Chapter 8

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Assessment of health status by molecular measures in adults ranging from middle-aged to old: ready for clinical use?

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Experimental Gerontology. 2016 Apr 2. pii: S0531-5565(16)30088-2

Abstract

In addition to measures already used in clinical practice, molecular measures have been proposed to assess health status, but these have not yet been introduced into clinical practice. We aimed to test the association of functional capacity measures used in current practice and molecular measures with age and health status.

The cohort consisted of 178 middle-aged to old participants of the Leiden Longevity Study (range 42-82 years). We tested associations between functional capacity measures (physical tests: grip strength, 4-meter walk, chair stand test; cognitive tests: Stroop test, digit symbol substitution test and 15-picture learning test) with age and with cardiovascular or metabolic disease as a measure of the health status. These associations with age and health status were also tested for molecular measures (C reactive protein (CRP), numbers of senescent p16INK4a positive cells in the epidermis and dermis and putative immunosenescence (presence of CD57+ T cells)).

All functional capacity measures were associated with age. CRP and epidermal p16INK4a positivity were also associated with age, but with smaller estimates. Grip strength and the Stroop test were associated with cardiovascular or metabolic disease, as was epidermal p16INK4a positivity. All associations with cardiovascular or metabolic disease attenuated when adjusting for age.

In conclusion, in middle-aged to old persons, the molecular measures tested here were more weakly associated with age and health status than functional capacity measures. Whether these molecular measures associate more closely with health status in the elderly or in specific groups of patients needs to be explored further.

Introduction

Markers that characterize the rate of aging in humans are important in an era of increasing lifespan and emerging novel opportunities for interventions to potentially prolong lifespan. Ideally, these measures should associate with age and with prevalence of age-related disease as a measure of an individual's health status. In clinical practice, functional capacity can be assessed by testing e.g. muscle strength, balance and walking speed. Performance on physical performance tests are associated with age [1-3], as well as health outcomes such as disability [4], disease [5] and mortality [6;7], even at middle age [2]. The cognitive domain is evaluated by making use of the Mini Mental State Examination (MMSE) assessing global cognitive functioning but also testing specific cognitive domains such as executive functioning, recall and attention. Performance on cognitive tests is associated with age [8], as well as disease [9;10] and mortality [11]. These functional capacity measures have proven their use in clinical practice.

Molecular mechanisms which are causally related to the aging process have also been reported to provide insights into an individual's health [12]. Molecular measures associated with age and health status include telomere length, transcriptomic and epigenetic parameters, inflammatory markers and cellular senescence [13-15]. Low grade systemic inflammation, measured by e.g. C-reactive protein (CRP) has been associated with age [16], disease [17;18] and mortality [17-19;19]. Cellular senescence, the phenomenon of permanent cell cycle arrest of somatic cells after a certain number of cell divisions or particular insults such as DNA damage, is found to be more prevalent at higher age in many tissues [20-23]. Immunosenescence, which may involve cellular senescence of T lymphocytes, has been linked to mortality [24;25]. Furthermore, cellular senescence has been related to many age-related diseases such as diabetes [26], glomerular disease [27] and chronic obstructive pulmonary disease [28], which strengthens the rationale to use senescence as a potential marker for the human aging process. A small number of studies has used such senescence measures to predict clinical outcome, but with inconsistent results [29-31]. Overall, molecular measures have not yet found their way into clinical practice.

We aimed to evaluate the associations between functional capacity measures and molecular measures (focussing specifically on t CRP, skin senescence and immunosenescence) from middle age onwards (age range 42-82 years), and to associate these with the presence of cardiovascular or metabolic disease as a measure for health status.

Methods

Study design and participants

In the Leiden Longevity Study, factors contributing to familial longevity are studied in long-lived families; the study design has been described previously [32]. Offspring of nonagenarian siblings as well as their partners, who act as environmentally-matched controls, participated in this study. Subjects were recruited from July 2002 to May 2006. Over several years (November 2006 to May 2008, and September 2009 to December 2010) data on functional capacity and molecular measures were acquired from these participants, many of which have been published previously [2;8;33-35]. The study was approved by the Medical Ethics Committee of Leiden University Medical Center and all participants gave their informed consent.

Medical history

Medical history on myocardial infarction, cerebrovascular accident, hypertension, diabetes mellitus, malignancy, chronic obstructive pulmonary disease and rheumatoid arthritis was obtained from general practitioners. Presence of any of these diseases was defined as one or more disease in the medical history. Presence of a cardiovascular or metabolic disease was defined as one or more of four cardiovascular or metabolic diseases (presence of myocardial infarction, cerebrovascular accident, hypertension or diabetes mellitus).

Functional capacity measures

Functional capacity measures were based on measures used in clinical practice, and included measures of physical and cognitive domains. Grip strength of the dominant hand was measured in the upright position with maximal force using a hand dynamometer (Jamar, Sammons Preston Inc., Bolingbrook, IL, USA) [33]. The best of three attempts, expressed in kilograms, was used for analysis. Four meter walking speed was measured twice across a 4-meter course starting from a standing position. Participants were asked to walk at usual pace. The fastest time in seconds was used for analyses. The time to stand up and sit down on a chair as fast as possible five times formed the chair stand test [2]. Cognition was assessed by neuropsychological testing [8]. For this study, part 3 of the Stroop test (time in seconds needed to read coloured words printed in an incongruous ink colour), the digit symbol substitution test (correct number of symbols) and delayed recall (correct number after 20 minutes) of the 15-picture learning test were used because of their known associations with age in larger cohorts [8].

Molecular measures

From the diverse set of existing molecular measures, we here studied specific measures of inflammation, skin senescence and immunosenescence.

High sensitive C-reactive protein (hsCRP) was measured in serum (Hitachi Modular P 800 from Roche, Almere, the Netherlands) [36]. Participants with a hsCRP>10 mg/L were excluded from the analyses due to possible acute infection.
Skin biopsies from the upper inner arm were taken and stained for senescence-associated p16INK4a expression by immunohistochemistry, as described previously [34]. p16INK4a-positive cells were counted separately in the epidermis (positive staining cells per mm length of the epidermal-dermal junction) and in the dermis (positive staining cells per 1mm^2 dermis). Peripheral blood mononuclear cells (PBMCs) were analysed by flow cytometry for T-cell differentiation phenotypes [35] to study immunosenescence. The frequency of T cells bearing the 'senescence-associated' marker CD57 was previously found to be higher in elderly than in young individuals [37;38]. In the present study, we therefore selected the proportion of CD4+CD57+ and CD8+CD57+ T-cells for analysis. Cytomegalovirus (CMV) serostatus was taken into account as a possible confounder, and measured by ELISA using the CMV-IgG-ELISA PKS assay (Medac GmbH, Wedel, Germany), as per the manufacturer's instructions [35].

Analyses - Datasets
Analysis of associations of the measures of the functional capacity and molecular measures with age and cardiovascular/ metabolic disease were conducted using data from 178 participants. The number of included participants was limited by the measure with the lowest number of available data (epidermal and dermal p16INK4a positivity). Not all measures were available in this exact same group of 178 participants, and therefore data from randomly selected participants of the cohort in whom the measures were available were added to complement the dataset (4-meter walk test and chair stand test random subset N=95, Stroop test, digit symbol substitution test and 15-picture learning test random subset N=57, CRP random subset N=15, proportion of CD8+CD57+ and CD4+CD57+ T-cells random subset N=98). The functional capacity and molecular measures were divided into tertiles of worst, average and best test results. Values of these tertiles are shown in Supplementary Table 1.

Analyses - Statistics
Statistical analyses were performed using IBM SPSS Statistics 20. Graphs were drawn with Prism Graphpad version 5. First we tested whether functional capacity and molecular measures were associated with age using linear regression (with estimated means via linear mixed models). The first model was adjusted for sex (grip strength analyses used sex-specific tertiles) and the immunosenescence associations for CMV serostatus. The second model additionally adjusted for the presence of one or more diseases. Next, we tested whether functional capacity and molecular measures associate with health status, determined by history of one or more cardiovascular or metabolic diseases using logistic regression. The first model was adjusted for sex (except grip strength analysis) and for CMV serostatus for

immunosenescence associations. The second model additionally adjusted for age. Adjustment for membership of a long-lived family ('offspring') is not reported here as this did not change results. Lastly, we tested which measures were associated independently of other measures by using a multivariable model (linear regression for age and logistic regression for health status). Variables were included that were associated with age in the first analysis model (grip strength, 4-meter walk test, chair stand test, Stroop test, digit symbol substitution test, 15-picture learning test, CRP and epidermal p16INK4a positivity). For this analysis only those participants with data available on of all these measures were included (N=83). Within this group, we also used combined test results by computing the average tertile of functional capacity measures (grip strength, 4-meter walk test, chair stand test, Stroop test, digit symbol substitution test, 15-picture learning test) and the average tertile of molecular measures (CRP, epidermal p16INK4a positivity). These averaged test results were used as the independent variable in the linear regression with age, and logistic regression with health status analyses.

Results

Table 1 depicts the characteristics of the participants per functional capacity and molecular measure, including age, gender and diseases of participants.

Associations between functional capacity and molecular measures with age are shown in Supplementary Table 2. All functional capacity measures were significantly associated with age. Participants in the tertile of worst test results were older than those in the average and best test result tertiles. Except for the 4-meter walk test and the 15-picture learning test, all functional capacity measures were also significantly associated with age after further adjustment for disease. For the molecular measures, CRP and epidermal p16INK4a positivity were significantly associated with age. Participants in the tertile of worst test results were older than those in the average and best test result tertiles. CRP and epidermal p16INK4a positivity had smaller estimates and were less significantly associated with age than functional capacity measures. Dermal p16INK4a positivity and the proportion of CD57+ T cells within CD8+ and CD4+ T cells were not significantly associated with age. CRP was significantly associated with age independently of disease, whereas the association between epidermal p16INK4a positivity and age was attenuated upon further adjustment for disease. For visualisation purposes, measures that were most strongly associated with age are shown in Figure 1.

The associations between functional capacity and molecular measures with cardiovascular or metabolic disease are shown in Supplementary Table 3. Grip strength and the Stroop test as functional capacity measures were significantly associated with cardiovascular or metabolic

Table 1. Characteristics of study participants per functional capacity and molecular measure

	Functional capacity measures			Molecular measures		
	Grip strength	4-meter walk & chair stand test	Cognitive tests	CRP	Skin senescence	Immunosenescence
Participant overlap with skin senescence dataset, no.	178	83	121	163	178	80
Randomly selected participants, no.	0	95	57	15	0	98
Age, years, mean (SD)	63.4 (6.62)	62.7 (7.02)	63.3 (6.82)	62.9 (6.62)	63.4 (6.62)	61.9 (7.51)
Female, no. (%)	90 (50.6)	95 (53.4)	93 (52.2)	90 (50.6)	90 (50.6)	89 (50.0)
Offspring, no. (%)	90 (50.6)	91 (51.1)	95 (53.4)	90 (50.6)	90 (50.6)	87 (48.9)
≥1 disease, no. (%)	58 (35.4)	56 (31.5)	58 (38.4)	49 (31.8)	58 (35.4)	42 (28.0)
≥1 cardiovascular/metabolic disease, no. (%)	51 (31.1)	48 (30.6)	50 (32.1)	44 (27.7)	51 (31.1)	32 (21.1)

All subsets N=178. SD: standard deviation. No.: number. %: valid percentage. CRP: C-reactive protein. Missing data on diseases for 14-28 participants. ≥1 disease: presence of one or more of the following diseases: myocardial infarction, cerebrovascular accident, hypertension, diabetes mellitus, malignancy, chronic obstructive pulmonary disease or rheumatoid arthritis. ≥1 cardiovascular/metabolic disease: presence of one or more of the following diseases: myocardial infarction, cerebrovascular accident, hypertension or diabetes mellitus.

disease, whereas the other measures were not. Participants in the worst test result tertile of grip strength and of the Stroop test had a history of cardiovascular or metabolic disease more frequently than those in the average and best test result tertiles. Results did not remain statistically significant after further adjustment for age, although a trend remained for grip strength. Epidermal p16INK4a positivity was significantly associated with cardiovascular or metabolic disease, but did not remain significant after adjustment for age. Other molecular measures were not associated with cardiovascular or metabolic disease. Associations between the functional capacity and molecular measures and presence of cardiovascular or metabolic disease are shown in Figure 2.

Multivariable regression analyses of functional and molecular measures with age and health status demonstrated that grip strength, the Stroop test and digit symbol substitution test were significantly associated with age, independently of other measures, shown in Table 2. Grip strength and the 15-picture learning test were significantly associated with health status independently of the other measures.

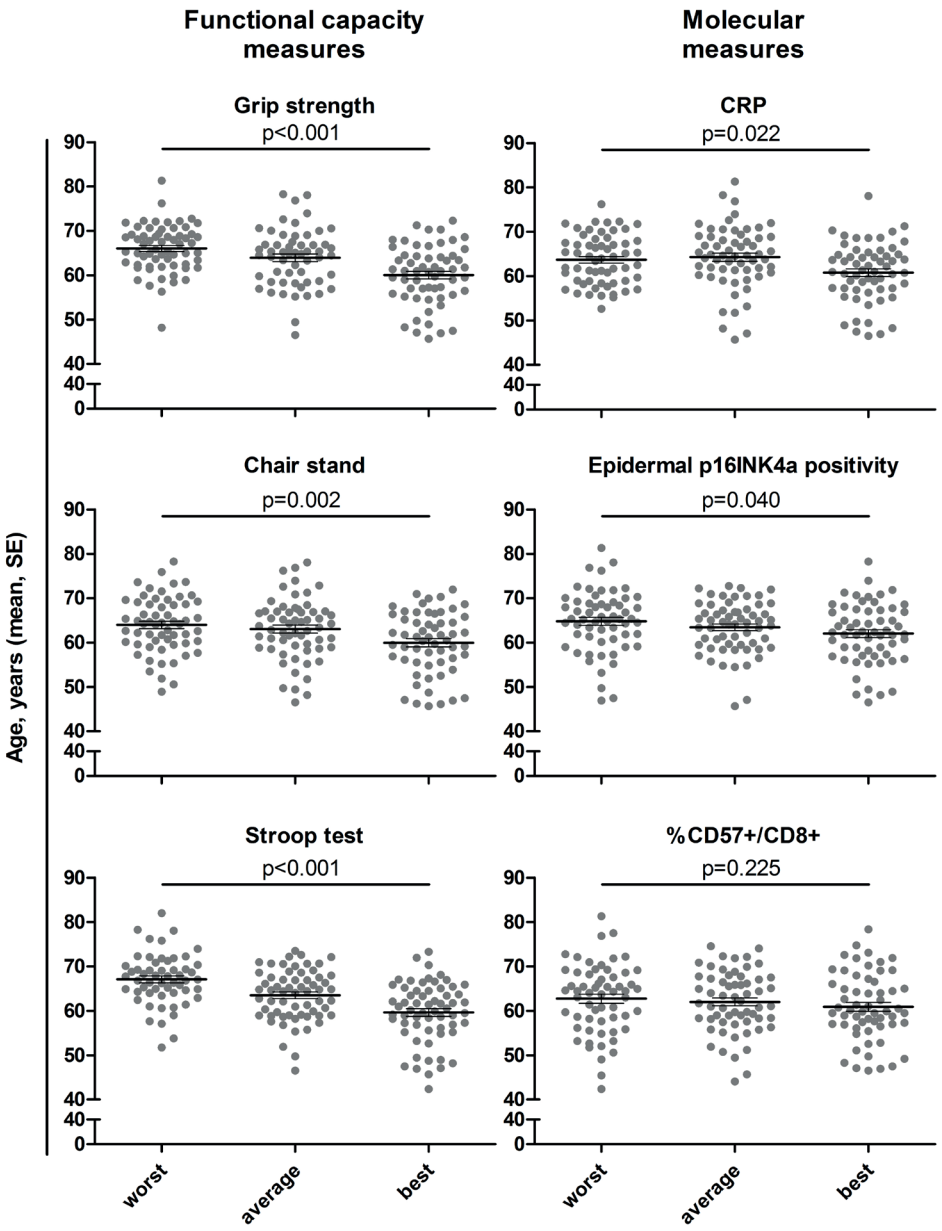

Figure 1. Age of participants dependent on functional capacity and molecular measures given in tertiles. SE: standard error. Single data points and the mean age per tertile of measure (worst, average, best test result) are given. P for trends are derived from linear mixed models, adjusted for sex (plus CMV serostatus in the CD57+/CD8+ association). N=178 for all measures.

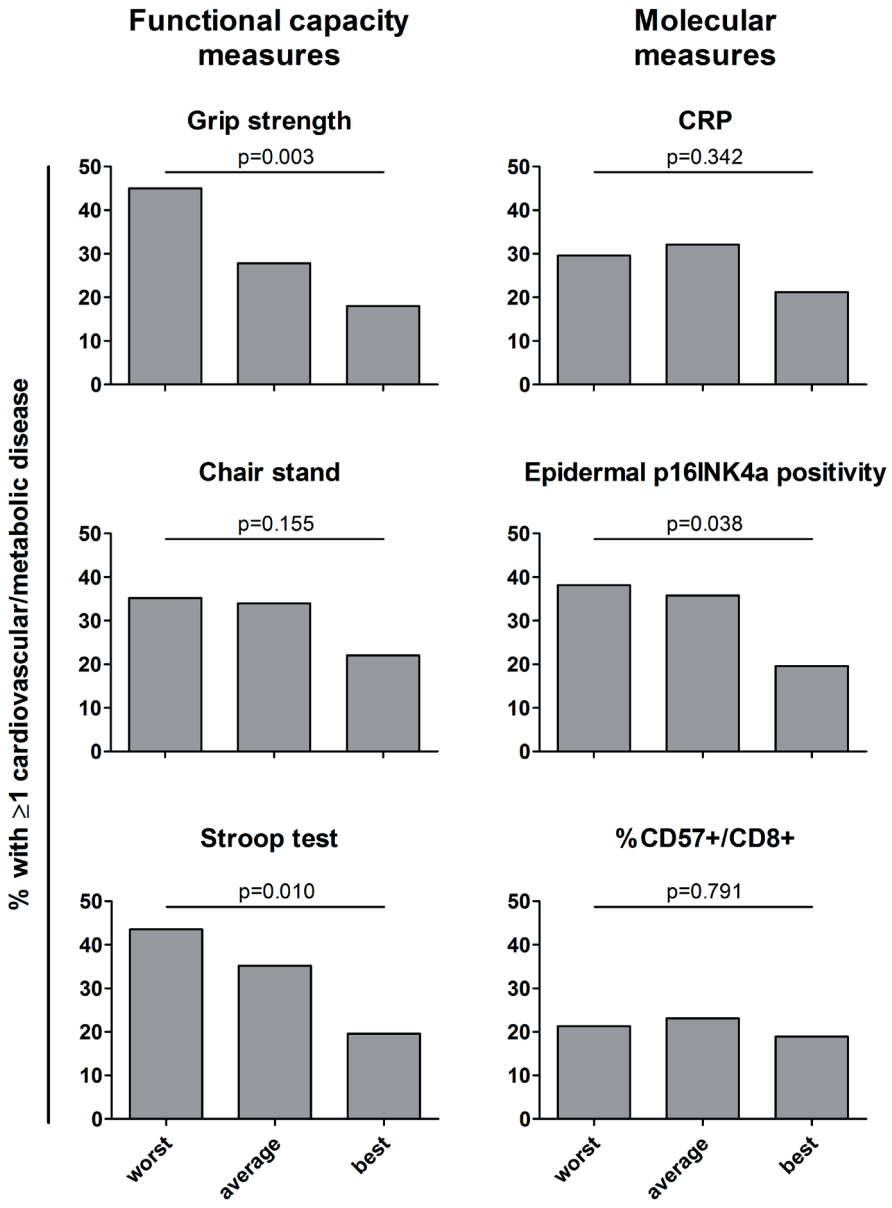

Figure 2. Presence of cardiovascular/ metabolic disease dependent on functional capacity and molecular measures given in tertiles.
Percentage of participants with one or more cardiovascular/metabolic diseases (myocardial infarction, cerebrovascular accident, hypertension or diabetes mellitus) are given per tertile of measure (worst, average, best test result). P-values were calculated with logistic regression; adjusted for sex (plus CMV serostatus in the CD57+/CD8+ association). N=178 for all measures.

Table 2. Associations between age and cardiovascular/metabolic disease with functional capacity and molecular measures

	Age		Cardiovascular/metabolic disease	
	β (SE)	P-value	β (SE)	P-value
a. Multivariable model				
Functional capacity measures				
Physical tests				
Grip strength	-3.17 (0.85)	<0.001	-1.28 (0.48)	0.008
4-meter walk test	0.79 (0.81)	0.333	0.53 (0.42)	0.208
Chair stand test	0.03 (0.83)	0.967	0.60 (0.43)	0.159
Cognitive tests				
Stroop test	-3.12 (0.90)	0.001	0.20 (0.47)	0.679
Digit symbol substitution test	-1.91 (0.95)	0.047	-0.34 (0.46)	0.460
15-picture learning test	-0.80 (0.86)	0.355	-1.09 (0.47)	0.019
Molecular measures				
CRP	-0.99 (0.79)	0.212	-0.16 (0.40)	0.689
Epidermal p16INK4a	-0.56 (0.82)	0.495	0.10 (0.40)	0.800
b. Univariate - combined measures				
Combined functional capacity measures	-8.88 (1.48)	<0.001	-1.04 (0.60)	0.085
Combined molecular measures	-2.86 (1.37)	0.039	-0.87 (0.50)	0.543

N=83 for all measures. SE: standard error. CRP: C-reactive protein. Multivariable model: tertiles of all measures were included as independent variable in a linear regression model for the association with chronological age; in a logistic regression model for the association with cardiovascular/metabolic disease. Univariate model: the average tertile of all functional capacity measures combined, and all molecular measures combined was used as the independent variable.

Discussion

In this study of middle-aged to old adults (range 42-82 years), functional capacity measures are associated with age, whereas molecular measures are inconsistently and weakly associated with age. Associations of all measures were stronger with age than with cardiovascular or metabolic disease as measure of health status.

These observed associations between different functional capacity measures and age are in line with the literature. Both physical and cognitive tests have been shown to be a reflection of the aging process through their links with mortality [6;7;11;39] and disability [4;40] in older people. Some measures of physical and cognitive performance are known to be different in men and women [41-46], which we therefore accounted for in our analyses. Even in this smaller group of

178 participants (to aid comparison to the molecular measure analyses) the associations were statistically significant, highlighting the strong links between these measures and aging in men and women.

Recently, several different measures of the underlying biological aging process have been studied, but here we have focussed on low-grade systemic inflammation and cellular senescence in the skin and in the immune system. We found low-grade systemic inflammation to be associated with age, similar to findings from other larger studies [19;47;48], but not to cardiovascular or metabolic disease within this study and sample size. Cellular senescence has been linked in the literature to both higher age [20-23] and to age-related pathologies [20;26-28], but most studies have a modest sample size and studies on disease rarely adjusted for age. Within the Leiden Longevity Study, epidermal but not dermal p16INK4a positivity also linked to age [49]. However, this association is not fully independent of disease, as estimates became smaller and the significance diminished after adjustment for disease. Epidermal p16INK4a is also linked to cardiovascular disease or metabolic disease in skin of middle-aged to old persons, but not independently of age. The associations of senescence measures (e.g. p16INK4a epidermal positivity) with age and health status were weaker than for the functional capacity measures (e.g. grip strength), indicating a need for large number of participants when using senescence measures, which are therefore in the current state less appropriate for clinical practice. Indeed, in a recent study p16INK4a positivity in T-cells was not predictive for length of stay in hospitalized middle-aged to old patients after a coronary artery bypass graft surgery [31]. In the present study, T cell CD57 positivity was not associated with either age or disease in these middle-aged to old persons. One of the reasons for this lack of association might be the more limited age range of these participants compared to other studies [37;38]. However, it is more likely that the history of pathogen exposures in these subjects over-rode any potential effects of age. When a multivariable model was performed, only grip strength was associated with both age and health status independently of all other measures. Thus, no clear added value of the molecular measures tested here was found when taking functional capacity measures into account.

More molecular markers have been proposed in the literature to capture the aging process. These markers are based on biomaterial, and are therefore more invasive than functional capacity measures. However, efforts should be made to measure molecular markers in a minimally-invasive manner in routinely clinically-obtained biomaterial such as blood, or other easily accessible material such as urine or saliva. Measurement of molecular markers has the potential to be optimized and routinized, whereas measurement of functional capacity measures is usually dependent on trained health care workers and is more time-consuming. With expanding knowledge of the biology of the aging process, more molecular markers are

likely to be found in addition to current known markers which could better describe the pathophysiology of underlying systems. Within the Leiden Longevity Study markers like telomere length in leukocytes and expression levels of IL7R were shown to be markers of familial longevity and mortality [50;51], and several loci associated with longevity or familial longevity were identified [52;53]. When studying the value of markers for prediction of health status, most studies focus on older persons, or specific groups of patients. For example, various markers such as grip strength, vitamin D and brain natriuretic peptide were associated with health status in persons aged 85 years and above [54]. Sex-differences in molecular markers are rarely reported and should be more often considered in future studies. In addition to the search for single markers of the aging process, also multiple markers have been used to describe biological age [55;56]. This approach focusses on the correlated aging in multiple organs and might already be useful at a younger age [57]. However, the comparison of molecular measures with functional measures in terms of the strength of association with age and health status had not yet been performed.

A strength of the present study is that participants of the Leiden Longevity Study were uniquely phenotyped for both functional capacity and molecular measures. This allowed us to compare these measures dependent on both age and cardiovascular or metabolic disease (as a measure for health status). While not all participants had all phenotypes measured over the years of study participation, we ensured equally sized datasets by completing all datasets up to 178 participants by random selection. A limitation of this study its cross-sectional nature, which does not allow drawing firm conclusions on predictive properties of the measures on e.g. disease incidence, next to the limited sample size. In addition, only a limited number of molecular measures were tested, and the assessment of the numbers of senescent skin cells was done using only one marker of cellular senescence and in only a small section of sun-protected skin. It is still unclear whether senescence in a section can adequately reflect the whole organ. Another limitation is the use of middle-aged to old participants who were still relatively healthy.

In conclusion, currently used functional capacity measures show stronger associations with age and health status in middle-aged to old persons compared to tested molecular measures. Further studies are required to determine the value of other molecular measures of aging in addition to functional capacity measures, and in older persons or in specific groups of patients.

Acknowledgements

The Leiden Longevity Study was funded by the Innovation Oriented Research Program on Genomics (SenterNovem; IGE01014 and IGE5007), the Centre for Medical Systems Biology, and The Netherlands Genomics Initiative/Netherlands Organization for Scientific Research (05040202 and 050-060-810). The work was further funded by Unilever PLC, European Union-funded Network of Excellence LifeSpan FP6 036894, DFG-PA 361/14-1, DFG-PA 361/11-1, the seventh framework programme MYOAGE (HEALTH-2007-2.4.5-10), and Switchbox (FP7, Health-F2-2010-259772).

Supplementary Table 1. Values for tertiles of functional capacity and molecular measures.

	Tertiles of measures		
	Worst	Average	Best
Functional capacity measures			
Physical tests			
Grip strength (female), kg	<28	28-32	>32
Grip strength,(male), kg	<42	42-50	>50
4-meter walk test, s	>3.90	3.33-3.90	<3.33
Chair stand test, s	>13.4	11.4-13.4	<11.4
Cognitive tests			
Stroop test, trial 3, s	>54	42-54	<42
Digit symbol substitution test, no. correct	<40	40-49	>49
15-picture learning test, delayed recall, no. correct	<11	11-12	>12
Molecular measures			
Inflammation			
CRP, mg/L	>1.80	0.79-1.80	<0.79
Senescence – skin			
Epidermal p16INK4a, no./mm	>1.30	0.30-1.30	<0.30
Dermal p16INK4a, no./mm2	>2.05	0.72-2.05	<0.72
Senescence - immune system			
CD57+/CD8+, %	>32.4	18.5-32.4	<18.5
CD57+/CD4+, %	>1.82	0.64-1.82	<0.64

No.: number. CRP: C-reactive protein. Tertiles are given as worst, average and best test results.

Supplementary Table 2. Association of functional capacity and molecular measures with age.

	Age (years) per tertile of measures								
	worst		average		best				
	mean	SE	mean	SE	mean	SE	Stand. β[a]	P for trend[a]	P for trend[b]
Functional capacity measures									
Physical tests									
Grip strength	66.0	0.77	64.0	0.82	60.0	0.81	-0.372	<0.001	<0.001
4-meter walk test	65.1	0.87	61.2	0.86	62.3	0.87	-0.168	0.022	0.052
Chair stand test	64.2	0.87	64.0	0.86	60.3	0.87	-0.105	0.002	0.006
Cognitive tests									
Stroop test	67.0	0.82	63.6	0.78	59.7	0.78	-0.439	<0.001	<0.001
Digit symbol substitution test	65.7	0.76	64.7	0.84	59.2	0.81	-0.396	<0.001	<0.001
15-picture learning test	64.6	0.9	63.4	0.84	61.8	0.93	-0.163	0.035	0.068
Molecular measures									
Inflammation									
CRP	63.6	0.84	64.3	0.83	60.9	0.84	-0.170	0.022	0.037
Senescence – skin									
Epidermal p16	64.7	0.85	63.4	0.84	62.2	0.85	-0.154	0.040	0.133
Dermal p16	63.5	0.86	63.2	0.85	63.5	0.86	-0.006	0.992	0.822
Senescence - immune system									
%CD57+/CD8+	62.7	1.04	62.0	0.97	60.8	1.02	-0.103	0.225	0.285
%CD57+/CD4+	62.4	1.09	62.1	0.98	61.0	1.05	-0.077	0.375	0.165

N=178 for all measures. SE: standard error. Stand: standardized. Linear regression (estimated means via linear mixed models), age as dependent variable. Model 1: adjustment for sex (and CMV serostatus for immunosenescence). Model 2: as model 1 plus the presence of any disease. Estimated means from model 1 are given. P-value [a] for model 1, p-value [b] for model 2.

Supplementary Table 3. Association of functional capacity and molecular measures with presence of cardiovascular/metabolic disease.

	Percentage of disease per tertile of measures				
	worst	average	best	P-value[a]	P-value[b]
Functional capacity measures					
Physical tests					
Grip strength	45.0	27.8	18.0	0.003	0.057
4-meter walk test	29.4	26.8	36.0	0.466	0.797
Chair stand test	35.2	34.0	22.0	0.155	0.434
Cognitive tests					
Stroop test	43.5	35.2	19.6	0.010	0.203
Digit symbol substitution test	31.0	43.5	23.1	0.405	0.715
15-picture learning test	38.0	33.3	24.5	0.182	0.376
Molecular measures					
Inflammation					
CRP	29.6	32.1	21.2	0.342	0.660
Senescence – skin					
Epidermal p16INK4a	38.2	35.8	19.6	0.038	0.115
Dermal p16INK4a	32.7	33.3	27.3	0.537	0.603
Senescence - immune system					
%CD57+/CD8+	21.3	23.1	18.9	0.791	0.892
%CD57+/CD4+	15.1	26.0	22.4	0.436	0.851

N=178 for all measures. Number and valid percentages are given; P-values are derived from logistic regression, with measures as tertiles. The outcome is the presence of one or more of the following diseases: myocardial infarction, cerebrovascular accident, hypertension or diabetes mellitus. P-value [a] for model 1, p-value [b] for model 2 . Model 1 adjusted for sex (and CMV serostatus for immunosenescence), model 2 additionally for age.

References

(1) Beenakker KG, Ling CH, Meskers CG et al. Patterns of muscle strength loss with age in the general population and patients with a chronic inflammatory state. *Ageing Res Rev* 2010;9:431-436.

(2) Stijntjes M, Meskers CGM, de Craen AJM et al. Effect of calendar age on physical performance: a comparison of standard clinical measures with instrumented measures in middle-aged to older adults. *Gait & Posture*. In press.

(3) Verlinden VJ, van der Geest JN, Hoogendam YY, Hofman A, Breteler MM, Ikram MA. Gait patterns in a community-dwelling population aged 50 years and older. *Gait Posture* 2013;37:500-505.

(4) Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med* 1995;332:556-561.

(5) Abellan van KG, Rolland Y, Andrieu S et al. Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an International Academy on Nutrition and Aging (IANA) Task Force. *J Nutr Health Aging* 2009;13:881-889.

(6) Ling CH, Taekema D, de Craen AJ, Gussekloo J, Westendorp RG, Maier AB. Handgrip strength and mortality in the oldest old population: the Leiden 85-plus study. *CMAJ* 2010;182:429-435.

(7) Studenski S, Perera S, Patel K et al. Gait speed and survival in older adults. *JAMA* 2011;305:50-58.

(8) Stijntjes M, de Craen AJ, van HD et al. Familial longevity is marked by better cognitive performance at middle age: the Leiden Longevity Study. *PLoS One* 2013;8:e57962.

(9) Reis JP, Launer LJ, Terry JG et al. Subclinical atherosclerotic calcification and cognitive functioning in middle-aged adults: the CARDIA study. *Atherosclerosis* 2013;231:72-77.

(10) Lee M, Saver JL, Hong KS et al. Cognitive impairment and risk of future stroke: a systematic review and meta-analysis. *CMAJ* 2014;186:E536-E546.

(11) Gussekloo J, Westendorp RG, Remarque EJ, Lagaay AM, Heeren TJ, Knook DL. Impact of mild cognitive impairment on survival in very elderly people: cohort study. *BMJ* 1997;315:1053-1054.

(12) Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;153:1194-1217.

(13) Pallis AG, Hatse S, Brouwers B et al. Evaluating the physiological reserves of older patients with cancer: the value of potential biomarkers of aging? *J Geriatr Oncol* 2014;5:204-218.

(14) Peters MJ, Joehanes R, Pilling LC et al. The transcriptional landscape of age in human peripheral blood. *Nat Commun* 2015;6:8570.

(15) Horvath S, Pirazzini C, Bacalini MG et al. Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. *Aging (Albany NY)* 2015.

(16) Bruunsgaard H. The clinical impact of systemic low-level inflammation in elderly populations. With special reference to cardiovascular disease, dementia and mortality. *Dan Med Bull* 2006;53:285-309.

(17) De MM, Franceschi C, Monti D, Ginaldi L. Inflammation markers predicting frailty and mortality in the elderly. *Exp Mol Pathol* 2006;80:219-227.

(18) Akbaraly TN, Hamer M, Ferrie JE et al. Chronic inflammation as a determinant of future aging phenotypes. *CMAJ* 2013;185:E763-E770.

(19) Bruunsgaard H, Ladelund S, Pedersen AN, Schroll M, Jorgensen T, Pedersen BK. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. *Clin Exp Immunol* 2003;132:24-31.

(20) Bhat R, Crowe EP, Bitto A et al. Astrocyte senescence as a component of Alzheimer's disease. *PLoS One* 2012;7:e45069.

(21) Liu Y, Sanoff HK, Cho H et al. Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of human aging. *Aging Cell* 2009;8:439-448.

(22) Melk A, Schmidt BM, Takeuchi O, Sawitzki B, Rayner DC, Halloran PF. Expression of p16INK4a and other cell cycle regulator and senescence associated genes in aging human kidney. *Kidney Int* 2004;65:510-520.

(23) Ressler S, Bartkova J, Niederegger H et al. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. *Aging Cell* 2006;5:379-389.

(24) Wikby A, Johansson B, Ferguson F, Olsson J. Age-related changes in immune parameters in a very old population of Swedish people: a longitudinal study. *Exp Gerontol* 1994;29:531-541.

(25) Ferguson FG, Wikby A, Maxson P, Olsson J, Johansson B. Immune parameters in a longitudinal study of a very old population of Swedish people: a comparison between survivors and nonsurvivors. *J Gerontol A Biol Sci Med Sci* 1995;50:B378-B382.

(26) Verzola D, Gandolfo MT, Gaetani G et al. Accelerated senescence in the kidneys of patients with type 2 diabetic nephropathy. *Am J Physiol Renal Physiol* 2008;295:F1563-F1573.

(27) Sis B, Tasanarong A, Khoshjou F, Dadras F, Solez K, Halloran PF. Accelerated expression of senescence associated cell cycle inhibitor p16INK4A in kidneys with glomerular disease. *Kidney Int* 2007;71:218-226.

(28) Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in patients with pulmonary emphysema. *Am J Respir Crit Care Med* 2006;174:886-893.

(29) Koppelstaetter C, Schratzberger G, Perco P et al. Markers of cellular senescence in zero hour biopsies predict outcome in renal transplantation. *Aging Cell* 2008;7:491-497.

(30) Gingell-Littlejohn M, McGuinness D, McGlynn LM et al. Pre-transplant CDKN2A expression in kidney biopsies predicts renal function and is a future component of donor scoring criteria. *PLoS One* 2013;8:e68133.

(31) Pustavoitau A, Barodka V, Sharpless NE et al. Role of senescence marker p16INK4a measured in peripheral blood T-lymphocytes in predicting length of hospital stay after coronary artery bypass surgery in older adults. *Experimental Gerontology*. In press.

(32) Schoenmaker M, de Craen AJ, de Meijer PH et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. *Eur J Hum Genet* 2006;14:79-84.

(33) Ling CH, de Craen AJ, Slagboom PE, Westendorp RG, Maier AB. Handgrip strength at midlife and familial longevity : The Leiden Longevity Study. *Age (Dordr)* 2012;34:1261-1268.

(34) Waaijer ME, Parish WE, Strongitharm BH et al. The number of p16INK4a positive cells in human skin reflects biological age. *Aging Cell* 2012;11:722-725.

(35) Derhovanessian E, Maier AB, Beck R et al. Hallmark features of immunosenescence are absent in familial longevity. *J Immunol* 2010;185:4618-4624.

(36) Rozing MP, Mooijaart SP, Beekman M et al. C-reactive protein and glucose regulation in familial longevity. *Age (Dordr)* 2011;33:623-630.

(37) McNerlan SE, Rea IM, Alexander HD, Morris TC. Changes in natural killer cells, the CD57CD8 subset, and related cytokines in healthy aging. *J Clin Immunol* 1998;18:31-38.

(38) Di Benedetto S, Derhovanessian E, Steinhagen-Thiessen E, Goldeck D, Muller L, Pawelec G. Impact of age, sex and CMV-infection on peripheral T cell phenotypes: results from the Berlin BASE-II Study. *Biogerontology* 2015;16:631-643.

(39) Bohannon RW. Hand-grip dynamometry predicts future outcomes in aging adults. *J Geriatr Phys Ther* 2008;31:3-10.

(40) Taekema DG, Gussekloo J, Maier AB, Westendorp RG, de Craen AJ. Handgrip strength as a predictor of functional, psychological and social health. A prospective population-based study among the oldest old. *Age Ageing* 2010;39:331-337.

(41) Guralnik JM, Simonsick EM, Ferrucci L et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994;49:M85-M94.

(42) Guralnik JM, Ferrucci L, Pieper CF et al. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. *J Gerontol A Biol Sci Med Sci* 2000;55:M221-M231.

(43) Vereeck L, Wuyts F, Truijen S, Van de Heyning P. Clinical assessment of balance: normative data, and gender and age effects. *Int J Audiol* 2008;47:67-75.

(44) Callisaya ML, Blizzard L, Schmidt MD, McGinley JL, Srikanth VK. Sex modifies the relationship between age and gait: a population-based study of older adults. *J Gerontol A Biol Sci Med Sci* 2008;63:165-170.

(45) Hardy R, Cooper R, Aihie SA et al. Body mass index, muscle strength and physical performance in older adults from eight cohort studies: the HALCyon programme. *PLoS One* 2013;8:e56483.

(46) Matthews F, Marioni R, Brayne C. Examining the influence of gender, education, social class and birth cohort on MMSE tracking over time: a population-based prospective cohort study. *BMC Geriatr* 2012;12:45.

(47) Rumley A, Emberson JR, Wannamethee SG, Lennon L, Whincup PH, Lowe GD. Effects of older age on fibrin D-dimer, C-reactive protein, and other hemostatic and inflammatory variables in men aged 60-79 years. *J Thromb Haemost* 2006;4:982-987.

(48) Ballou SP, Lozanski FB, Hodder S et al. Quantitative and qualitative alterations of acute-phase proteins in healthy elderly persons. *Age Ageing* 1996;25:224-230.

(49) Waaijer ME, Gunn DA, Adams PD et al. P16INK4a Positive Cells in Human Skin Are Indicative of Local Elastic Fiber Morphology, Facial Wrinkling, and Perceived Age. *J Gerontol A Biol Sci Med Sci* 2015.

(50) Deelen J, Beekman M, Codd V et al. Leukocyte telomere length associates with prospective mortality independent of immune-related parameters and known genetic markers. *Int J Epidemiol* 2014;43:878-886.

(51) Passtoors WM, van den Akker EB, Deelen J et al. IL7R gene expression network associates with human healthy ageing. *Immun Ageing* 2015;12:21.

(52) Deelen J, Beekman M, Uh HW et al. Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age. *Hum Mol Genet* 2014;23:4420-4432.

(53) Beekman M, Blanche H, Perola M et al. Genome-wide linkage analysis for human longevity: Genetics of Healthy Aging Study. *Aging Cell* 2013;12:184-193.

(54) Martin-Ruiz C, Jagger C, Kingston A et al. Assessment of a large panel of candidate biomarkers of ageing in the Newcastle 85+ study. *Mech Ageing Dev* 2011;132:496-502.

(55) Li Q, Wang S, Milot E et al. Homeostatic dysregulation proceeds in parallel in multiple physiological systems. *Aging Cell* 2015.

(56) Arbeev KG, Ukraintseva SV, Akushevich I et al. Age trajectories of physiological indices in relation to healthy life course. *Mech Ageing Dev* 2011;132:93-102.

(57) Belsky DW, Caspi A, Houts R et al. Quantification of biological aging in young adults. *Proc Natl Acad Sci U S A* 2015;112:E4104-E4110.