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ORIGINAL ARTICLE

Circulating interleukin-37 declines with aging in healthy humans: relations to healthspan indicators and *IL37* gene SNPs

Vienna E. Brunt · Akpevweoghene P. Ikoba · Brian P. Ziemba · Dov B. Ballak · Alexander Hoischen · Charles A. Dinarello · Marissa A. Ehringer · Douglas R. Seals

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Abstract Aging is characterized by declines in physiological function that increase risk of age-associated diseases and limit healthspan, mediated in part by chronic low-grade inflammation. Interleukin (IL)-37 suppresses inflammation in pathophysiological states but has not been studied in the context of aging in otherwise healthy humans. Thus, we investigated associations between IL-37 and markers of healthspan in 271 young (18–39 years; $n=41$), middle-aged (40–64 years; $n=162$), and older (65 + years; $n=68$) adults free of overt clinical disease. After conducting a thorough validation of AdipoGen's IL-37 ELISA, we found that plasma IL-37 is lower in older adults (young: 339 ± 240, middle-aged: 345 ± 234; older: 258 ± 175 pg/mL; $P=0.048$), despite elevations in pro-inflammatory markers. As such, the ratios of circulating IL-37 to pro-inflammatory markers were considerably lower in older adults (e.g., IL-37 to C-reactive protein: young, 888 ± 918 vs. older, 337 ± 293; $P=0.02$), indicating impaired IL-37 responsiveness to a pro-inflammatory state with aging and consistent with the notion of immunosenescence. These ratios were related to multiple indicators of healthspan, including positively to cardiorespiratory fitness ($P<0.01$) and negatively to markers of adiposity, blood pressure, and blood glucose (all $P<0.05$). Lastly, we correlated single-nucleotide polymorphisms (SNPs) in the *IL37* and *ILR8* (the co-receptor for IL-37) genes and found that variants in *IL37* SNPs tended to be associated with blood pressure and adiposity ($P=0.08$–0.09) but did not explain inter-individual variability in circulating IL-37 concentrations

Vienna E. Brunt and Akpevweoghene P. Ikoba share co-first authorship.

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V. E. Brunt · A. P. Ikoba · B. P. Ziemba · D. B. Ballak · M. A. Ehringer · D. R. Seals
Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO, USA

V. E. Brunt (✉) · D. B. Ballak · C. A. Dinarello
Department of Medicine, University of Colorado Denver Anschutz Medical Campus, CO 80045 Aurora, USA
e-mail: vienna.brunt@cuanschutz.edu

A. Hoischen · C. A. Dinarello
Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands

A. Hoischen
Department of Human Genetics & Radboud Institute of Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands

M. A. Ehringer
Institute for Behavioral Genetics, University of Colorado Boulder, Boulder, CO, USA

across age ($P \geq 0.23$). Overall, our findings provide novel insights into a possible role of IL-37 in biological aging in humans.

Keywords IL37 · Single-nucleotide polymorphism · Anti-inflammatory · Cytokines · Immunosenescence

Introduction

Aging is characterized by declines in physiological function and increased risk of age-associated diseases [25, 34]. Collectively these changes limit human healthspan [26], defined as the period of life free of major impairments in function (disability, frailty), serious chronic diseases, and poor quality of life [56]. Markers of healthspan include cardiorespiratory fitness (aerobic exercise capacity), lean body mass, markers of adiposity, blood pressure, vascular function, and clinical blood markers such as plasma lipids, fasting glucose and insulin, and measures of insulin resistance.

One of the most remarkable and clinically important phenomena of physiological aging is the variability in function, disease risk, and healthspan among individuals of similar age. These observations have resulted in a growing interest in "biological" compared with "chronological" aging and the underlying mechanisms influencing one's biological age [11, 19, 55]. One mechanism believed to play a key role in influencing physiological aging and healthspan in humans is chronic low-grade inflammation or "inflammaging" [17, 51]. Inflammaging is characterized by increases in pro-inflammatory signaling and markers of systemic inflammation, including age-related increases in circulating concentrations of inflammatory cytokines [7]. However, our understanding of the signaling molecules and pathways linking inflammaging to physiological function and healthspan in humans is limited.

Interleukin (IL)-37 is an intriguing, but to date understudied, member of the IL-1 family originally characterized in 2010 [5, 39] that has been implicated in modulating inflammation in several pathophysiological states, including obesity, insulin resistance, and the metabolic syndrome, as well as in aging (e.g., [3, 4, 13]. Administration of recombinant IL-37 suppresses inflammation across a variety of pro-inflammatory states and reduces morbidity [2, 36, 39, 58]. However, genetic variants in *IL37* that interfere with protein function can result in impaired anti-inflammatory responses [27, 60] and are linked to increased risk of severe pro-inflammatory diseases [27, 68]. In old mice, we recently demonstrated that 10–14 days of administration of human recombinant IL-37 improved vascular function, whole-body glucose metabolism, and exercise tolerance [4]. Presently there is no information on circulating concentrations of IL-37, *IL37* gene variants, nor the potential influence of IL-37 on physiological function or other markers of healthspan with aging in healthy humans.

Accordingly, the overarching goal of the present study was to gain insight into the association between plasma levels of IL-37, *IL37* gene variants, and markers of healthspan with aging in adults free of overt clinical disorders and disabilities. First, because we have observed cross-reactivity of some first-generation IL-37 ELISAs with other IL-1 family members, we performed comprehensive experiments to validate the AdipoGen ELISA for measuring IL-37 concentrations in human plasma and serum; although this ELISA has been used in several prior studies, here we are the first to confirm its accuracy and specificity in an independent setting (i.e., besides the manufacturer's validation). Second, we studied a cohort of 271 young, middle-aged, and older adults, and found that plasma concentrations of IL-37 are, on average, reduced in older adults without clinical disease and are related to multiple physiological and clinical markers of healthspan. We also identified common coding single-nucleotide polymorphisms (SNPs) in the *IL37* and *ILR8* genes, a cell membrane co-receptor for IL-37 [40]. Although the 9 SNPs studied were not obviously associated with plasma concentrations of IL-37, certain common *IL37* haplotypes did tend to predict healthspan markers. Overall, we provide initial evidence that IL-37 concentrations decline in older age and are related to selective markers of healthspan in healthy humans.

Methods

All procedures involving human subjects were approved by the Institutional Review Board at the University of Colorado Boulder (protocol #'s B5062, B5074, B5081, B5082, B5094, B5097, B5114, B6006, B6060, B6209, B6310, and

B6398), and were conducted in accordance with the Declaration of Helsinki. All subjects had participated in previous clinical studies conducted in the Seals Laboratory [12, 21, 22, 24, 44, 45, 49, 53, 61, 62] and had given informed consent for biological and genetic samples to be stored and used for future investigations. Biological samples and phenotypic data used for the present study were collected under baseline conditions for cross-sectional and parallel-design studies ($n = 200$) or at the end of the placebo phase for crossover design studies ($n = 47$). There were no carry over effects reported for any intervention [21, 62]. All subjects were healthy (free of overt clinical disease) based on medical history and physical evaluation conducted by a physician. Subjects over 60 years of age underwent incremental treadmill testing with ECG and blood pressure to ensure the absence of overt heart disease. Inclusion criteria for the present study were as follows: (1) 18 + years of age, (2) having stored useable plasma and DNA samples, and (3) having previously collected deep physiological phenotyping measures. Previous clinical studies, however, had varying inclusion/exclusion criteria related to prescription medications, physical activity, baseline vascular function, and body mass index (BMI). Please see Supplemental Table S1 (medications) and Table 1 (physiological phenotypes) for in-depth characterization of our subject cohort.

Table 1 Healthspan markers

	Overall cohort	Young (18–39 years)	Middle-aged (40–64 years)	Older (65 + years)
VO_2max (mL/min/kg)	33 (32, 34)	43 (40, 47)	33 (32, 34)*	28 (26, 30)*†
Body composition				
Body mass index (kg/m^2)	25 (25, 26)	24 (23, 25)	26 (25, 26)*	25 (24,26)
Fat mass (kg)	23 (22, 25)	18 (14, 21)	24 (22, 26)*	25 (22, 28)*
Fat mass (% total mass)	29 (28, 31)	23 (20, 27)	30 (28, 31)*	32 (29, 34)*
Lean mass (kg)	50 (49,51)	54 (50, 57)	50 (48, 52)	47(45, 50)*
Lean mass (% total mass)	67 (66, 69)	73 (70, 77)	67 (66, 69)*	65 (62, 67)*
Vascular function markers				
Systolic blood pressure (mmHg)	122 (120, 124)	112 (109, 116)	122 (120, 125)*	127 (124,131)*†
Diastolic blood pressure (mmHg)	74 (73, 75)	66 (63, 69)	75 (74, 77)*	74 (72, 76)*
Brachial artery flow-mediated dilation (%Δ)	5.4 (5.0, 5.8)	7.8 (6.5, 9.2)	5.4 (5.0, 5.8)*	4.2 (3.7, 4.8)*†
Clinical blood-based markers				
Total cholesterol (mg/dL)	195 (190,199)	161 (152,170)	203 (198, 208)*	194 (186, 202)*
LDL cholesterol (mg/dL)	115 (112,119)	94 (87, 102)	122 (117, 127)*	112 (105, 119)*
HDL cholesterol (mg/dL)	56 (54, 58)	48 (45, 51)	56 (54, 59)*	61 (56, 66)*
Triglycerides (mg/dL)	108 (101, 115)	94 (79, 109)	115 (105, 124)	101 (90,111)
Glucose (mg/dL)	88 (87, 89)	87 (84, 90)	87 (86, 89)	90 (88, 93)
Insulin (mg/dL)	8 (7,8)	7 (6, 9)	8 (7,8)	8 (6, 9)
HOMA-IR	1.6 (1.5,1.8)	1.6 (1.2, 2.0)	1.6 (1.5, 1.8)	1.7 (1.5, 2.0)
Circulating inflammatory cytokines				
WBC (/L)	5.2 (5.0,5.4)	5.6 (5.1, 6.1)	5.1 (4.9,5.3)	5.2 (4.9, 5.5)
IL-6 (pg/mL)	1.2 (1.2, 1.3)	0.9 (0.8, 1.1)	1.2 (1.1, 1.4)	1.4 (1.2, 1.6)*
C-reactive protein (mg/L)	0.9 (0.8, 1.0)	0.7 (0.5, 0.9)	0.8 (0.7, 0.9)	1.2 (1.0, 1.5)*†
TNFα (pg/mL)	1.3 (1.2, 1.4)	1.1 (0.9, 1.2)	1.4 (1.2, 1.5)	1.3 (1.1, 1.5)

Data are mean with 95% confidence interval

Abbreviations: *LDL* low-density lipoprotein; *HDL* high-density lipoprotein; *HOMA-IR* homeostatic model assessment for insulin resistance; *WBC* white blood cell count; *IL-6* interleukin-6; *TNF*α tumor necrosis factor-alpha

*$P < 0.05$ vs. young adults

†$P < 0.05$ vs. middle-aged adults

Deep physiological phenotyping of human subjects

Cardiorespiratory fitness (VO_2max) was measured during incremental treadmill testing using open-circuit spirometry, on a separate day from screening or vascular function assessments. Body mass index was calculated from body mass and height, which were determined using anthropometry. Body fat and lean mass were determined by dual-energy X-ray absorptiometry.

Blood pressure and vascular function were measured, and venous blood was collected, following an 8–12-h overnight fast, > 20 h without alcohol, caffeine, or vigorous physical activity, and > 48 h without dietary supplements or over-the-counter medications. Subjects refrained from taking prescription medications the morning of the study. Arterial blood pressure was measured in triplicate over the brachial artery (arm supported at heart level) following at least 5 min of seated rest using a validated semi-automated device. Vascular endothelial function was assessed by brachial artery flow-mediated dilation (FMD_{BA}), in accordance with established guidelines [9]. FMD_{BA} is expressed as a percent change in diameter from baseline. Venous blood was collected for the following: (1) analysis of lipid, lipoprotein, and blood glucose concentrations and complete blood count in a Clinical Laboratory Improvement Amendments-certified laboratory (Boulder Community Hospital Clinical Laboratory, Boulder, CO); (2) analysis of inflammatory markers IL-6, tumor necrosis factor (TNF)α, and C-reactive protein (CRP) by commercial ELISA by the University of Colorado Anschutz Medical Campus Clinical Translational Research Center Core Laboratory; (3) analysis of blood insulin by commercial ELISA (Mercodia, Uppsala, Sweden); and (4) isolation of DNA (see below). Additional samples of EDTA plasma and serum were stored at − 80 °C for use in future investigations (see the "Circulating IL-37 concentrations" section below). In a subset of subjects, venous blood was also collected for isolation of peripheral blood mononuclear cells (PBMCs) by centrifugation with Histopaque-1077 (Sigma-Aldrich, Corp., St. Louis, MO).

Circulating IL-37 concentrations

Concentrations of IL-37 in buffer, EDTA plasma, serum, and PBMC lysates were measured according to manufacturer's instructions using a commercially available ELISA (AdipoGen, San Diego, CA).

AdipoGen ELISA validation To assess accuracy and specificity, IL-37 concentrations were determined in the provided assay buffer, first, with a range of concentrations of human recombinant IL-37 protein added (0.0–1.8 ng/mL), second with human recombinant IL-1 receptor antagonist (IL-1ra) alone (100–5000 pg/mL), and third with a range of human recombinant IL-37 protein (0.65–1.30 ng/mL) + either 0, 700, or 1200 pg/mL of IL-1ra added. For these experiments, we used a recombinant human IL-37 construct composed of amino acid residues 46–218 that was expressed in *E. coli* and purified to homogeneity [29]. IL-1ra was selected to assess assay specificity, as this protein exhibits similar tertiary structure and chemical conservation at the purported receptor-binding interface (reference structures PDBID 1LIT and 5HN1—all atom RMSD of 0.701 Å). To assess reproducibility, IL-37 concentrations were measured in plasma samples from 10 healthy subjects on two separate days. In these same 10 subjects, IL-37 concentrations were measured in PBMC lysates, lysed in RIPA buffer by sonication and diluted in the provided assay buffer to normalize for total protein content (measured by BCA assay), and in serum (to determine if IL-37 can be measured in plasma and serum interchangeably). Subjects used for these comparisons were selected as the most recently studied subjects with useable stored aliquots of all three sample types.

Main cohort Once validated, the AdipoGen IL-37 ELISA was used to measure circulating IL-37 in EDTA plasma in our full cohort of 271 young, middle-aged, and older adults.

DNA isolation

Venous whole blood samples were stored at 4 °C for no more than 1 week after collection from human subjects prior to DNA isolation. High molecular weight DNA was purified from whole blood using a commercially available kit (Gentra Puregene Kit for whole blood; Qiagen, Hilden, Germany) according to manufacturer's instructions. DNA samples were flash frozen and stored at − 80 °C until sequencing.

SNP sequencing and genotyping

The coding exons of *IL37* and *ILR8* were sequenced using targeted molecular inversion probe (MIP) resequencing, as previously described [27, 60]. Raw sequencing data were produced by the Illumina Next-Seq 500 system and aligned to the hg19 reference genome, after which variant genotypes were determined. Variants were observed in 15 SNPs. For each SNP, the minor allele was identified as that expressed in the lowest number of subjects in our cohort.

In total, 269 DNA samples were available for sequencing, with 248 samples passing quality control; 9 samples were excluded as they did not have sufficient DNA to process for MIPs, and 12 samples were excluded as X-chromosome coverage over autosomes did not match subject-reported sex (i.e., suspected sample swap). Based on the number of samples with sex mismatch, the error rate of our genotyping is estimated to be 4.6%. Position-based coverage depth (i.e., DNA quality) was calculated for all samples. There were 6 individuals for *IL37* and 66 individuals for *ILR8* with position-based coverage depth < 100× for at least one of the selected coding exon regions for these genes. However, we chose to still include these individuals in subsequent analyses, as there was high correlation between the number of minor alleles possessed across certain SNPs (see linkage disequilibrium analyses described below) and individuals with low position-based coverage matched the patterns observed in the overall group.

The frequencies of minor alleles for each SNP were compared to published frequencies in dbSNP for cohorts with similar ethnic backgrounds to our subjects, i.e., majority European descent. For all subsequent analyses, we focused on *common* coding variants (minor allele fraction > 1%). We identified 1 rare coding variant in *IL37* that was non-synonymous and a frameshift. We have reported genotypes for this SNP but, as it was only observed in 1 individual, we did not include it in any other analyses. Although 5 rare coding variants were observed in *ILR8*, we have not reported these due to the lower position-based coverage of this gene.

Statistical analyses

The majority of statistical analyses were conducted in R (version 3.6.3; The R Foundation, Vienna, Austria). Some of the simpler analyses (*t*-tests, ANOVAs, simple linear regressions) were conducted in Prism (version 9.2.0; GraphPad Software, LLC, La Jolla, CA). Unless noted otherwise, statistical significance was set to $\alpha = 0.05$.

Validation of the AdipoGen IL-37 ELISA Relations between different methods of determining IL-37 concentrations (e.g., actual vs. measured; Fig. 1, panels A-B, E) were determined using simple linear regression. Concentrations of IL-37 in matched human plasma vs. PBMC lysates were compared using Student's paired *t*-test. The effect of adding IL-1ra to varying concentrations of IL-37 was assessed using 2-way repeated measures ANOVA (IL-37 × IL-1ra concentration).

Circulating IL-37 and healthspan markers across age groups Plasma concentrations of IL-37, IL-6, TNFα, and CRP were first assessed for outliers (ROUT; $Q = 0.1–1.0\%$ as specified in the "Results" section) and outliers were removed prior to all subsequent analyses. Data were assessed for normality using the Shapiro–Wilk test. Clinical and physiological healthspan markers, inflammatory markers, both non-normalized and log-transformed plasma concentrations of IL-37, and the ratios of IL-37 to both IL-6 and CRP were compared across young (18–39 years), middle-aged (40–64 years), and older (65 + years) adults by one-way ANOVA, with Tukey's post hoc test when significant main effects were observed. Relations between age and the ratios of IL-37 to both IL-6 and CRP were assessed using linear regression, both unadjusted and adjusted for sex and race/ethnicity.

Circulating IL-37 vs. healthspan markers Relations between all healthspan markers and IL-37 or the ratios of IL-37 to IL-6 or CRP were assessed using linear regression, both unadjusted and adjusted for age, sex, and race/ethnicity. Pearson correlation coefficients were estimated for all comparisons. Given the relatively low sample sizes once outliers were removed, alpha values were not corrected for multiple comparisons.

SNP analyses Hardy Weinberg equilibriums were calculated for each SNP using the genetics package in R. Linkage disequilibrium (LD) between all SNPs

in each gene were also calculated using the genetics package in R. As the minor alleles of rs3811046 and rs3811047 were observed in near-perfect LD, while the minor alleles of rs2708943, rs2723183, rs2723187, rs2708947, and rs2723192 were observed in a separate nearly perfect LD block, we used rs3811046 to represent haplotype 1 (HAP1) in the *IL37* gene and rs2723192 to represent HAP2 in the *IL37* gene for all subsequent analyses. Rs7947 was used to represent the *ILR8* gene HAP, because the minor alleles of the two *ILR8* gene SNPs were also observed in nearly perfect LD. For all HAPs, data are presented as having 0, 1, or 2 copies of the minor allele.

Associations between *IL37* and *ILR8* gene HAPs (0, 1, or 2 copies of the minor alleles) and plasma IL-37 concentrations were determined using linear regression, with adjustment for age, sex, and race/ethnicity.

Associations between *IL37* and *ILR8* gene HAPs (0, 1, or 2 copies of the minor alleles) and healthspan markers were determined as follows. Because there was a total of 20 phenotypic measures (i.e., healthspan markers) collected, many of which show some degree of correlation, we reduced complexity of the data and the need for multiple testing correction by using principal component analysis followed by an exploratory factor analysis to identify common underlying factors. First, missing values for all phenotypic measures were imputed using the multivariate imputation by chained equations (MICE) package in R [59]. With the exception of inflammatory markers, *Z*-scores were then calculated for all phenotypic measures by standardizing values to a normal curve (mean = 0, standard deviation = 1) and inflammatory markers (plasma IL-6, CRP, TNFα, and IL-37) and were instead log-transformed to normalize for their skewed distribution. Factor loadings were applied to raw phenotypic scores to generate overall factor scores for each subject. The top 3 factors were selected for use in linear regression analysis based on principal component analysis, specifically by examining the scree plot, and confirmed by exploratory factor analysis. Then, multiple linear regression analysis was used to test for associations between the top 3 factor scores and the three genotypic HAPs, adjusted for age, sex, and race/ethnicity. To account for multiple testing, the significance level was set as $P < 0.00555$ (0.05/9, as 9 statistical tests were run across 3 phenotypic factors × 3 HAPs).

Results

Validation of AdipoGen ELISA for measuring IL-37 concentrations in serum and plasma

A major limitation of investigations of IL-37 to date has been the lack of a sufficiently specific assay for quantifying circulating concentrations of IL-37. In addition, there have been concerns that some assays may detect IL-37 consistently in serum but not in plasma. The AdipoGen ELISA has been most widely used to measure IL-37 concentrations [23, 57, 69, 70], but to our knowledge, has not yet been thoroughly validated in an independent setting. As such, we conducted experiments to validate the accuracy and specificity of the AdipoGen IL-37 ELISA, as well as whether it can be used reliably in human plasma samples.

First, to confirm accuracy of this ELISA for detecting IL-37, we tested concentrations of human recombinant IL-37 protein ranging from 0.0 to 1.8 ng/mL diluted in the provided assay buffer and observed a near-perfect correlation between the known and measured concentrations of IL-37 ($r^2 = 0.97$, $P = 0.0004$; Fig. 1A).

To confirm reproducibility, we measured IL-37 in plasma samples (collected with EDTA) from 10 healthy subjects (aged 19–71 years; mean ± SD: 54 ± 18 years) on two separate days, with two separate kits (separated by months with samples returned to − 80 °C storage in between). We observed that measured IL-37 on each day was almost perfectly reproducible ($r^2 = 0.98$, $P < 0.0001$; Fig. 1B). We additionally measured IL-37 concentrations in peripheral blood mononuclear cell (PBMC) lysates from these same 10 subjects, as both plasma and PBMCs have been commonly used to determine IL-37 levels [23, 57, 69, 70], but to our knowledge, IL-37 protein levels in these two tissues have never been directly compared. We found that IL-37 concentrations in our subjects were higher in plasma than in PBMC lysates ($P = 0.005$,Fig. 1C).

Next, we addressed the possibility of the ELISA detecting other interleukins or related proteins using

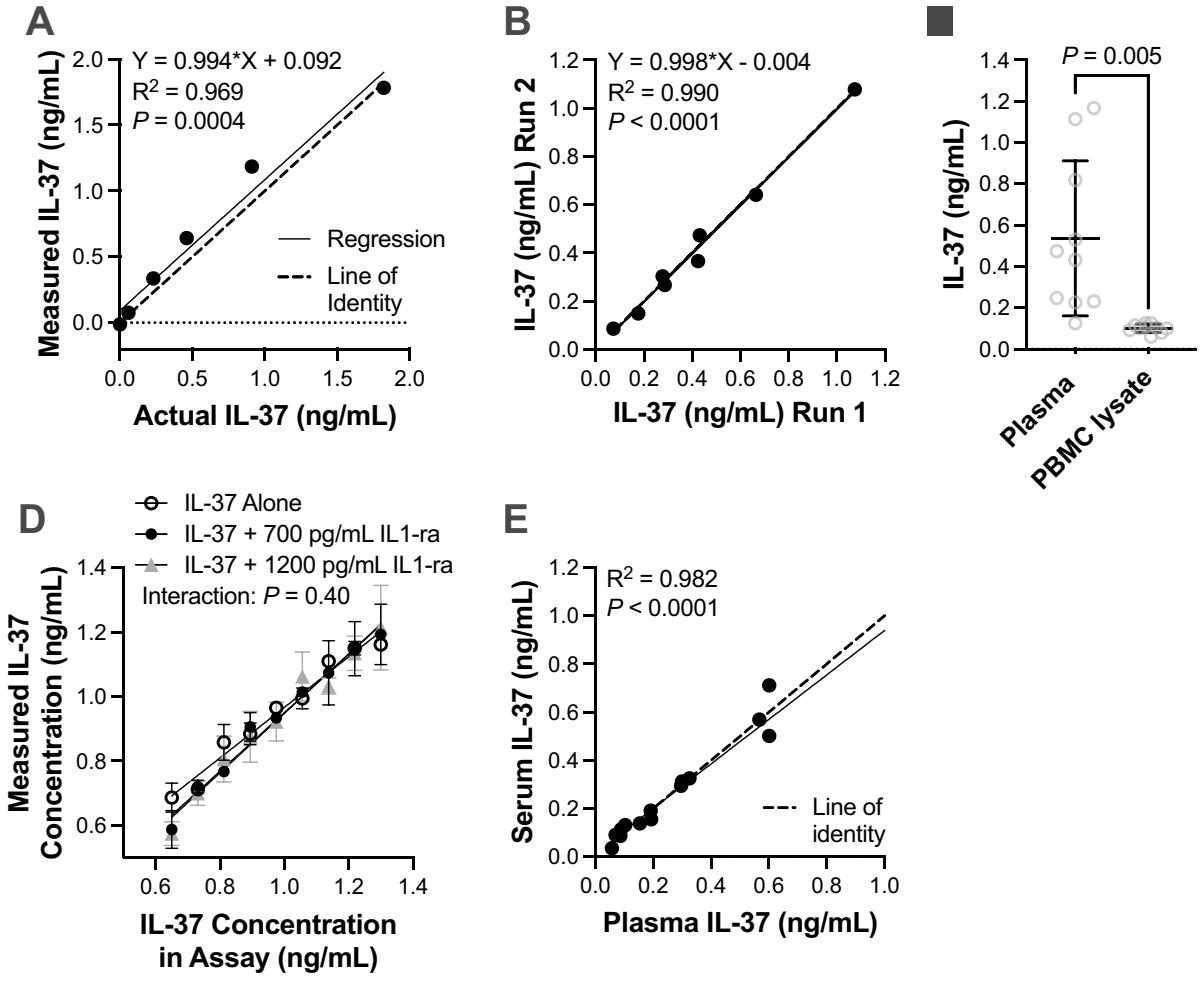

Fig. 1 Validation of the AdipoGen interleukin (IL)-37 ELISA. **A** Measured vs. actual concentrations of human IL-37 recombinant protein were very similar. **B** IL-37 measured in human plasma is reproducible across days and assay kits ($n=8$). **C** Concentrations of IL-37 were higher in plasma than in peripheral blood mononuclear cell (PBMC) lysates from the same subjects ($n=10$; data are mean ± SD with individual data). **D** Adding IL-1 receptor antagonist (IL-1ra) to assay buffer across a range of IL-37 concentrations did not affect IL-37 measured by the ELISA, confirming specificity for IL-37 ($n=9$/condition). **E** IL-37 concentrations were similar between plasma and serum from the same subjects ($n=17$)

IL-1ra, which is among the more similar proteins to IL-37 in terms of structural and chemical homology [5] and is a protein we had observed cross-reaction to with earlier commercially available IL-37 ELISAs. We tested binding specificity of the ELISA for detecting IL-37, first, by testing IL-1ra protein alone, in concentrations ranging from 100 to 5000 pg/mL diluted in the provided assay buffer and did not observe any nonspecific signal above the background measurement of the assay buffer alone (data not shown). We next tested 700 and 1200 pg/mL of IL-1ra (700 pg/mL of IL-1ra is representative of that typically measured in human plasma) [20] added to a range of IL-37 concentrations from 0.65 to 1.30 ng/mL and observed no effect of IL-1ra at any concentration of IL-37 (IL-37 × IL-1ra interaction: $P=0.40$,Fig. 1D), confirming specificity to IL-37 of the AdipoGen ELISA.

Lastly, to determine if the AdipoGen ELISA can be used reliably in either serum or plasma, at least in healthy subjects, we compared IL-37 concentrations in matched plasma and serum samples from 17 healthy adult subjects (aged 21–68; mean ± SD: 53 ± 13 years). IL-37 concentrations were nearly

identical between plasma and serum using this assay ($R^2 > 0.98$, $P < 0.0001$; Fig. 1E).

Overall, our data indicate that the AdipoGen IL-37 ELISA is accurate, reproducible, specific for IL-37, and can be used interchangeably with either plasma or serum from human subjects.

Characteristics and healthspan markers in main human cohort

Various clinical and physiological healthspan markers were measured in a cohort of 271 young (18–39 years; $n = 41$), middle-aged (40–64 years; $n = 162$), and older (65 + years; $n = 68$) healthy adults. Subject characteristics and prescription medications are provided in Supplemental Table S1. Roughly equal numbers of male and female subjects were studied across all age groups. The types and number of subjects taking each type of prescription medication were typical for healthy young, middle-aged, and older adults (subjects abstained from all medications the morning of vascular testing and blood draws). Subjects of all racial and ethnic backgrounds were included, and the distribution of races/ethnicities was representative of the Boulder/Denver metropolitan area (Supplemental Table S1).

Clinical and physiological healthspan markers are provided in Table 1. As expected, we observed a stepwise reduction in cardiorespiratory fitness across young, middle-aged, and older adults. Both middle-aged and older adults had higher fat mass, serum cholesterol (total, LDL and HDL), and diastolic blood pressure than young adults (all $P < 0.02$ vs. young), but there were no significant differences in these markers between the two older groups (all $P > 0.11$). Consistent with previous studies from our laboratory [6, 15, 22], we observed a stepwise increase in systolic blood pressure and a stepwise reduction in brachial artery FMD, an established measure of endothelium-dependent dilation (vascular endothelial function), across young, middle-aged, and older adults (main effect for both, $P < 0.001$). There were no significant differences in fasted serum triglycerides, glucose, insulin, or the HOMA measure of insulin resistance (ANOVA main effect: all $P > 0.05$) across these 3 age groups of healthy adults.

Relative to young adults, two circulating markers of inflammation, IL-6 and C-reactive protein, were significantly higher in the older compared with the young adults (both $P < 0.004$). In middle-aged adults, IL-6 also tended to be higher than in young adults ($P = 0.06$), whereas CRP did not differ ($P = 0.77$). Circulating levels of TNFα also tended to increase with age (main effect: $P = 0.13$). There were no differences in circulating white blood cell counts among age groups (main effect: $P = 20$).

Overall, in general, our cohort demonstrated the expected group differences in established markers of healthspan with aging.

Circulating concentrations of IL-37 with aging

After validating the AdipoGen IL-37 ELISA, we measured plasma IL-37 concentrations in our cohort of young, middle-aged, and older adults (Fig. 2A). Using non-transformed data, IL-37 concentrations did not differ between young and middle-aged adults but were significantly lower in older adults ($P = 0.048$ vs. MA). However, when plasma IL-37 concentrations were log-transformed, this difference was no longer statistically significant at $P < 0.05$, although a distinct trend remained (MA vs. O: $P = 0.10$; Shapiro–Wilk test of non-transformed data: $P < 0.001$). Because IL-37 levels are reported to increase in response to pro-inflammatory signaling [13, 46, we also calculated ratios of plasma IL-37 to two key pro-inflammatory markers, circulating C-reactive protein and IL-6. On average, the ratios of IL-37 to C-reactive protein (Fig. 2B) and IL-6 (Fig. 2C) were only 38% and 49%, respectively, of that in young adults (both $P < 0.05$), indicating that older adults, at least chronically, do not mount an appropriate plasma IL-37 response to combat age-related increases in circulating pro-inflammatory markers. Importantly, the inverse relations between age and the ratios of IL-37 to C-reactive protein ($R = -0.18$, $P < 0.05$) and IL-6 ($R = -0.20$, $P < 0.05$) remained statistically significant even when adjusted for sex and race/ethnicity.

Relations between circulating IL-37 concentrations and healthspan markers

To investigate whether circulating IL-37 influences physiological function and healthspan, we calculated Pearson correlation coefficients between IL-37 and each of the markers in Table 2. Plasma IL-37 was not significantly correlated with any of these markers, neither when regressions were unadjusted nor

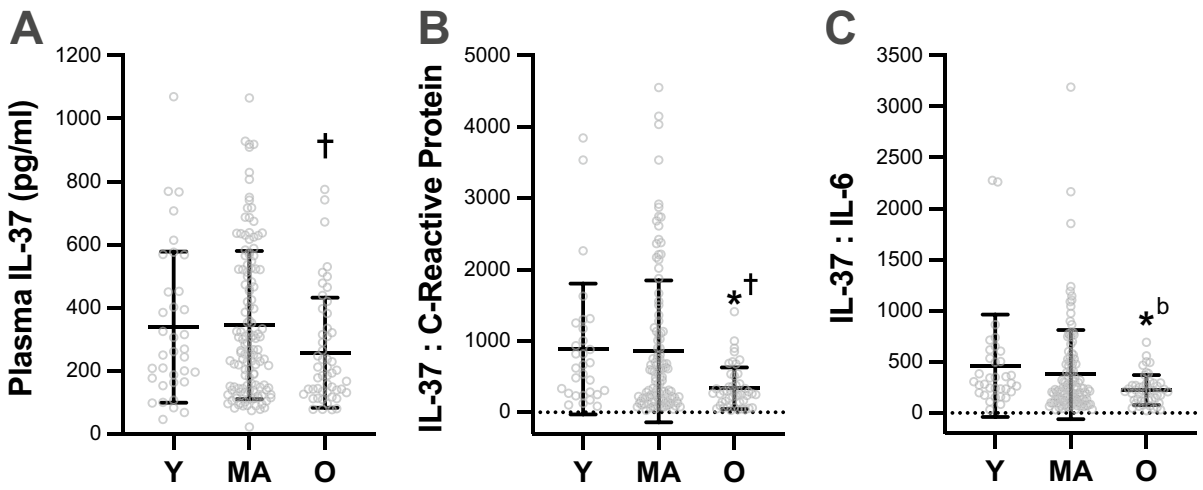

Fig. 2 Circulating concentrations of interleukin (IL)-37 are reduced with aging and insufficient to balance age-related increases in pro-inflammatory markers. Plasma IL-37 (**A**) and the ratios of IL-37 to pro-inflammatory serum C-reactive protein (**B**) and IL-6 (**C**) in young (Y; 18–39 years; $n = 34–38$), middle-aged (MA; 40–64 years; $n = 130–145$), and older (O; 65 + years; $n = 51–61$) adults. Outliers for each marker were removed using ROUT ($Q = 0.1\%$); subjects included in ratios if data for both markers remained after outlier removal. Graphs are mean ± SD with individual data. *$P < 0.05$ vs. young adults. †$P < 0.05$ vs. middle-aged adults. $^{b}P < 0.10$ vs. middle-aged adults (one-way ANOVA with Tukey's post hoc test)

Table 2 *IL37* and *ILR8* genotype frequencies and Hardy Weinberg equilibrium (HWE) *P* values

Gene	SNP ID	Genotype frequency			HWE *P* value
IL37	rs3811046	T/T: 124 (50%)	T/G: 102 (41%)	G/G: 21 (8%)	1
	rs3811047	G/G: 124 (50%)	G/A: 102 (41%)	A/A: 21 (8%)	1
	rs2708943	C/C: 201 (81%)	C/G: 43 (17%)	G/G: 3 (1%)	0.72
	rs2723183	A/A: 201 (81%)	A/G: 43 (17%)	G/G: 3 (1%)	0.72
	rs2723187	C/C: 204 (83%)	C/T: 40 (16%)	T/T: 3 (1%)	0.45
	rs2708947	T/T: 199 (81%)	T/C: 45 (18%)	C/C: 3 (1%)	0.73
	rs2723192	G/G: 200 (81%)	G/A: 44 (18%)	A/A: 3 (1%)	0.72
	rs758200167	CC: 246 (> 99%)	C*: 1 (0.4%)		N/A
ILR8	rs7947	A/A: 139 (56%)	A/G: 94 (38%)	G/G: 14 (6%)	0.86
	rs3210908	C/C: 137 (55%)	C/T: 99 (40%)	T/T: 11 (4%)	0.23

Data for genotype frequency are genotype, *N* (%). Genotypes are presented in order of having 0, 1, or 2 copies of the minor allele

controlled for age, sex, and race/ethnicity (Supplemental Table S2).

As the ratio of anti-inflammatory to pro-inflammatory signaling may have a greater influence on physiology than one aspect of this signaling alone, we also calculated Pearson correlation coefficients between the ratios of IL-37 to C-reactive protein and IL-6 and each of the healthspan markers. Higher ratios of IL-37 to pro-inflammatory markers were favorably associated with several healthspan markers; significant relations from unadjusted correlations are presented in Fig. 3. The ratio of IL-37 to C-reactive protein was positively correlated with cardiorespiratory fitness (VO_2max) and % lean mass, and inversely correlated with body mass, multiple markers of adiposity (BMI, % fat mass), and white blood cell counts. Each of these markers was also significantly correlated with C-reactive protein alone.

The ratio of IL-37 to IL-6 was positively correlated with cardiorespiratory fitness and % lean mass, and inversely correlated with markers of greater adiposity (body mass, BMI, % fat mass), systolic and mean arterial blood pressure, and fasting blood glucose. Diastolic blood pressure and blood glucose were not

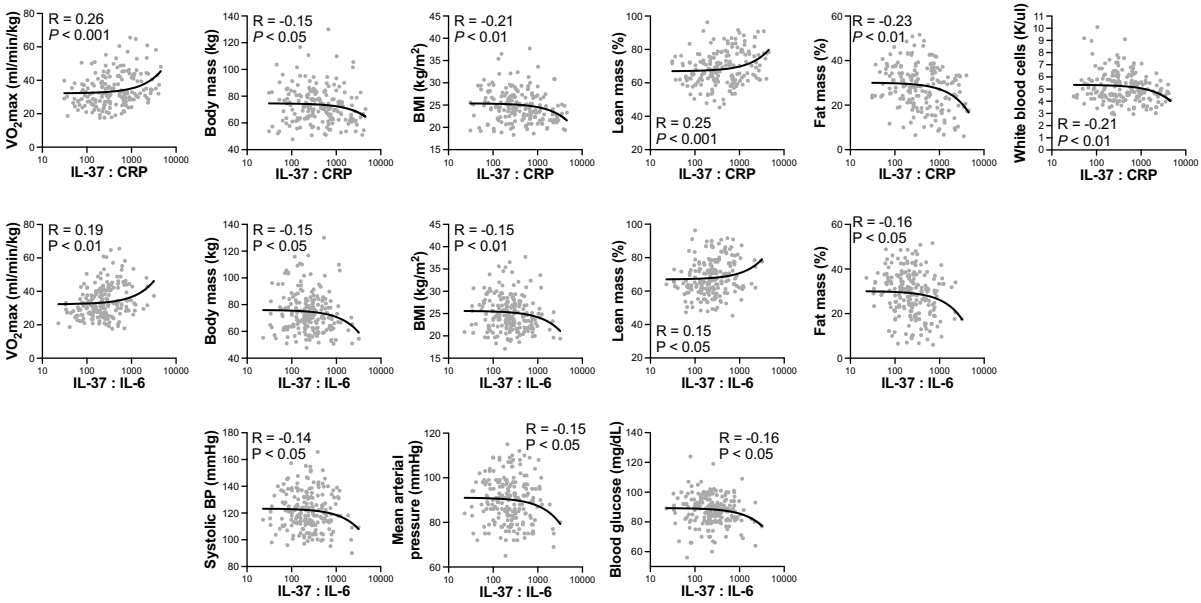

Fig. 3 Significant correlations between healthspan markers and the ratios of plasma interleukin (IL)-37 and C-reactive protein (CRP) or IL-6 in young (18–39 years; $n = 34–38$), middle-aged (40–64 years; $n = 130–145$), and older (65 + years; $n = 51–61$) adults. Regression coefficients and P values shown are the results of unadjusted Pearson correlations. Only statistically significant correlations ($P < 0.05$) are shown

significantly correlated with IL-6 alone ($P = 0.06$ and 0.73, respectively), suggesting that the ratio of anti-inflammatory to pro-inflammatory signaling specifically (vs. pro-inflammatory signaling alone) may be an important influencer of at least some aspects of changes in physiological function and healthspan status with advancing age. Correlations were also adjusted for age, sex, and race/ethnicity, with full results provided in Supplemental Table S2. Ratios of IL-37 to TNFα were not calculated due to missing data (insufficient number of subjects with both cytokines measured).

Genotypes of SNPs in *IL37* and *ILR8*

Despite observing significant reductions in plasma IL-37 concentrations (both absolute and normalized to pro-inflammatory markers) and declines in select healthspan markers with aging, there was significant variation in each of these outcomes within age groups, consistent with the notion of successful vs. non-successful aging [28]. We next sought to determine if SNP genotypes in *IL37* and *ILR8* (co-receptor for IL-37) may account for the following: (a) variation in plasma IL-37 concentrations,and (b) variation in healthspan markers, with aging. Genotypes of the following common coding SNPs were determined in DNA isolated from whole blood in 38 young, 153 middle-aged, and 56 older adults (total $n = 247$): rs3811046, rs3811047, rs2708943, rs2723183, rs2723187, rs2708947, rs2723192, rs7947, and rs3210908. All common coding SNPs used were non-synonymous, i.e., they alter the amino acid sequence of the protein. We additionally identified 1 rare coding *IL37* variant that was non-synonymous and a frameshift (C deletion): rs758200167. However, this rare variant was only observed in 1 subject and there was nothing remarkable about this subject, i.e., all phenotypic data fell within normal ranges for their age.

The frequency of each *IL37* and *ILR8* genotype in our cohort of healthy adults and Hardy Weinberg equilibrium P values are provided in Table 2. For common SNPs, the frequency of subjects possessing the minor allele ranged from 16 to 41% for 1 copy and from 1 to 8% for 2 copies, which was comparable to published frequencies on dbSNP for individuals of European descent (specifically, the 1000 genomes project). The distribution of common *IL37* and *ILR8* genotypes were in accordance

with the Hardy Weinberg equilibrium, i.e., they were consistent with stable genotype frequencies across generations.

To identify non-random associations between SNPs, we calculated linkage disequilibrium (LD) between SNPs using the genetics package in R (Supplemental Figure S1). We found that the minor alleles of *IL37* SNPs rs3811046 and rs3811047 occurred in near-perfect LD (i.e., the same genotype at rs3811046 could nearly perfectly predict the genotype at rs3811047). The minor alleles of *IL37* SNPs rs2723192, rs2708943, rs2723183, rs2723187, and rs2708947 also occurred in a separate nearly perfect LD block. Therefore, in all further analyses, we used the number of copies of the minor allele for rs3811046 to represent haplotype 1 (HAP1) in *IL37* and the number of copies of the minor allele for rs2723192 to represent haplotype 2 (HAP2) in *IL37*. The minor alleles of *ILR8* gene SNPs also occurred in near-perfect LD. For further analyses, the number of copies of the minor allele for rs7947 was used to represent the *ILR8* HAP.

Relations between *IL37* and *ILR8* haplotypes and circulating IL-37 concentrations

We next sought to determine if *IL37* and/or *ILR8* haplotypes might account for differences in circulating IL-37 protein levels in healthy adults differing in age. Plasma IL-37 was log-transformed to account for skewness. First, we compared plasma IL-37 concentrations across the number of copies of the minor allele (0–2) in *IL37* HAP1 and HAP2, and the *ILR8* HAP. There were no significant differences in plasma IL-37 concentrations across the number of copies in *IL37* HAP1 (ANOVA main effect: $p=0.88$; Fig. 4A). The average concentration of IL-37 protein was higher in individuals with 2 minor allele copies of *IL37* HAP2; however, this did not reach statistical significance due to the low number of subjects with this genotype (only 3) (ANOVA main effect: $p=0.60$; Fig. 4B). There was a trend toward individuals with 1 minor allele copy of the *ILR8* HAP having lower plasma IL-37 protein concentrations (0 vs. 1 copy: $p=0.13$; Fig. 4C).

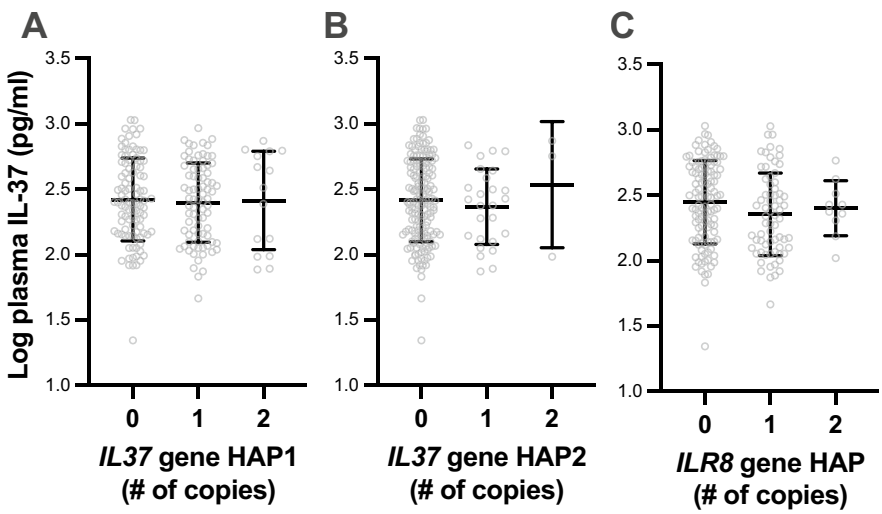

Fig. 4 Genotypes for SNPs in *IL37* and *ILR8* do not predict plasma concentrations of IL-37 protein. Plasma IL-37 protein concentrations in individuals with 0, 1, or 2 copies of the minor allele in **A** HAP1 in the *IL37* gene (rs3811046; minor allele: G); **B** HAP2 in the *IL37* gene (rs2723192; minor allele: C); and **C** the *ILR8* HAP (rs7947; minor allele: G). Plasma IL-37 protein concentrations are log-transformed to account for skewness (outliers excluded; ROUT: $Q=1\%$). Graphs are mean ± SD with individual data. The final numbers of subjects included in this analysis with 0, 1, and 2 copies, respectively, of each haplotype are as follows: $n=91$, 81, and 15 for HAP1 in the *IL37* gene; $n=166$, 26, and 3 for HAP2 in the *IL37* gene; and $n=116$, 69, and 10 for the *ILR8* HAP

In addition, we performed multiple linear regression between *IL37* or *ILR8* HAPs and circulating IL-37 protein concentrations, adjusting for age, sex, and race/ethnicity. These analyses did not show any significant relations between plasma IL-37 levels and *IL37* and *ILR8* HAPs ($P \geq 0.23$; Supplemental Table S3).

Overall, although it is possible that we would see differences in a larger cohort, our data indicate that variant genotypes in *IL37* and *ILR8* do not obviously explain the variability in circulating IL-37 protein concentrations observed.

Relation between genotypes for SNPs in *IL37* and *ILR8* and healthspan markers

To determine how *IL37* and *ILR8* haplotypes may be related to physiological function, we conducted a principal component analysis (PCA) followed by an exploratory factor analysis (EFA) in the cohort of healthy adults ($n = 247$). Any missing values for phenotypic measures were imputed using the multivariate imputation by chained equations (MICE) package in R. The PCA analysis revealed support for the presence of three distinct factors. This was confirmed using an exploratory factor analysis, which yielded a three-factor structure. Supplemental Table S4 shows the loadings of individual items onto each of the three factors. Fat mass (%), lean mass (%), BMI, and VO_2max were the primary items that loaded on factor 1 ("adiposity and fitness"; a higher factor 1 score indicates greater adiposity and lower cardiorespiratory fitness). Systolic, diastolic, and mean arterial blood pressure strongly loaded on factor 2 ("blood pressure"; a higher factor 2 score indicates higher blood pressure). Diastolic blood pressure, lean mass (%), and fat mass (%) were the strongest items loading on factor 3 ("blood pressure and adiposity"; a higher factor 3 score indicates higher blood pressure and greater adiposity).

Factor scores were then generated for each subject based on the item loadings and used in a standard linear regression analysis. Multiple linear regression models were used to determine the extent to which the three factors were related to the *IL37* and *ILR8* haplotypes (number of copies of the minor allele), with adjustment for age, sex, and race/ethnicity (see Supplemental Tables S5 and S6 for full results). Sex significantly influenced factor score 1 (positively, i.e., women on average had higher adiposity and lower VO_2max) and factor score 2 (inversely, i.e., women on average had lower blood pressure) in regression models for both genes.

When controlling for multiple comparisons, there were no significant correlations between any of these factors and the *IL37* and *ILR8* HAPs. However, *IL37* HAP1 showed a trend toward an inverse association with factor 3, "the blood pressure and adiposity" factor ($P = 0.08$; Fig. 5A), suggesting that individuals with 1 or 2 copies of this haplotype's minor alleles may be better protected against increased diastolic blood pressure and/or adiposity. *IL37* HAP2 tended to be positively associated with factor 2, which strongly loaded systolic, diastolic, and mean arterial blood pressures ($P = 0.09$; Fig. 5B), suggesting that individuals with 1 or 2 copies of this minor haplotype may be predisposed to higher blood pressures. The *ILR8* gene HAP was not associated with any of the healthspan factors (all $P > 0.17$). All non-trending relations are presented in Supplemental Figure S2.

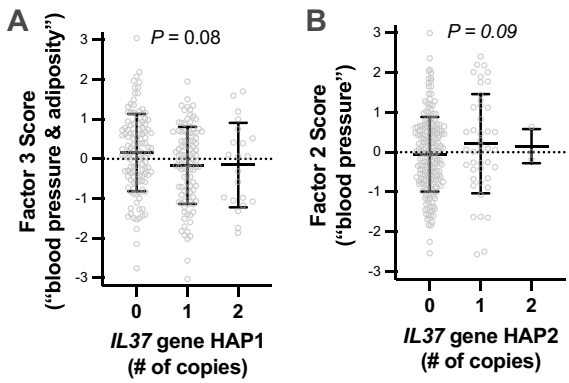

Fig. 5 Associations between factors for healthspan markers and haplotypes in *IL37* and *ILR8*. **A** *IL37* HAP1 tended to be inversely associated with factor 3, which loaded diastolic blood pressure, lean mass (%), and fat mass (%). **B** *IL37* HAP2 tended to be positively associated with factor 2, which strongly loaded systolic, diastolic, and mean arterial blood pressure. Graphs are mean ± SD with individual data points. The final numbers of subjects included in this analysis with 0, 1, and 2 copies, respectively, of each haplotype are as follows: $n = 124$, 102, and 21 for HAP1 in the *IL37* gene; and $n = 200$, 44, and 3 for HAP2 in the *IL37* gene. See Table 2 for further details of SNPs and text in the "Statistical analyses" section for determination of haplotypes. Factor loading was determined by principal component analysis; see Supplemental Table S4 for full details on factor loading

Discussion

Here, we investigated for the first time the role of IL-37 in the context of human physiological aging. Our most important results include four novel findings. We first established the accuracy, reproducibility, and specificity of the AdipoGen IL-37 ELISA, which had not previously been performed despite the use of this assay in earlier studies in the field. Second, we found that circulating concentrations of IL-37 are lower on average in older compared with younger adults, despite chronic low-grade elevations in pro-inflammatory cytokines. Third, IL-37 concentrations across age were associated with several key physiological and clinical markers of human healthspan, including those related to cardiorespiratory fitness, adiposity, blood pressure, and glucose tolerance. Finally, we also assessed the prevalence of various common, coding SNP genotypes in the *IL37* and *ILR8* genes and found that the minor allele of certain *IL37* gene SNPs tended to be associated with blood pressure and adiposity. Overall, we provide the first evidence for changes in circulating IL-37 with aging in adults free of major health disorders and possible links with indicators of human healthspan.

Validation of the IL-37 AdipoGen ELISA

Although the AdipoGen ELISA has been widely used in human studies to assess circulating IL-37 concentrations in various disease states [16, 18, 52, 67, 69], there has been concern in the field regarding its validity and specificity [52]. Using multiple approaches, we systematically established, for the first time in an independent setting, that the assay is accurate, reproducible, and specific to IL-37 (i.e., it did not detect IL-1ra, a cytokine with very similar structural and chemical homology). We further established that the AdipoGen ELISA provides similar values of circulating IL-37 obtained from samples of human EDTA plasma and serum. Our results confirm not only the validity of this assay in the present study, but also its use in prior investigations.

Of note, the plasma IL-37 concentrations that we measured in our cohort of young, middle-aged, and older adults were on average higher and spanned a wider range (mean ± SD: 324 ± 225 pg/mL; range: 22–1070 pg/mL) than previously reported concentrations in healthy adults. For example, Santarelli et al. reported a reference range of 41.1–126.8 pg/mL in plasma, determined from a meta-analysis of 7 studies ($n = 388$) of which 4 out of 7 used the AdipoGen ELISA. However, the studies included in this meta-analysis for plasma were all in primarily Han Chinese subjects who may have been more homogeneous than our cohort. Our subjects ranged widely in adiposity, blood pressure, age, and other phenotypic markers. As such, especially given our extensive validation, the "healthy" range of plasma IL-37 concentrations may be wider than previously thought and/or may differ across ethnicities.

Because PBMCs represent the most accessible cell type in studies of humans, particularly large cohort investigations, as part of validating the assay, we also compared IL-37 concentrations in plasma and PBMC lysates in matched samples obtained from young, middle-aged, and older subjects. We found that IL-37 levels were much lower in PBMCs than plasma sampled from the same subjects. To our knowledge, this is the first direct comparison of IL-37 protein levels in plasma compared with PBMCs in humans and these data suggest that the circulating IL-37 "signal" may be more accessible in plasma, at least in populations without overt disease. This finding has practical importance for large human trials because of the additional time and expense associated with isolation of PBMCs from blood samples.

Circulating IL-37 declines with aging

In the present study, plasma concentrations of IL-37 were, on average, lower in healthy adults over 65 years of age compared with younger adults. Importantly, the lower levels of plasma IL-37, an anti-inflammatory cytokine, in older adults were observed in the face of elevated plasma concentrations of the pro-inflammatory proteins IL-6, TNFα, and CRP, common markers of age-associated chronic low-grade inflammation (inflammaging) [17].

Most studies of IL-37 in humans have focused on its role in inflammatory disorders, each of which is characterized by excessive immune activation, e.g., rheumatoid arthritis, systemic lupus erythematosus, and atherosclerosis. In these states, circulating IL-37 levels are *elevated* relative to healthy controls [16, 32, 33, 35, 38, 46, 50, 54, 64, 66, 67, 69, 71], presumably as a compensatory physiological response to mitigate the impact of increases in circulating

pro-inflammatory cytokines [16, 66, 67]. Indeed, under such conditions, circulating IL-37 often correlates with concentrations of IL-6, TNFα, CRP, and/or other pro-inflammatory cytokines [16, 31, 32, 46, 66, 69], as well as with markers of disease severity or progression [31, 38, 38, 67, 71].

Here we report for the first time the *opposite* direction of association between IL-37 and circulating pro-inflammatory cytokines in the context of healthy aging. The data suggest that not only did our healthy older subjects not demonstrate increases in IL-37 in response to chronically elevated levels of pro-inflammatory cytokines, but plasma concentrations of IL-37 were lower than in young and middle-aged adults. This apparent lack of an appropriate IL-37 compensatory response to low-grade inflammation was most evident when assessing the ratios of plasma IL-37 to IL-6 and CRP, which were much lower in older adults compared to the other age groups. These results are consistent with the notion of systemic immunosenescence with aging in the absence of chronic disease and disability [51]. Our findings suggest that circulating IL-37 might be explored as a novel marker of immunosenescence in the setting of biological aging in humans, and that strategies to restore circulating IL-37 concentrations in older adults may reduce chronic low-grade inflammation and thereby extend healthspan. Importantly, this finding also indicates that older adults may be at greater risk of severe outcomes with acute inflammatory insults or infections. For example, a lesser increase in circulating IL-37 concentrations early in SARS-CoV-2 infection is associated with worse clinical prognosis in hospitalized patients [32]. These authors also observed that treating SARS-CoV-2-infected mice with recombinant IL-37 reduced pulmonary inflammation and pathology.

Circulating IL-37 and healthspan markers

Although circulating IL-37 was lower in the older (aged 65 + years) group in our cohort compared with the two younger groups, we observed considerable inter-individual variability in absolute concentrations among our older subjects. Because substantial variability also has been reported in various physiological and clinical measures of healthspan in older adults [28], we sought to determine if circulating IL-37 concentrations might be related to these indicators of healthspan within our study sample.

We found that absolute concentrations of plasma IL-37 were not significantly related to any of the diverse set of healthspan measures assessed, possibly due to the low sample size. However, the ratios of plasma IL-37 to both plasma IL-6 and CRP, i.e., our indirect markers of circulating IL-37 responsiveness to systemic pro-inflammatory signaling, were related to several indicators of healthspan, including positive associations with cardiorespiratory fitness and negative associations with markers of adiposity, blood pressure (IL-37:IL-6 only), fasting blood glucose (IL-37:IL-6 only), and white blood cell counts (IL-37:CRP only). As an important biochemical role of IL-37 is to counter IL-6 pro-inflammatory signaling, the plasma IL-37:IL-6 ratio, in particular, may be of greater physiological relevance than IL-6 concentrations alone. Consistent with this idea, diastolic blood pressure and fasting blood glucose were related to the ratio of IL-37 to IL-6, but not to IL-6 alone.

Collectively, these findings are in agreement with studies in mice showing that higher IL-37, achieved via treatment with human recombinant IL-37 or transgenic overexpression of the human *IL37* gene, improves exercise endurance capacity [4, 8] and enhances glucose tolerance and insulin sensitivity with aging [4] and high-fat diet feeding [2]. In humans, adipose tissue IL-37 expression increased in morbidly obese adults after extreme weight loss and IL-37 was negatively correlated with BMI [37]. Moreover, among elderly patients with type-2 diabetes, those with higher IL-37 had better insulin sensitivity [30]. Thus, our results, combined with observations from previous studies in mice and humans, indicate that IL-37 may serve as a marker, if not a mediator, of physiological and health status, especially in relation to adiposity and metabolic function and the process of biological aging.

IL37 and *ILR8* SNP genotypes

We measured the frequency of genotypes in 7 common, coding SNP alleles in the *IL37* gene and 2 common, coding SNP alleles in the *ILR8* gene. In *IL37*, the minor alleles of 2 SNPs, rs3811046 and rs3811047, were observed in nearly perfect linkage disequilibrium with one another (i.e., individuals who had 1 or 2 copies of the minor allele for 1 SNP, also

had 1 or 2 copies for the other). As such, we grouped these into HAP1. The minor alleles of the remaining 5 SNPs in *IL37* were observed in nearly perfect linkage disequilibrium with one another and were grouped into HAP2. Similarly, the minor alleles of the two SNPs that we measured in the *ILR8* gene also were observed in near-perfect linkage disequilibrium (*ILR8* HAP).

Contrary to our initial hypothesis, we did not observe any significant relations between the studied haplotypes and circulating IL-37 concentrations. However, although our sample size of 247 is large for studies involving deep physiological phenotyping of healthy adult humans, it is quite small for genetic studies, which often include hundreds to thousands of subjects. As such, we cannot definitively rule out an effect of these SNP variants on IL-37 levels, nor did we study other common (e.g., non-coding/regulatory) or rare genetic variants. Despite the low sample size, we did observe trends toward associations with these haplotypes and healthspan markers, as discussed more below.

IL37 **gene haplotype 1** HAP1 contained the rs3811046 and rs3811047 SNPs in the *IL37* gene. These SNPs are the most commonly studied in the literature for the *IL37* gene [1, 10, 14, 42, 43, 63, 65, 68]. We observed that having more copies of the minor allele of HAP1 tended to be related to lower diastolic blood pressure and adiposity, indicating a protective effect of these SNPs. Of note, this allele (G in rs3811046) is the major allele in African and African American populations, but minor for all other ethnicities. Consistent with the notion of these alleles being protective, El-Sayed et al. found that patients with rheumatoid arthritis possessing 1 or 2 copies of the minor allele of rs3811037 had lower disease severity than patients with 0 copies [14],however, the sample size in this study was low ($n = 100$). The minor allele of rs3811047 has also been associated with decreased risk of gastric cardiac adenocarcinoma [63] and peptic ulcers [10]. However, contrary to our findings, the minor allele of rs3811047 has also been associated with lower PBMC mRNA expression of IL-37 and higher incidence of coronary artery disease [68].

IL37 **gene haplotype 2** HAP2 contained the rs2708943, rs2723183, rs2723187, rs2708947, and rs2723192 SNPs in the *IL37* gene. We observed that having more copies of the minor allele of HAP2 tended to be related to higher blood pressure, indicating a potentially detrimental effect of these SNPs. These SNPs were much less common than those contained in HAP1. Only 18% of our cohort had 1 copy of the minor allele and only 1% had 2 copies. To our knowledge, only one other paper has reported on any of these SNPs [41]. They observed that the minor allele of rs2708943 was associated with lower IL-37 mRNA expression, which is consistent with our findings of this SNP possibly being detrimental for specific indicators of healthspan.

ILR8 **gene haplotype** The *ILR8* gene haplotype contained SNPs rs7947 and rs3210908. We found that having 1 copy of the minor allele was very common (38% of our cohort; however, we acknowledge that position-based coverage of *ILR8* was low in 27% of subjects in our cohort). IL-R8 is a co-receptor necessary for IL-37 to initiate cellular signaling. As such, we were not expecting *ILR8* genotypes to affect circulating IL-37 levels but rather hypothesized that these variants in these SNPs might be related to certain indicators of healthspan. Contrary to this hypothesis, we observed no trends between the *ILR8* haplotype and healthspan markers. To our knowledge, this is the first time these SNPs in the *ILR8* gene have been examined in relation to measures physiological function or healthspan.

Limitations and experimental considerations

As previously mentioned, a sample size of 247 subjects is impressive for studies involving deep physiological phenotyping but low for genotyping studies, which typically include thousands of individuals. Thus, larger cohorts will likely be required to more definitively associate *IL37* and *ILR8* SNPs with healthspan markers. Secondly, heterogeneity in factors besides differing rates of biological aging likely contributed to the observed variability and may have prevented us from observing stronger correlations. For example, the type and frequency of prescription medications varied across age groups. Some of these medications are known to have anti-inflammatory effects (e.g., aspirin and statins) and could have affected circulating IL-37 concentrations and/or

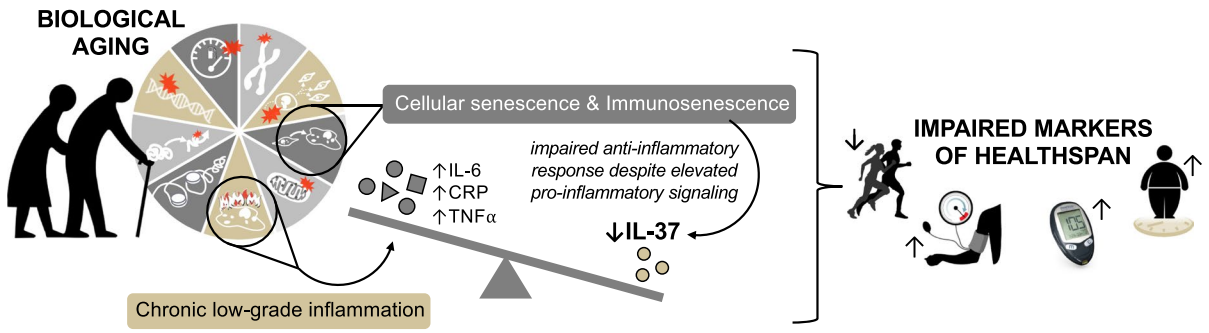

Fig. 6 IL-37 in the context of biological aging. Chronic low-grade inflammation and immunosenescence are two hallmark mechanisms of biological aging. In the present study, circulating concentrations of IL-37 were lower in healthy older adults, compared to young and middle-aged adults, which may both reflect immunosenescence (i.e., impaired ability to mount an anti-inflammatory response) and contribute to chronic low-grade inflammation (i.e., reduced suppression of production of pro-inflammatory cytokines). Importantly, reduced ratios of IL-37 to key pro-inflammatory markers were associated with impairments in several markers of healthspan, suggesting that both pro-inflammatory and anti-inflammatory processes should be considered in future studies and models of biological aging and healthspan

healthspan markers. Furthermore, incidence of other health conditions, such as elevated blood pressure, elevated cholesterol, and thyroid disorders, likely increased with aging. It is important to note that all of our subjects underwent rigorous health screening and those with established chronic disease or any underlying cardiovascular disease were excluded; however, subclinical changes in these processes were likely present in middle-aged and older adults. That said, these are all physiological changes that are part of the process of biological aging. Thus, we do feel that our cohort is representative in general of healthy aging. Lastly, distribution of race/ethnicity did differ, albeit not significantly, across age groups, which could have affected the incidence of SNPs and associations between SNPs and healthspan markers. We were unfortunately underpowered to test effects of race/ethnicity of these variables.

Summary and conclusions

The findings of the present investigation establish the validity of the AdipoGen IL-37 ELISA for assessing circulating concentrations of IL-37 in either EDTA plasma or serum from healthy humans varying in age. Compared to PBMCs, we also observed higher levels of IL-37 in plasma (i.e., a more readily obtainable tissue) in our population of relatively healthy adults. As such, this assay appears to have considerable utility for use in future studies of IL-37 and human aging.

We also report for the first time that older adults free of any overt clinical disorders demonstrate lower circulating concentrations of IL-37 compared with younger healthy adults, despite being faced with systemic low-grade inflammation (inflammaging). This novel observation suggests that, in contrast to prior reports of compensatory increases in IL-37 in settings of pro-inflammatory clinical disorders, primary human aging appears to reduce the circulating anti-inflammatory IL-37 response to a pro-inflammatory milieu, consistent with the concept of immunosenescence with primary aging in humans. Although absolute plasma concentrations of IL-37 were not related to diverse physiological and clinical indicators of human healthspan, we found that the ratios of IL-37 to IL-6 and/or CRP correlated significantly with several such markers, including cardiorespiratory fitness, body fatness, blood pressure, and fasting blood glucose. Nevertheless, we were unable to identify any obvious associations between the studied *IL37* or *ILR8* haplotypes and plasma concentrations of IL-37 in our overall cohort. However, we found that selective haplotypes tended to be associated with specific indicators of healthspan. Overall, our results shed new light on a possible role of IL-37 in biological aging with primary (healthy) aging in humans (Fig. 6). Accordingly, our findings indicate that anti-inflammatory processes (e.g., circulating IL-37 concentrations) should be incorporated in studies and integrated models of biological aging and healthspan, as they may play as large a role as pro-inflammatory

signaling. Furthermore, strategies to supplement and/or restore IL-37 concentrations in older adults could slow the rate of biological aging, improve healthspan, and/or reduce risk of chronic age-related diseases, consistent with results of recent inflammation-suppressing clinical trials [47, 48].

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Author contribution V.E.B., A.P.I., B.P.Z., D.B.B., C.A.D., M.A.E., and D.R.S. conceived of and designed the experiments. V.E.B., A.P.I., B.P.Z., D.B.B., A.H., and M.A.E. collected and analyzed data. V.E.B., A.P.I., B.P.Z., M.A.E., and D.R.S. interpreted data. V.E.B. and D.R.S. wrote the initial draft of the manuscript. All authors revised the manuscript critically for intellectual content, approved the final version, and agree to be accountable for all aspects of the work.

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Availability of data and material All data are available from the corresponding author upon reasonable request.

Code availability Code for statistical analyses is available from the corresponding author upon reasonable request.

Declarations

Ethics approval All procedures involving human subjects were approved by the Institutional Review Board at the University of Colorado Boulder and were conducted in accordance with the Declaration of Helsinki.

Consent to participate All human subjects provided oral and written informed consent prior to participation.

Consent for publications As part of the informed consent process, all human subjects consented to publication of all data resulting from their participation in the studies, including use of samples for publication of future studies.

Competing interests The authors declare no competing interests.

References

1. Allam G, Mohamed IAA, Alswat KA, Abbadi SH, Nassif R, Alharthi BJ, Nasr A. Association of IL-37 gene polymorphisms with susceptibility to tuberculosis in Saudi subjects. Microbiol Immunol. 2016;60:778–86.
2. Ballak DB, et al. IL-37 protects against obesity-induced inflammation and insulin resistance. Nat Commun. 2014;5:4711.
3. Ballak DB, Li S, Cavalli G, Stahl JL, Tengesdal IW, van Diepen JA, Klück V, Swartzwelter B, Azam T, Tack CJ, Stienstra R, Mandrup-Poulsen T, Seals DR, Dinarello CA. Interleukin-37 treatment of mice with metabolic syndrome improves insulin sensitivity and reduces pro-inflammatory cytokine production in adipose tissue. J Biol Chem. 2018;293:14224–36.
4. Ballak DB, Brunt VE, Sapinsley ZJ, Ziemba BP, Richey JJ, Zigler MC, Johnson LC, Gioscia-Ryan RA, Culp Hill R, Eisenmesser EZ, D'Alessandro A, Dinarello CA, Seals DR. Short-term interleukin-37 treatment improves vascular endothelial function, endurance exercise capacity, and whole-body glucose metabolism in old mice. Aging Cell. 2020;19: e13074.
5. Boraschi D, Lucchesi D, Hainzl S, Leitner M, Maier E, Mangelberger D, Oostingh GJ, Pfaller T, Pixner C, Posselt G, Italiani P, Nold MF, Nold-Petry CA, Bufler P, Dinarello CA. IL-37: a new anti-inflammatory cytokine of the IL-1 family. Eur Cytokine Netw. 2011;22:127–47.
6. Brunt VE, Casso AG, Gioscia-Ryan RA, Sapinsley ZJ, Ziemba BP, Clayton ZS, Bazzoni AE, VanDongen NS, Richey JJ, Hutton DA, Zigler MC, Neilson AP, Davy KP, Seals DR. Gut microbiome-derived metabolite trimethylamine N-oxide induces aortic stiffening and increases systolic blood pressure with aging in mice and humans. Hypertension. 2021;78:499–511.
7. Bruunsgaard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokines. Curr Opin Hematol. 2001;8:131–6.
8. Cavalli G, Justice JN, Boyle KE, D'Alessandro A, Eisenmesser EZ, Herrera JJ, Hansen KC, Nemkov T, Stienstra R, Garlanda C, Mantovani A, Seals DR, Dagna L, Joosten LAB, Ballak DB, Dinarello CA. Interleukin 37 reverses the metabolic cost of inflammation, increases oxidative respiration, and improves exercise tolerance. Proc Natl Acad Sci USA. 2017;114:2313–8.
9. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002;39:257–65.
10. Davarpanah E, Jafarzadeh A, Nemati M, Bassagh A, Abasi MH, Khosravimashizi A, Kazemipoor N, Ghazizadeh M, Mirzaee M. Circulating concentration of interleukin-37 in Helicobacter pylori-infected patients with peptic ulcer: its association with IL-37 related gene polymorphisms and bacterial virulence factor CagA. Cytokine. 2020;126: 154928.
11. Deelen J, Beekman M, Capri M, Franceschi C, Slagboom PE. Identifying the genomic determinants of aging and

longevity in human population studies: progress and challenges. BioEssays. 2013;35:386–96.
12. DeVan AE, Johnson LC, Brooks FA, Evans TD, Justice JN, Cruickshank-Quinn C, Reisdorph N, Bryan NS, McQueen MB, Santos-Parker JR, Chonchol MB, Bassett CJ, Sindler AL, Giordano T, Seals DR. Effects of sodium nitrite supplementation on vascular function and related small metabolite signatures in middle-aged and older adults. J Appl Physiol. 2016;120:416–25.
13. Dinarello CA, Nold-Petry C, Nold M, Fujita M, Li S, Kim S, Bufler P. Suppression of innate inflammation and immunity by interleukin-37. Eur J Immunol. 2016;46:1067–81.
14. El-Sayed EH, Saleh MH, Al-Shahaly MH, Toraih EA, Fathy A. IL-37 gene variant (rs3811047): a marker of disease activity in rheumatoid arthritis: a pilot study. Autoimmunity. 2018;51:378–85.
15. Eskurza I, Monahan KD, Robinson JA, Seals DR. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. J Physiol. 2004;556:315–24.
16. Feng M, Kang M, He F, Xiao Z, Liu Z, Yao H, Wu J. Plasma interleukin-37 is increased and inhibits the production of inflammatory cytokines in peripheral blood mononuclear cells in systemic juvenile idiopathic arthritis patients. J Transl Med. 2018;16:277–310.
17. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci. 2014;69:S4–9.
18. Godsell J. Clinical associations of IL-10 and IL-37 in systemic lupus erythematosus. Nature Publishing Group; 2016. p. 1–10.
19. Hamczyk MR, Nevado RM, Barettino A, Fuster V, Andres V. Biological versus chronological aging: JACC focus seminar. J Am Coll Cardiol. 2020;75:919–30.
20. Hurme M, Santtila S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1beta genes. Eur J Immunol. 1998;28:2598–602.
21. Jablonski KL, Racine ML, Geolfos CJ, Gates PE, Chonchol M, McQueen MB, Seals DR. Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. J Am Coll Cardiol. 2013;61:335–43.
22. Jablonski KL, Donato AJ, Fleenor BS, Nowlan MJ, Walker AE, Kaplon RE, Ballak DB, Seals DR. Reduced large elastic artery stiffness with regular aerobic exercise in middle-aged and older adults: potential role of suppressed nuclear factor κ B signalling. J Hypertens. 2015;33:2477–82.
23. Ji Q, Zeng Q, Huang Y, Shi Y, Lin Y, Lu Z, Meng K, Wu B, Yu K, Chai M, Liu Y, Zhou Y. Elevated plasma IL-37, IL-18, and IL-18BP concentrations in patients with acute coronary syndrome. Mediators Inflamm. 2014;2014:165742.
24. Kaplon RE, Hill SD, Bispham NZ, Santos-Parker JR, Nowlan MJ, Snyder LL, Chonchol M, LaRocca TJ, McQueen MB, Seals DR. Oral trehalose supplementation improves resistance artery endothelial function in healthy middle-aged and older adults. Aging (albany NY). 2016;8:1167–83.
25. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA, Richardson A, Schadt EE, Wyss-Coray T, Sierra F. Geroscience: linking aging to chronic disease. Cell. 2014;159:709–13.
26. Kirkland JL, Peterson C. Healthspan, translation, and new outcomes for animal studies of aging. J Gerontol A Biol Sci Med Sci. 2009;64:209–12.
27. Klück V, van Deuren RC, Cavalli G, Shaukat A, Arts P, Cleophas MC, Crișan TO, Tausche AK, Riches P, Dalbeth N, Stamp LK, Hindmarsh JH, Jansen TLTA, Janssen M, Steehouwer M, Lelieveld S, van de Vorst M, Gilissen C, Dagna L, Van de Veerdonk FL, Eisenmesser EZ, Kim S, Merriman TR, Hoischen A, Netea MG, Dinarello CA, Joosten LA. Rare genetic variants in interleukin-37 link this anti-inflammatory cytokine to the pathogenesis and treatment of gout. Ann Rheum Dis. 2020;79:536–44.
28. Kuo PL, Schrack JA, Shardell MD, Levine M, Moore AZ, An Y, Elango P, Karikkineth A, Tanaka T, de Cabo R, Zukley LM, AlGhatrif M, Chia CW, Simonsick EM, Egan JM, Resnick SM, Ferrucci L. A roadmap to build a phenotypic metric of ageing: insights from the Baltimore Longitudinal Study of Aging. J Intern Med. 2020;287:373–94.
29. Li S, Neff CP, Barber K, Hong J, Luo Y, Azam T, Palmer BE, Fujita M, Garlanda C, Mantovani A, Kim S, Dinarello CA. Extracellular forms of IL-37 inhibit innate inflammation in vitro and in vivo but require the IL-1 family decoy receptor IL-1R8. Proc Natl Acad Sci USA. 2015;112:2497–502.
30. Li T, Li H, Li W, Chen S, Feng T, Jiao W, Wu C, Dong J, Li Y, Li S, Feng M, Wei X. Interleukin-37 sensitize the elderly type 2 diabetic patients to insulin therapy through suppressing the gut microbiota dysbiosis. Mol Immunol. 2019;112:322–9.
31. Li H, Shen C, Chen B, Du J, Peng B, Wang W, Chi F, Dong X, Huang Z, Yang C. Interleukin-37 is increased in peripheral blood mononuclear cells of coronary heart disease patients and inhibits the inflammatory reaction. Mol Med Rep. 2020;21:151–60.
32. Li A, Ling Y, Song Z, Cheng X, Ding L, Jiang R, Fu W, Liu Y, Hu H, Yuan S, Chen J, Zhu C, Fan J, Wang J, Jin Y, Zhang M, Zhu L, Sun P, Zhang L, Qin R, Zhang W, Qiu C, Shen Y, Zhang L, Shi Z, Zhao C, Zhu T, Lu H, Zhang X, Xu J. Correlation between early plasma interleukin 37 responses with low inflammatory cytokine levels and benign clinical outcomes in severe acute respiratory syndrome coronavirus 2 infection. J Infect Dis. 2021;223:568–80.
33. Lin P-C, Chiou S-S, Hsu W-Y, Liao Y-M, Tsai S-P, Su H-L, Lu P-T, Tseng Y-H. Plasma levels of IL-1β and IL-37 in patients with severe haemophilia. J Int Med Res. 2020;48:300060520940512.
34. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–217.
35. Lotfy H, Moaaz M, Moaaz M. The novel role of IL-37 to enhance the anti-inflammatory response of regulatory T cells in patients with peripheral atherosclerosis. Vascular. 2020;28:629–42.
36. Moretti S, Bozza S, Oikonomou V, Renga G, Casagrande A, Iannitti RG, et al. IL-37 inhibits inflammasome

activation and disease severity in murine aspergillosis. ed. Klein BS. PLoS Pathog. 2014;10:e1004462.
37. Moschen AR, Molnar C, Enrich B, Geiger S, Ebenbichler CF, Tilg H. Adipose and liver expression of interleukin (IL)-1 family members in morbid obesity and effects of weight loss. Mol Med. 2011;17:840–5.
38. Mountford S, Effenberger M, Noll-Puchta H, Griessmair L, Ringleb A, Haas S, Denk G, Reiter FP, Mayr D, Dinarello CA, Tilg H, Bufler P. Modulation of liver inflammation and fibrosis by interleukin-37. Front Immunol. 2021;12: 603649.
39. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol. 2010;11:1014–22.
40. Nold-Petry CA, et al. IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. Nat Immunol. 2015;16:354–65.
41. Offenbacher S, Jiao Y, Kim SJ, Marchesan J, Moss KL, Jing L, Divaris K, Bencharit S, Agler CS, Morelli T, Zhang S, Sun L, Seaman WT, Cowley D, Barros SP, Beck JD, Munz M, Schaefer AS, North KE. GWAS for Interleukin-1β levels in gingival crevicular fluid identifies IL37 variants in periodontal inflammation. Nat Commun. 2018;9:3686.
42. Özgüçlü S, Duman T, Ateş FSÖ, Küçükşahin O, Çolak S, Ölmez Ü. Serum interleukin-37 level and interleukin-37 gene polymorphism in patients with Behçet disease. Clin Rheumatol. 2019;38:495–502.
43. Pei B, Xu S, Liu T, Pan F, Xu J, Ding C. Associations of the IL-1F7 gene polymorphisms with rheumatoid arthritis in Chinese Han population. Int J Immunogenet. 2013;40:199–203.
44. Pierce GL, Beske SD, Lawson BR, Southall KL, Benay FJ, Donato AJ, Seals DR. Weight loss alone improves conduit and resistance artery endothelial function in young and older overweight/obese adults. Hypertension. 2008;52:72–9.
45. Pierce GL, Eskurza I, Walker AE, Fay TN, Seals DR. Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults. Clin Sci. 2011;120:13–23.
46. Ragab D, Mobasher S, Shabaan E. Elevated levels of IL-37 correlate with T cell activation status in rheumatoid arthritis patients. Cytokine. 2019;113:305–10.
47. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017a;377:1119–31.
48. Ridker PM, MacFadyen JG, Thuren T, Everett BM, Libby P, Glynn RJ, et al. Effect of interleukin-1β inhibition with canakinumab on incident lung cancer in patients with atherosclerosis: exploratory results from a randomised, double-blind, placebo-controlled trial. Lancet. 2017b;390:1833–42.
49. Rossman MJ, Kaplon RE, Hill SD, McNamara MN, Santos-Parker JR, Pierce GL, Seals DR, Donato AJ. Endothelial cell senescence with aging in healthy humans: prevention by habitual exercise and relation to vascular endothelial function. Am J Physiol Heart Circ Physiol. 2017;313:H890–5.
50. Ruggeri RM, Cristani M, Vicchio TM, Alibrandi A, Giovinazzo S, Saija A, Campennì A, Trimarchi F, Gangemi S. Increased serum interleukin-37 (IL-37) levels correlate with oxidative stress parameters in Hashimoto's thyroiditis. J Endocrinol Invest. 2019;42:199–205.
51. Salvioli S, et al. Immune system, cell senescence, aging and longevity–inflammaging reappraised. Curr Pharm Des. 2013;19:1675–9.
52. Santarelli DM, Vincent FB, Rudloff I, Nold-Petry CA, Nold MF, Russo MA. Circulating interleukin-37 levels in healthy adult humans - establishing a reference range. Front Immunol. 2021;12: 708425.
53. Santos-Parker JR, Strahler TR, Bassett CJ, Bispham NZ, Chonchol MB, Seals DR. Curcumin supplementation improves vascular endothelial function in healthy middle-aged and older adults by increasing nitric oxide bioavailability and reducing oxidative stress. Aging (albany NY). 2017;9:187–208.
54. Satiroglu O, Gürlek B, Durakoglugil ME, Duman H, Erdogan T, Çetin M, Ergül M, Yilmaz A, Uydu HA. The role of serum interleukin-37 levels, inflammation and blood pressure in patients with preeclampsia. Clin Exp Hypertens. 2020;42:669–74.
55. Seals DR, Melov S. Translational geroscience: emphasizing function to achieve optimal longevity. Aging (albany NY). 2014;6:718–30.
56. Seals DR, Justice JN, LaRocca TJ. Physiological geroscience: targeting function to increase healthspan and achieve optimal longevity. J Physiol. 2016;594:2001–24.
57. Shou X, Lin J, Xie C, Wang Y, Sun C. Plasma IL-37 elevated in patients with chronic heart failure and predicted major adverse cardiac events: a 1-year follow-up study. Dis Markers. 2017;2017:9134079.
58. Teng X, et al. IL-37 ameliorates the inflammatory process in psoriasis by suppressing proinflammatory cytokine production. J Immunol. 2014;192:1815–23.
59. van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. J Stat Softw. 2011;45:1–67.
60. van Deuren RC, Arts P, Cavalli G, Jaeger M, Steehouwer M, van de Vorst M, Gilissen C, Joosten LAB, Dinarello CA, Mhlanga MM, Kumar V, Netea MG, van de Veerdonk FL, Hoischen A. Impact of rare and common genetic variation in the interleukin-1 pathway on human cytokine responses. Genome Med. 2021;13:94.
61. Walker AE, Kaplon RE, Lucking SMS, Russell-Nowlan MJ, Eckel RH, Seals DR. Fenofibrate improves vascular endothelial function by reducing oxidative stress while increasing endothelial nitric oxide synthase in healthy normolipidemic older adults. Hypertension. 2012;60:1517–23.
62. Walker AE, Kaplon RE, Pierce GL, Nowlan MJ, Seals DR. Prevention of age-related endothelial dysfunction by habitual aerobic exercise in healthy humans: possible role of nuclear factor κB. Clin Sci. 2014;127:645–54.
63. Wang X, Yin J, Zheng L, Wang L, Shi Y, Tang W, Ding G, Liu C, Liu R, Chen S, Gu H. The variant interleukin 1f7 rs3811047 G>A was associated with a decreased risk of gastric cardiac adenocarcinoma in a Chinese Han population. Tumour Biol. 2014;35:3509–15.

64. Wu G-C, Li H-M, Wang J-B, Leng R-X, Wang D-G, Ye D-Q. Elevated plasma interleukin-37 levels in systemic lupus erythematosus patients. Lupus. 2016;25:1377–80.
65. Wu Q, Zhou J, Yuan ZC, Lan YY, Xu WD, Huang AF. Association between IL-37 and systemic lupus erythematosus risk. Immunol Invest. 2021;17:1–12.
66. Xia L, Shen H, Lu J. Elevated serum and synovial fluid levels of interleukin-37 in patients with rheumatoid arthritis: attenuated the production of inflammatory cytokines. Cytokine. 2015;76:553–7.
67. Ye L, Ji L, Wen Z, Zhou Y, Hu D, Li Y, Yu T, Chen B, Zhang J, Ding L, Du J, Huang Z. IL-37 inhibits the production of inflammatory cytokines in peripheral blood mononuclear cells of patients with systemic lupus erythematosus: its correlation with disease activity. J Transl Med. 2014;12:69–78.
68. Yin D, et al. Genomic variant in IL-37 confers a significant risk of coronary artery disease. Sci Rep. 2017;7:42175.
69. Yu K, Min X, Lin Y, Huang Y, Huang S, Liu L, Peng Y, Meng K, Li D, Ji Q, Zeng Q. Increased IL-37 concentrations in patients with arterial calcification. Clin Chim Acta. 2016;461:19–24.
70. Zhang F, Zhu X-J, Zhu X-J, Liu Y-X, Yuan T, Yao Q-M. Plasma levels and expression of interleukin-37 in patients with immune thrombocytopenia. Exp Ther Med. 2019;18:2739–45.
71. Zhang Y, Xu C, Wang H, Nan S. Serum interleukin-37 increases in patients after ischemic stroke and is associated with stroke recurrence. Oxid Med Cell Longev. 2021;2021:5546991.