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Research Article

Physical fitness and frailty index in developing biological age prediction model

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Abstract

The global increase in the older population has resulted in escalating healthcare costs and burdens on governments and families. Understanding biological age (BA) as distinct from chronological age (CA) holds significant potential in accurately assessing individuals' health status and susceptibility to diseases. During exercise, myokines like irisin and lactate are released from skeletal muscles, facilitating cross-talk with organs such as the brain and heart. This may improve physical fitness, reducing frailty and BA. This research aimed to develop a comprehensive BA prediction model integrating genetic and epigenetic factors. The study involved 59 healthy adults, comprising 31 males and 28 females, with average ages of 58.2 ± 7 years and 50.1 ± 8.5 years, respectively. Assessments of physical fitness and completion of the Frailty Index (FI34) questionnaire were conducted to capture genetic and epigenetic influences. Feature selection, principal component analysis (PCA), and multiple linear regression (MLR) were employed to tailor BA prediction models for each gender. We identified seven significant biomarkers for males, including FI34, percent of skeletal muscle mass (SM), handgrip strength (GS), flexibility via sit-and-reach test (SR), peak torque of quadriceps muscles (PTQ), cardiopulmonary fitness (VO_{2max}), and basal metabolic rate (BMR). Conversely, females exhibited six key biomarkers: FI34, SM, GS, waist-to-hip ratio (WHR), peak torque of hamstring muscles (PTH), and percentage of body fat (PBF). We have successfully developed a comprehensive model for estimating BA by integrating key biomarkers representing epigenetic and genetic impacts. Estimating BA is crucial for precise health evaluations and disease risk assessments.

Key Words: **Healthy aging, Biological age, Lifestyle, Exercise physiology, Epigenetic factors**

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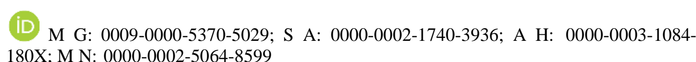
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Introduction

The older population in the world is growing rapidly due to low birth rates, increasing life expectancy, improvement in healthcare conditions, and reduction in mortality rates in recent years. The number of people aged 65 and older is projected to rise dramatically worldwide in the next several decades, from 730 million in 2020 to nearly two billion by 2060. Of that 1.25 billion increase, about 800 million (or 64 percent) is expected to occur in Asia (He et al., 2022). In the US, this statistic will rise from 58 million in 2022 to 82 million by 2050 (a 47% increase), and the 65-and-older age group's share of the total population is projected to rise from 17% to 23% (U.S.CensusBureau., 2023), and in Europe, the median age will increase from 37.7 years old in 2003 to 52.3 years old by 2050 (Rogers et al., 2015).

Aging is characterized by a progressive decline in organ function, physical function as well as the body's ability to withstand damage and stress (Kim et al., 2013; Maffei et al., 2017). The way individual's age is crucial for healthy aging, independence, disease prevention, and reducing healthcare costs for governments and families. Population aging is linked to higher healthcare spending, with about 20% of the growth expected to be due to aging by 2025 (Chen et al., 2023). In China, for instance, healthcare expenditure rose by almost 10.47 times from 2000 to 2017. In Beijing, medical expenses for older adults are expected to exceed 66.7% by 2030 (Chen et al., 2023). In the United States, healthcare spending for older persons increased six times from 1978 to 2018. In high-income countries like those in Europe, the increase in healthcare costs for the older population is not significant (Chen et al., 2023). This shows that healthy, active older people do not impose a significant financial burden on themselves or governments, exemplifying healthy aging.

The World Health Organization (WHO) defines "healthy aging" as a process of maintaining the functional ability to promote well-being in older age (Rudnicka et al., 2020), and this increases disparities between chronological and biological age.

Chronological age (CA) measures the time elapsed since birth and provides a framework to estimate normal developmental phases or rates (Jee et al., 2012). Genetic and epigenetic factors cause people of the same CA to age differently, complicating accurate assessment of their health (Zhang et al., 2024). On the other hand, biological age (BA) is an objective assessment of the decremented progression of biological or physiological vitality and incremental vulnerability to morbidity and mortality concerning CA (Jee et al., 2012). It serves to track the trajectory of damage over time (Jia et al., 2017).

Various methods have been proposed to estimate biological age, such as epigenetic clocks and telomere length measurements (Jylhävä et al., 2017). Since aging processes vary across organs, it's vital to collect diverse biomarkers from multiple systems and integrate them with statistical models to accurately assess overall aging status (Bae et al., 2013). Designing a biological age model is a reliable method for estimating BA (Jee et al., 2012; Jia et al., 2017; Kimura et al., 2012), encompassing a range of biomarkers from different organs. The BA prediction model and biomarkers should mathematically capture these changes and swiftly assess BA to predict future physiological capability (Jee et al., 2012).

Researchers developed a quantitative method to define healthy aging by assessing lifelong physical and functional decline and integrating it into terms like "frailty". A broader view of frailty was introduced with the frailty index (FI), reflecting the ratio of accumulated deficits across various biomarkers (Mitnitski et al., 2001). Kim et al., 2013; developed FI34 with 34 health-related variables as a compound phenotype, including diseases, symptoms, physical abilities, mental and depression state (MMSE & GDS), and self-assessed health. FI34 effectively predicts survival and mortality (Kim et al., 2013).

It has been shown that exercise training and fitness play a crucial role in enhancing longevity and promoting healthy aging in the older population (Fathi et al., 2015). During exercise, skeletal muscles release peptides called myokines, which enter the bloodstream and communicate with distant organs, facilitating crosstalk between muscles and these organs. Physical exercise, via this mechanism, is viewed as a treatment for many diseases, particularly those linked to lifestyle. Hence, it's often referred to as the 'real polypill' (Hashimoto et al., 2021). Studies have demonstrated that systemic vascular function improves significantly after physical exercise, likely due to enhanced blood flow, strain, and shear stress, which lead to calcium influx in hyperpolarized endothelial cells, thereby increasing the production of endothelial nitric oxide synthase (Calverley et al., 2020). Moreover, exercise training has been found to lower low-density lipoprotein, elevate high-density lipoprotein, and enhance insulin sensitivity (Søgaard et al., 2018). Enhancing cerebral blood flow and vasoreactivity throughout life, reduce the risk of stroke-related death and dementia, and improve cognitive function and disassociating the brain's 'biological' from 'chronological' age, reducing the former by up to as much as a decade (Calverley et al., 2020). Exercise-induced molecular interactions alter gene expression regulating endothelial and smooth muscle cell fate, while also influencing the release of key mediators like brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF), which are crucial for neurogenesis, synaptic plasticity, and brain angiogenesis (Calverley et al., 2020).

Irisin, a myokine regulated by muscle contractions with both peripheral and central effects, increases in response to exercise (Inyushkin et al., 2023). It is released into the bloodstream, converting white adipocytes into 'brite' (beige) cells. This increases brown fat, boosts thermogenesis, reduces white fat, and improves body composition in older adults (Wu et al., 2012). Irisin crosses the blood-brain barrier (BBB) and promotes the positive effects of physical activity on brain function. It stimulates BDNF production and neurogenesis in various brain regions, helping prevent and manage Alzheimer's disease in older adults (Inyushkin et al., 2023).

Lactate plays a key role in crosstalk between skeletal muscles and organs like the brain and heart. Lactate, as a metabolic myokine, may elevate neural activity and regulate the cerebrovascular system in response to exercise (Hashimoto et al., 2021). It has been shown that lactate released from skeletal muscles during exercise can serve as a significant fuel source for the heart and contribute to improved heart health (Brooks, 2021). Subsequently, it is demonstrated that higher levels of physical fitness are linked to lower mortality rates (Ahlund et al., 2019). In contrast, it is highlighted that lifestyle-related diseases like hypertension contribute to increased mortality rates in older adults (Qu et al., 2023). Physical fitness biomarkers can measure epigenetic conditions and the ability and functionality of different parts of the body (Jee et al., 2012; Kimura et al., 2012). Conversely, FI34's substantial genetic basis makes it suitable for analyzing healthy aging and longevity (Kim & Jazwinski, 2015), but this index alone may not accurately estimate BA. No study has yet considered both genetic and epigenetic factors, which could enhance the model's comprehensiveness and applications. This study aims was to (1) identify BA biomarkers, (2) integrate the frailty index into the BA model, and (3) develop a comprehensive BA estimation model considering genetic and epigenetic factors.

Materials and Methods

Participants

Healthy adults (31 males and 28 females) volunteered. Males had a mean age of 58.2±7 years (50–72 years), and females 50.1

±8.5 years (39–67 years). Participants aged older than 30 years old, were chosen due to the decline in major organs and physiological functions after this age (Jee et al., 2012). Healthy adults without any cardiovascular diseases, musculoskeletal abnormalities, and clinical disorders, as per the American Colleague of Sports Medicine (ACSM) guidelines (Ferguson, 2014), were included in the study. All the research was approved by the ethical committee of the Iran University of Medical Sciences (Ethical code: IR.IUMS.REC.1400.197) and informed consent was taken from all the participants.

Test items and procedures

Each subject first completed the Frailty Index Questionnaire-34 (FI34) during an interview. Then, they underwent a comprehensive physical fitness session. Figure 1 shows the test items and procedures. Body composition was measured with the Inbody-770 device. Balance was evaluated using a Biodex device. Cardiopulmonary fitness ($VO_{2\ max}$) was assessed via a treadmill test. Flexibility was measured with an electronic sit-and-reach test. Quadriceps and hamstring peak torque were measured using a Biodex device by isokinetic protocol. Finally, handgrip strength was measured with a digital dynamometer.

Frailty index questionnaire-34

During an interview, the 34-item Frailty Index Questionnaire (FI34) was completed for each subject. This questionnaire encompassed various assessments, including (i) records of possible diseases of the individuals (23 items), (ii) uni-lateral sta--nce with eyes open, (iii) ability to perform daily activities independently (3 items), (iv) calculating Body Mass Index (BMI),(v) getting up from a chair without using their hands, (vi) the history of cancer in first-degree relatives, (vii) Geriatric Depression scale Questionnaire (GDS), (viii) measuring systolic blood pressure (SBP) and diastolic blood pressure (DBP), (ix) Mini-Mental State Exam Questionnaire (MMSE) and, (x) Self-rating of health Status (Kim & Jazwinski, 2015).

BMI was calculated using an automatic stadiometer (BSM370) and Inbody-770 (BPM040S12FXX, Korea). Blood pressure was measured with a digital sphygmomanometer (BPB10320, Korea) after 10 minutes of rest, following specific preliminary conditions (see body composition test section). SBP and DBP were measured from the brachial artery of an upper arm. After completing the questionnaire, the scores were summed and divided by 34 for a total score. Scores closer to zero indicated better health, while scores closer to one indicated greater frailty (Kim & Jazwinski, 2015).

Body composition test

Participants followed specific preparation guidelines as outlined by the Inbody protocol for accurate outcomes (McLester et al., 2020). Various metrics, including percent of skeletal muscles (SM), body mass index (BMI), percent of body fat (PBF), basal metabolic rate (BMR), waist-to-hip ratio (WHR), and visceral fat area (VFA) were measured carefully.

Balance test

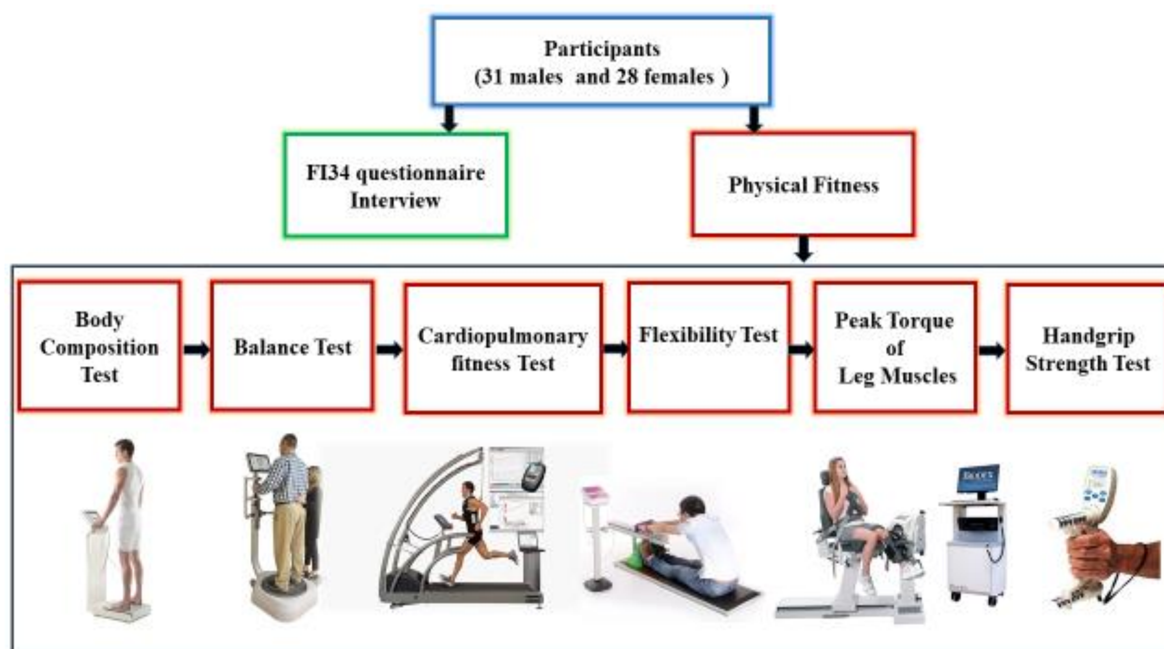

Figure 1. Overview of participants, FI34 frailty index questionnaire-34, and physical fitness assessment procedures.

The balance test used a Biodex Balance System SD (Model 950-302, New York). Participants stood barefoot, and after adjusting foot coordinates, the plates moved to induce imbalance, challenging participants to maintain balance. A lower index indicated better balance (Siddiqi & Masood, 2018).

Cardiopulmonary fitness test

Cardiopulmonary fitness (VO_{2max}) was assessed via a graded treadmill exercise test (h/p/cosmos Pulsar 3p, Germany) using the Bruce protocol, with an endpoint at 85% or greater of the age-predicted maximal heart rate (Loh et al., 2021).

Flexibility test

General flexibility was assessed using the sit-and-reach test (SR). Participants sat with their feet flat against the flexometer, bent forward three times with extended hands, and held the horizontal plate with their fingertips for about two seconds each time. The average of the three distances was recorded (Nokariya et al., 2023).

Peak torque of leg muscles test

Peak torque of leg muscles was measured using a Biodex device (US). After warming up by extending and flexing their dominant knee, participants performed four maximum knee extensions and flexions at 60 deg/sec in an isokinetic protocol. Peak torque of the quadriceps (PTQ) and hamstrings (PTH) was recorded (Tuominen et al., 2023).

Handgrip strength test

Handgrip strength (GS) was measured using a digital dynamometer, testing each hand three times. The mean strength of each hand was calculated, and the higher score was recorded as the dominant hand grip strength (Carson, 2020; Meysami et al., 2023; Zammit et al., 2019). Tests were stopped immediately if there were significant changes in vital signs or a termination request. Data from terminated tests were excluded from further analysis (Jee et al., 2012).

Analysis for biological age prediction model

Figure 2 shows the process of constructing biological age prediction models for males and females. This process involved three major steps: (1) Feature selection, (2) Principal component analysis (PCA), and (3) Model construction using multiple linear regression analysis (MLR).

Feature selection

After gathering features including FI34 and physical fitness metrics (height, weight, SM, BMI, PBF, BMR, WHR, VFA, VO2max, BAL, PTQ, PTH, SR, and GS), we calculated Pearson correlation coefficients (CC) between these 15 features and chronological age (CA) for both females and males. Features with CC > 0.15 and p-values < 0.05 were selected (Jia et al., 2017).

Next, we identified features capturing similar physiological concepts and exhibiting high inter-correlation, indicating redundancy. We prioritized features with high correlation to CA, excluding those with lower correlation from further analysis (Jia et al., 2017).

Principal component analysis (PCA)

Relevant features were selected based on their correlation with

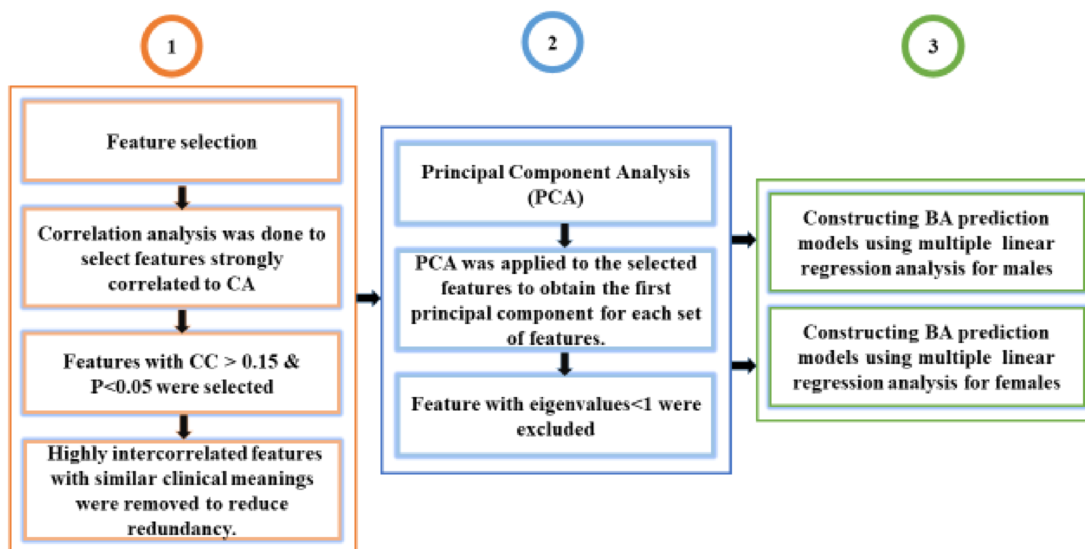

Figure 2. Process of constructing BA prediction models. BA biological age, CA chronological age, CC correlation coefficient, PCA principal component analysis

CA, and PCA was used to derive the first principal components for each set of features. To assess the stability of these components, we evaluated the first principal component by loading and unloading CA values into the PCA. This ensured that the selected features maintained significant factor loadings without CA. Additionally; we analyzed the relationship between the first principal components and CA through factor loading analysis, where high factor loadings indicated strong associations with CA (Jia et al., 2017).

Multiple linear regression analysis for biological age (BA) estimation

We employed the multiple linear regression (MLR) approach for BA estimation. This method involves determining aging features based on their correlation with CA using the following equation:

$$BA_i = Predicted\ CA_i = b_0 + \sum_{j=1}^{m} b_j x_{ji} \qquad (1)$$

Here, BAi represents the BA of certain individuals, and m represents the number of aging biomarkers in the MLR model. Xji (i =1…n, j =1…m) represents the jth biomarker of ith individual and b is the coefficient of each feature. By applying MLR, we aimed to ascertain the impact of each aging feature on BA estimation. This approach enabled the identification of feature that exhibit significant correlations with CA, offering insights into the aging process and its associated physiological changes (Jia et al., 2017).

Results

Results of Feature Selection

From the initial set of 15 biomarkers listed in Table 1, we applied a selection criterion based on correlation coefficient with CA greater than 0.15 and a significance level (p) below 0.05. As a result, we identified 10 biomarkers for males and 10 biomarkers for females. For males, the selected biomarkers were GS, SR, PTH, PTQ, VO_{2max}, BMR, SM, weight, height, and FI34 and for females, the selected biomarkers were GS, PTH, PTQ, BAL, VFA, WHR, PBF, BMI, SM, and FI34 (Table 1).

Table 1. Biomarkers and their correlation coefficient with chronological age

	Males			Females		
Biomarkers	**Mean ± SD**	**CC**	**P-Value**	**Mean ± SD**	**CC**	**P-Value**
Age *(Years)*	58.2±7.0	1	----	50.1±8.5	1	----
FI34 *(0-1)*	0.08±0.04	0.33*	0.044	0.12±0.08	0.47*	0.01
GS *(kg)*	44.9±9.3	-0.46**	0.009	26.9±3.9	-0.39*	0.04
SR *(cm)*	31.4±6.6	-0.37*	0.04	38.8±12.8	-0.003	----
PTH *(N.M)*	76.5±19.7	-0.49**	0.005	46.9±15.8	-0.64**	0.001
PTQ *(N.M)*	171.2±38.2	-0.61**	0.001	104.2±22.7	-0.53**	0.004
BAL	1.8±0.9	0.51	----	1.2±0.5	0.48**	0.009
VO_{2max}, *(ml/kg/min)*	44.9±8.3	-0.51**	0.003	39.9±8.2	----	----
VFA *(cm^2)*	98.1±34.8	-0.003	----	128.4±38.3	0.51**	0.006
WHR	0.92±0.06	-0.07	----	0.9±0.04	0.34*	0.048
BMR *(kcal)*	1668±190	-0.64**	0.001	1260±119	----	----
PBF *(%)*	26.4±6.1	0.29	----	38.3±5.3	0.58**	0.001
BMI *(kg/m^2)*	27.7±2.7	-0.24	----	26.7±3.5	0.42*	0.03
SM *(%)*	41.2±3.6	-0.37*	0.04	33.3±3.1	-0.58**	0.001
Wt. *(kg)*	82.0±12.7	-0.47**	0.008	67.2±10.6	0.18	----
Ht. *(cm)*	171.6±8.3	-0.51**	0.003	158.5±6.7	-0.32	----

BAL balance, BMI body mass index, BMR basal metabolic rate, CA chronological age, CC Correlation Coefficient, FI34 frailty index-34, GS handgrip strength, PBF percent of body fat, PTH peak torque of hamstrings, PTQ peak torque of quadriceps, SM percent of skeletal muscle, SR sit-and-reach test, VFA visceral fat area, VO2max maximal oxygen consumption (cardiopulmonary fitness), WHR waist-to-hip ratio, Wt. Weight, Ht. height. Levels of probability (* $p < 0.05$; ** $p < 0.01$).

In the process of redundant feature elimination based on similar physiological concepts for males, we observed a notable correlation between PTH and PTQ, with a coefficient of 0.73. While PTH exhibited a correlation of -0.49 with CA, PTQ demonstrated a stronger correlation of -0.61. Hence, PTQ was chosen as the representative feature. Additionally, weight and height were deemed redundant as they were encompassed within BMI, and BMI, in turn, was covered in the FI34 questionnaire. Therefore, weight, height, and BMI were eliminated from further consideration. So seven biomarkers remained. In the feature selection process for females, we observed a strong correlation between PTH and PTQ, with a coefficient of 0.79. While PTH exhibited a correlation of -0.64 with CA, PTQ showed a correlation of -0.53. Consequently, PTH was chosen as the preferred biomarker. Moreover, we identified a high correlation between PBF and VFA, with a coefficient of 0.93. While PBF demonstrated a correlation of 0.58 with CA, VFA showed a correlation of 0.51. Hence, PBF was included. Additionally, BAL, despite its correlation of 0.48, was eliminated as a redundant feature (correlation between BAL and SM with a coefficient of 0.51). Furthermore, BMI was considered redundant as it was represented in the FI34 questionnaire and was excluded.

Results of PCA

PCA was applied to these selected features to acquire the first principal component for each series of biomarkers. Eigenvalues greater than 1.0 were used as selection criteria (Jia et al., 2017).

For the first analysis, CA was included to confirm the relationship between CA and the principal component identified. Considering a dataset comprised of seven candidate biomarkers for males, the first principal component which accounted for 46.84% of the total variance and an eigenvalue of 3.87 was acquired. Out of a dataset consisting of six candidate biomarkers for females, the first principal component which accounted for 53.17% of the total variance and an eigenvalue of 3.86 was obtained (Table 2).

For the second analysis, CA was excluded to specify whether the relationships of the candidate biomarkers of aging to the principal components were maintained without an effect of CA. The first principal component accounted for 45.37% of the total variance and an eigenvalue of 3.29 for males and 52.71% of the total variance and an eigenvalue of 3.28 for females were confirmed (Table 2).

Results of MLR

(1) After selecting and confirming biomarkers (seven for males and six for females), that reflect the real process of aging, we constructed two formulae using MLR for males and females (Jia et al., 2017). In the first step of the construction BA prediction model, we labeled our predicted CA as fitness age score (FAS). Our formulae were as follows:

FAS (males)=58.226+(1.108 ×FI34)+(1.373 ×SM)+(-0.351 ×GS)+(-1.032 ×SR)+(-1.741 ×PTQ)+(-1.966 ×VO_2 max)+(-2.778 ×BMR) (2)

FAS (females)=50.107+(2.107 ×FI34)+(-2.345 ×SM)+(0.466 ×GS)+(2.258 ×WHR)+(-4.311 ×PTH)+(-1.369 ×PBF) (3)

Table 2. First principal component loadings of selected biomarkers.

Males			Females		
Biomarkers	**PCA Load Including CA**	**PCA Load Excluding CA**	**Biomarkers**	**PCA Load Including CA**	**PCA Load Excluding CA**
GS, ***(kg)***	-0.36	-0.39	**GS,** *(kg)*	-0.33	-0.37
SR, ***(cm)***	-0.24	-0.25	**PTH,** *(N.M)*	-0.37	-0.38
PTQ, ***(N.M)***	-0.42	-0.45	**WHR**	0.26	0.29
VO_{2max}, ***(ml/kg/min)***	-0.37	-0.40	**PBF,** *(%)*	0.47	0.52
BMR, ***(kcal)***	-0.37	-0.38	**SM,** *(%)*	-0.47	-0.52
SM, ***(%)***	-0.38	-0.44	**CA,** *(years)*	0.41	----
FI34, ***(0-1)***	0.25	0.28	**FI34,** *(0-1)*	0.30	0.31
CA, ***(years)***	0.42	----	**Eigenvalue**	3.86	3.28
Eigenvalue	3.87	3.29	**Total Variance,** *(%)*	53.17	52.71
Total variance, ***(%)***	46.84	45.37			

BAL balance, BMI body mass index, BMR basal metabolic rate, CA chronological age, FI34 frailty index-34, GS handgrip strength, PBF percent of body fat, PTH peak torque of hamstrings, PTQ peak torque of quadriceps, SM percent of skeletal muscle, SR sit-and-reach test, VFA visceral fat area, VO2max maximal oxygen consumption (cardiopulmonary fitness), WHR waist-to-hip ratio, Wt. weight, Ht. height.

-(2) In the next step, since our FAS had not been so accurate yet, we transformed to BAS which represents the physiological function of an individual. BAS formula is the standardized form of FAS. We implemented the below template (Jia et al., 2017).

$$BAS=a\times \frac{X_1-mean_1}{SD_1}+b\times \frac{X_2-mean_2}{SD_2}+\cdots+n\times \frac{X_n-mean_n}{SD_n} \quad (4)$$

So the formulae transformed as below:

$$BAS\ (males)=90.122+(25.009\times FI34)+(0.378\times SM)+(-0.038\times GS)+(-0.157\times SR)+(-0.046\times PTQ)+(-0.239\times VO_2max)+(-0.015\times BMR) \quad (5)$$

$$BAS\ (females)=45.160+(26.092\times FI34)+(-0.765\times SM)+(0.119\times GS)+(52.115\times WHR)+(-0.272\times PTH)+(-0.258\times PBF) \quad (6)$$

(3) It was quite challenging to compare BAS and CA as BAS was not expressed in years. As per the formulae (1), BA was regarded as the anticipated CA in an ideal aging process. The objective was to compute BA based on the CA of the participants. This meant that the anticipated CA was calculated based on the CA of the participants. The mean and standard deviation of the CA of the participants, along with BAS (standard deviation of the predicted CA of the participants), were determined. Consequently, BAS transformed to BA using the T-score retransformation (Jia et al., 2017):

$$BA=BAS\times SD_CA+mean_CA \quad (7)$$

The transformed formulae looked different:

$$BA\ (males)=100.351+(33.030\times FI34)+(0.500\times SM)+(-0.050\times GS)+(-0.207\times SR)+(-0.060\times PTQ)+(-0.316\times VO_2max)+(-0.019\times BMR) \quad (8)$$

$$BA\ (females)=43.376+(35.505\times FI34)+(-1.041\times SM)+(0.162\times GS)+(70.916\times WHR)+(-0.371\times PTH)+(-0.351\times PBF) \quad (9)$$

(4) In equation (7), SDCA represented the standard deviations of CA values for all participants, while meanCA represents the average CA values for all participants. The PCA technique is a variation of MLR, which can help in minimizing the effects of over- or under-estimated BA. The systematic error in BA calculation arises due to the deviation of BA from the mean population, as described in equation (4). To remove the end effect of the BA formulae, some researchers have adjusted BA by incorporating the Z score into the calculation (Jia et al., 2017).

$$Z=(CA_i-mean_CA)\times(1-b) \quad (10)$$

Equation (10) utilized CAi to represent an individual's CA, while b was a coefficient derived from the MLR analysis conducted between BA and CA. Subsequently, we applied a specific equation to rectify BA.

$$Corrected\ BA\ (BA_C)=BA+Z \quad (11)$$

In designing the BA estimation model, we focused on correcting and enhancing our formulae in four stages, and finally, in the last stage, we arrived at a corrected BA (BA_C). Further, we proceeded to examine and compare CA, BA_C, the difference between BA_C and CA, and FI34 in males and females (Table 3).

According to Table 3, the interpretation of the difference (D) BA and CA is as follows:

If $D > 0$, the BA of the individual exceeds the CA, indicating a potential frailty or accelerated aging. if $D = 0$, the BA and CA are similar, and if $D < 0$, the BA is less than CA, indicating that the individual is younger than his/her CA, potentially indicating resilience or slower aging.

Discussion

Modeling biological age estimation can be very effective and valuable in predicting individuals' health status and subsequently in clinical applications. After evaluating individuals' health status and their aging process, recommendations supported by evidence can be advised to improve their lifestyles. Afterward, individuals can periodically repeat these tests, monitoring their biological aging more eagerly. We constructed a comprehensive model of BA estimation including both FI34 and physical fitness biomarkers as independent variables. In this study, we identified seven biomarkers for males including, FI34, SM, GS, SR, PTQ, VO2max, and BMR, and six biomarkers for females including FI34, SM, GS, WHR, PTH, and PBF. The selected biomarkers of physical fitness represent the capabilities of various parts of the body such as body composition elements, arm and leg strength, whole-body flexibility, and cardiopulmonary fitness.

Researchers showed that FI34 has a substantial genetic basis, which renders it suitable for genetic analysis of healthy aging and longevity (Kim & Jazwinski, 2015). Conversely, some studies implemented physical fitness biomarkers to design BA prediction models, representing epigenetics and individuals' lifestyles (Jee et al., 2012; Kimura et al., 2012). Our method encompasses both genetic and epigenetic impacts making it more comprehensive than the previous research. This method is rapid, reproducible, and non-invasive, encouraging individuals to participate.

We found FI34 as a significant biomarker in our formulae for both males and females. Some researchers have implemented several physical fitness and physiological biomarkers in their BA prediction models (Husted et al., 2022; Jee et al., 2012; Kimura et al., 2012), but none of them used FI34 simultaneously. Kim and Jazwinski, 2015; found a genomic region on chromosome 12, related to healthy aging and longevity and also concerning FI34. Since FI34 can mostly include genetic effects in estimating

BA, we assessed its impacts and included as an important genetic biomarker for estimating BA in our formulae. Also, sex differences were observed in FI34 relative involvement. Correlation coefficient for females was more than males. This can be interpreted as females having relatively lower physical fitness compared with men, and being frailer (Kimura et al., 2012). The FI34 questionnaire assesses various health indicators and disease risks, so enhancing physical fitness can reduce the FI34 score, thereby lowering BA.

Some studies used FI34 as a genetic measure of BA in both males and females, but they did not consider the epigenetic and lifestyle-related effects (Kim & Jazwinski, 2015; Kim et al., 2013). To achieve an efficient estimating model of BA, we included these epigenetic factors in our model, too.

We identified SM as a significant biomarker for both males and females. However, previous studies found it significant only for males (Jee et al., 2012). For both males and females, SM can be a reliable biomarker of aging. The terms "sarcomalacia" and "sarcopenia" suggested before, express the idea of skeletal muscle mass loss with advancing age (Nascimento et al., 2019). Increasing the regular secretion of myokines from skeletal muscles can significantly help control the rate of aging process.

Table 3. Comparing chronological age, biological age, and frailty index in all participants.

	Males					Females			
	CA	**BA_C**	**D**	**FI34**		**CA**	**BA_C**	**D**	**FI34**
1	71	74.53	3.53	0.127	1	63	72.04	9.04	0.257
2	71	72.57	1.57	0.088	2	53	48.98	-4.02	0.099
3	71	75.24	4.24	0.189	3	48	43.09	-4.91	0.059
4	50	49.49	-0.51	0.039	4	43	45.76	2.76	0.098
5	68	69.51	1.51	0.093	5	39	40.83	1.83	0.015
6	57	52.77	-4.23	0.061	6	53	40.88	-12.12	0.054
7	56	49.48	-6.52	0.039	7	61	55.61	-5.39	0.169
8	56	62.25	6.25	0.051	8	42	40.96	-1.04	0.061
9	54	51.28	-2.72	0.14	9	45	44.99	-0.01	0.029
10	53	46.66	-6.34	0.032	10	43	23.77	-19.23	0.029
11	51	58.97	7.97	0.041	11	56	54.99	-1.01	0.133
12	53	51.78	-1.22	0.017	12	57	59.18	2.18	0.17
13	52	48.77	-3.23	0.093	13	62	61.91	-0.09	0.135
14	54	57.77	3.77	0.13	14	53	54.60	1.60	0.142
15	58	59.53	1.53	0.059	15	45	46.90	1.90	0.118
16	54	63.65	9.65	0.186	16	64	65.15	1.15	0.306
17	62	62.70	0.70	0.096	17	41	35.57	-5.43	0.051
18	53	51.99	-1.01	0.046	18	60	67.16	7.16	0.12
19	70	73.13	3.13	0.061	19	44	48.20	4.20	0.096
20	52	57.29	5.29	0.039	20	46	45.27	-0.73	0.051
21	67	63.87	-3.13	0.054	21	46	47.77	1.77	0.076
22	53	49.41	-3.59	0.081	22	43	46.37	3.37	0.076
23	54	56.97	2.97	0.081	23	67	65.41	-1.59	0.127
24	53	58.99	5.99	0.032	24	40	44.40	4.40	0.301
25	61	58.86	-2.14	0.152	25	56	57.79	1.79	0.287
26	55	51.37	-3.63	0.054	26	39	46.68	7.68	0.162
27	52	49.23	-2.77	0.088	27	51	50.34	-0.66	0.098
28	72	66.30	-5.70	0.074	28	43	48.39	5.39	0.110
29	59	55.86	-3.14	0.066	-	--	-----	----	-----
30	57	56.08	-0.92	0.115	-	--	-----	----	-----
31	56	48.72	-7.28	0.059	-	--	-----	----	-----

BA biological age (years), CA chronological age (years), FI34 frailty index-34 (0-1), BA_C corrected BA (Years), D(years)=(BA_C)-CA.

Irisin, as an important myokine, is associated with telomere length, a reliable indicator of aging (Khavinson et al., 2016; Rana et al., 2014). It is shown that circulating irisin, produced by skeletal muscle contractions, can pass through BBB, elevating BDNF and activating neurogenesis, synaptogenesis, and improving cognition, sleep, mood and supporting brain health (Delezie & Handschin, 2018). As body composition is a key factor in health-related fitness, increasing SM through the mentioned mechanism can enhance physical fitness and lower BA.

We identified GS as a significant biomarker in our males and females' formulae, consistent with some previous BA studies (Jee et al., 2012; Kimura et al., 2012). It is a commonly used measure of frailty in older adults (Zammit et al., 2019), and there is a robust association between GS and brain volume decline with advancing age (Meysami et al., 2023). Age-related decline in GS is commonly interpreted as indicating of sarcopenia. GS is related not only to the strength of upper body and finger flexor muscles, but also to their coordination with each other, the extensor muscles, and the CNS, engaging these muscles in a highly selective manner (Carson, 2020; Zammit et al., 2019). We included GS in our formulae for both males and females, whereas a similar study did not (Husted et al., 2022).

We observed SR as a significant biomarker only for males, consistent with the study by (Jee et al., 2012). A previous study observed significantly decreased flexibility (SR) with increasing CA in men (Jee et al., 2012). Increasing muscular fascia thickness may lead to reduced joint flexibility with age causing musculoskeletal disorders (Wilke et al., 2019). Females are significantly more flexible than males (Höög & Andersson, 2021), so flexibility may not be a significant physical fitness biomarker when compared to males.

We identified WHR and PBF as significant adipose tissue factors only for females, consistent with previous research (Jee et al., 2012). Whole body fat is a major health factor due to several adipokines secreted by adipose tissue. There is significant cross-talk between adipose tissue, the brain, and other organs. Altered adipokine levels can lead to type-2 diabetes, cardiovascular diseases, and neurodegenerative diseases, and are considered key mediators of communication between the periphery and the CNS in both health and disease (Forny-Germano et al., 2018).

Some previous studies included vertical jumps in their BA formulae to represent leg power (Jee et al., 2012; Kimura et al., 2012). In another relevant study, PTQ was evaluated but not selected (Husted et al., 2022). This study identified PTQ and PTH as significant biomarkers in both males and females. We selected one of these two items for males and females, aligning with their anatomy. Falls are common among older individuals and can result in hip fractures (Kleiven, 2020). Therefore, maintaining good balance is essential for fall prevention, as demonstrated in our FI34 items. Additionally, strong leg muscles are vital for balance control. Considering the decline in skeletal muscle strength as one ages (Freitas et al., 2024), we identified PTQ and PTH as significant indicators of leg muscle strength and balance.

We identified and chose VO_{2max} as a reliable biomarker specifically for males. It's well-established that cardiopulmonary function is a predictor of mortality (Kokkinos et al., 2022), with mortality rates potentially increasing due to declining lung function, and alterations in respiratory muscles, and motor neurons (Jee et al., 2012). While previous studies utilized VO_{2max} for both males and females (Husted et al., 2022; Jee et al., 2012), we found it not significant for females, possibly due to relatively low cardiopulmonary fitness among our participants.

In our study, BMR was specifically identified and chosen for men due to its relatively high correlation with CA in males compared to the low correlation observed in females. Previous studies have demonstrated a negative correlation between BMR and advancing age in participants over thirty years old (Nikooyeh et al., 2022). BMR is likely proportional to cellular metabolism and thus to the number of mitochondria within a standard cell (Kitazoe et al., 2019). Physical exercise increases lactate, promoting mitochondrial biogenesis and enhancing metabolism (Park et al., 2021; Zhou et al., 2021). Improved physical fitness from exercise training boosts daily energy in older people and may reduce frailty.

In Table 3, we identified three categories for the disparity between BA_C and CA (D factor). There appears to be a connection between the D factor and FI34. Past research suggests that a higher FI34 score correlates with increased frailty, higher BA, and closer proximity to death. Their results suggest that with each 0.1-unit rise in FI34, the probability of nearing death rises by 25 percent (Kim & Jazwinski, 2015).

Limitations and future studies

While the current study presented formulae for estimating BA and examining physical and mental health-span, there are some limitations to be considered. One key limitation is the relatively moderate sample size used in this study. A larger sample size could potentially enhance the robustness and generalizability of the findings. Additionally, this study did not predict life-span or time to death, as it focused on estimating BA and health-span. Longitudinal studies tracking individuals over time will be essential for forecasting mortality risk through BA.

To address the limitations of the current study and further advance our understanding of biological aging, future research could focus on the following areas. Firstly, conducting longitudinal studies with larger sample sizes to track individuals over time would provide valuable insights into how biological age changes and its relationship to mortality risk. It is suggested to investigate

the relationship between exercise-induced lactate, changes in brain gray and white matter volume, and brain BA. Additionally, the optimal exercise dose-response for enhancing brain volume and reducing its BA should be determined.

Furthermore, incorporating a multi-dimensional approach by including a broader range of biomarkers, genetics, and epigenetic factors can offer a more comprehensive view of biological aging. Additionally, utilizing advanced computational techniques such as machine learning and artificial intelligence can help improve models for predicting mortality and lifespan based on biological age. Lastly, intervention studies focusing on lifestyle factors like nutrition and sleep and their impact on biological age and mortality outcomes could provide insights into strategies for healthy aging. By addressing these areas in future research; we can strengthen our understanding of biological aging and its implications for health and longevity.

Conclusion

In this study, we have successfully developed a model for estimating biological age by integrating key biomarkers of physical fitness and frailty, along with considerations for both epigenetic and genetic impacts. The inclusion of these factors has strengthened the reliability of our biological age estimation model for both males and females. Our findings support the applicability of this model in clinical settings for evaluating individuals' physical and mental health-span.

Our study highlights the importance of personalized approaches to assessing biological age and underscores the potential for utilizing this model in diverse healthcare contexts. By continuing to refine and validate our model through longitudinal studies and larger sample sizes, we can further enhance its predictive accuracy and utility in predicting health outcomes and mortality risks. This research lays the foundation for future investigations into biological aging and its implications for personalized healthcare interventions and longevity.

What is already known on this subject?

Several methods including epigenetic clock, measuring telomere length, frailty index and using physical fitness biomarkers have been proposed for estimating BA. Physical fitness biomarkers can measure epigenetic conditions and the ability and functionality of different parts of the body. Conversely, FI34's substantial genetic basis makes it suitable for analyzing healthy aging and longevity, but this index alone may not accurately estimate BA.

What this study adds?

This study incorporates both genetic and epigenetic factors, which could enhance BA model's comprehensiveness and applications.

Organ Cross-Talk Tips:

- Muscle and biological age are interconnected through a complex network of signaling molecules, where age-related changes in muscle secretions, such as myokines

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All the research was approved by the ethical committee of the Iran University of Medical Sciences (Ethical code: IR.IUMS.REC.1400.197).

Informed consent Not applicable

Author contributions

Conceptualization: M.G., M.N.; Methodology: M.G., S.A.; Software: S.A.; Validation: M.N; Formal analysis: M.N; Investigation: M.N., A.H.; Resources: M.G., A.H.; Data curation: M.N., S.A.; Writing - original draft: M.G.; Writing - review & editing: M.N., S.A.; Visualization: M.N; Supervision: M.N; Project administration: M.N; Funding acquisition: M.N.

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