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Association between body size and selected hematological parameters in men and women aged 45 and above from a hospitalized population of older adults: an insight from the Polish Longitudinal Study of Aging (1960–2000)

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Abstract: In elderly people, anemia occurs with increasing frequency with each advancing decade and can be a harbinger of very serious health conditions, including gastrointestinal bleeding, gastric and duodenal ulcers, and cancer. Therefore, age-dependant changes in hematological parameters deserve special attention. Nonetheless, very few longitudinal studies of aging have focused on possible associations between basic anthropometric characteristics and hematological parameters in older people. Here, we present some evidence that body size can be associated with red blood cell count as well as some other selected hematological parameters in adults aged 45 to 70 years. Longitudinal data on anthropometric and hematological parameters have been obtained from physically healthy residents at the Regional Psychiatric Hospital for People with Mental Disorders in Cibórz, Lubuskie Province, Poland (142 individuals, including 68 men and 74 women). The residents who took psychoactive drugs were excluded from the study. To evaluate the studied relationships, three anthropometric traits were used and three dichotomous divisions of the study sample were made. The medians of body height, body weight, and body mass index at the age of 45 years were used to divide the sample into: shorter and taller, lighter and heavier, and slimmer and stouter individuals, respectively. Student's t-test, Pearson's correlation, and regression analysis were employed. The results of the present study suggest that the relationship between body size and red blood cell count is slightly more pronounced in men and its strength depends on age. However, the correlations between body size and red blood cell count proved to be weak in both sexes. With aging, the strength of the relation decreased gradually, which might have been caused by the aging-associated changes in the hematopoietic system, anemia, or was an artifact. Further studies are needed to elucidate the unclear association between body size and hematological parameters in older adults.

Key words: aging, anthropometric characteristics, blood, hematological parameters, longitudinal studies, senescence

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Introduction

Red blood cells (RBCs, erythrocytes) are continuously produced throughout ontogeny in the red bone marrow from hematopoietic stem cells, starting from the gestational age of 4–5 months onward, though at earlier stages of the prenatal development, the process of erythropoiesis mainly takes place in several different anatomical sites, i.e. in the yolk sac, the liver, and spleen (Mathur et al. 2011; Pimkin and Weiss 2012; Mescher 2013; Naeim et al. 2013). As erythroblasts mature, they lose their nuclei and most organelles, but acquire oxygen-carrying pigment, i.e. metalloprotein hemoglobin, about 29 pg in each cell. The creation of a terminally differentiated red blood cell takes approximately 5–6 days. The mature red cells function as carriers of oxygen and carbon dioxide. They pick up oxygen in the lungs and ferry it to the cells of the body. Thus, they constitute the only blood cells whose function does not require them to leave the vasculature. The structure of red blood cells is well suited to their primary function. The red blood cells of normal size and shape are called normocytes. They show the form of small (around 7.5 μm in diameter, range 6.5–8.5 μm), flattened, biconcave disks whose centers are thinner that their edges since they are approximately 2.5 μm thick at the rim and only 0.8–1.0 μm thick in the middle (Huang et al. 2011; Mescher 2013). RBCs that are smaller and larger than normocytes are called microcytes and macrocytes, respectively. The shape of red blood cells makes these cells unusually flexible, which permits them to bend as well as squeeze through tiny capillaries. Other functions of red blood cells include: playing certain role in the immune response, modulating T cell proliferation and survival, releasing free radicals from hemoglobin after lysis caused by bacteria or by other intruders in order to destroy the pathogens, protecting against oxidative damage, inactivating some types of free radicals, acting as acid-base buffer, producing nitric oxide, S-nitrosothiol, and certain enzymes, promoting normal blood flow through releasing substances that relax and dilate blood vessels, etc. (Richards et al. 1998; Fonseca et al. 2003; Sprague et al. 2007; Morera and MacKenzie 2011; Jelkmann 2012).

Red blood cells are the most numerous cells in the blood. They constitute around 45% of the total blood volume and 95% of the volume of the formed elements (Jelkmann 2012). Every second, $2–3 \times 10^6$ new cells are formed in the red bone marrow and released into the circulation (Dean 2005). It is estimated that 2.5×10^9 red blood cells are produced throughout the day per kilogram of body weight in normal conditions (Naeim et al. 2013). In total, an adult has approximately $20–30 \times 10^{12}$ RBCs, which comprises roughly one quarter of the total number of cells in the human body (Dean 2005; Bianconi et al. 2013). It should be remembered that normal red blood cell count for a given individual depends on age, sex, and some specific conditions, including altitude, climate, and diet. In newborns, the normal RBC count ranges from 4.9 to 7.5×10^6/μl, whereas in infants up to 2 months of age, it decreases to about 4.5×10^6/μl. After the age of 14, RBC count, blood hemoglobin concentration, and hematocrit are typically higher in males compared with females. Between the ages of 17 and 20, RBC count increases to 4.9×10^6/μl in adolescent males and 4.7×10^6/μl in adolescent females. In adults, the average

normal RBC count is $5.4 \times 10^6/\mu l$ (range, $4.5–5.9 \times 10^6/\mu l$) in men and $4.8 \times 10^6/\mu l$ (range, $4.1–5.1 \times 10^6/\mu l$) in women (Vajpayee et al. 2011). Since mature red blood cells do not have nuclei, they are unable to replenish proteins or repair cellular damage. Therefore, they can survive in the circulation for about 100–120 days and then after covering roughly 240 km of their journey through the vasculature, they must be phagocytized by macrophages in the liver and spleen; the average daily rate of loss of RBCs is 2.08×10^{11} (Nozaki et al. 1995; Dean 2005; Adamson 2008; Bosman et al. 2008; Huang et al. 2011; Jelkmann 2012). In the case of some chronic diseases, including sickle-cell disease, spherocytic anemia, thalassemia, chronic renal failure, and hypersplenism, the lifespan of these cells can be markedly reduced. Some recent studies have shown that the lifespan of red blood cells may be shorter in patients with poor glycemic control, although the results remain mixed (Cohen et al. 2008). RBC count that is significantly lower or higher than expected may result from many different conditions or diseases, such as bone marrow disease, cardiovascular disease, cigarette smoking, dehydration, overhydration, malnutrition, hypoxia, renal disease, bleeding, pregnancy, toxins, neoplasm, drugs, and so forth.

It is well known that RBC count depends on sex and changes significantly with age, but we know of no studies that have examined longitudinal changes with increasing age in RBC count in both sexes, depending on their body size, i.e.: height (m), weight (kg), and relative weight expressed as body mass index, BMI (kg/m^2). The lack of such investigations presumably results from the fact that longitudinal data on changes with advancing age in hematological parameters remain scarce. We have overcome these difficulties by collecting longitudinal data on anthropometric and hematological parameters from the Polish Longitudinal Study of Aging (PLSA), carried out in the years 1960–2000. The present study aimed to evaluate the relationship between body size and RBC count in a hospitalized population of Polish adult men and women from the PLSA.

Materials and methods

The data on anthropometric and hematological parameters have been obtained from the registry at the Regional Psychiatric Hospital for People with Mental Disorders in Cibórz, Lubuskie Province, Poland. In the years 1960–2000, this medical institution functioned also as long-term sheltered accommodation and a residential home for people from the lower socioeconomic strata as well as for those who were there because of a socially motivated decision. In the Polish People's Republic, these residents were at this institution and they underwent routine physicals. Thus, their health was continuously monitored. The hospital staff had their consent to take all these measurements as well as to set up the database for medical and scientific purposes.

After the end of communism in Poland, these residents stayed voluntarily at this hospital until the year 2000, when the system of mental hospitals in Poland was reformed and deeply reorganized. The whole study was conducted in accordance with the Declaration of Helsinki and consisted in observation only. No experiments were conducted. None of the residents was mistreated or tortured. The process of exploring the med-

ical records and collecting the data was performed in the years 2005–2007 with permission and consent of both local and hospital authorities. The health status of the inmates was evaluated systematically during regular physicals at the hospital on its premises by medical staff. The direct access to written records and files that had been stored at the hospital archives of case history, gave our research team a unique opportunity to study longitudinal and cross-sectional changes with age in numerous morphological, physiological, and biochemical traits (Borysławski et al. 2015). The study was carried out in accordance with the Declaration of Helsinki and consisted in observation only. The process of data collection was performed with permission and consent of hospital authorities. All files were anonymized so as not to divulge any personal or confidential information.

Out of the total number of residents (N=3,500), who stayed at the hospital in the analyzed period, we have carefully selected solely data from physically healthy individuals who stayed there continuously for at least 25 years. Thus, the majority (74%) of the chosen individuals were physically and mentally healthy. The rest (26%) had mild mental disorders. The patients who took powerful psychoactive drugs were excluded from this study. Thus longitudinal data were available from 142 subjects, including 68 men and 74 women, whose health status and aging profiles were evaluated for 25 years, starting from the age of 45 onward. The longitudinal part of the study lasted for over 40 years. All the chosen subjects were 45 years of age at the beginning of the study and 70 years of age at the end of the study. It should be stressed that the inmates lived for several decades under very similar and relatively prosperous environmental conditions at the hospital and maintained roughly the same lifestyle. This fact boosts the value of the study sample and makes it quite unique. Further details of the study sample and data collection are described elsewhere (Borysławski et al. 2015; Chmielewski et al. 2015a; 2015b; 2016). To analyze changes with age in the selected parameters as well as test possible differences in the individuals who differed in body size, three anthropometric measures were used and three dichotomous divisions of the study sample were made. The medians of body height (169.5 cm for men, 155.8 cm for women) at the age of 45 years were used to divide the sample into shorter and taller subjects. The medians of body weight (65.7 kg for men, 61.4 kg for women) at the age of 45 years were employed to divide the sample into lighter and heavier subjects. The medians of body mass index, BMI (22.9 kg/m^2 for men, 24.0 kg/m^2 for women) at the age of 45 years were used to divide the sample into slimmer and stouter subjects, N=34 for each subgroup of men and N=37 for each subgroup of women.

All measurements were performed in accordance with internationally accepted standards and requirements. Research techniques and methods of the analysis were standardized. The measurements of body height were performed for a very long time by generally the same nurses using a standard stadiometer, graduated to the nearest 0.1 cm. Height was measured when an individual was standing barefoot with heels together, upper extremities at the side, lower extremities and back straight, shoulders relaxed and the head adjusted to the Frankfort plane, according to the technique described by Martin (1928). Body weight was measured to the nearest 0.1 kg using calibrat-

ed digital scales. The BMI was calculated as body weight (kg) divided by the square of the height (m^2). Blood samples from the median cubital vein were drawn several times a year by a nurse in order to conduct routine examinations such as blood cell counting, erythrocyte sedimentation rate, hematocrit, blood hemoglobin concentration, and some other hematological parameters. Complete blood tests were performed from 10 up to 18 times over a five-year period, for a very long time, i.e. at least 25 years. Therefore, mean values of each analyzed trait were calculated for the consecutive five-year periods, starting from the age of 45 years onward. Blood cell counting was performed manually by medical laboratory scientists. Hemoglobin concentration level was determined with Sahli's hemoglobinometer method. Hematocrit value was estimated using Wintrobe's method.

The normality of the data distribution was tested with the K-S test. To determine and compare the rate and patterns of changes with age in the analyzed parameters in the compared groups of subjects as well as derive mathematical formulae describing these changes and differences, analysis of variance (ANOVA), Student's t-test, Pearson's correlation, and regression analysis were run. To find the best approximation of the function, the method of least squares was applied. A given function of regression was confirmed as the best fitting model of longitudinal changes with aging only when a coefficient of determination (R^2) reached the highest value as well as an unknown parameter (β_0) along with a coefficient of regression (β_1) were statistically significant at $P<0.05$. For the purpose of the study, five types of regression functions were tested: (1) linear function, $y=\beta_1 x + \beta_0$, (2) logarithmic function, $y=\beta_1 \mathrm{Ln}(x) + \beta_0$, (3) polynomial function, $y=\beta_1 x^2 + \beta_2 x + \beta_0$, (4) exponential function type I, $y=\beta_1 x^a$, and (5) exponential function type II, $y=\beta_1 e^{a(x)}$, where x is age (an independent variable), y is a value of an analyzed morphological characteristic (a dependant variable), β_2 stands for the second coefficient of regression, *a* represents the exponent, and *e* denotes the base of the natural logarithm.

Results

The baseline characteristics of the anthropometric and hematological parameters of the subjects at the onset of the study are shown in Tables 1 and 2. All tested traits were normally distributed (K-S test, $p>0.2$). The analysis showed that mean values of the most important morphological parameters generally fell within the normal range for healthy adults. In each age category, men were significantly taller than women. In the first three age categories, men were also heavier than women (t-test, $p<0.05$), whereas women were constantly stouter than men ($p<0.01$ in the first three age categories and $p<0.001$ in the last three age categories). The statistical analysis revealed that at the age of 45 years, body height was positively correlated with body weight in men ($r=0.52$, $p<0.001$) and in women ($r=0.36$, $p=0.02$). At this age, body weight was strongly correlated with BMI in both sexes ($r=0.83$, $p<0.001$ for men; $r=0.88$, $p<0.001$ for women) but no significant correlations were found between body height and body mass index ($r=0.05$, $p=0.703$ for men; $r=0.11$, $p=0.365$ for women).

Men had higher red blood cell count compared with women in each age category (t-test, $p<0.001$; Fig. 1). Likewise, blood hemoglobin concentration level

Table 1. Basic anthropometric parameters analyzed in the PLSA: description, units of measurement and baseline characteristics at the onset of the analysis (all individuals aged 45 years). Statistical significances of the differences were determined using *t*–test

Trait	Unit	Mean	*SD*	Mean	*SD*	*t*–test	*p*–value
		Men (N=68)		Women (N=74)			
Body height	cm	169.7	6.7	157.1	7.2	11.13	0.000
Body weight	kg	66.4	8.9	61.7	11.6	2.67	0.009
BMI	kg/m²	23.0	2.7	25.0	4.4	–3.27	0.001
		Shorter men (N=34)		Taller men (N=34)			
Body height	cm	164.6	4.8	174.8	3.7	–9.82	0.000
Body weight	kg	62.9	8.6	69.8	7.9	–3.43	0.001
BMI	kg/m²	23.2	2.7	22.9	2.7	0.49	0.627
		Shorter women (N=37)		Taller women (N=37)			
Body height	cm	151.8	2.8	162.2	5.8	–9.68	0.000
Body weight	kg	57.1	10.2	66.4	11.1	–3.76	0.000
BMI	kg/m²	24.8	4.4	25.3	4.4	–0.53	0.598
		Lighter men (N=34)		Heavier men (N=34)			
Body height	cm	166.4	6.5	173.0	5.1	–4.72	0.000
Body weight	kg	59.4	4.7	73.4	6.2	–10.46	0.000
BMI	kg/m²	21.5	1.7	24.6	2.6	–5.89	0.000
		Lighter women (N=37)		Heavier women (N=37)			
Body height	cm	155.4	7.6	158.6	5.9	–2.05	0.044
Body weight	kg	52.3	5.2	71.2	7.8	–12.19	0.000
BMI	kg/m²	21.7	2.2	28.4	3.4	–10.06	0.000
		Slimmer men (N=34)		Stouter men (N=34)			
Body height	cm	170.0	7.6	169.5	5.7	0.33	0.745
Body weight	kg	60.8	6.2	72.0	7.6	–6.69	0.000
BMI	kg/m²	21.0	1.2	25.1	2.1	–9.81	0.000
		Slimmer women (N=37)		Stouter women (N=37)			
Body height	cm	156.2	7.9	157.8	5.8	–0.96	0.340
Body weight	kg	52.7	5.9	70.8	8.3	–10.80	0.000
BMI	kg/m²	21.6	2.0	28.5	3.3	–10.84	0.000

and hematocrit value were continuously greater in men compared with women throughout the 25-year study period. There were no significant age-related changes in red blood cell count in men over the period under study, while an increase in red blood cell count in women was observed, as revealed by the best fitting regression functions. The best-fit model of regression was polynomial in men and logarithmic in women. Taller men had significantly higher red blood cell count than shorter men in the age categories of 45 (4.4×10^6/μl vs. 4.2×10^6/μl, t=2.26, p=0.027) and 50 years (4.4×10^6/μl vs. 4.1×10^6/μl, t=3.22, p=0.002; Fig. 2). The age-dependent changes in red blood cell count were nonsignificant in both subgroups of men. During the 25-year period under study, taller and shorter women did not differ in red blood cell count (Fig. 3). The regression function was fitting only in taller women (the logarithmic model was well fitted).

Table 2. Basic hematological parameters analyzed in the PLSA: description, units of measurement and baseline characteristics at the age of 45 in both sexes (N=142)

Analyte	Unit	Mean	*SD*	Mean	*SD*	*t*–test	*p*–value
		Men (N=68)		Women (N=74)			
RBC	10^6/µl	4.3	0.4	3.9	0.4	6.46	0.000
WBC	10^3/µl	6.8	1.5	6.3	2.0	1.50	0.135
Hematocrit	%	43.4	3.4	40.4	2.9	5.57	0.000
Hemoglobin	g/dl	13.4	1.2	12.2	1.2	6.21	0.000
Color index	–	1.0	0.0	1.0	0.0	1.01	0.312
MCV	fl	102.0	7.0	104.9	10.1	–2.01	0.047
MCH	pg	31.5	1.6	31.5	2.1	0.04	0.966
MCHC	g/dl	31.0	2.0	30.2	2.7	1.96	0.052
Lymphocytes	%	30.6	8.1	31.8	6.8	1.00	0.317
Monocytes	%	2.0	1.7	2.3	2.4	0.68	0.500
Eosinophils	%	3.3	2.5	3.3	2.5	0.06	0.953
		Shorter men (N=34)		Taller men (N=34)			
RBC	10^6/µl	4.2	0.4	4.4	0.3	–2.26	0.027
WBC	10^3/µl	7.1	1.6	6.5	1.5	1.46	0.150
Hematocrit	%	42.7	3.3	44.1	3.5	–1.67	0.099
Hemoglobin	g/dl	13.2	1.2	13.6	1.2	–1.39	0.168
Color index	–	1.0	0.0	1.0	0.0	0.03	0.978
MCV	fl	102.7	7.6	101.3	6.3	0.83	0.285
MCH	pg	31.7	1.5	31.2	1.6	1.17	0.562
MCHC	g/dl	31.0	2.0	30.9	2.1	0.07	0.943
Lymphocytes	%	29.4	8.1	31.7	8.1	–1.17	0.246
Monocytes	%	2.0	1.7	2.0	1.7	0.13	0.910
Eosinophils	%	3.5	3.1	3.1	1.7	0.68	0.500
		Lighter men (N=34)		Heavier men (N=34)			
RBC	10^6/µl	4.2	0.4	4.4	0.3	–2.26	0.027
WBC	10^3/µl	7.1	1.6	6.5	1.4	1.54	0.128
Hematocrit	%	42.6	3.5	44.1	3.3	–1.79	0.070
Hemoglobin	g/dl	13.2	1.2	13.7	1.2	–1.80	0.076
Color index	–	1.0	0.0	1.0	0.0	0.75	0.456
MCV	fl	102.6	7.7	101.4	6.3	0.72	0.475
MCH	pg	31.6	1.2	31.4	1.9	0.47	0.637
MCHC	g/dl	30.9	2.0	31.0	2.2	–0.29	0.771
Lymphocytes	%	30.0	9.3	31.1	6.8	–0.58	0.562
Monocytes	%	2.3	2.0	1.8	1.3	1.17	0.247
Eosinophils	%	3.4	2.7	3.3	2.3	0.06	0.952
		Slimmer men (N=34)		Stouter men (N=34)			
RBC	10^6/µl	4.2	0.4	4.3	0.3	–2.07	0.043
WBC	10^3/µl	7.0	1.5	6.6	1.6	0.96	0.340
Hematocrit	%	42.6	3.4	44.2	3.3	–1.91	0.061
Hemoglobin	g/dl	13.1	1.1	13.7	1.2	–2.26	0.027
Color index	–	1.0	0.0	1.0	0.0	–0.11	0.913
MCV	fl	102.3	8.1	101.7	5.7	0.39	0.696
MCH	pg	31.4	1.3	31.6	1.8	–0.56	0.579
MCHC	g/dl	30.8	2.2	31.1	1.9	–0.64	0.525
Lymphocytes	%	29.8	8.8	31.4	7.5	–0.82	0.418
Monocytes	%	2.2	2.0	1.9	1.3	0.69	0.491
Eosinophils	%	3.4	2.6	3.3	2.5	0.14	0.887

Analyte	Unit	Mean	*SD*	Mean	*SD*	*t*–test	*p*–value
		Shorter women (N=37)		Taller women (N=37)			
RBC	10^6/μl	3.9	0.4	3.9	0.4	–0.11	0.912
WBC	10^3/μl	6.5	2.0	6.2	1.9	0.48	0.631
Hematocrit	%	40.2	2.9	40.6	2.9	–0.64	0.522
Hemoglobin	g/dl	12.1	1.3	12.3	1.1	–0.76	0.450
Color index	–	1.0	0.1	1.0	0.1	0.20	0.845
MCV	fl	104.5	9.5	105.4	10.8	–0.40	0.689
MCH	pg	31.2	1.4	31.7	2.6	–1.14	0.257
MCHC	g/dl	30.0	2.6	30.3	2.7	–0.39	0.694
Lymphocytes	%	32.3	6.6	31.3	7.0	0.66	0.514
Monocytes	%	2.0	1.6	2.5	3.0	–0.88	0.379
Eosinophils	%	3.2	2.4	3.4	2.6	–0.23	0.822
		Lighter women (N=37)		Heavier women (N=37)			
RBC	10^6/μl	3.8	0.4	3.9	0.4	–1.23	0.222
WBC	10^3/μl	6.4	2.0	6.3	2.0	0.37	0.713
Hematocrit	%	40.3	3.0	40.5	2.8	–0.22	0.828
Hemoglobin	g/dl	12.0	1.1	12.4	1.2	–1.29	0.201
Color index	–	1.0	0.1	1.0	0.1	–0.42	0.678
MCV	fl	106.2	10.8	103.7	9.3	1.08	0.285
MCH	pg	31.4	2.2	31.5	2.0	–0.06	0.954
MCHC	g/dl	29.8	2.9	30.5	2.4	–1.10	0.274
Lymphocytes	%	32.4	7.5	31.2	6.0	0.74	0.464
Monocytes	%	2.0	1.7	2.6	2.9	–1.12	0.268
Eosinophils	%	3.0	1.7	3.6	3.1	–1.10	0.273
		Slimmer women (N=37)		Stouter women (N=37)			
RBC	10^6/μl	3.8	0.4	4.0	0.4	–1.99	0.050
WBC	10^3/μl	6.5	2.0	6.2	2.0	0.77	0.445
Hematocrit	%	40.2	3.1	40.6	2.7	–0.63	0.533
Hemoglobin	g/dl	11.9	1.1	12.4	1.2	–2.05	0.044
Color index	–	1.0	0.1	1.0	0.1	–0.25	0.804
MCV	fl	106.4	10.6	103.5	9.5	1.26	0.212
MCH	pg	31.4	2.3	31.5	2.0	–0.16	0.869
MCHC	g/dl	29.7	2.8	30.6	2.4	–1.41	0.163
Lymphocytes	%	32.0	7.7	31.7	5.8	0.16	0.871
Monocytes	%	2.2	2.4	2.4	2.3	–0.38	0.706
Eosinophils	%	3.3	2.8	3.4	2.2	–0.18	0.860

Heavier men had higher red blood cell count compared with lighter men in the first three age categories (Fig. 4), i.e. at the ages of 45 (4.4×10^6/μl vs. 4.2×10^6/μl, t=2.26, P=0.027), 50 (4.4×10^6/μl vs. 4.1×10^6/μl, t=3.29, p=0.002), and 55 years (4.5×10^6/μl vs. 4.2×10^6/μl, t=2.99, p=0.004). No significant differences between red blood cell count in heavier and lighter women were observed (Fig. 5). There were no significant age-related changes in red blood cell count in these subgroups, except for a gradual increase in red blood cell count in heavier women (the logarithmic model was best fitted). Stouter men had higher red blood cell count compared with slimmer men at the age of 45 years (4.4×10^6/μl vs. 4.2×10^6/μl, t=2.07, p=0.043; Fig. 6). In women, red blood cell count was higher in stouter individuals compared with slimmer ones at the ages of 45 (4.0 ×

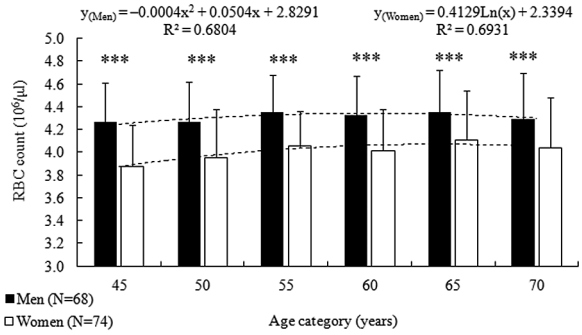

Fig. 1. Longitudinal changes with age in red blood cell count in the consecutive age categories in men and women from the PLSA, expressed as the regression lines: arithmetic means, standard deviations, and differences between the subjects from the two compared groups, tested with the Student's t-test (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)

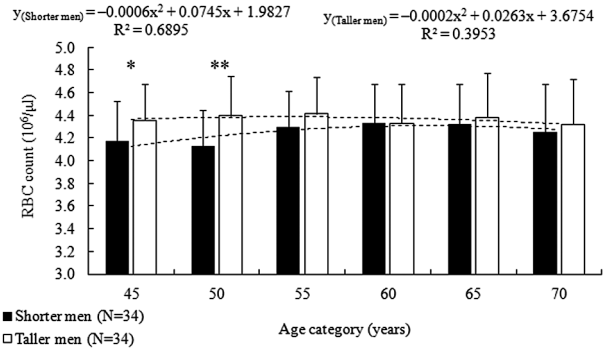

Fig. 2. Longitudinal changes with age in red blood cell count in the consecutive age categories in shorter and taller men from the PLSA, expressed as the regression lines

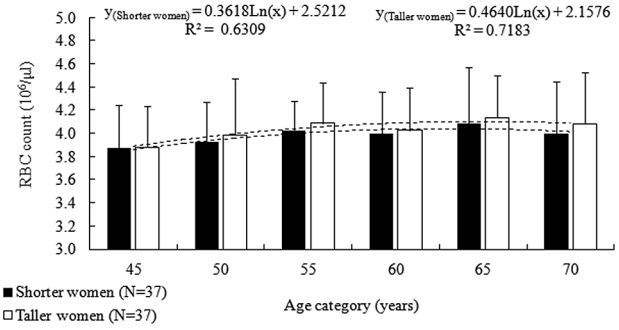

Fig. 3. Longitudinal changes with age in red blood cell count in the consecutive age categories in shorter and taller women from the PLSA, expressed as the regression lines

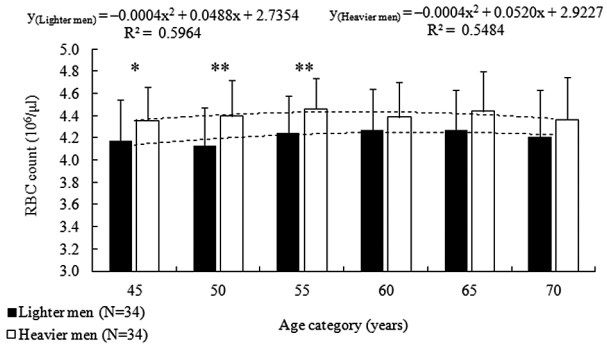

Fig. 4. Longitudinal changes with age in red blood cell count in the consecutive age categories in lighter and heavier men from the PLSA, expressed as the regression lines

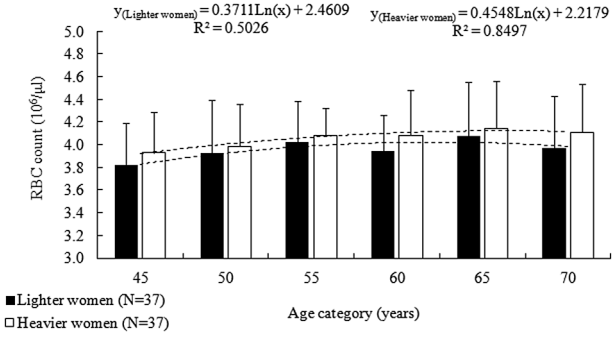

Fig. 5. Longitudinal changes with age in red blood cell count in the consecutive age categories in lighter and heavier women from the PLSA, expressed as the regression lines

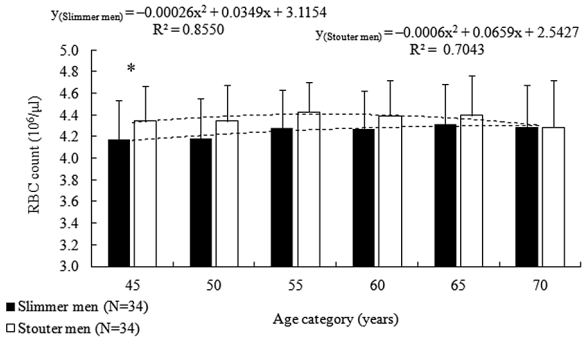

Fig. 6. Longitudinal changes with age in red blood cell count in the consecutive age categories in slimmer and stouter men from the PLSA, expressed as the regression lines

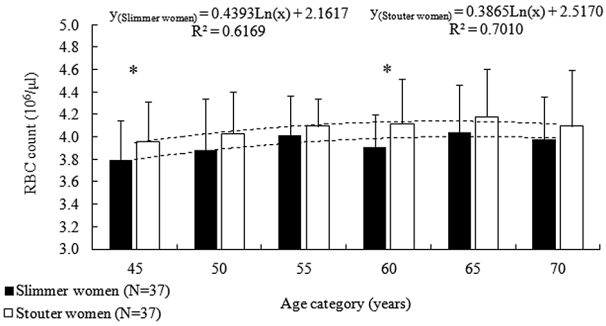

Fig. 7. Longitudinal changes with age in red blood cell count in the consecutive age categories in slimmer and stouter women from the PLSA, expressed as the regression lines

10^6/µl vs. 3.8 × 10^6/µl, t=1.99, p=0.05) and 60 years (4.1 × 10^6/µl vs. 3.9 × 10^6/µl, t=2.59, p=0.012; Fig. 7).

At the onset of the study, i.e. in the age category of 45 years, a moderate positive relationship between body height and red blood cell count was found for men (r=0.4, p=0.02; Fig. 8A), but not for women (r=0.2, p=0.08; Fig. 8B). Moreover, there was a moderate positive correlation between body weight and red blood cell count in men (r=0.4, p<0.001; Fig. 9A) and a weak correlation in women (r=0.3, p=0.028; Fig. 9B). The body mass index was positively related to red blood cell count in men (r=0.3, p=0.018; Fig. 10A), but not in women (r=0.2, p=0.176; Fig. 10B). At the age of 50, the correlations were moderate and significant in men (r=0.5, p<0.001 with respect to height, r=0.4, p<0.001 with respect to weight, and r=0.2, p=0.049 with respect to the BMI). In women, no such relationships were found, except for the weak positive correlation between height and red blood cell count (r=0.3, p=0.021). In men, at the age of 55, there was a weak positive correlation between body height and red blood cell count (r=0.2, p=0.048) as well as between body weight and red blood cell count (r=0.3, p=0.025), but we found no correlation between the BMI and RBC count (r=0.2, p=0.2). In women, at the age of 55, only body height was weakly associated with red blood cell count (r=0.3, p=0.02). In

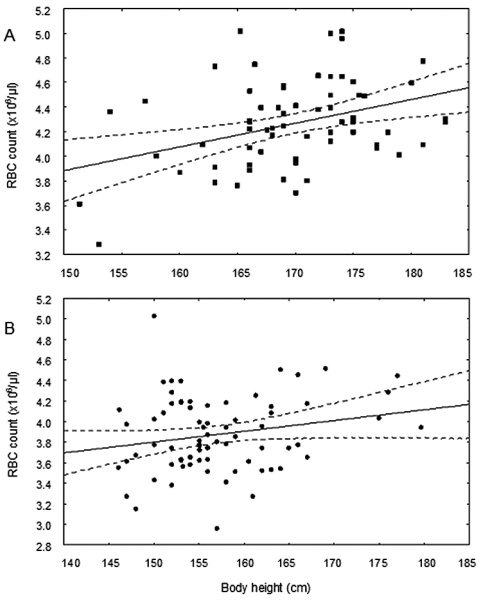

Fig. 8. The correlation between body height and red blood cell count in men (A) and women (B) at the onset of the study (all subjects aged 45 years)

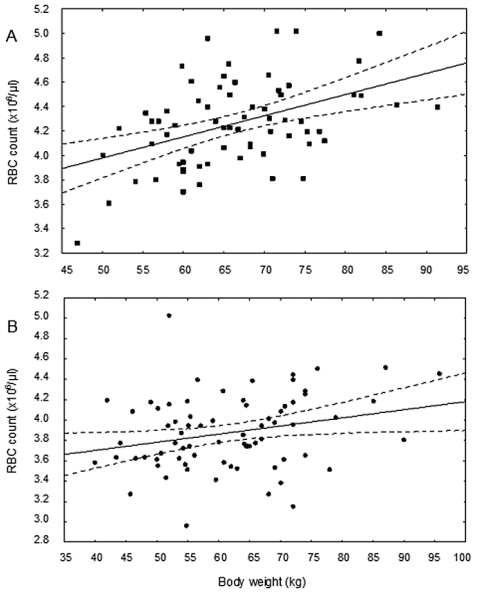

Fig. 9. The correlation between body weight and red blood cell count in men (A) and women (B) at the onset of the study (all subjects aged 45 years)

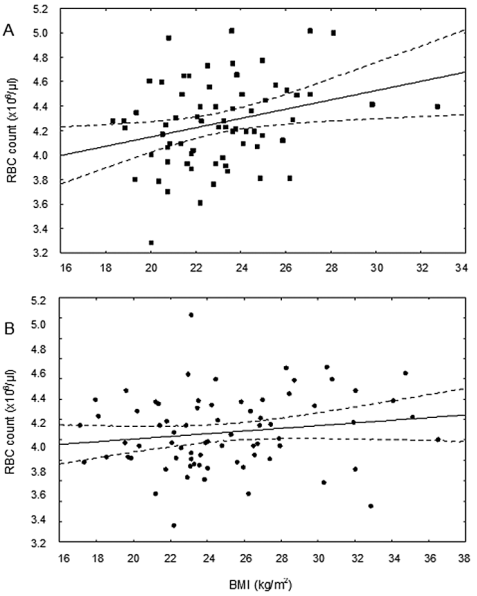

Fig. 10. The correlation between body mass index and red blood cell count in men (A) and women (B) at the onset of the study (all subjects aged 45 years)

general, in later age categories there were no such associations, except for a weak positive correlation between height and red blood cell count at the ages of 65 and 70 years in both sexes ($r \approx 0.3$, $p \approx 0{,}05$).

In men and women, there were significant age-related changes in hematocrit value. The curve of regression assumed an inverted U-shaped pattern in both cases and the models were polynomial (for men: $y = -0.0142x^2 + 1.5917x$, $R^2 = 0.857$; for women: $-0.0134x^2 + 1.4897x$, $R^2 = 0.594$). The highest values occurred at the age of 55 in men (44.2%) and at the age of 50 in women (41.3%). In each age category, hematocrit levels were higher in men than in women (t-test, $p < 0.001$).

In both sexes, there were no significant differences between shorter and taller individuals through the period under study (t-test, $p > 0.05$). In these four groups of subjects, the models of regression were polynomial (for shorter men: $y = -0.0119x^2 + 1.342x + 6.2468$, $R^2 = 0.914$; for taller men: $y = -0.0087x^2 + 0.9393x + 19.344$, $R^2 = 0.951$; for shorter women: $y = -0.0071x^2 + 0.75x + 20.949$, $R^2 = 0.912$; for taller women: $y = -0.0079x^2 + 0.86x + 17.891$, $R^2 = 0.711$).

There were no significant differences in hematocrit level between lighter and heavier men in any of the six age categories (t-test, $p > 0.05$). However, in first three age categories, i.e. 45, 50, and 55 years, heavier men tended to have higher hematocrit levels than lighter men and the significance of the differences was on the border of statistical significance (at age 45, $t = 1.79$, $p = 0.070$; at age 50, $t = 1.87$, $p = 0.066$; at age 55, $t = 1.94$, $p = 0.056$). In both these groups of men,

the best-fit models turned out to be polynomial (for lighter men: $y = -0.0115x^2 + 1.278x + 8.082$, $R^2=0.880$; for heavier men: $y = -0.0092x^2 + 1.0032x + 17.5088$, $R^2=0.931$). At the age of 55 years, heavier women had significantly higher hematocrit level than lighter women ($t=2.02$, $p=0.047$). For these two groups of women, the best fitting models of regression proved to be polynomial as well (for lighter women: $y = -0.0060x^2 + 0.6323x + 24.1566$, $R^2=0.933$; for heavier women: $y = -0.0089x^2 + 0.9779x + 14.6835$, $R^2=0.805$).

In each age, the differences in hematocrit level between slimmer and stouter men were nonsignificant (t-test, $p>0.05$). However, at the onset of the study (age 45), the difference was on the border of statistical significance (44.2% in stouter men vs. 42.6% in slimmer men, $t=1.91$, $p=0.061$). In both these groups of men, the best-fit models were polynomial (for slimmer men: $y = -0.0086x^2 + 0.981x + 15.8336$, $R^2=0.92$; for stouter men: $y = -0.0120x^2 + 1.3003x + 9.7573$, $R^2=0.908$). Stouter women had higher hematocrit level than slimmer ones at the age of 55 ($t=2.25$, $p=0.027$) as well as at the age of 60 ($t=2.57$, $p=0.012$). In both slimmer and stouter groups of women, the best fitting models of regression were polynomial (for slimmer women: $y = -0.0036x^2 + 0.3597x + 31.3765$, $R^2=0.769$; for stouter women: $y = -0.0136x^2 + 1.5138x$, $R^2=0.912$).

As for blood hemoglobin concentration, changes with age in both sexes and in each group of subjects are very similar to those observed in red blood cell count. In men, there was a steady but very slight and slow age-related decrease in blood hemoglobin level. However, these age-dependent changes turned out to be statistically nonsignificant (ANOVA, $p>0.05$). No age-related changes in hemoglobin level in women were found. In each age, men had higher blood hemoglobin concentration than women (t-test, $p<0.001$). In men, the best-fit model of regression was linear ($y = -0.0091x + 13.8588$, $R^2=0.287$). In women, the model was polynomial ($y = -0.0014x^2 + 0.1618x + 7.6632$, $R^2=0.702$).

Taller men had higher hemoglobin level than shorter men at the age of 50 years (13.6 vs. 13.0 g/dl, $t=2.20$, $p=0.032$), which is in agreement with the previous observation concerning the differences in red blood cell count between shorter and taller men. In both groups of women, no significant differences in hemoglobin level were found. In men, the best fitting models of regression were linear (for shorter men: $y = -0.003x + 13.3543$, $R^2=0.027$; for taller men: $y = -0.0152x + 14.3633$, $R^2=0.443$). In women, the models were polynomial (for shorter women: $y = -0.0011x^2 + 0.1363x + 8.2223$, $R^2=0.609$; for taller women: $y = -0.0016x^2 + 0.1874x + 7.1041$, $R^2=0.715$).

Heavier men had higher hemoglobin level than lighter men at the ages of 50 (13.7 vs. 12.9 g/dl, $t=2.89$, $p=0.005$) and 55 years (14.0 vs. 13.3 g/dl, $t=2.16$, $p=0.034$). No such differences were found in women (t-test, $p>0.05$ in each age category). In both groups of men, the best-fit models were linear (for lighter men: $y = -0.0026x + 13.2222$, $R^2=0.038$; for heavier men: $y = -0.0155x + 14.4954$, $R^2=0.400$). In women, the models of regression turned out to be polynomial (for lighter women: $y = -0.0008x^2 + 0.1006x + 9.2074$, $R^2=0.408$; for heavier women: $y = -0.0019x^2 + 0.2231x + 6.1190$, $R^2=0.823$).

Stouter men had higher blood hemoglobin level than slimmer ones at the age

of 45 years (13.7 vs. 13.1 g/dl, t=2.26, p=0.027). Interestingly, the analysis revealed that stouter women had significantly higher blood hemoglobin level than slimmer women at the ages of 45 (12.4 vs. 11.9 g/dl, t=2.05, p=0.044), 60 (12.7 vs. 12.0 g/dl, t=2.40, p=0.019), and 65 years (12.9 vs. 12.2 g/dl, t=2.12, p=0.037). In both groups of men, the best fitting models were linear (for slimmer men: y= 0.0055x + 12.8601, R^2=0.144; for stouter men: y= –0.0236x + 14.8575, R^2=0.612). In both groups of women, the models of regression were polynomial (for slimmer women: y= $–0.0007x^2$ + 0.0893x + 9.41, R^2=0.318; for stouter women: y= $–0.0020x^2$ + 0.2344x + 5.9165, R^2=0.74). Thus, we found that at the age of 45, both red blood cell count and blood hemoglobin concentration were significantly higher in stouter men compared with slimmer men. Similarly, stouter women had higher red blood cell count than slimmer ones at the ages of 45 and 60 and they had higher blood hemoglobin concentration than slimmer women at the ages of 45, 60, and 65 years.

In men, there was a significant decline with age in color index of the blood and the best fitting model was exponential type I (y= $1.361x^{-0.00826}$, R^2=0.79). The color index decreased with age in women as well, but the best-fit model of regression turned out to be linear (y= –0.0014x + 1.0469, R^2=0.93).

In each age category, there were no statistically significant differences in the color index between shorter and taller men (t-test, p>0.05). In women, however, the color index was higher in taller women compared with the shorter ones at the age of 55 (0.985 vs. 0.965, t=2.12, p=0.038). In both groups of men, the best fitting models were exponential type I (for shorter men: y= $1.4741x^{-0.1027}$, R^2=0.805; for taller men: y= $1.2561x^{-0.0624}$, R^2=0.592). In both groups of women, the models of regression were linear (for shorter women: y= –0.0014x + 1.0476, R^2=0.702; for taller women: y= –0.0014x + 1.0462, R^2=0.776).

In men and women, no significant differences in the color index were observed between lighter and heavier subjects in each age category (t-test, p>0.05). In both groups of men, the best-fit models of regression were exponential type I (for lighter men: y= $1.4583x^{-0.1001}$, R^2=0.77; for heavier men: y=$1.2705x^{-0.0651}$, R^2=0.646). In both groups of women, however, the best-fit models were again linear (for lighter women: y= –0.0017x + 1.0682, R^2=0.925; for heavier women: y= –0.001x + 1.0256, R^2=0.714).

There were also no significant differences in the color index between slimmer and stouter subjects in both sexes and in each age category (t-test, p>0.05). In both groups of men, the best fitting models were exponential type I (for slimmer men: y= $1.3767x^{-0.0862}$, R^2=0.745; for stouter men: y= $1.3454x^{-0.079}$, R^2=0.758). In women, the models of regression were linear and well adjusted (for slimmer women: y= –0.0013x + 1.0383, R^2=0.843; for stouter women: y= –0.0015x + 1.056, R^2=0.934).

Discussion

Hematological parameters, such as RBC count, hematocrit level, and blood hemoglobin concentration, are partially related to each other by biochemical and physiological factors like oxygen deprivation (hypoxia), erythropoietin, interleukin-3, and other erythropoiesis-stimulating agents, including androgens, thyroid hormones, cortisol, and growth hormone

(Meineke and Crafts 1968; Peschle et al. 1972, 1978; Golde et al. 1977a, 1977b; Goodman et al. 1985; Merchav et al. 1988; Umemura et al. 1989; Bijlani and Manjunatha 2011; Mathur et al. 2011; Pimkin and Weiss 2012). Hypoxia is probably the most powerful physiological mechanism that promotes erythropoiesis, and the kidneys are the most sensitive oxygen sensors involved in mediating the hypoxic induction of the production of RBCs by the red bone marrow (Goldberg et al. 1988, 1989; Haase 2010).

The results of the present study suggest that in adulthood there is a positive relationship between body height and RBC count in both sexes. Since there were no significant differences between the residents in terms of place of living, altitude, diet, lifestyle, etc., these findings could be tentatively interpreted as suggesting that shorter and taller individuals differ in the rate of the process of erythropoiesis due to different levels of growth hormone (GH), insulin-like growth factor 1 (IGF-1), and some other intrinsic factors associated with the difference with hormonal milieu between shorter and taller individuals, rather than due to some extrinsic factors, such as diet, nutrition, lifestyle, and so forth. However, no direct analysis of hormone profiles in the subjects was performed and, therefore, this is only a tentative explanation of the observed relationships.

Growth hormone (GH) is released in a pulsatile fashion by the anterior lobe of the pituitary gland (adenohypophysis) during the process of growth but its level and possible differences in the rate of secretion between shorter and taller adults as well as their long-term consequences have not been fully studied (Gardner and Shoback 2011). Some authors reported that erythropoiesis is impaired in adult patients with growth hormone deficiency and growth hormone stimulates erythropoiesis in adults with such deficiency (Christ et al. 1997). It was hypothesized that there is a permissive role of growth hormone in the hematopoietic system because when growth hormone deficiency is associated with multiple pituitary hormone deficiency, there are some pathological effects on erythropoiesis which are not corrected until growth hormone treatment is started (Valerio et al. 1997). It is well known that hypopituitarism is often accompanied by normocytic anemia.

On the other hand, adults with growth hormone deficiency are generally not anemic. Furthermore, growth hormone treatment does not increase blood hemoglobin concentration or hematocrit value (Cuneo et al. 1991). Likewise, young individuals with growth hormone deficiency are usually not anemic, which seems to contradict the statement that normal level of growth hormone is indispensable to erythropoiesis *in vivo* (Vihervuori et al. 1996). However, different compensatory mechanisms may play an important role here. Be that as it may, growth hormone has a stimulatory effect on erythropoiesis, by and large, and it was demonstrated that growth hormone treatment increases plasma erythropoietin level in anemic patients with growth hormone deficiency (Sohmiya and Kato 2001). It is commonly believed that growth hormone stimulates erythropoiesis by increasing the oxygen consumption of tissues and thereby promoting tissue hypoxia, which in turn accelerates erythropoietin production by the kidneys (Bijlani and Manjunatha 2011).

The statistical analysis has also revealed that both absolute body weight and relative body weight, expressed as

body mass index, are positively related to red blood cell count in adults of both sexes. Thus we found a positive correlation between body size and red blood cell count. In general, this relationship was more pronounced in men and depended on age since it was gradually waning with increasing age in both sexes. Many endocrinological and hematological studies have shown that individuals with high body mass index tend to have elevated levels of steroid hormones produced by the zona fasciculata of the adrenal cortex, mainly cortisol. This hormone stimulates the stem cells in the red bone marrow and its higher level in the blood is associated with increased production of hemoglobin, red blood cells, white blood cells, and platelets (Lodish et al. 2010; Hattangadi et al. 2011). Our observation that stouter individuals tend to have higher blood hemoglobin concentration, elevated hematocrit value, and higher red blood cell count compared with slimmer individuals can be attributed to some extent to the increased incidence of mutations in the glucocorticoid receptors and higher sensitivity of these receptors. The polymorphism of the glucocorticoid receptor has been linked to central obesity in men (Dobson et al. 2001). Moreover, the polymorphism of gene coding for glucocorticoid receptor is more prevalent among individuals with high body mass index. The *N363S* polymorphism is associated with increased individual glucocorticoid sensitivity, which means that stouter people are relatively more prone and susceptible to cortisol than slimmer people. As a consequence, potentially higher concentration of biologically active cortisol in the blood and greater susceptibility to this glucocorticoid in stouter individuals compared with slimmer ones can result in increased red blood cell production and elevated red blood cell count. Furthermore, the production of androgens by the adrenal gland is usually increased in overweight and obese individuals, mainly due to increased peripheral clearance but also because of disorders of the dynamics of the adrenal cortical response to adrenocorticotropic hormone (Simkin 1961).

As to the observed sex differences, higher values of the discussed hematological parameters in each age category in men compared with women (t-test, $p<0.05$) can be attributed to some extent to the sex difference in the hormonal milieu. Most importantly, androgens like testosterone promote erythropoiesis by several different mechanisms, while estrogens have inhibitory effects on the process of erythropoiesis (Dukes and Goldwasser 1961; Mirand and Gordon 1966; Naets and Wittek 1966; Lindemann 1973; Shahidi 1973; Shahani et al. 2009). Estrogens suppress erythropoietin production as well as inhibit the hematopoietic stem cell response to this glycoprotein. Moreover, testosterone and other androgens play an important role in the development of red blood cells. These sex hormones enhance erythropoietin secretion and stimulate the development of erythroid progenitor cells. Moreover, testosterone modulates and increases iron assimilation as well as improves red blood cell iron uptake. Androgens can also act directly on the red bone marrow to accelerate the process of erythropoiesis (Rishpon-Meyerstein et al. 1968). Androgens potentiate the action of erythropoietin by increasing the population of hematopoietic stem cells on which erythropoietin can act (Bijlani and Manjunatha 2011). Thus these sex hormones can increase the plasma erythropoietin level, red blood cell count,

blood hemoglobin concentration, and hematocrit value. It was hypothesized that testosterone increases hematocrit by suppressing the iron regulatory peptide hepcidin, thereby resulting in increased bioavailable iron (Bachman et al. 2010). Interestingly, testosterone inhibits hepcidin transcription and testosterone administration is linked to increased iron incorporation into red blood cells (Guo et al. 2013). However, the mechanisms by which androgens along with testosterone increase hemoglobin concentration and hematocrit remain poorly understood.

It was earlier established that the correlation between body weight and blood volume is linear, while the relation between body height and blood volume is nonlinear. The relationship between age and blood volume is nonlinear as well (Wolański 2012). Although biological mechanisms of control of erythropoiesis do not change significantly with age, serum erythropoietin level increases with aging to compensate the subclinical blood loss in elderly people. With age, some natural regressive changes in the hematopoietic system occur. As a result, average values of red blood cell count, blood hemoglobin concentration, hematocrit value, ferritin and serum iron levels, decrease slightly with age, whereas erythropoietin resistance eventually develops, especially in older individuals with diabetes mellitus, heart disease, and chronic kidney failure (Beers and Berkow 2000). Anemia is a common condition in older men (9–18%) and older women (8–13%) and its prevalence among individuals aged 85 years and above exceeds 20% (Patel and Guralnik 2009). Normocytic anemia is also more prevalent among older people. Furthermore, iron-deficiency anemia might occur in elderly people, which often signals other serious health problems such as chronic gastrointestinal bleeding, gastric ulcers, duodenal ulcers, and cancer. Since the period under study was not long enough to embrace the later stages of ontogeny and the age-dependent changes during senescence, no significant age-related changes in red blood cell count in men and women were observed. Nonetheless, men experienced significant age-related decrease in blood hemoglobin concentration starting from the age of 55 years onward, whereas age-dependent decline in hematocrit value occurred in both sexes, starting from the same age category onward.

Authors' contributions

PC conceived and designed the study, was a principal investigator for the research project, analyzed the data, interpreted the results and wrote the paper; BS helped analyze the data; JC and KC collected the data and performed initial statistical analyses; KB collected the data and supervised the research.

Conflict of interest

The authors declare that there is no conflict of interests.

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