Effect of Donor Age on the Deformability and Aggregability of Density-Separated Red Blood Cells

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ABSTRACT

Deformability of density-separated red blood cells (RBC) was determined in individuals, in three different age groups. The "young" group was composed of eight individuals between 22-30 years of age; there were eight individuals between 35-45 years of age in the "mid-age" group and 8 individuals between 60-85 years of age in the "old" group. Density separation was performed using discontinuous, Iodixanol (Optiprep®) density gradients. Five layers of Iodixanol with densities between 1.075 and 1.115 g/mL were used. The density distribution of RBC was estimated by determining the hemoglobin concentration in each layer, after centrifugation. The RBC deformability was measured by ektacytometry in least and most dense RBC fractions. In all age groups, the RBC were mostly accumulated in the layers with 1095 and 1105 g/mL densities. In the "young" group, about 80% of RBC were distributed in each of these gradients almost equally. In the "old" group, 50% of RBC were in 1095 g/mL density layer, while 28% was present in the 1105 g/mL layer. The values for "mid-age" group were in between "young" and "old" groups. In all age groups, EI of denser RBC were smaller, in comparison with the RBC in less dense layers. However, the difference between the EI of less and more dense RBC was more pronounced in the "old" group. RBC aggregation was found to be higher in denser RBC fraction, but the difference was less pronounced in "old" group. These results suggest that, RBC circulating in the vasculature of "aged" individuals exhibit more pronounced rheological alterations, during the aging process of RBC.

Key Words: Age, Red blood cell.

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INTRODUCTION

It is well known that in vivo aging of red blood cells (RBC) is associated by increased cellular density[1]. Density separation has been widely used to obtain RBC at various ages accepting that the older RBC get denser[2-9]. It has been previously shown that rheological properties of RBC may vary significantly according to their density. Several groups reported that denser RBC have decreased deformability and increased aggregability[8-11]. Such alterations together with consequent metabolic and functional alterations in RBC might be related to metabolic deteriorations as they age. Alternatively, RBC might be damaged by the factors in their microenvironment through out their life span, including the effects of neighboring cell (e.g., leukocytes) and local metabolic/chemical factors (e.g., oxygen free radicals)[12,13]. Such mechanisms of RBC aging might be altered by the general aging process of the individual. This study aims at investigating the differences in density separated RBC in humans in various age groups.

MATERIALS and METHODS

Donors and blood samples: Twenty four healthy volunteers were included in the study. These volunteers were divided into three groups according to their age as follows:

1. Young group: 8 individuals between 22-30 years of age; 4 female and 4 male.

2. Mid-age group: 8 individuals between 35-45 years of age; 1 female and 7 male.

3. Old group: 8 individuals between 60-85 years of age; 4 female and 4 male. The blood samples were obtained from an antecubital vein and anticoagulated by heparin (15 IU/mL).

Density seperation of RBC: RBC were separated according to their density using discontinuous Iodixanol (Optiprep®) density gradients. Iodixanol with densities between 1.075 and 1.115 g/mL (2 mL each) were layered on top of each other in a test tube. One mL of whole blood was carefully layered on top of the least dense layer and the tube was centrifuged at 2500 g for 25 min, at 22°C. Each layer was carefully obtained separately and the density distribution of RBC was estimated by determining the hemoglobin concentration in each layer.

The two density fractions in which RBC were mostly accumulated were used for the analysis of deformability and aggregability. The RBC density accumulated in 1.095 g/mL layer were used as least dense fraction, whereas the RBC in 1.105 g/mL were accepted as most dense fraction.

Measurement of RBC deformability: RBC deformability (i.e., the ability of the entire cell to adopt a new shape in response to a deforming force) in least and most dense fractions was determined at various fluid shear stresses. The ektacytometer based on a laser diffraction system (LORCA, RR Mechatronics, Hoorn, The Netherlands) used in this study has been described elsewhere in detail[14]. Briefly, RBC are suspended at a low volume concentration in an isotonic, high-viscosity solution of polyvinylpyrolidone (PVP, 360 kDa molecular mass), then subjected to defined shear stresses in a transparent coaxial Couette system. Laser light is directed through the suspension and is diffracted by the cells; at stasis the diffraction pattern is circular and becomes elongated with cell deformation. The laser diffraction pattern is analyzed by a microcomputer and RBC elongation indexes (EI) are calculated for shear rates between 0.5-50 Pascal (Pa); an increased EI indicates greater cell deformation. The measurements were carried out at 25°C.

Determination of RBC aggregation indexes: RBC aggregation was also determined in least and most dense RBC fractions using a custom-built photometric aggregometer interfaced to a digital computer. The measurement is based upon the increase of light transmission through a RBC suspension consequent to aggregation[15]. The RBC suspension is initially subjected to high shear to disperse preexisting aggregates, following which shear is abruptly stopped and the increase of light transmission integrated over a fixed time period. The data obtained via this method yield a dimensionless aggregation index that increases with the extent and rate of aggregation. RBC aggregation was measured in autologous plasma, with hematocrit value adjusted to 0.4 L/L by adding or removing suspending medium. Measurements were done at 37°C in triplicate for each sample and the mean of these three measurements was used for that sample.

Statistics: Results are expressed as mean ± standard error. Statistical comparisons were done by one-way and two-way ANOVA where appropriate.

RESULTS

The density distributions of RBC in young, mid-age and old groups are presented in Figure 1. RBC in all age groups were mostly accumulated in the layers with densities between 1.095-1.105 g/mL. However, the percentage of RBC with densities between 1.095 to 1.105 g/mL was significantly higher in the old group, in comparison with the young group. In contrast, the percentage of RBC with densities between 1.105 to 1.115 g/mL was found to be significantly lower in the old group than the young group.

Elongation indexes measured in less dense RBC fractions were higher than more dense RBC fractions in all age groups (Figure 2). It should be noted that the difference of elongation indexes between the least and most dense RBC fractions was also dependent on the shear stress at which the measurement was carried out (Figure 3). It is also obvious from Figure 3 that the differences in the elongation indexes of the most and least dense RBC were much more pronounced in the older groups.

In all age groups, aggregation of denser RBC were higher, in comparison with the RBC in less dense layer. However, the differences between the aggregation of less and more dense RBC were more pronounced in the "young" and "mid-age" group (Figure 4).

DISCUSSION

It has been observed that RBC density in the old group was lower than young and mid-age groups. The process of RBC aging might be altered by the general aging process[16]. Aged individuals show increased percentage of low density, young RBC and almost twice as many reticulocytes in their circulation as young adults and a heavy density fraction of erythrocytes decreases with increasing donor age[16-18]. The cause of this is unknown but may be due to decreased RBC lifespan in older individuals[16,18]. Therefore, the shift of RBC density to lower values in the old group is consistent with the literature reports.

Previous studies demostrated that aged, denser RBC have decreased deformability and increased aggregability[8-11]. The results of this study are in accordance with these findings. The measurement of EI using ektacytometer is a sensitive and reliable method to assess RBC deformability[14]. The applied shear stress is one of the critical factors in detecting the differences in deformability, especially if the alterations are small. The dependence of the EI differences between various RBC populations on the applied shear stress has been previously observed and confirmed in this study. It can be seen in Figure 3 that the differences in EI between the groups with various age ranges get smaller and less significant as the shear stresses applied during the measurement of EI get higher[19,20]. This dependence can be explained if by assuming that if the applied shear stress is big enough, RBC with slightly impaired deformability can also deform at a degree close to normal RBC, yielding similar EI.

Furthermore, it has been observed that the deformability difference between least and most dense RBC becomes more significant as the age of the donors increase. The RBC mechanical properties are closely related to the structure and physiological status of this cell and are sensitive to the alterations in their enviroment as well as to metabolic disturbances[21-23]. The reason

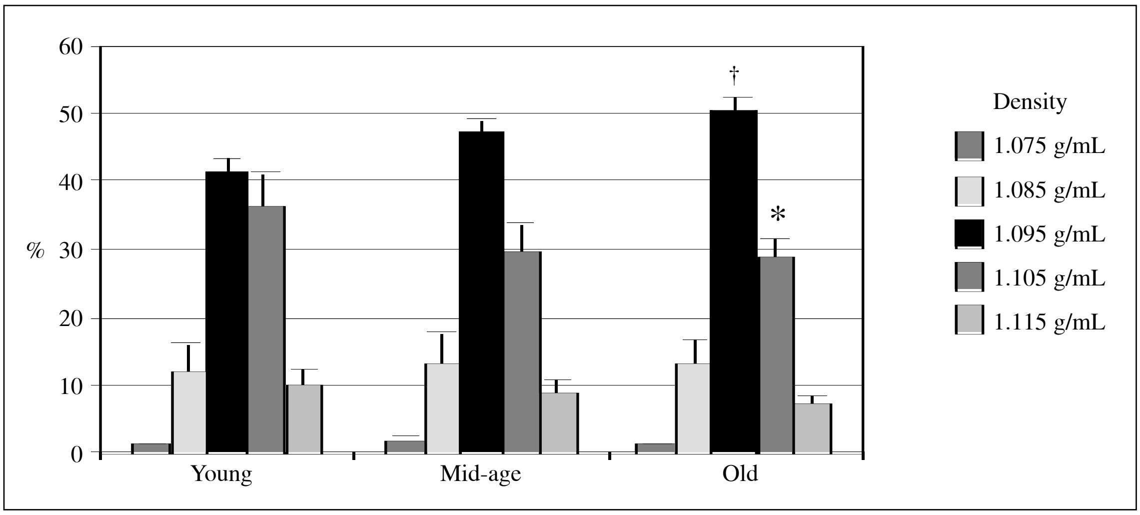

Figure 1. Percentage of RBC distributed in density fractions between 1.075-1.115 g/mL in young, mid-age and old groups. Difference from young by "One-way ANOVA"; *: p< 0.05; †: p< 0.01.

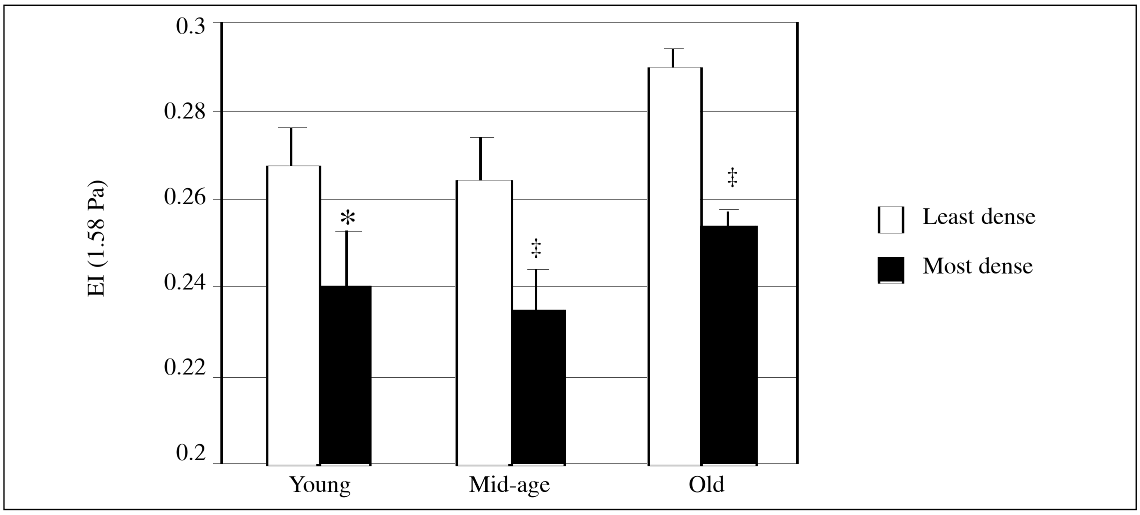

Figure 2. Elongation indexes of the least and most dense RBC fractions measured at 1.58 Pa shear stress in the young, mid-age and old groups. Difference from the "least dense"; *: p< 0.05, ‡: p< 0.001.

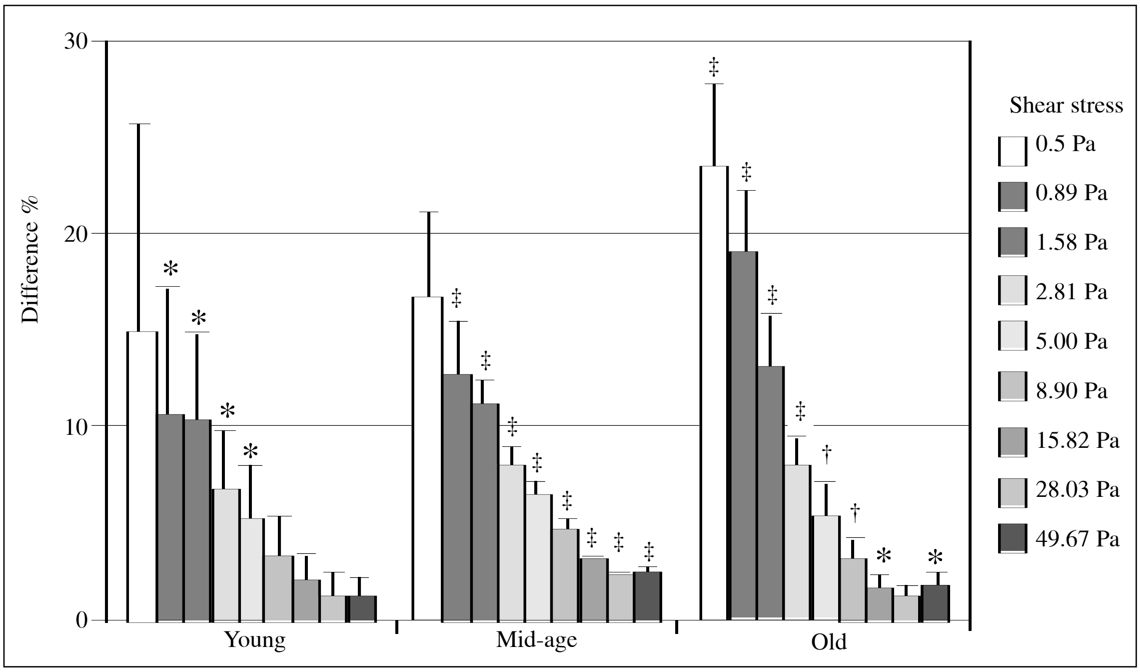

Figure 3. Difference between the elongation indexes of the least and most dense RBC measured at various shear stresses in the young, mid-age and old groups. Difference between least and most dense RBC by "Two-way ANOVA"; *: p< 0.05, †: p< 0.01, ‡: p< 0.001.

for the more pronounced alteration of RBC mechanical properties in aged individuals is not clearly known. It can be suggested that the change in density mostly reflects the "pre-programmed" metabolic detorioration, that is directly related to the length of the time spended in the circulation by a given RBC. Alternatively, changes in the mechanical properties may result from the microenvironmental influences. The effectiveness of these microenvironmental factors may differ according to the age of individuals (e.g., more pronounced vascu-

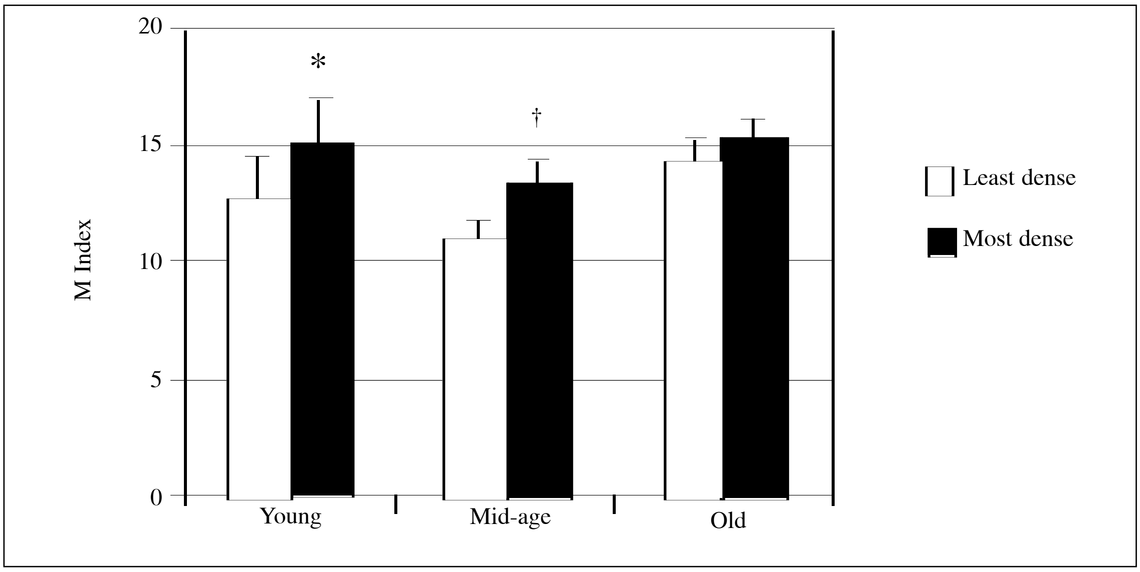

Figure 4. Agregations of the least and most dense RBC fractions measured in the young, mid-age and old groups. Difference from the "least dense"; *: p< 0.05, †: p< 0.001.

lar problems in the aged individuals).

Alternatively, the difference in the aggregability of least and most dense RBC was found to be smaller and nonsignificant in the group with the age range of 60-85, unlike the young and mid-age groups. However, it should be noted that also the least dense RBC in the old group exhibited a high degree of aggregability in comparison to the young and mid-age groups. Therefore, the smaller aggregability difference between least and most dense RBC in this group should not be considered as a decreased deterioration of RBC surface properties in the circulatory system of the elderly, but as increased aggregability of whole RBC population in this group.

The observed higher degree of deterioration of RBC rheological properties may contribute to the circulatory disorders that have a significantly higher insidence in older people.

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