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Fitness age outperforms body mass index in differentiating aging patterns and health risk profiles of healthy adults aged 51–80 years

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Abstract Physical fitness has been extensively shown to strongly associate with general health status and major health risks. Here we tested the ability of a novel estimate of fitness age (FitAge) to differentiate aging trajectories.

This study aimed at (1) testing the ability of Fit-Age to differentiate aging patterns among decelerated, normal, and accelerated agers in selected health domains, (2) estimating the risk for developing major health issues depending on the aging trajectory, and (3) comparing FitAge to body mass index (BMI) categorization in differentiating healthy from unhealthy aging patterns.

A total of 176 volunteers participated in this cross-sectional study. Participants underwent clinical screening and a comprehensive assessment of body composition, nutritional and health-related status, cognitive functioning, and haematochemical analyses with routine tests, oxidative stress, and inflammation markers. Scores for major health risks were also computed.

FitAge outperformed BMI in estimating major health risk scores and was able to differentiate decelerated from normal and accelerated agers for health risk profile and several physiological domains. Body composition, immune system activation, and inflammation markers emerged as those variables flagging the largest differences between decelerated and accelerated aging patterns.

The novel estimate of biological aging can accurately differentiate both in women and men decelerated from accelerated agers in almost all the domains scrutinized. Overall, decelerated aging is linked to positively oriented features which associate with reduced risk of developing major health issues.

The present findings have potential relevance and practical implications to identify individuals at higher risk of accelerated aging according to their FitAge estimated via simple and cost-effective motor tests.

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Introduction

Converging evidence from epidemiological studies suggests that an active lifestyle is associated with a lower risk of cardiovascular events and mortality [1]. A recent cohort study of 4840 US adults aged 40 to 85 years estimated that approximately 110,000 deaths per year could be prevented if physical activity is increased by a small amount (*i.e.*, 10 min per day) [2]. In a population-based cohort study of 14,599 UK healthy participants, it was found that healthy middle-aged and older adults, and also individuals with cardiovascular disease and cancer, could gain substantial longevity benefits by becoming more physically active, irrespective of past physical activity levels and established risk factors [3]. Thus, physical fitness (PF) is considered a major determinant of health status that even surpasses other cardiovascular or sociodemographic determinants classically associated with adverse health outcomes [4]. PF declines with age at different individual rates [5]. It has been shown that those who preserve PF while aging are at lower risk for morbidity and tend to live longer [6] [7]. The relationship between PF and aging quality seems of utmost importance as the level of physical activity is a modifiable factor that can act as a potent modulator of the aging trajectory. Considerable impact on population health can be attained with consistent engagement in physical activity during mid to late life. Consequently, health status can be predicted and monitored by assessing PF [8].

A recent cross-sectional study of Italian healthy adults aged 51–80 years introduced a comprehensive and novel measure of biological aging based on different components of fitness. Specifically, using a linear regression model with elastic net penalization previously employed to define the epigenetic age [9], participants' biological/fitness age (FitAge) was predicted considering the results of six motor-functional fitness tests (Four Square Step Test, Timed Up and Go, 10-Meter Timed Walk, 6-Minute Walk Test, maximum voluntary isometric contraction of the dominant quadriceps, handgrip test) as predictors [10]. Accordingly, age acceleration (FitAA) was defined as the residuals of the regression of FitAge

on chronological age, considered dependent variable. This allowed categorization of the participants into three categories: (1) decelerated agers: individuals with FitAA scores lower than -2.5 years; (2) normal agers: individuals with FitAA scores ranging from -2.5 to 2.5 years; and (3) accelerated agers: those with FitAA higher than 2.5 years [10].

Based on the above background, the objective of the present study was to test the ability of the newly introduced categorization of biological aging (FitAge) to differentiate among decelerated, normal, and accelerated agers over a comprehensive set of health domains (body composition, nutritional status, general health, cognitive status, hematochemical parameters). Our starting hypothesis was that decelerated agers would exhibit significantly healthier features and scores than accelerated agers in most of the domains tested. Additionally, drawing on the findings of a recent systematic review (11), which indicated a stronger role for fitness levels than fatness as estimated with body weight and height, we also expected that FitAge would outperform the body mass index (BMI), which is universally employed to estimate body weight and fatness, and used to predict major health risks including cardiovascular diseases, comorbidities, and all-causes mortality [11]. With such hypotheses being confirmed, the novel FitAge estimates might represent a robust index to monitor both PF status and the aging trajectory, employing a small set of non-invasive, low-cost validated motor tests.

Methods

Study design, participants selection, and clinical examination

The study design was set as observational cross-sectional. The study had an overall duration of 2 years and took place at the Department of Biomedical Sciences, University of Sassari, Italy from January 2021 to December 2023. The current study was advertised via public engagement events to obtain volunteers. Eligible participants were required to be over 50 years old at the time of the examination (from February 2021 to December 2021) and have no medical, physical, or cognitive condition that would interfere with participation in the functional assessments. After a preliminary telephone interview the first 300

respondents deemed apparently eligible were recalled to undergo clinical examinations. All subjects were evaluated by a geriatric specialist against the eligibility criteria. Respiratory, rheumatologic, neurological, cardiovascular, musculoskeletal, neoplastic, and metabolic conditions were thoroughly investigated and set as exclusion criteria. Additional confounding factors that may influence health outcomes, such as the participant's smoking history, level of education, and current pharmacological therapy, were also checked and recorded.

One hundred and seventy-six volunteers were deemed eligible and participated in the study. The Institutional Review Board and the Clinical Research Ethics Committee approved all procedures involving human subjects (ID: PG/2020/16846), which were carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant before inclusion and participation in the tests.

Participants' assessments

Body composition

Body composition was estimated employing BMI calculations (weight/height^2 in meters) and quantified by bioelectrical impedance vector analysis (BIVA) (BIA 101, Akern, Italy). The subjects were tested supine, after a 5-min rest, on a non-conducting surface with their arms slightly abducted away from their trunk and the legs separated. Absolute and percent body cell mass (BCM), fat mass (FM), fat free mass (FFM), total body water (TBW), extracellular water (ECW) and intracellular water (ICW), skeletal muscle mass (SMM), and appendicular skeletal muscle mass (ASMM) were estimated.

Nutritional status

Nutritional status was assessed by the Mini Nutritional Assessment (MNA) inventory and dietary habits evaluation. MNA was administered to evaluate the risk of malnutrition (17–23.5 points), protein-calorie malnutrition (< 17 points), or adequate nutritional status (> 23.5 points) [12]. Dietary habits were assessed through validated questionnaires of adherence to Mediterranean Diet [13, 14] and through the 7-Day Food Diary which captures data immediately after principal meals and snacks throughout the day, overcoming potential memory bias.

Health and cognitive status

Health-related status was investigated trough a set of self-reported scales. The Italian validated version of the Geriatric Depression Scale (GDS) [15] was used to assess the subject's depressive symptoms. Quality of life, functional status, and well-being were measured using the Italian short-form health survey (SF-36) [16]. Cognitive status was assessed using the Italian version [17] of the Montreal Cognitive Assessment (MOCA) [18]. The sleep quality was assessed using the Italian version [19] of the Pittsburgh Sleep Quality Index (PSQI) [20].

Hematochemical tests

Blood samples were drawn by the laboratory analysis of the University Hospital of Sassari. A complete haematochemical analysis was performed to appraise the main blood constituents such as white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), eosinophils (EOS), large unstained cells (LUC), urea, creatinine, alkaline phosphatase, serum iron, and C-reactive protein (CPR).

ELISA assays—The concentrations of interleukin 6 (IL-6), leptin, tumor necrosis factor (TNF-α), and insulin-like growth factor 1 (IGF-1) were determined using Standard ABTS EDK Human IL-6 Kit (PeproTech EC, Ltd., London, UK), Standard ABTS EDK Human Leptin Kit (PeproTech EC, Ltd., London, UK), Human TNF-α Mini TMB ELISA Development Kit (PeproTech EC, Ltd., London, UK), and Human IGF-I ELISA Kit (Sigma-Aldrich Chemie GmbH, Germany), respectively. One hundred microliters of each sample was incubated in a pre-treated plate for 2 h at room temperature (RT). After three washing steps in Phosphate-Buffered Saline (PBS), detection antibody was added in each well for 2 h at RT and then removed and replaced by Streptavidin–Horseradish Peroxidase (HRP) for 30 min at RT. Antibody was then washed three times and liquid substrate incubated at RT for 20 min. Color development was analyzed at 450 nm using a plate reader (Akribis Scientific, Common Farm, Frog Ln, Knutsford, UK). Standard curves were prepared according to the manufacturer's

instructions. Each sample was assayed in duplicate, and values were expressed as the mean ± standard deviation (SD) of 2 measures per sample.

Oxidative stress indicators—Blood samples were collected using tubes containing ethylenediaminetetraacetic acid at room temperature. Immediately after collection, the samples were centrifuged at $1500 \times g$ for 10 min, and the plasma was removed and stored at −80 °C until tested. Paraoxonase (PON-1) activity was determined by using paraoxon (O,O-diethyl-O-p-nitrophenyl phosphate) as a substrate and measuring the increase in absorbance at 412 nm due to the formation of 4-nitrophenol. Enzyme activity was calculated from the molar extinction coefficient $(17,000 \text{ M}^{-1} \text{ cm}^{-1})$ with 1 nmol of 4-nitrophenol formed per minute used as the unit of enzyme activity. Protein-SH (PSH) determination was performed spectrophotometrically at 405 nm using Ellman's reagent (DTNB, 5,5′-dithiobis-2-nitrobenzoic acid) and a standard curve obtained using standard solutions of reduced glutathione (GSH). PSH levels were normalized against the total amount of plasma proteins measured by Lowry's method at 750 nm. Malondialdehyde (MDA) and other aldehydes produced by lipid peroxidation induced by hydroxyl free radicals were measured spectrophotometrically by the thiobarbituric acid reactive substances (TBARS) method forming an MDA-TBA2 adduct that absorbs strongly at 532 nm. Plasma homocysteine levels were assessed by ELISA kit (antibodies-online GmbH, Germany), according to the manufacturer's instructions.

Health risk scores

To estimate the risk for cardiovascular diseases (CVD) three composite risk scores predictive of 10-year risk were computed: the Framingham Risk Score (FRS) for coronary heart disease [21], the CVD risk from the American College of Cardiology (ACC) and American Heart Association (AHA) [22], and the 10-year risk of atherosclerosis based on the Multi-Ethnic Study of Atherosclerosis (MESA) study [23].

The risk of diabetes type 2 [24], the 10-year mortality risk score [25], and the Charlson Comorbidity Index (CCI) [26] were also calculated.

Statistical analysis

Data analyses were performed using SPSS 26.0 software (IBM, New York, USA). Power analyses were conducted using G*Power 3.1.9.2 software [27]. Normality of data was analyzed with the Shapiro–Wilk test. Levene's test was performed to test for homogeneity of variances. General linear model univariate analysis of variance (ANOVA) was used to test differences in the outcomes of interest depending on the FitAge categorization over three levels: decelerated, normal, and accelerated aging [10]. Briefly, individuals were divided into the above categories based on the age acceleration displayed through a coreset of fitness tests (FitAA). As introduced in the background, decelerated agers were those individuals with FitAA scores lower than −2.5 years, normal agers were individuals with FitAA scores ranging from −2.5 to 2.5 years, and accelerated agers were those with FitAA higher than 2.5 years [10].

The main effect of Sex and FitAge*Sex interactions was also tested, followed by Bonferroni-adjusted pairwise comparisons. The direct effect of body composition based on body weight and height and the derived BMI categories (≤18, underweight; 18.1 to 24, normoweight; 24.1 to 29.9, overweight; ≥30, obese) was also tested along with the potential FitAge*BMI and sex interaction. Bonferroni-corrected post hoc tests were conducted to locate the main source of the observed significant difference. The non-parametric Kruskal–Wallis test was employed when the assumptions of normality of the data were violated. Correlation analysis was also run to measure the bivariate association of FitAge and BMI with the calculated scores for major health risks (FRS; ACC; AHA; MESA; diabetes; CCI). Pearson's or Spearman's r coefficients were computed depending on data distribution.

For all tests, statistical significance was set at the conventional threshold of 0.05.

Results

Participants

The analyzed sample included 176 volunteers (72 men and 104 women; mean age 66.48 ± 7.77 years) divided in three groups according to the

categorization based on FitAA ($n = 43$ decelerated, $n = 97$ normal, and $n = 36$ accelerated). Table 1 summarizes the study sample characteristics including anthropometric and sociodemographic variables such as polypharmacy, smoking status, and educational level at study entry. Tables 2–5 report the main results of the study according to the domain examined, reporting only data for those variables showing significant differences among groups according to FitAA. Data from all those variables for which no significant differences were observed are presented in the Supplementary Materials 1–3.

Overall, one-way ANOVA (factor: three-level BMI categorization: normoweight, overweight, obese) revealed significant main effects for BMI on few selected variables (WBC_count: $p = 0.005$; LUC%: $p = 0.04$), unlike FitAge for which significant main effects were detected for most of the outcomes tested, as detailed below and in Table 3–5. No BMI*FitAA interactions were observed for any of the outcomes nor in women neither in men.

Associations of FitAge and BMI with health risk scores

The results of bivariate correlations run for FitAge and BMI with health risk scores are summarized in Table 2. FitAge outperformed BMI in all the bivariate correlations, particularly when testing the association with the Mortality score, the only exception being the Stern Diabetes risk for which BMI, unlike FitAge, was significantly associated.

Comparing decelerated, normal, and accelerated aging trajectories

A significant main effect of Sex was detected for most of the variables tested over the four domains considered. However, no significant Sex*FitAge interactions emerged.

Body composition—One-way ANOVA (factor: three-level aging trajectory: decelerated, normal, accelerated) revealed a significant difference between the three groups in all the descriptors of body composition. Post hoc pairwise comparisons with Bonferroni correction for multiple comparisons detected a significant difference favoring the decelerated group over both the normal and accelerated groups in BCM, FM, FFM, TBW, ECW, ICW, ECW to ICW ratio, SMM and

ASMM; a significant difference was revealed in ICW to FFM ratio between the decelerated and accelerated groups. All these results are described with statistical details in Table 3.

Health and cognitive status—As detailed in Table 4, one-way ANOVA detected significant differences in the Physical functioning, Vitality, and Social Functioning SF-36 sub-scales and in the PSQI. Bonferroni-adjusted pairwise comparisons showed significantly higher functionality scores in decelerated agers as compared to normal and accelerated agers in the Physical Functioning SF-36 sub-scale. For Vitality and Social Functioning SF-36 sub-scales a significant difference emerged between decelerated and normal agers but not between decelerated and accelerated nor normal and accelerated agers. In the PSQI score decelerated agers had significantly higher scores than the accelerated group.

No other significant differences emerged for GDS, MoCA, and the other SF-36 subscales (Supplementary Material 1).

Nutritional status—No significant differences in MNA nor dietary habits emerged among groups (Supplementary Material 2).

Hematochemical parameters—Overall, regardless of the Fit_AA group, all participants displayed hematochemical values and scores falling within the physiological ranges. ANOVA showed a significant difference among groups in WBC, HGB, hematocrit, EOS (%), LUC, serum creatinine, cPR, and Il-6. Bonferroni-adjusted pairwise comparisons detected a significant difference between the accelerated and decelerated groups in WBC (lower count in decelerated agers), hematocrit (higher in decelerated agers) and EOS (lower count in decelerated agers), LUC (lower count in decelerated agers), HGB (higher concentration in decelerated agers), serum creatinine (higher concentration in decelerated agers), IL-6 (higher concentration in decelerated agers), and CRP (higher concentration in accelerated agers). All these results and their statistics are detailed in Table 5. No other significant differences were detected in the other hematochemical parameters (Supplementary Material 3).

Discussion

The main objective of the present study was to compare three groups of healthy adults aged

Table 1 Main anthropometric, clinical, and sociodemographic characteristics of the participants

Variable			FitAA status			
			Total (*n* = 176)	Decelerated (*n* = 43)	Normal (*n* = 97)	Accelerated (*n* = 36)
Sex	Women		*n* = 104	*n* = 19	*n* = 63	*n* = 22
	Men		*n* = 72	*n* = 24	*n* = 34	*n* = 14
Age (*years*)	**Whole sample**		**66.48 ± 7.77**	**68.14 ± 5.62**	**65.84 ± 7.40**	**65.47 ± 10.14**
	Women		66.97 ± 7.46	68.42 ± 5.71	66.48 ± 6.74	66.0 ± 9.79
	Men		65.74 ± 8.2	67.92 ± 5.66	64.65 ± 8.47	64.5 ± 11.13
Fitness age (*years*)	**Whole sample**		**66.33 ± 4.57**	**63.59 ± 2.36**	**66.12 ± 3.70**	**70.40 ± 5.98**
	Women		66.73 ± 4.41	63.56 ± 2.37	66.35 ± 3.48	70.55 ± 5.49
	Men		65.74 ± 4.77	63.62 ± 2.4	65.7 ± 4.10	70.11 ± 7.04
Fitness age acceleration (*years*)	**Whole sample**		**0.01 ± 3.04**	**− 3.54 ± 0.92**	**+ 0.01 ± 1.40**	**+ 4.44 ± 2.27**
	Women		+ 0.22 ± 2.96	− 3.70 ± 1.03	− 0.05 ± 1.41	+ 4.37 ± 2.0
	Men		− 0.33 ± 3.17	− 3.42 ± 0.83	+ 0.12 ± 1.39	+ 4.59 ± 2.78
Weight (*kg*)	**Whole sample**		**69.44 ± 13.29**	**68.76 ± 13.24**	**68.78 ± 13.74**	**72.40 ± 12.30**
	Women		63.55 ± 10.41	58.32 ± 8.36	63.29 ± 10.16	68.67 ± 11.10
	Men		78.35 ± 12.22	77.02 ± 10.22	78.96 ± 13.81	79.25 ± 11.83
Height (*m*)	**Whole sample**		**1.595 ± 0.011**	**1.623 ± 0.095**	**1.585 ± 0.120**	**1.597 ± 0.010**
	Women		1.530 ± 0.09	1.537 ± 0.046	1.531 ± 0.106	1.535 ± 0.05
	Men		1.692 ± 0.072	1.690 ± 0.064	1.686 ± 0.08	1.711 ± 0.066
Body mass index (*kg/m²*)	**Whole sample**		**26.97 ± 3.95**	**25.93 ± 3.29**	**26.81 ± 4.11**	**28.36 ± 3.72**
	Women		26.77 ± 4.17	24.7 ± 3.57	26.4 ± 4.06	29.01 ± 3.65
	Men		27.27 ± 3.61	26.9 ± 2.75	27.57 ± 4.15	27.15 ± 3.68
Blood pressure—diastolic (*mmHg*)	**Whole sample**		**82.49 ± 8.83**	**80.93 ± 8.54**	**82.48 ± 8.07**	**84.18 ± 11.08**
	Women		81.34 ± 8.93	78.42 ± 8.0	81.49 ± 8.2	82.86 ± 11.46
	Men		84.23 ± 8.43	82.92 ± 8.59	84.32 ± 7.6	86.58 ± 10.41
Blood pressure—systolic (*mmHg*)	**Whole sample**		**130.76 ± 15.08**	**132.81 ± 13.74**	**130.44 ± 15.72**	**129.26 ± 15.28**
	Women		128.66 ± 15.39	128.47 ± 13.18	128.89 ± 15.68	128.32 ± 17.38
	Men		133.93 ± 14.12	136.25 ± 13.45	133.32 ± 15.61	131.0 ± 10.88
Polypharmacy	**Whole sample**		**1 (0.14)**	**1 (0.26)**	**1 (0.17)**	**1.50 (0.39)**
	Women		2 (0.18)	1 (0.43)	2 (0.22)	2 (0.44)
	Men		1 (0.21)	2 (0.30)	1 (0.24)	1 (0.78)
Smoking status	*Smoking*	**Whole sample**	**14.53% (25)**	**24%**	**52%**	**24%**
		Women	72% (18)	100.0%	76.92%	33.33%
		Men	28% (7)	0.0%	23.8%	66.66%
	Not smoking	**Whole sample**	**85.47%**	**25.17%**	**54.42%**	**19.05%**
		Women	57.82%	35.14%	62.5%	71.43%
		Men	42.17%	64.86%	37.5%	28.57%
	Never smoked	**Whole sample**	**65.99%**	**19.59%**	**57.73%**	**21.65%**
		Women	58.76%	12.28%	59.65%	26.32%
		Men	41.24%	30.0%	55.0%	15.0%
	Have smoked	**Whole sample**	**43.5%**	**32.4%**	**50.0%**	**17.6%**
		Women	56.0%	21.43%	57.14%	17.86%
		Men	44.0%	54.55%	36.36%	9.09%

Table 1 (continued)

Variable			FitAA status			
			Total (*n*=176)	Decelerated (*n*=43)	Normal (*n*=97)	Accelerated (*n*=36)
Educational level	*Primary school*	**Whole sample**	**5.3%**	**23.53%**	**46.47%**	**24.7%**
		Women	66.66%	75.0%	75.0%	50.0%
		Men	33.33%	25.0%	25.0%	50.0%
	Middle school	**Whole sample**	**23.8%**	**17.5%**	**52.5%**	**30.0%**
		Women	70.0%	28.57%	71.43%	91.66%
		Men	30.0%	71.43%	28.57%	8.33%
	High school	**Whole sample**	**46.4%**	**21.8%**	**60.3%**	**17.9%**
		Women	55.7%	35.29%	61.7%	57.14%
		Men	44.3%	64.71%	38.3%	42.9%
	Tertiary +	**Whole sample**	**25.0%**	**33.3%**	**52.4**	**14.3%**
		Women	59.5%	57.14%	68.18%	33.33%
		Men	40.48%	42.86%	31.82%	66.66%

All data are presented as mean ± standard deviation, except for *Sex, Polypharmacy, and smoking and educational levels*, shown as count (*n*), median and (standard error), percentage, respectively

Abbreviations: kg kilograms, *m* meters, *mmHg* millimeters of mercury

Table 2 Correlation matrix of FitAge and BMI with health risk scores

Variables	FRS	ACCAHA	Stern diabetes risk	MESA	CCI	Mortality score
FitAge	**.444*****	**.672*****	.181	**.439*****	**.504*****	**.660*****
BMI	**.293*****	**.337*****	**.440*****	**.287*****	.117	**.224****

Abbreviations: FRS Framingham risk score coronary heart disease, *ACCAHA* American College of Cardiology and American Heart Association cardiovascular diseases risk score, *MESA* Multi-Ethnic Study of Atherosclerosis risk score, *CCI* Charlson Comorbidity Index, *FitAge* fitness age, *BMI* body mass index

* Significant for *p*<0.05

** Significant for *p*<0.005

*** Significant for *p*<0.001

51–80 years according to their aging trajectory, *i.e.*, decelerated, normal, or accelerated aging over a range of selected health domains. This categorization based on FitAA was introduced in a previous work from the same laboratory where performance in a comprehensive set of motor tests was found to predict biological age in a meaningful and accurate manner [10]. The three groups were contrasted over a range of domains including body composition, hematochemical parameters, cognitive performance, mood, sleep, global health, physical functioning, and nutritional status.

The first finding of this study was that the proposed categorization could differentiate decelerated from accelerated aging, with these trajectories corresponding to significant differences in body composition and hematochemical profile. Indeed, even though all participants were healthy and fell within the physiological range for the variables assessed, older adults with decelerated aging trajectories exhibited significant, positively oriented differences from accelerated agers in almost all the domains scrutinized (e.g., lower cRP; higher HGB, etc.). Conversely, categorization in normoweight, overweight, and obese participants,

Table 3 Significant group differences in body composition domain according to FitAA status and sex

Variable		FitAA status				Statistics		Post hoc multiple comparisons
		Total (n=176)	Decelerated (n=43)	Normal (n=97)	Accelerated (n=36)	FitAA main effect		
						F value	p value	
BCM (%)	**Whole sample**	**38.35±5.36**	**41.61±4.95**	**37.71±5.16**	**35.97±4.62**	13.641	<0.0005	Normal<Decel p<0.0005
	Women	35.5±3.96	37.98±4.01	35.37±3.77	33.7±3.48	6.357	0.002	Accel<Decel p<0.0005
	Men	42.64±4.23	44.48±3.56	42.03±4.59	4.52±2.92	4.797	0.009	-
	FitAA*Sex					0.016	0.98	
FM (%)	**Whole sample**	**27.13±7.93**	**22.92±7.65**	**27.85±7.4**	**30.46±7.76**	10.403	<0.0005	Normal<Decel p=0.001
	Women	31.02±6.67	27.89±8.08	30.97±6.11	34.39±5.29	6.907	0.001	Accel<Decel p<0.0005
	Men	21.25±5.77	19.46±5.25	22.07±6.01	22.61±5.65	1.612	0.20	-
	FitAA*Sex					0.949	0.39	
FFM (%)	**Whole sample**	**72.87±7.93**	**77.08±7.65**	**72.15±7.4**	**69.54±7.76**	10.403	<0.0005	Normal<Decel p=0.001
	Women	68.98±6.67	72.71±8.08	69.03±6.11	65.61±5.29	6.907	0.001	Accel<Decel p<0.0005
	Men	78.75±5.77	80.54±5.25	77.93±6.01	77.39±5.65	1.612	0.203	-
	FitAA*Sex					0.949	0.389	
TBW (%)	**Whole sample**	**53.47±5.86**	**56.49±5.73**	**52.93±5.40**	**51.09±5.93**	9.709	<0.0005	Normal<Decel p=0.002
	Women	50.49±4.84	53.13±6.00	50.54±4.40	48.08±3.82	6.610	0.002	Accel<Decel p<0.0005
	Men	57.94±4.19	59.15±3.9	57.36±4.17	57.13±4.66	1.357	0.260	-
	FitAA*Sex					1.060	0.35	
ECW (%)	**Whole sample**	**46.84±3.02**	**45.55±2.29**	**47.15±3.17**	**47.6±3.01**	5.745	0.004	Normal<Decel p=0.010
	Women	47.84±2.55	47.04±1.73	48.05±2.71	47.95±2.63	1.012	0.366	Accel<Decel p=0.009
	Men	45.33±3.07	44.37±1.98	45.49±3.32	46.89±3.68	3.327	0.038	-
	FitAA*Sex					0.951	0.39	
ICW (%)	**Whole sample**	**53.16±3.02**	**54.45±2.29**	**52.85±3.17**	**52.40±3.01**	5.745	0.004	Normal<Decel p=0.010
	Women	52.16±2.55	52.96±1.73	51.95±2.71	52.05±2.63	1.012	0.366	Accel<Decel p=0.009
	Men	54.67±.3.07	55.63±1.98	54.51±3.32	53.11±3.68	3.327	0.038	-
	FitAA*Sex					0.951	0.389	

Table 3 (continued)

Variable		FitAA status				Statistics		Post hoc multiple comparisons
		Total (n=176)	Decelerated (n=43)	Normal (n=97)	Accelerated (n=36)	FitAA main effect		
						F value	p value	
ECW/ICW ratio	**Whole sample**	**0.89±0.11**	**0.84±0.78**	**0.9±0.12**	**0.91±0.11**	5.852	0.003	Normal<Decel p=0.009 Accel<Decel p=0.009
	Women	0.92±0.1	0.80±0.65	0.93±0.10	0.93±0.1	1.171	0.313	
	Men	0.84±0.11	0.84±0.08	0.84±0.12	0.89±0.14	3.276	0.04	
	FitAA*Sex					0.890	0.412	
ICW/FFM ratio	**Whole sample**	**0.74±0.08**	**0.71±0.07**	**0.74±0.08**	**0.76±0.09**	3.946	0.021	Accel<Decel p=0.018
	Women	0.76±0.08	0.74±0.08	0.76±0.08	0.8±0.07	4.017	0.2	
	Men	0.7±0.06	0.69±0.05	0.70±0.06	0.69±0.08	0.183	0.833	
	FitAA*Sex					1.870	0.16	
SMM (%)	**Whole sample**	**32.36±6.41**	**35.25±6.58**	**31.72±6.03**	**30.51±6.23**	6.639	0.002	Normal<Decel p=0.007 Accel<Decel p=0.004
	Women	28.27±3.83	29.82±5.18	28.28±3.41	26.88±3.25	2.876	0.059	
	Men	38.54±4.13	39.54±3.8	38.1±4.39	37.75±3.95	1.233	0.294	
	FitAA*Sex					0.229	0.8	
ASMM (%)	**Whole sample**	**26.53±3.29**	**28.01±3.10**	**26.2±3.2**	**25.59±3.29**	6.606	0.002	Normal<Decel p=0.007 Accel<Decel p=0.004
	Women	24.53±2.04	25.59±2.26	24.51±1.97	23.69±1.7	3.925	0.022	
	Men	29.54±2.41	29.93±2.20	29.32±2.65	29.38±2.19	0.592	0.554	
	FitAA*Sex					0.845	0.43	

Table 4 Significant group differences in health-related outcomes according to FitAA status and sex

Variable		FitAA status				Statistics		
		Total ($n=176$)	Decelerated ($n=43$)	Normal ($n=97$)	Accelerated ($n=36$)	FitAA main effect		Post hoc multiple comparisons
						F value	p value	
PSQI	**Whole sample**	**8.08 ± 3.48**	**7.35 ± 3.11**	**7.96 ± 3.54**	**9.32 ± 3.51**	3.270	0.04	Decel < Accel $p=0.039$
	Women	8.93 ± 3.70	8.10 ± 3.63	8.59 ± 3.65	10.63 ± 3.53	3.911	0.022	
	Men	6.78 ± 2.64	6.75 ± 2.54	6.76 ± 3.0	6.92 ± 1.88	0.012	0.988	
	FitAA*Sex					1.288	0.28	
SF-36 Physical Functioning	**Whole sample**	**88.99 ± 11.09**	**94.07 ± 7.43**	**87.60 ± 11.03**	**86.47 ± 13.29**	6.535	0.002	Decel > Normal $p=0.004$ Decel > Accel $p=0.007$
	Women	87.36 ± 11.74	95.0 ± 8.16	85.87 ± 11.02	85.0 ± 13.97	6.049	0.003	
	Men	91.45 ± 9.59	93.33 ± 6.70	90.91 ± 10.42	89.17 ± 12.03	0.694	0.501	
	FitAA*Sex					1.460	0.23	
SF-36 Vitality	**Whole sample**	**65.29 ± 16.13**	**70.23 ± 15.88**	**63.13 ± 15.48**	**65.15 ± 17.3**	2.951	0.055	Normal < Decel $p=0.049$
	Women	63.94 ± 16.03	69.74 ± 12.96	62.30 ± 16.09	63.64 ± 17.74	1.574	0.21	
	Men	67.32 ± 16.17	70.63 ± 18.14	64.7 ± 14.36	67.92 ± 16.85	0.959	0.385	
	FitAA*Sex					0.10	0.90	
SF-36 Social Functioning	**Whole sample**	**80.36 ± 19.59**	**88.08 ± 15.66**	**76.98 ± 21.33**	**80.15 ± 16.32**	4.991	0.008	Normal < Decel $p=0.006$
	Women	77.67 ± 21.8	86.18 ± 20.37	74.25 ± 22.89	80.11 ± 17.95	3.08	0.049	
	Men	84.42 ± 14.91	88.08 ± 15.66	82.2 ± 17.13	80.21 ± 13.54	1.394	0.251	
	FitAA*Sex					0.549	0.579	

All data are scores, which are shown as mean ± standard deviation

Abbreviations: FitAA Fitness Age Acceleration*, PSQI* Pittsburgh Sleep Quality Index*, SF-36* 36-Item Short Form Survey

as estimated by BMI, was not fully capable to differentiate healthy from unhealthy patterns. While BMI scores associated with risk scores for developing major health conditions including type-2 diabetes, and cardiovascular diseases, this estimate was outperformed by the newly introduced categorization based on FitAge. Importantly, FitAge had similar capabilities of differentiating decelerated from accelerated agers in women and men, as suggested by the absence of significant interactions with sex and in spite of the observed differences in most of the domains here evaluated.

Health-related domain

In our cohort the quality of sleep assessed by PSQI proved poor, with scores exceeding the cutoff of 7 points in all groups, which indicates disturbed sleep [28]. However, significantly lower scores were detected for decelerated compared to accelerated agers, in line with the established knowledge that being physically active improves sleep quality in middle-aged and older adults [29–31]. While decelerated and normal agers displayed a significantly lower BMI (25.9 and 26.3, respectively) than accelerated agers (28.4), all groups fell within the overweight range. Some potential underlying mechanisms that link poor sleep to overweight/obesity and vice versa have been previously described. Poor quality and quantity of sleep lead to altered metabolic and endocrine function [32], for instance in the stress response system as well as in the secretion of hormones such as ghrelin and leptin, which play important roles in appetite regulation and influence food choice and caloric intake [33]. In our study, however, ghrelin was not measured, and leptin levels were comparable among groups, and not

Table 5 Significant group differences in hematochemical parameters according to FitAA status and sex

Variable		FitAA status				Statistics		
		Total (n=176)	Decelerated (n=43)	Normal (n=97)	Accelerated (n=36)	FitAA main effect		Post hoc multiple comparisons
						F value	p value	
WBC (×10^3/ µL)	**Whole sample**	**5.95 ± 1.41**	**5.73 ± 1.34**	**5.86 ± 1.18**	**6.48 ± 1.91**	3.161	0.045	Accel < Decel p = 0.021
	Women	5.64 ± 1.19	5.34 ± 1.42	5.63 ± 0.98	5.95 ± 1.48	1.051	0.352	
	Men	6.41 ± 1.58	6.05 ± 1.21	6.3 ± 1.4	7.42 ± 2.27	4.407	0.014	
	FitAA*Sex					1.09	0.339	-
HGB (g/dL)	**Whole sample**	**13.95 ± 1.34**	**14.46 ± 1.27**	**13.76 ± 1.3**	**13.86 ± 1.4**	4.291	0.015	Normal < Decel p = 0.013
	Women	13.45 ± 1.11	13.7 ± 0.96	13.45 ± 1.17	13.22 ± 1.03	0.823	0.441	
	Men	14.72 ± 1.29	15.09 ± 1.15	14.35 ± 1.34	14.98 ± 1.28	3.013	0.052	
	FitAA*Sex					1.707	0.18	-
HCT (%)	**Whole sample**	**43.56 ± 4.66**	**45.17 ± 3.31**	**43.28 ± 3.5**	**42.28 ± 7.68**	4.061	0.019	Accel < Decel p = 0.022
	Women	41.91 ± 4.81	42.75 ± 2.54	42.24 ± 3.18	40.19 ± 8.68	2.372	0.097	
	Men	46.04 ± 3.09	47.18 ± 2.4	45.27 ± 3.24	45.96 ± 3.41	1.418	0.245	
	FitAA*Sex					1.318	0.27	-
EOS (%)	**Whole sample**	**3.00 ± 1.66**	**2.60 ± 1.12**	**2.99 ± 1.57**	**3.54 ± 2.30**	2.982	0.053	Decel < Accel p = 0.047
	Women	2.86 ± 1.67	2.27 ± 1.01	2.92 ± 1.56	3.2 ± 2.29	1.710	0.184	
	Men	3.22 ± 1.64	2.87 ± 1.15	3.14 ± 1.59	4.12 ± 2.3	2.383	0.095	
	FitAA*Sex					0.577	0.56	-
EOS (×10^3/ µL)	**Whole sample**	**0.19 ± 0.14**	**0.16 ± 0.08**	**0.18 ± 0.12**	**0.26 ± 0.21**	5.181	0.007	Decel < Normal p = 0.007
	Women	0.17 ± 0.12	0.13 ± 0.07	0.16 ± 0.08	0.22 ± 0.20	2.661	0.073	
	Men	0.23 ± 0.16	0.18 ± 0.08	0.23 ± 0.17	0.32 ± 0.21	3.992	0.02	
	FitAA*Sex					0.217	0.81	-
LUC (×10^3/ µL)	**Whole sample**	**0.14 ± 0.09**	**0.12 ± 0.04**	**0.13 ± 0.05**	**0.18 ± 0.18**	4.552	0.012	Decel < Accel p = 0.024 Normal < Accel p = 0.018
	Women	0.13 ± 0.1	0.11 ± 0.03	0.12 ± 0.05	0.17 ± 0.2	2.955	0.055	
	Men	0.15 ± 0.08	0.13 ± 0.05	0.14 ± 0.06	0.19 ± 0.14	1.831	0.164	
	FitAA*Sex					0.019	0.98	-
Creatinine (mg/dL)	**Whole sample**	**0.85 ± 0.15**	**0.93 ± 0.13**	**0.82 ± 0.14**	**0.84 ± 0.14**	5.243	0.007	Normal < Decel p = 0.006
	Women	0.77 ± 0.07	0.8 ± 0.05	0.77 ± 0.08	0.75 ± 0.06	0.462	0.632	
	Men	0.97 ± 0.14	0.99 ± 0.11	0.94 ± 0.18	0.97 ± 0.13	0.673	0.513	
	FitAA*Sex					0.275	0.76	-
CRP (mg/dL)	**Whole sample**	**0.38 ± 0.16**	**0.37 ± 0.14**	**0.36 ± 0.14**	**0.45 ± 0.20**	3.311	0.039	Normal < Accel p = 0.035
	Women	0.39 ± 0.16	0.39 ± 0.16	0.37 ± 0.14	0.44 ± 0.23	2.937	0.056	
	Men	0.37 ± 0.14	0.37 ± 0.14	0.34 ± 0.13	0.45 ± 0.17	0.169	0.845	
	FitAA*Sex					0.672	0.01	-
IL-6 (ng/mL)	**Whole sample**	**733.29 ± 269.86**	**819.16 ± 287.58**	**686.14 ± 238.57**	**755.52 ± 305.60**	3.762	0.025	Normal < Decel p = 0.024
	Women	693.66 ± 247.58	707.40 ± 236.53	698.71 ± 250.48	666.784 ± 258.77	0.153	0.858	
	Men	792.44 ± 292.04	911.40 ± 297.77	662.56 ± 216.30	910.81 ± 329.54	7.816	0.001	
	FitAA*Sex					4.891	0.01	-

All data are shown as mean ± standard deviation

Abbreviations: FitAA Fitness Age Acceleration, *WBC* white blood cells, *Hgb* hemoglobin, *HCT* hematocrit test, *EOS* eosinophils, *LUC* large unstained cells, *CRP* C-reactive protein, *IL-6* interleukin 6, *µL* microliters, *mg/dL* milligrams/deciliters, *ng/mL* nanograms/milliliters

associated with poor sleep patterns. It has also been proposed that insufficient sleep, sleep disturbances, and the consequent daytime sleepiness and fatigue may lead to reduced physical activity [33]. Overall, these influences can lead to an imbalance between energy intake and expenditure potentially promoting weight gain.

Regarding self-reported quality of life assessed by the SF-36 questionnaire, decelerated agers displayed significantly higher scores, i.e., higher quality of life, of physical and social functioning. When checking the observed scores against the largest normative dataset available [34], physical functioning of our total cohort was approximately 28% higher, raising to a 36% difference for decelerated agers. Conversely, for the social functioning sub-score, both normal and accelerated agers were below the normative value, whereas decelerated agers showed a 9% higher score.

Body composition

It is well-established that aging is associated to changes in body composition including increased FM and decreased lean mass [35, 36]. Participants in the decelerated aging group likely counteracted such process as they displayed significantly less FM and more SMM than accelerated or normal agers. The relationship between body composition and frailty has been mainly related to higher levels of inflammation [37] and impairment of muscle quality due to fat infiltration [38]. These two factors have been demonstrated to interact, jointly contributing to the development of insulin and catecholamine resistance, eventually reducing metabolic flexibility, i.e., the cellular capacity to switch freely between oxidative substrates in response to nutritional and physiological cues [37, 39]. Our data also revealed that decelerated agers displayed more favorable cellular water compartmentalization, as shown by the significantly lower ECW, higher ICW, and, consequently, lower ECW/ICW ratio. ICW reflects muscle cell mass, whereas ECW represents the sum of interstitial fluid and blood plasma in the extracellular space [40]. An increase in the ECW/ICW ratio, as detected in our normal and accelerated agers, indicates a relative increase in non-contractile tissue, which has been linked to reduced muscle strength and functional capacity, eventually leading to dynapenia and sarcopenia [41, 42]. This ratio, which is now recognized as a valid predictor of

global health and functional status, being the lower the better [41, 43], stood out in our cohort as a highly sensitive parameter for distinguishing decelerated from accelerated agers, and decelerated from normal agers, as well.

Hematochemical domain

While all participants displayed values and scores falling within the physiological range, significant differences emerged for several hematochemical parameters. Decelerated agers displayed significantly higher HGB content and hematocrit. Such findings were expected since these are well-known adaptations for increasing oxygen-carrying capacity that are associated to a physically active lifestyle [44]. The possible underlying mechanisms are proposed to come mainly from bone marrow, including stimulated erythropoiesis with hyperplasia of the hematopoietic bone marrow, improvement of the hematopoietic microenvironment, and hormone- and cytokine-accelerated erythropoiesis induced by cardiovascular exercise training [45].

While WBC count was within the normality intervals (4500–11,000 WBC per microliter) for all three groups, the number of leukocytes was significantly higher among accelerated agers. Even though a 10–13% increase is not suggestive per se of health issues, the results of the Baltimore Longitudinal Study of Aging [46] showed a significant linear increase on the WBC vs. mortality curve in all-causes and cardiovascular mortality, starting from WBC count > 3500/μL. In that study the threshold for increased risk was identified for WBC count $\geq$ 6000/mm^3. Four categories were thus identified ($\leq$ 3500; 3501–6000; 6001–10,000; $\geq$ 10,000). Based on this categorization, the three groups of our cohort of healthy aged adults polarized into "low risk" (decelerated agers: 5729 $\pm$ 1340; normal agers: 5859 $\pm$ 1180) and "moderate risk" (accelerated agers: 6484 $\pm$ 1910).

Even though within normality values for all groups (< 5%), eosinophil fraction was found significantly higher in accelerated compared to normal and decelerated agers, suggesting that a subclinical state of low-grade chronic inflammation was present. The same pattern applied to CRP levels, which were found elevated in accelerated agers by 19–23% compared to decelerated and normal groups, respectively. CRP participates in the systemic response to

inflammation, and its levels are known to be proportional to cardiovascular and all-causes mortality risks [47]. Also, LUC count was found to differ according to the aging trajectory, with decelerated agers showing a lower count than normal and accelerated agers. LUC are large-sized peroxidase-negative blood cells that tend to rise in number when markers of inflammation in the body increase, i.e., when lymphocytes or monocytes are activated [48]. The same lymphocytes, when activated, are enlarged in size, and can be considered LUC. Overall, the findings of lower LUC counts and other inflammatory markers in decelerated agers are in line with the results of a large report of 51,623 participants from the US National Health and Nutrition Examination Survey, which showed that the rise of inflammatory scores contributes to accelerated aging and multi-morbidity [49].

Decelerated agers presented significantly higher mean serum creatinine. Creatinine is an endogenous substance generated from the nonenzymatic conversion of creatine and creatine phosphate, 95% of which is found in muscles [50]. While serum creatinine is commonly used to estimate glomerular filtration rate and monitor kidney functioning, elevated levels are also found in healthy individuals with increased muscle mass or high meat consumption [51]. Due to the correlation between its levels and muscle mass, serum creatinine has long been used as a surrogate of muscle mass measurements [52, 53]. Creatinine generation is low among individuals who have reduced muscle mass, either constitutionally or disease related. Hence, low creatinine level could be regarded as a proxy of muscle mass and protein-energy wasting, which is a relevant issue in older adults due to the increasing prevalence of sarcopenia and dynapenia beginning from the fifth decade of life.

IL-6 was found significantly higher in decelerated agers. While IL-6 has context-dependent pro- and anti-inflammatory properties [54], the view in the context of exercise is that it has primarily anti-inflammatory effects [55]. IL-6 directly inhibits the expression of TNF-α and IL-1β and is a potent inducer of the interleukin-1 receptor antagonist, which exerts anti-inflammatory activity by blocking IL-1 receptors and thereby preventing signal-transduction of the pro-inflammatory IL-1 [55]. Unlike IL-1β and TNF-α, IL-6 does not upregulate major inflammatory mediators such as nitric oxide or matrix metalloproteinases. Rather, IL-6 appears to be the primary inducer of the hepatocyte-derived acute-phase proteins, many of which have anti-inflammatory properties. Skeletal muscles appear to be a key organ in IL-6 production. Research has demonstrated that IL-6 is produced by contracting skeletal muscles [56] and is released to the circulation in large quantities, being subjected to a very high turnover during muscular exercise [57].

Aging trajectories and health risk scores

While also BMI was significantly associated with major health risk scores, FitAge emerged as a stronger predictor for most of the calculated risks (5 out of 6), in line with the now widely accepted notion that higher fitness is associated with better health outcomes [8]. Substantial evidence during the last two decades indicates that PF status markedly alters the relationship between body fatness and subsequent major health outcomes [58]. Although clearly excess body weight and low PF are synergistically associated with worse CVD risk factors and increased prevalence of CVD, compared to normal weight-fit individuals, unfit individuals have a twofold higher risk of mortality regardless of BMI. Overweight-fit and obese-fit individuals have a similar mortality risk as normal weight individuals [11], suggesting that researchers, clinicians, and public health officials, while not discarding BMI, should devote more attention to physical activity and fitness-based interventions rather than narrowing strategies to weight-loss driven approaches to reduce CVD and mortality risk.

Study limitations

This work is not free of limitations. First, cross-sectional designs like the one here employed do not allow to draw causal inferences as they are devised to test potential associations rather than conclusively establishing cause-and-effect relationships. Future studies with a longitudinal observation will have to confirm whether the observed associations hold a clinical meaning. Second, we did not set the study focus only on older adults as also middle-aged adults transitioning into senescence were included. Third, data from a sample of 176 Italian participants from one single center may limit the generalizability and external validity of our findings, thus warranting additional research to explore whether the study findings hold true across a broader population

spectrum in terms of more diverse age groups and demographics. Additionally, our aging trajectories were determined by relying mainly on the fitness levels displayed by the participants. Further studies should improve the accuracy and ecological validity of our novel biomarker—the FitAA—by including age accelerators derived from other relevant domains, such as cognitive, metabolic, and hematochemical ones, as well as by validating it against established descriptors of physical fitness such as maximal oxygen uptake and muscle strength. Finally, while a strength of the present study resides in the profiling of cellular senescence for muscle mass and other cells in the body based on fitness status, genetic and molecular analyses could more adequately allow to validate FitAA as well as test experimentally the mechanisms underpinning the multi-level aging process.

Conclusions

The newly introduced categorization based on Fit-Age acceleration of biological aging was able to differentiate decelerated from normal and accelerated agers in terms of health risk profile and several physiological domains. In particular, body composition and hematochemical parameters describing immune system activation and inflammation markers emerged as those variables flagging the largest differences between decelerated and accelerated aging trajectories. FitAA, which is a resource efficient index based on simple and cost-effective motor tests that combines different types of performance, all functionally relevant to the aging person (walking ability, dynamic balance, executive functions, muscle strength, and cardiorespiratory fitness, may represent a promising tool for monitoring agers over time, potentially allowing early detection of even subtle motor-functional deteriorations. Prospectively, if interventions are administered to manage age-related functional decline, FitAA could also serve as a relevant endpoint of treatment to evaluate the effectiveness of the proposed therapeutic approaches.

Declarations

Ethical approval The Institutional Review Board and the Clinical Research Ethics Committee approved all procedures involving human subjects (ID: PG/2020/16846). All study procedures were carried out in accordance with the Declaration of Helsinki.

Consent to participate Written informed consent was obtained from each participant before inclusion and participation in the tests.

Conflict of interest The author declare no competing interests.

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