.65 (−1.53 to 0.23)	0.15	−0.08 (−1.30 to 1.14)	0.90	−0.03 (−1.24 to 1.17)	0.53
EAA Horvath units: years	−0.70 (−2.01 to 0.62)	0.30	−0.75 (−1.97 to 0.47)	0.23	0.35 (−1.09 to 1.80)	0.63	0.39 (−1.03 to 1.81)	0.59
EAA SkinBloodClock units: years	−0.49 (−1.31 to 0.34)	0.25	−1.06 (−1.86 to −0.27)	0.01	−1.16 (−2.28 to −0.03)	0.04	−0.92 (−2.05 to 0.20)	0.11
Intrinsic EAA (IEAA) units: years	−0.40 (−1.51 to 0.71)	0.48	−0.49 (−1.53 to 0.54)	0.35	0.16 (−1.22 to 1.55)	0.82	0.18 (−1.19 to 1.54)	0.80
Extrinsic EAA (EEAA) units: years	0.07 (−1.26 to 1.41)	0.92	−0.72 (−1.87 to 0.44)	0.22	−0.16 (−1.79 to 1.46)	0.85	−0.07 (−1.68 to 1.53)	0.93
EAA PhenoAge units: years	0.44 (−0.88 to 1.76)	0.51	0.23 (−0.99 to 1.45)	0.71	−0.24 (−1.93 to 1.44)	0.78	−0.29 (−1.97 to 1.37)	0.73
EAA GrimAge units: years	0.48 (−0.59 to 1.55)	0.38	−0.03 (−1.02 to 0.96)	0.95	0.29 (−0.86 to 1.44)	0.62	0.24 (−0.90 to 1.38)	0.68
DNAm TL age adjusted units: kb	0.01 (−0.03 to 0.05)	0.65	0.01 (−0.03 to 0.06)	0.59	0.01 (−0.05 to 0.07)	0.83	0.01 (−0.05 to 0.07)	0.82

Adjusted models contain covariates for occupational years of service, race, sex, BMI, and fire department. The baseline model consists of first visits only. The longitudinal models include first and second study visits. These models include data from two fire departments. The table depicts beta values for recruits compared with incumbents. For instance, for EAA SkinBloodClock in longitudinal adjusted models, recruits are on average 1.16-years younger in epigenetic age than incumbent firefighters.

BMI, body mass index; CI, confidence interval.

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effects,[30–32] and respiratory disease.[33] DNA methylation studies suggest that firefighting may also impact the epigenome and may have some role in some of these disease processes. For example, in a subset of the participants from the present study, we identified 680 loci throughout the genome with altered DNA methylation approximately 2 years after starting work in the fire service when compared with the recruits' own baseline samples.[34] Methylation levels of 140 of these loci also associated with cumulative time spent at fires in between baseline and follow-up. In another example, an Ohio-based study of 18 firefighters reported that the promoter of *DUSP22*, a gene that encodes a phosphatase and is associated with lymphoma, was significantly hypomethylated in firefighters compared with nonfirefighting controls.[7] To further demonstrate relationships of the epigenome with firefighting, we have previously used machine learning to identify DNA methylation sites highly correlated with years of firefighting.[9] In addition, the one existing study of firefighting and epigenetic age relationships in 197 US firefighters (a subset of the present study) reported that toxic, persistent per- and polyfluoroalkyl substances (PFAS) were associated with several epigenetic clocks.[10] Although the study reported inverse associations of perfluorodecanoate and perfluoroundecanoate with GrimAge, multiple other PFAS were positively associated with epigenetic aging. Perfluorohexane sulfonate was positively associated with three clocks (EEAA, Hannum and SkinBlood). Linear perfluorooctanoate was associated with 6 clocks (Hannum, Horvath, IEAA, EEAA, SkinBlood, and PhenoAge). In addition, the sum of perfluoromethylheptane sulfonate isomer was associated with acceleration of two other clocks, IEAA and Horvath.[10] Given these findings and previously reported occupational health risks, we hypothesized that years of firefighting would be associated with accelerated epigenetic age—a surrogate of worsened morbidity and mortality risk.

The results of our analyses examining relationships between incumbents (mean chronological age of 41.7 years, mean 14.5 years as a firefighter) compared with recruits (mean chronological age of 29.1 years, mean 0.3 years as a firefighter) agreed with our hypothesis for the SkinBloodClock biomarker. Incumbents, who have greater cumulative occupational hazard exposures, had an EAA compared with recruits by an average of 1.16 years. Still, it is important to consider why we do not observe significant relationships with the other epigenetic age markers. Different epigenetic aging markers represent different aspects of biological aging, but also in the settings of different environmental exposures or disease processes, existing research has demonstrated that epigenetic aging markers have different sensitivities.[16,35] For example, the SkinBloodClock is primarily viewed as a predictor of chronological age.[15] Comparably, of all epigenetic age measures in our study sample, it has the strongest correlation with chronological age and second lowest margin of error, behind GrimAge, which incorporates chronological age in its predictions. Thus, although EAA accounts for chronological age, there could still be residual confounding by chronological age impacting our results. Even so, because the SkinBloodClock has been associated with all-cause mortality,[15] this finding merits further investigation.

Our results from analyses exploring relationships of years of service and epigenetic age were largely contrary to our original hypothesis. The strength of this association in incumbents was relatively small (0.05–0.08 years) and stronger in recruits, who have less cumulative occupational hazard exposures than incumbents. These findings may be influenced by the healthy worker effect (HWE), a phenomenon observed in studies of occupational exposure and disease where people who remain actively employed for longer periods have a consistent tendency to exhibit better health than the population at large.[36] This effect is difficult to characterize statistically due to the inherent absence of information for individuals who do not enter the workforce or whose employment status changes; however, the presence of the HWE in firefighting has been characterized in a cohort of German firefighters[37] and in studies of firefighters in the US.[38] It may occur when susceptible subpopulations or individuals experiencing adverse health effects leave the workforce (often these are individuals who may be highly exposed) or if a firefighter's job and task or years of service are associated with exposure levels over time.[39]

Other factors may contribute to the unexpected finding. Additional years of service as a firefighter is frequently associated with advancement in rank, which in turn is associated with different job functions on the fire ground that often result in a lower burden of occupational exposures. For example, a typical career track in large municipal and county fire departments is for a firefighter-EMT assigned to an engine or truck company to become a firefighter-paramedic assigned to a paramedic rescue unit, and/or an apparatus engineer or company officer on an engine or truck company. Both promoted positions are likely to have a lower burden of hazardous occupational exposures on fire incidents than the firefighter EMT, given that exterior responses to a fire have lower exposure than interior responses.[40] The reduction in epigenetic age with reduction in exposures has been reported elsewhere. A pilot investigation of the impact of smoking cessation on biological age found that smoking cessation was associated with a "significant improvement" in Hannum's and Horvath's aging clocks.[41] Furthermore, a recent review noted that preliminary evidence suggests that specific interventions such as changes in diet and exercise can slow or reverse aging clocks and that noninterventional studies have linked age deceleration to sleep quality, diet, and physical activity.[42] This information highlights the importance of conducting future longitudinal epigenetic age studies with more specific firefighter occupational exposure classification to determine whether additional years of service and a lower burden of hazardous exposures, along with the high level of physical exertion associated with firefighting, is associated with a slowing or reversing of aging clocks. In our study, we were unable to account for rank or lifestyle factors mentioned previously because of limitations of the data. Future research is needed to further refine analyses of epigenetic aging in firefighters that includes details on current and former rank and lifestyle factors associated with health status. This is particularly important in our sensitivity analyses that excluded BMI as a covariate. The results of our years of service analyses in incumbents and recruits respectively were largely unchanged. However, the results comparing EAA in recruits versus incumbents was no longer statistically significant in the sensitivity analysis. It is reassuring that the effect estimates were in the same direction and were of similar magnitude (with recruits being on average epigenetically younger), but the finding merits further investigation. Existing work suggests that higher BMIs may be misleading if muscle mass is not taken into context.[43] Hence in our cohort, higher BMI may not necessarily translate to adiposity. Still, future work with more health-related data including central adiposity measures will be necessary to fully understand this finding.

Our study has several strengths including the utilization of a broad panel of epigenetic aging markers to better understand the occupational risks of firefighters. Still, we have some limitations. First, there may be limits to the generalizability of our results given that most of the study subjects were White and male. Moreover, there may be some residual confounding in our study because participants came from two different studies, and we do not have consistent information on physical activity or lifestyle factors to include in our models. Technical and day-to-day variation may reduce precision and add noise to epigenetic clock data, yet this is not expected to bias results in a particular direction. Future studies should explore these occupational epigenetic age relationships in more diverse populations and longitudinally with data that better characterizes occupational health exposures and risks, which can vary by job type/rank.

In conclusion, our findings support the use of epigenetic age markers to measure the cumulative effects of exposures in firefighters. Additional studies, including longitudinal studies, will be important for better characterizing occupational exposure and epigenetic aging relationships in US firefighters.

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