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Remodelling of biological parameters during human ageing: evidence for complex regulation in longevity and in type 2 diabetes

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Abstract Factor structure analyses have revealed the presence of specific biological system markers in healthy humans and diseases. However, this type of approach in very old persons and in type 2 diabetes (T2DM) is lacking. A total sample of 2,137 Italians consisted of two groups: 1,604 healthy and 533 with T2DM. Age (years) was categorized as adults (≤65), old (66–85), oldest old (>85–98) and centenarians (≥99). Specific biomarkers of routine haematological and biochemical testing were tested across each age group. Exploratory factorial analysis (EFA) by principal component method with Varimax rotation was used to identify factors including related variables. Structural equation modelling (SEM) was applied to confirm factor solutions for each age group. EFA and SEM identified specific factor structures according to age in both groups. An age-associated reduction of factor structure was observed from adults to oldest old in the healthy group (explained variance 60.4% vs 50.3%) and from adults to old in the T2DM group

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(explained variance 57.4% vs 44.2%). Centenarians showed three-factor structure similar to those of adults (explained variance 58.4%). The inflammatory component became the major factor in old group and was the first one in T2DM. SEM analysis in healthy subjects suggested that the glucose levels had an important role in the oldest old. Factorial structure change during healthy ageing was associated with a decrease in complexity but showed an increase in variability and inflammation. Structural relationship changes observed in healthy subjects appeared earlier in diabetic patients and later in centenarians.

Keywords Ageing · Exploratory factor analysis · Structural equation modelling · Centenarians · Diabetic patients

Introduction

Human ageing, characterized by a complex remodelling of thousands of biological variables necessary for maximizing the probability of survival, is the product of genetic, environmental and stochastic factors. Studies have underlined that age-related effects on specific biological functioning variables arise from common causal mechanism and may explain many of the manifesting outcomes predominantly observed in old age (Mackinnon et al. 2006; Cevenini et al. 2010). In order to understand the causal mechanisms of the human ageing process, the model of 'centenarians', a representative group of successful ageing, has been identified (Franceschi et al. 2000). Data obtained from these centenarian studies showed that the ageing process is characterized by the remodelling of some important networks, as suggested by the peculiar clinical and anthropometric characteristics present in very old (Barbieri et al. 2003). According to the remodelling theory of age, the peculiar characteristics of centenarians might be the net result of the continuous adaptation of the body to deleterious changes over time (Barbieri et al. 2001). Recently, Yashin et al. showed that exceptional survival requires dynamic maintenance of physiological variables at 'optimal values'. These indices are not fixed for the entire life course but change over ageing, suggesting that the determinants of exceptional survival could have 'age-trajectories' rather than fixed values (Yashin et al. 2009). It is now becoming evident that while genetic differences contribute modestly to life expectancy before the age of 60 years, their impact on survival becomes more prominent at the extreme ages (Franceschi et al. 2007a, b; Gögele et al. 2011).

Studies of the genetics of human longevity have consistently found that two genes—APOE (Schachter et al. 1994) and FOXO3 (Willcox et al. 2008)—are associated with human longevity. Both of these genes and the majority of other potential findings that have emerged during the past decade (de Magalhães et al. 2009a, b; Capri et al. 2006, 2008; Pawlikowska et al. 2009; Bonafè and Olivieri 2009; Singh et al. 2007) are related mainly to inflammation, stress response or lipid and glucose metabolism.

These results also confirmed that the imbalance between inflammatory and anti-inflammatory networks result in low-grade chronic pro-inflammation, 'inflammaging' which is mainly due to a genetic predisposition (Franceschi et al. 2007a, b; Franceschi 2007; Salvioli et al. 2006). Interestingly, a recent meta-analysis of age-related gene expression profiles revealed several common signatures of ageing, including overexpression of inflammation and immune response genes and an

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underexpression of genes associated with lipid and glucose metabolism (de Magalhães et al. 2009a, b).

Interestingly, even if ageing is associated with a significant rise in oxidative stress mainly due to a decline in anti-oxidant activity and a rise in pro-oxidant factors, centenarians have a significantly lower degree of oxidative stress (Rabini et al. 2002; Suzuki et al. 2010). This finding has led investigations to reveal that specific biomarkers are strongly linked to glucose metabolism in longevity (Barbieri et al. 2003). Furthermore, glucose metabolism worsens over ageing due to an age-related decline in insulin-mediated glucose uptake. However, glucose metabolism is maintained in healthy centenarians and has been shown to be better than that of old adults (Barbieri et al. 2003). Therefore, one may hypothesize that adults with type 2 diabetes (T2DM) may have insulin pathway dysfunctions as older healthy persons. and specific biomarkers may reveal such trajectory patterns.

In addition to specific markers of glucose and lipid metabolism, other biomarkers have been included as useful tools in the evaluation of health status over ageing (Frisoni et al. 1994; Nakamura and Miyao 2003; Yashin et al. 2009). In particular, total cholesterol (TC), triglycerides (TG), plasma glucose levels (GLU), C reactive protein (CRP), fibrinogen (FIBR), white blood cell count (WBCC) and haemoglobin (Hb) have been shown to vary over the ageing process (Frisoni et al. 1994; Nakamura and Miyao 2003).

Previous publications have applied factor or principal components analysis, a statistical technique used to study interrelating variables on human ageing phenotypes and the risk factor clusters in common age-related human diseases (Nakamura and Miyao 2003; Choi et al. 2003; Hasegawa et al. 2005; Wang et al. 2004; Noale et al. 2006; Passarino et al. 2007; Khader et al. 2011; O'Hea et al. 2009). Moreover, this type of analysis has also been applied to measure systemic inflammatory status over ageing mainly in middle-aged adults (Hsu et al. 2009; Riechelmann et al. 2005).

Therefore, the aim of this study was to investigate the variability using exploratory factorial analysis (EFA) of specific biomarkers related to inflammation, glucose and lipid metabolism across different age groups in a large sample of healthy subjects, including centenarians and old persons with T2DM. We also applied structural equation modelling (SEM) to test for the complexity of these biomarkers over ageing and disease status.

Materials and methods

Study population

A total of 2,137 people from different Italian regions, aged from 18 to 111 years, were enrolled to participate in this study that was financed by Italian Health Ministry and by the Ministry of Education, University and Research from 2007 to 2009. The study sample consisted of 1,325 healthy subjects (n=577 men and n=748 women, age range 18–98), 279 centenarians (n=53 men and n=226 women, age range 99–111 years) and 533 participants with T2DM (n=288 men and n=245 women, age range 40–86 years). Participants were recruited from the I.N.R.C.A. and five Italian universities including University of Bologna, University of Florence, University of Milan, University of Parma and University of Palermo.

Inclusion criteria of the healthy subjects were the absence of acute (such as acute myocardial infarction) and chronic diseases (such as Alzheimer and T2DM) at the time of blood collection and clinical examination. Information on their health status was assessed by questionnaires, laboratory assays and physical examination. The sample of 1,325 healthy subjects was categorized according to age in four groups as follows: adults (≤65 years) (n=421), old (66–85 years) (n=642), oldest old (86–98 years) (n=262) and centenarians (>99 years) (n=279).

T2DM was diagnosed according to the American Diabetes Association Criteria (American Diabetes Association 2007). T2DM group was categorized according to age as follows: adults (≤65 years) (n=235) and old (66–85 years) (n=298). All subjects gave their informed consent to participate in the study, which was approved by the Ethics Committee of the I.N.R.C.A. and by the Ethical Committee of the Sant'Orsola-Malpighi Hospital, University of Bologna for centenarians.

Seven biochemical and haematological parameters related to lipid, glucose and inflammatory immunological pathways of ageing were analysed and included fasting plasma GLU (milligrams per decilitre), TC (milligrams per decilitre), TG (milligrams per decilitre), FIBR (milligrams per decilitre), CRP (milligrams per decilitre), WBCC (10^3/mm^3) and Hb (grams per decilitre). Haematology and blood chemistry assays were determined by routine laboratory methods that did not require standardization of such parameters.

All tests were performed at the Medical Laboratory of the I.N.R.C.A. Hospital, Ancona, Italy and at the Medical Laboratory of the Sant'Orsola-Malpighi Hospital, Bologna, Italy.

Overnight fasting venous blood samples of all subjects were collected from 8:00 to 9:00 a.m. The samples were either analysed immediately or stored at −80°C for no longer than 30 days.

Statistical analysis

Statistical analyses were performed according to the following steps: In the first step, differences among the groups were assessed by univariate analysis using ANOVA with multiple comparisons (Dunnett's *t* test) in healthy subjects (Dunnett 1980) and centenarians while a *t* test in T2DM patients. TG and CRP levels were not normally distributed and log-transformed for statistical analyses.

In the second step, EFA was used to investigate the relationships between several correlated variables by identifying underlying factors for each age group, and principal component analysis (PCA) was used to extract the initial set of components (Costello and Osborne 2005). PCA transformed a set of correlated variables into a linear combination which accounted for both the maximum proportion of the total variance and a new set of uncorrelated components. The eigenvalues with a value >1.0 were retained in the analysis. A Varimax orthogonal rotation was used in this analysis to obtain the factors. To interpret the results from factor analysis, the pattern of the factor loading was examined to determine which original variables represented primary constituents of each factor.

Lastly, in the third step, SEM was applied to confirm the factor solutions for each age group. A model representative of the biological parameters (indicators) and the corresponding factors coming from EFA solutions was constructed. After evaluating the improvement of fit from an independent to a hypothesized model, the primary goal was to determine the 'goodness of fit' between the hypothesized structural model and the sample data. The confirmatory approach allowed to test for a priori hypothesis and explicitly defines the association between indicators and factors (latent variables) (Tabachnick and Fidel 2001).

SEM is a statistical method employing factor analysis and linear regression techniques to assess the 'fit' of a model to the data. SEM is an extension of general linear model that enables to examine more complex relationships. The hypothesized model is presented in path diagrams where circles represent latent variables and residuals and rectangles represent measured variables (indicators). Causal effects are represented by single-headed arrows in the path diagram while correlations are associated with double-headed curved arrows. Absence of a line connecting variables implies no hypothesized direct effect (Bollen 1989).

Maximum likelihood estimation was employed to estimate all models. The first step of the SEM analysis was to evaluate the independency of the model by testing the hypothesis that there was no correlation among all variables. In the second step, the hypothesized model was tested and a chi-square difference test was calculated to verify an improvement in the 'fit' between independent and the hypothesized models.

The adequacy of model fit was evaluated using the following statistics to assess the degree of fit between estimated and observed variance: chi-square likelihood ratio statistic, Normed Fit Index (NFI), the goodness-of-fit index (GFI) and root mean square error of approximation (RMSEA).

The NFI reflects the proportion by which the hypothesized model improves fit compared to the independence model. NFI values above 0.95 are good and between 0.80 and 0.95 are acceptable. GFI gives a measure of the accounted variance by the model varying from 0 to 1, and it should be equal to or greater 0.95 to accept hypothesized model. The RMSEA decreases with increasingly good fit. RMSEA provides a 'rule of thumb' cutoff for model adequacy of <0.08. Data analysis was performed using the SPSS/Win program version 17 (Spss Inc., Chicago, IL, USA) and AMOS version 6 (Analysis of Moment Structures). The level of statistical significance was defined by a two-tailed p value <0.05.

Results

Table 1 reports the age-related mean levels across age of selected biomarkers in healthy and T2DM participants. In healthy subjects, FIBR and CRP significantly increased with advancing age, while WBCC did not show any significant difference. GLU and Hb (males) were higher in old compared to adults. TC and Hb were lower in the oldest old, and GLU, TC and Hb were lower in centenarians

Table 1 Clinical biomarkers in healthy subjects according to age

	Adult ≤65 years (*n*=421)	Old 66–85 years (*n*=642)	Oldest old 86–98 years (*n*=262)	Centenarians >99 years (*n*=279)
Plasma glucose (mg/dl)	91.42±10.90	100.71±30.41**	95.93±34.37	88.25±25.31*
Total cholesterol (mg/dl)	211.81±40.56	214.08±42.09	196.35±39.82**	186.22±40.49**
Triglycerides (mg/dl)	104.96±67.98	129.24±66.59**	111.97±55.52**	113.75±57.67
Haemoglobin (g/dl) (men)	14.06±1.30	14.42±1.38**	13.45±1.71**	12.77±1.33**
Haemoglobin (g/dl) (women)	14.23±1.77	13.52±1.33**	12.82±1.71**	12.34±1.76**
Fibrinogen (mg/dl)	297.19±68.60	336.75±89.87**	391.86±103.12**	394.07±134.51**
White blood cells (10^3/mm^3)	6.29±1.62	6.22±1.76	6.47±1.98	6.11±2.02
C-reactive protein (mg/dl)	0.33±0.49	0.58±1.11**	0.98±1.70**	0.75±1.15**

Data are presented as mean ± standard deviation

*p<0.05; **p<0.01 vs ≤65 years

respect to adults. TG levels were increased in older and oldest old, but not in centenarians.

Table 2 shows plasma levels of the same biomarkers in participants with T2DM according to age. GLU levels were significantly lower and FIBR levels significantly higher in old compared to adults. There were no substantial differences observed for other biomarkers across age.

Exploratory factorial analysis

Table 3 reports findings from the exploratory factor analysis of all biomarkers. Three factors were observed in groups of adults and old healthy subjects, while the group of oldest old showed two distinct factors. In the group of adults, parameters were associated with three factors as factor 1 Hb, TG and GLU (explained variance 27.0%), factor 2 FIBR and CRP (explained variance 17.10%) and factor 3 TC and WBCC (explained variance 16.3%). TG and WBCC were loaded in more than one factor.

In the old group, parameters associated with three factors were factor 1 as FIBR, CRP and WBCC (explained variance 24.4%); factor 2 as TG, TC and Hb (explained variance 18.6%) and factor 3 as Hb, GLU and WBCC (explained variance 15.2%). In this group of subjects, WBCC was associated with factors 1 and 3, while Hb was associated with factor 2 and factor 3 (Table 3).

In the oldest old group, parameters associated with two factors were factor 1 as TC, TG, Hb and GLU (explained variance 26.7%) and factor 2 as WBCC, CRP and FIBR (explained variance 23.6%). GLU was loaded in both factors (Table 3).

Table 2 Clinical biomarkers in adults with T2DM according to age

	Adult diabetics ≤65 years (*n*=235)	Old diabetics 66–85 years (*n*=298)
Plasma glucose (mg/dl)	169.62±53.04	159.40±45.46*
Total cholesterol (mg/dl)	208.68±38.18	205.70±37.60
Triglycerides (mg/dl)	148.06±135.42	133.62±83.75
Haemoglobin (g/dl) (men)	14.96±1.11	14.83±1.28
Haemoglobin (g/dl) (women)	13.82±1.03	13.45±1.19
Fibrinogen (mg/dl)	293.54±70.75	311.92±83.26**
White blood cells (10^3/mm^3)	6.77±1.66	6.62±1.58
C-reactive protein (mg/dl)	0.42±0.53	0.46±0.73

Data are presented as mean ± standard deviation

*p<0.05; **p<0.01 vs. ≤65 years for diabetic patients

Table 3 Exploratory factor analysis for both study groups according to age

Variable	Adult ≤65 years (n=421)			Old 66–85 years (n=642)			Oldest old 86–98 years (n=262)		Centenarians (n=279)			Adult diabetics ≤65 years (n=235)			Old diabetics 66–85 years (n=298)	
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2
Haemoglobin (g/dl)	0.728	−0.109	0.048	−0.334	0.462	0.423	0.606	−0.121	0.509	0.009	−0.229	−0.110	0.610	−0.328	−0.381	0.270
Triglycerides (mg/dl)	0.681	0.279	0.405	0.171	0.643	0.256	0.732	0.053	0.772	0.214	−0.035	0.221	0.511	0.538	0.167	0.795
Plasma glucose (mg/dl)	0.579	0.044	−0.082	0.065	−0.042	0.792	0.506	0.379	0.258	0.426	−0.424	−0.018	0.732	0.161	0.054	0.666
Fibrinogen (mg/dl)	−0.185	0.780	0.190	0.758	0.014	−0.131	−0.186	0.750	0.234	0.135	0.836	0.621	−0.161	0.227	0.716	0.050
C-reactive protein (mg/dl)	0.203	0.768	−0.077	0.745	0.059	0.131	−0.241	0.744	−0.093	0.712	0.329	0.673	−0.030	0.161	0.725	0.078
Total cholesterol (mg/dl)	0.157	0.148	0.848	0.060	0.838	−0.283	0.670	−0.203	0.825	−0.139	0.227	0.033	−0.046	0.844	−0.074	0.671
White blood cells (10^3/mm^3)	0.447	0.384	−0.346	0.516	0.161	0.427	0.293	0.645	0.053	0.778	−0.057	0.766	0.167	−0.202	0.579	0.071
Explained variance	27.0%	17.1%	16.3%	24.4%	18.6%	15.2%	26.7%	23.6%	23.1%	19.7%	15.6%	23.9%	18.3%	15.2%	25.6%	18.6%

Findings from the PCA explained 60% of variance in adults and old and 50% in the group of oldest old, suggesting increased variability over ageing. Centenarians revealed a three-factorial structure explaining 58.5% of total variance. The first factor was related to TC, TG and Hb (explained variance 23.1%), the second one to WBCC CRP and GLU (explained variance 19.7%) and the third one to FIBR with CRP (factor loading=0.329) (explained variance 15.6%) (Table 3).

In the T2DM, adults had a factor structure that accounted for 57.4%, of the total explained variance, with three factors, where the first one loaded FIBR, CRP and WBCC (explained variance 23.9%); the second factor (explained variance 18.3%) was related to GLU, Hb and TG that also loaded the third one (explained variance 15.2%) together with TC. In the old T2DM group, we found a two-factorial structure explaining 44.2% of total variance, where the first was related to FIBR, CRP and WBCC (explained variance 25.6%) and the second one to GLU, TC and TG (explained variance 18.6%) (Table 3). Figure 1a–f depicts age-related cluster patterns of the variables in three age-groups of healthy subjects, centenarians and T2DM, respectively.

Structural equation modelling

Figure 2a–d shows the path diagrams for each age group of healthy subjects. The SEM statistics in the final models are shown in Table 4. On the basis of EFA results, three-factor models were hypothesized for adult and old subjects, while a two-factor model for the oldest old group.

The adult group showed that GLU, Hb and TG were indicators of factor 1, CRP and FIBR of factor 2 and TC and WBCC of factor 3. In Table 4, a chi-square difference test indicated a significant improvement in fit between the independent and the hypothesized model. All fit indices (NFI, GFI and RMSA) indicated well fit to the data.

The old group had the following results: CRP, WBCC and FIBR were indicators of factor 1, TC and TG of factor 2 and GLU and Hb of factor 3. The old group also had a structural model suggesting three pathways. Chi-square difference test (Table 4) indicates a significant improvement in fit between the independent and the hypothesized model. All fit indices show good fit to the data. The structures of

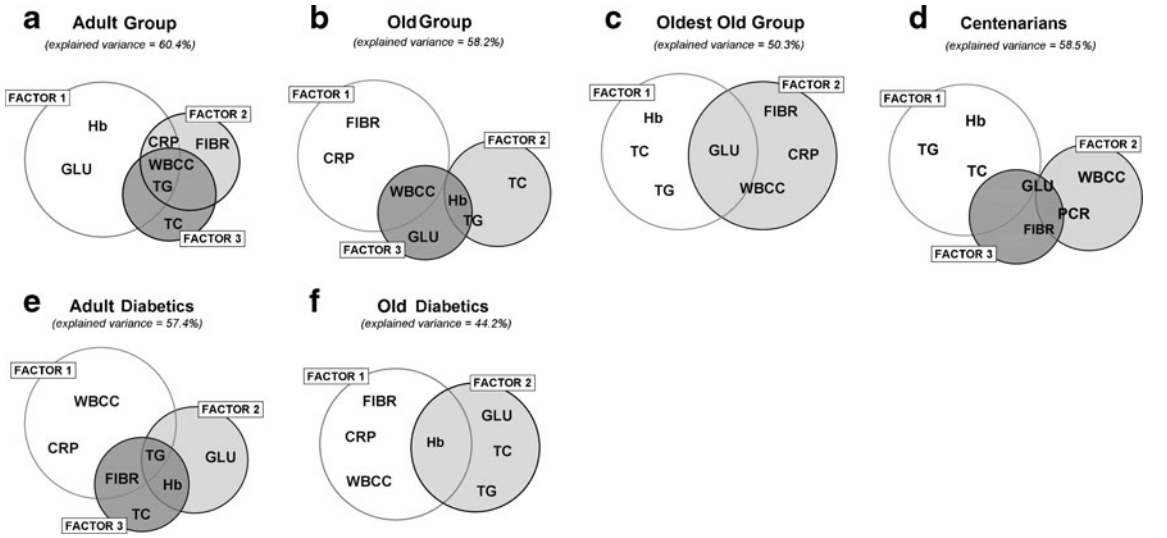


Fig. 1 Cluster pattern of selected variables in healthy people and diabetic patients according to age. Venn diagrams of the exploratory factor analysis for both study groups according to age. The *circle diameters* assess the explained variance for each factors and the *overlap pictures* the relationship between selected variables and factors shown on Table 3. Selected variables: fasting plasma glucose (*GLU*; milligrams per decilitre), total cholesterol (*TC*; milligrams per decilitre), triglycerides (*TG*; milligrams per decilitre), fibrinogen (*FIBR*; milligrams per decilitre), C-reactive protein (*CRP*; milligrams per decilitre), white blood cell count (*WBCC*; 103/mm^3) and haemoglobin (*Hb*; grams per decilitre). The sample of 1,325 healthy subjects was categorized according to age in four groups as follows: adult (≤65 years) (n=421), old (66–85 years) (n=642), oldest old (86–98 years) (n=262) and centenarians (>99 years) (n=279). T2DM group was categorized according to age as follows: adult (≤65 years) (n=235) and old (66–85 years) (n=298)

the relationship among the indicators are simpler respect to the adult group.

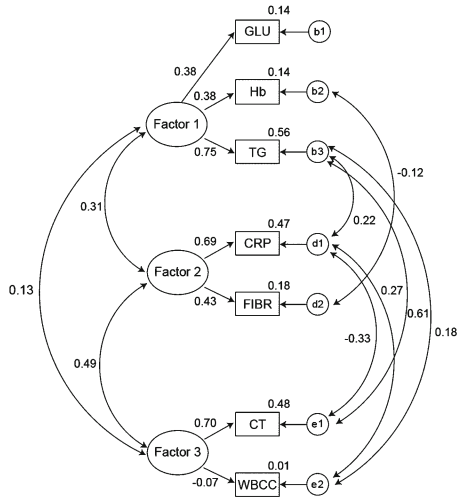
The oldest old group results showed that Hb, TG and TC were indicators of factor 1 and CRP, FIBR and WBCC of factor 2. Considering that from the EFA and GLU loaded as the first and second factor, we hypothesized a structural model with GLU that directly connected with other latent biomarkers. The path diagram shows the impact of glucose in the assessment of the first and second factors.

Path diagram of centenarians (panel d) suggests that TC, Hb and TG were indicators of factor 1, WBCC and GLU of factor 2 and PCR and FIBR of factor 3. SEM statistics in the centenarian model show good fit to the data. Centenarians revealed three structural pathway models similar to adults and old groups.

In Fig. 2e, f depicts path diagrams for each age group of T2DM patients. The adults of the T2DM group had a similar structure to old healthy group suggesting three pathways. The old T2DM group had a similar structure to oldest old healthy group suggesting two pathways. All fit indices showed good fit to the data (Table 4).

Conclusions

The use of exploratory factor analysis and structural equation modelling revealed an important factorial structure of population changes over ageing in a large sample of older individuals. In addition, the model structure of both the T2DM and the centenarian group was performed in order to observe model structure changes in the presence of an age-related disease and in centenarians such as a model of successful ageing. In particular, three-factor structures were identified in adults and old healthy subjects, while two-factor structures were found in the oldest old, suggesting reduced complexity of metabolic pathways during ageing. There was a parallel decline in the explained variance over ageing ranging from 60% to 50%, which suggests an increased level of variability. The factor including inflammatory variables, such as CRP, FIBR and WBCC, explained a higher level of variance in the old and in the oldest old respect to the adults. These findings confirm the inevitable increase in a pro-inflammatory state over human ageing.


a Adult Group ≤ 65 yrs
GLU
Hb
TG
CRP
FIBR
CT
WBCC
Factor 1
Factor 2
Factor 3
b1
b2
b3
d1
d2
e1
e2
0.14
0.38
0.38
0.56
0.75
0.47
0.69
0.18
0.43
0.48
0.70
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0.31
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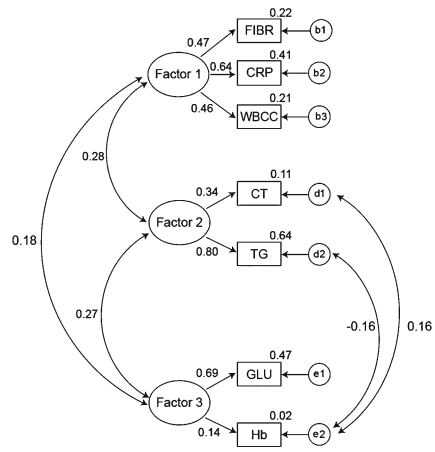
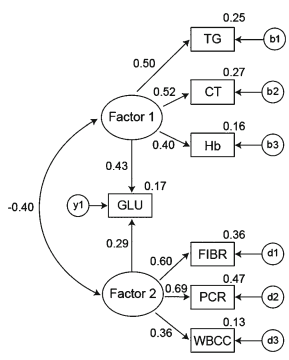
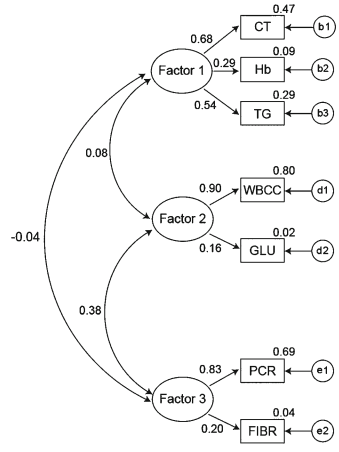
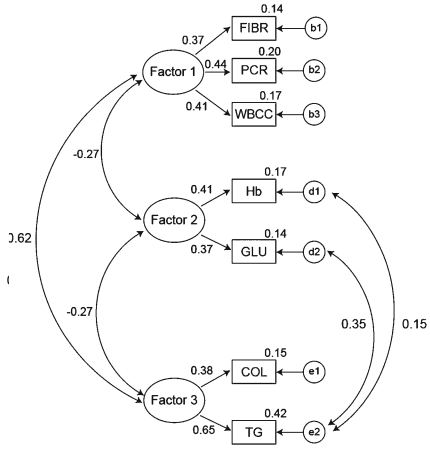
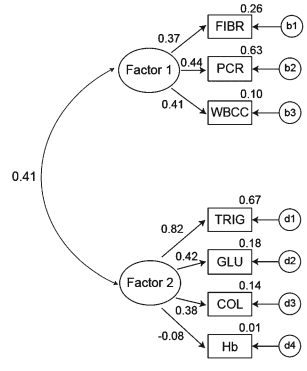
0.18
-0.33


b Older Group 66-85 yrs
FIBR
CRP
WBCC
CT
TG
GLU
Hb
Factor 1
Factor 2
Factor 3
b1
b2
b3
d1
d2
e1
e2
0.22
0.47
0.64
0.41
0.46
0.21
0.28
0.34
0.11
0.80
0.64
0.18
0.27
-0.16
0.16
0.69
0.47
0.14
0.02


c Oldest old Group 86-95 yrs
TG
CT
Hb
GLU
FIBR
PCR
WBCC
Factor 1
Factor 2
y1
b1
b2
b3
d1
d2
d3
0.25
0.50
0.27
0.52
0.16
0.40
0.43
0.17
-0.40
0.29
0.36
0.60
0.69
0.47
0.36
0.13


b Centenarians
CT
Hb
TG
WBCC
GLU
PCR
FIBR
Factor 1
Factor 2
Factor 3
b1
b2
b3
d1
d2
e1
e2
0.47
0.68
0.09
0.29
0.54
0.29
0.08
0.80
0.90
0.02
0.16
-0.04
0.38
0.69
0.83
0.04
0.20


e Adult Diabetics ≤ 65 yrs
FIBR
PCR
WBCC
Hb
GLU
COL
TG
Factor 1
Factor 2
Factor 3
b1
b2
b3
d1
d2
e1
e2
0.14
0.37
0.44
0.20
0.41
0.17
-0.27
0.41
0.17
0.62
0.37
0.14
-0.27
0.35
0.15
0.38
0.15
0.65
0.42


f Old Diabetic 66-85 yrs
FIBR
PCR
WBCC
TRIG
GLU
COL
Hb
Factor 1
Factor 2
b1
b2
b3
d1
d2
d3
d4
0.26
0.37
0.44
0.63
0.41
0.10
0.41
0.67
0.82
0.18
0.42
0.14
0.38
-0.08
0.01

◀ **Fig. 2** Path diagrams for each age group of healthy subjects and diabetic patients. SEM models of the exploratory factor analysis for both study groups according to age. *Circles* represent factors (1, 2, 3) and residuals (**b**, **d**, **e**,), and *rectangles* represent selected variables: fasting plasma glucose (*GLU*; milligrams per decilitre), total cholesterol (*TC*; milligrams per decilitre), triglycerides (*TG*; milligrams per decilitre), fibrinogen (*FIBR*; milligrams per decilitre), C-reactive protein (*CRP*; milligrams per decilitre), white blood cell count (*WBCC*; 103/mm3) and haemoglobin (*Hb*; grams per decilitre). The *single-headed arrow* represents causal relationship. *Double-headed curved arrow* represents the correlation. *Numbers on double-headed curved arrows* are correlation coefficients. *Numbers on single-headed curved arrows* are standardized regression coefficients. *Numbers over the rectangles* are R^2 values. The sample of 1,325 healthy subjects was categorized according to age in four groups as follows: adult (≤65 years) (n=421), old (66–85 years) (n=642), oldest old (86–98 years) (n=262) and centenarians (>99 years) (n=279). T2DM group was categorized according to age as follows: adult (≤65 years) (n=235) and old (66–85 years) (n=298)

Recently, different results were reported on inflammatory 'component' in older populations and its relationship with the measures of physical function (Bandeen-Roche et al. 2009; Hsu et al. 2009). Hsu et al. (2009) showed that inflammatory components have inconsistent associations with different aspects of physical performance, whereas Bandeen-Roche showed that an increased up-regulation of inflammatory mediators was significantly associated with a reduction in the mean mobility score. These authors analysed pro-inflammatory serum/plasma markers, such as IL-1β, TNF-α, IL-6, IL-18 and CRP. Unfortunately, these cytokines were not included in our protocol since their measures are not currently standardized. Indeed, CRP was included in our analysis since this protein is directly stimulated by pro-inflammatory cytokines and its measure is standardized for clinical diagnosis. We also included fibrinogen and white blood cell count which were obtained from standardized blood tests and are easily available. Interestingly, CRP and fibrinogen showed an age-related increase in healthy subjects, and fibrinogen was higher in young compared to older persons with T2DM. Moreover, the percentage of variability, explained by the inflammatory factor, increases with age supporting the hypothesis of 'inflammaging' (Franceschi et al. 2000; Salvioli et al. 2009). Interestingly, the inflammatory component was a significant determinant in the oldest old of healthy subjects and in old T2DM participants. This finding further highlights that a pro-inflammatory condition is accelerated in older persons with an age-related disease, such as T2DM. In the old and oldest groups, the inflammatory component was the first factor, whereas in centenarians it was the second one. This finding supports the hypothesis that a specific genetic anti-inflammatory pattern is present in centenarians which leads to lower production of pro-inflammatory levels in the general ageing population (Candore et al. 2010; Salvioli et al. 2006; Bonafè and Olivieri 2009).

Our data also showed that in the oldest old group of healthy subjects, glucose levels weighted the two factors in the same proportion (Fig. 2c), characterizing the importance of glucose metabolism in this population and suggesting that all selected variables were closely related to glucose levels. Intriguingly, glucose metabolism in healthy centenarians has been shown to be better than that of older individuals (Barbieri et al. 2003; Yashin et al. 2009). This observation could suggest that an efficient metabolic (energetic) pathway is needed to achieve longevity because all biological variables are directly related to glucose levels in the oldest age. It is hypothesized from studies of model organisms, short-term studies of humans and human epidemiologic data that two potent modulators of longevity in humans are insulin/insulin-like growth factor 1

Table 4 Fit indices for all models

Model	H_0 $\chi^{2\ (DF)}$	$\chi^{2\ (DF)}$	NFI	GFI	RMSA (90% CLs)
Adult ≤65 years	266.024 (21)	16.721 (6)	0.937	0.989	0.064 (0.029–0.103)
Old 66–85 years	313.978 (21)	27.170 (9)	0.913	0.987	0.067 (0.042– 0.095)
Oldest old 86–98 years	149.416 (21)	28.002 (9)	0.813	0.968	0.075 (0.039–0.112)
Centenarians	98.023 (21)	13.432 (11)	0.863	0.979	0.062 (0.000–0.114)
Adult diabetics ≥65 years	94.280 (21)	14.498 (10)	0.850	0.980	0.059 (0.000–0.107)
Old diabetics 66–85 years	183.696 (21)	13.434 (13)	0.927	0.988	0.011 (0.001–0.059)

(IGF-1) signalling and dietary restriction (Bonafè and Olivieri 2009; Fontana and Klein 2007; Roth et al. 2004; Willcox et al. 2007). It was suggested that these two pathways are intricately connected and both the insulin/IGF-1 pathway and the pathway that mediates the effects of dietary restriction have evolved to respond to the nutritional status of an organism, which in turn affects the lifespan (Narasimhan et al. 2009). Therefore, a fine regulation of glucose metabolism is required to maintain health as well as achieve longevity (Yashin et al. 2009).

All presented results are most likely explained by the combined effects of selective and remodelling forces towards achieving human longevity and are in accordance with the observation that healthy physiological processes require the complex interaction of multiple control systems operating over multiple time scales, while the ageing process itself and disease are associated with a loss of complexity in the dynamics of many physiological systems. This loss of complexity may reduce the ability to adapt to stress and lead to frailty. It can be hypothesized that the age-related diseases, such as T2DM, are states of critically impaired homeostasis resulting in an increased vulnerability towards stressors. On the contrary, genetically selected healthy older adults maintain a higher level of complexity and are more able to interact with environmental stressors. Future research will determine whether the creation of biomarker indices can improve multiple aspects of physiological complexity underlying human ageing process, screening and monitoring of clinical vulnerability in older adults.

Limitations

Our study included the most commonly used clinical biomarkers. However, we cannot exclude that additional biomarkers could be related to the factorial structure of the sample. Moreover, our data suggest an age-related decrease of complexity and a concomitant increase of variability that may be the result of the combined effects of selective and remodelling forces (genetic and environment) on reaching longevity. Considering the cross-sectional design of our study, we cannot discriminate between a genetic and a remodelling effect on factorial structure of the samples. A larger longitudinal study in older persons will be necessary.

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