

# Vitamin E Treatment Enhances Erythrocyte Deformability in Aged Rats

(aging / haemorheology / red blood cell aggregation / red blood cell deformability / oxidative stress)

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**Abstract.** The harmful effects of aging on blood rheology have been well known. These effects in the aging have been found to be associated with an increase in oxidative stress. The aim of this study was to seek whether treatment of vitamin E as a potent antioxidant could improve the age-related haemorheological abnormalities. For this purpose, male Wistar rats at the age of 3 and 24 months were used. The following parameters were evaluated: red blood cell (RBC) deformability, aggregation, plasma viscosity, vitamin E level, total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI), and the following results were obtained. First, aging was associated with a decrease in RBC deformability and increase in RBC aggregation and plasma viscosity. Second, compared with the young group, while plasma TOS levels and OSI were found to be significantly increased in aged rats, there was no significant change in their plasma TAS level. Third, vitamin E administration produced significant improvement in RBC deformability and decrement in TOS and OSI values in aged rats with respect to young and aged control groups. We did not find any significant effect of vitamin E treatment on RBC aggregation in both young and aged rats and finally, we found a signifi-

cantly lower plasma vitamin E level in aged rats than in young rats. In conclusion, these findings suggest that blood rheology impairs with age and vitamin E has ameliorating effects on age-induced haemorheological abnormalities especially in RBC deformability, probably by reducing the increased oxidative stress in old age.

## Introduction

Aging is characterized by a progressive decrease in various physiological functions of an individual organism over time. The aging process may be caused by many different mechanisms (Slater, 1984). One of the most popular of these is the free radical theory of aging, which was first introduced by Gerschman et al. (1954) and then developed by Harman (1956). According to Harman's theory of free radicals, aging results from the destructive effects of free radicals formed during cell metabolism (Harman, 1956). Reactive oxygen species (ROS) have been proposed as the main agents responsible for aging processes (Jin, 2010). It is well known that they can damage many biological molecules such as proteins, nucleic acids and lipids by interacting with and distorting their structure (Slater, 1984).

Haemorheology can be described as the science of blood flow. In general, it has long been known that the aging process produces several abnormalities in haemorheological parameters (Ajmani and Rifkind, 1998). For instance, increased fibrinogen concentrations (Papet et al., 2003; Schuitemaker et al., 2004), blood viscosity (Ajmani et al., 2000; Cheng et al., 2007; Coppola et al., 2000), plasma viscosity (Konstantinova et al., 2004), red blood cell (RBC) aggregation (Christy et al., 2010) and impaired RBC deformability (Cheng et al., 2007; Kucukatay et al., 2009) have been shown by using both animal and human models of old age. Moreover, all these changes are thought to be due to increased oxidative stress (Rifkind et al., 1997). Indeed, RBCs are very sensitive to the harmful effects of free radicals. First of all, RBCs are constantly exposed to oxidative stress due

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Abbreviations: AC – aged control group, AE – aged rats treated with vitamin E, AI – aggregation index, AMP – amplitude, BSO – buthionine sulphoximine, CD – conjugated diene, EI – elongation index, Hct – haematocrit, OSI – oxidative stress index, PUFA – polyunsaturated fatty acids, PV – plasma viscosity, RBC – red blood cell, ROS – reactive oxygen species, SNP – sodium nitroprusside, TAS – total antioxidant status, TOS – total oxidant status, YC – young control group, YE – young rats treated with vitamin E.

to continuously generated oxygen radicals by the autooxidation of haemoglobin (Misra and Fridovich, 1972). Secondly, RBC membranes contain relatively high levels of unsaturated fatty acids, which are especially good substrates for peroxidation reactions (Katherine et al., 1997). Studies have indicated that hypertension (Fowkes et al., 1993; Hacıoglu et al., 2002), cardiovascular (Lowe et al., 2002) and cerebrovascular diseases (Ajmani et al., 2000) are strongly correlated with haemorheological abnormalities. Therefore, the correction of the haemorheological abnormalities can hold an important place in the treatment of these pathologies.

Oxidative stress-induced membrane damage is usually initiated by any free radical. The process is summarized as follows: a free radical such as superoxide anion extracts a hydrogen ion from polyunsaturated fatty acids (PUFA) of biological membranes. This reaction converts PUFA to a PUFA radical (PUFA $\cdot$ ). Then a newly arranged molecule called conjugated diene (CD) arises. CD is an unstable molecule and very easily reacts with oxygen to form a peroxy radical (PUFAOO $\cdot$ ). These radicals are capable of removing the hydrogen ion from another lipid molecule. This vicious cycle continues in a chain reaction and cannot be terminated without a chain breaker molecule (Rimbach et al., 2002). Given the role of oxidative stress in haemorheological abnormalities in aging, antioxidant therapy may be an important treatment strategy in old age. Regarding this matter, various plant- and food-derived antioxidant compounds are drawing attention of investigators in the field of aging and related impairments (Carr and Frei, 1999; Chong-Han, 2010; Wojciech et al., 2010). One of these antioxidant compounds is vitamin E ( $\alpha$ -tocopherol). It is considered to be the most important chain-breaking lipid-soluble antioxidant in membranes. Hence, it is thought to have the ability to prevent the peroxidation of lipids in blood and biological membranes (Ingold et al., 1987).

Although previous studies have already presented that supplementation of several antioxidants may have preventive effects on age-related pathophysiological and clinical conditions, data on haemorheological and haematological alterations induced by aging are scarce. Moreover, no information is available about the effects of vitamin E, which is a widely used antioxidant, on haemorheological parameters during aging. Therefore, the present study was designed to explore the effects of vitamin E on haemorheological parameters in young and aged rats. In addition to that, the plasma vitamin E level, total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) were also determined to examine the contribution of these parameters to the possible reformatory role of vitamin E on age-induced alterations in haemorheological parameters.

## Material and Methods

### *Animals and experimental design*

Young (three months old) and aged (24 months old) male Swiss albino rats were used for the experiments. They were obtained from Pamukkale University Experimental Research Unit. Animals were housed in groups of four to five rats in stainless steel cages at standard conditions ( $24 \pm 2$  °C and  $50 \pm 5$  % humidity) with a 12 h light-dark cycle and fed *ad libitum* with standard rat chow and tap water. According to their age, animals were first divided into two main groups, namely young and aged groups. Each group was further divided (N = 10 rats/treatment group) into: young control group (YC), young rats treated with vitamin E (YE), aged control group (AC), and aged rats treated with vitamin E (AE). The study protocol was approved by the Pamukkale University Animal Care and Use Committee. The experiments were performed between 09:00 and 12:00 h.

### *Doses and treatment*

While vitamin E (i.e.,  $\alpha$ -tocopherol) dissolved in olive oil at a dose of 200 mg/kg/day was given intraperitoneally to rats of YE and AE groups for 6 weeks, the control groups (YC and AC) were only treated with the vehicle (olive oil) for the same period.

### *Sample preparation*

At the end of the experimental period, rats were anaesthetized deeply with intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg), and their abdomens were opened by a midline incision. Anticoagulated blood was collected from the abdominal aorta of rats under anaesthesia and used for the measurement of haemorheological (RBC deformability, aggregation and plasma viscosity) and biochemical parameters as stated below. The animals were then euthanased by exsanguinations while under ketamine and xylazine anaesthesia. In addition, pooled blank rat plasma was collected in heparinized tubes from rats hosted at an animal facility operating in our laboratory. Pooled rat plasma was used for preparation of plasma standards used in analysis of vitamin E.

### *RBC deformability measurements*

RBC deformability (i.e., the ability of the entire cell to adopt a new configuration when subjected to applied mechanical forces) was determined by laser diffraction analysis using an ektacytometer (LORCA, RR Mechatronics, Hoorn, The Netherlands). The system has been described elsewhere in detail (Hardeman et al., 1994). Briefly, a low Hct suspension of RBC in 4% polyvinylpyrrolidone 360 solution (MW 360 kD, Sigma P 5288, St. Louis, MI) (4 % in PBS, viscosity: 23.2 cP) was sheared in a Couette system composed of a glass cup and a precisely fitting bob with a gap of 0.3 mm between the cylinders. A laser beam was directed through the sheared sample, and the diffraction pattern

produced by the deformed cells was analysed by a computer attached to the ektacytometer. Based on the geometry of the elliptical diffraction pattern, the elongation index (EI) was calculated for nine shear stresses between 0.3 and 30 Pascal (Pa) as:  $EI = (L - W)/(L + W)$ , where L and W are the length and width of the diffraction pattern, respectively. An increased EI at the given shear stress indicates greater cell deformation and hence greater RBC deformability. All measurements were carried out at 37 °C.

#### *RBC aggregation measurements*

RBC aggregation was also determined by using LORCA described by Hardeman et al. (2001). The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then un-sheared (aggregating) blood, performed in a computer-assisted system at 37 °C. Back-scattering data were evaluated by the computer, and the aggregation index (AI), aggregation half time ( $t_{1/2}$ ), which shows the kinetics of aggregation, and the amplitude (AMP), which is a measure for the total extent of aggregation, were calculated on the basis that there is less back-scattered light from aggregating red cells. The haematocrit (Hct) of the samples used for aggregation measurements was adjusted to 40 % and blood was fully oxygenated.

#### *Determination of plasma viscosity*

Plasma viscosity (PV) was determined with a Wells-Brookfield cone-plate rotational viscometer (model DV-II + Pro, Brookfield engineering Labs, Middleboro, MA) at a shear rate of 375 s<sup>-1</sup> at 37 °C.

#### *Measurement of total oxidant status (TOS) of plasma*

TOS of plasma was measured using a novel automated colorimetric measurement method developed by Erel (2005). In this method, oxidants present in the sample oxidize the ferrous ion-O-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter ( $\mu\text{mol H}_2\text{O}_2 \text{ Eq/l}$ ).

#### *Measurement of the total antioxidant status (TAS) of plasma*

TAS of plasma was measured using a novel automated colorimetric measurement method developed by Erel (2004). In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and it reacts with the colourless substrate O-dia-

nisidine to produce the dianisyl radical, which is bright yellowish-brown in colour. Upon addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction are suppressed by the antioxidant components of the plasma, preventing the colour change and thereby providing an effective measurement of TAS. The assay results were expressed as mmol Trolox Eq/l, and the precision of this assay was lower than 3 %.

#### *Determination of oxidative stress index (OSI)*

The ratio of TOS to TAS was accepted as OSI. For calculation, the resulting unit of TAS was converted to mmol/l, and the OSI value was calculated according to the following formula:  $OSI (\text{arbitrary unit}) = \text{TOS} (\mu\text{mol H}_2\text{O}_2 \text{ Eq/l}) / \text{TAS} (\text{mmol Trolox Eq/l}) \times 100$ .

#### *Determination of plasma vitamin E level*

The measurement of vitamin E level in plasma was achieved by using the GC-FID method developed by Demirkaya and Kadioglu (2007). The amount of 0.5 ml of the plasma sample was mixed with 1 ml of ethanol as deproteinizing agent and then extracted with a mixture of hexane and dichloromethane (9 : 1, v/v). The mixture was then centrifuged at 3,000 rpm for 7 min. The organic layer was evaporated to dryness under vacuum and the residue was dissolved in ethanol. Two  $\mu\text{l}$  of the sample was injected into the GC-FID system for analysis. Chromatographic analysis was carried out in an Agilent 6890N Network gas chromatography system equipped with a flame ionization detector, an Agilent 7683 series autosampler, an Agilent chemstation. An HP-5 column with 0.25  $\mu\text{m}$  film thickness (30 m  $\times$  0.320 mm I.D., Agilent Technologies Inc., Hewlett-Packard Inc., Palo Alto, CA) was used for separation. Splitless injection was used and the carrier gas was nitrogen at a flow-rate of 2 ml/min. The injector and detector temperatures were 300 °C. The oven temperature was held at 150 °C for 1 min and then increased to 320 °C at a rate of 28 °C/min, where the temperature was held for 3 min.

For vitamin E analysis in plasma, calibration was performed by adding 0.1 ml standard solution of vitamin E to 0.5 ml of blank rat plasma up to the final vitamin E concentration of: 0.5, 1, 2, 5, 10, 20 and 40  $\mu\text{g/ml}$ . Each concentration level was extracted and assayed as described above. The mean regression equation was  $y = 3.2138x + 1.3463$  with a correlation coefficient (r) of 0.9998, where x is the concentration ( $\mu\text{g/ml}$ ) of vitamin E and y is the peak area of vitamin E. The standard error of intercept and slope of linear regression equations were 0.4748 and 0.0702, respectively. The LOQ defined as the lowest concentration of the measured value of standard solutions was 0.35  $\mu\text{g/ml}$ .

#### *Statistical analysis*

Results were presented as means  $\pm$  standard error (SE). Statistical comparisons between the groups were done by one-way analysis of variance (ANOVA) using the SPSS 10.0 software package for Windows. Post hoc

testing was performed for inter-group comparisons using the least significant difference (LSD) test. The significance level was set at  $P < 0.05$ .

**Results**

*RBC deformability at various shear stresses*

RBC deformability (i.e., the elongation index, EI) for young and aged controls was measured at nine shear stresses between 0.3 and 30.0 pascal (Pa). As expected, EI was found to increase with increasing applied stress levels in young and aged controls. At the same time,

RBCs of the aged control group were significantly less deformable at all stress levels (Fig. 1). The most obvious difference in the EI was seen between 0.95 and 3 Pa. Therefore, EI values measured at 0.95 Pa were chosen as the ability of RBC deformability in all experimental groups. Fig. 2 presents EI values measured at 0.95 Pa. RBC deformability of aged rats was found to be significantly impaired compared with that of young rats. While there was no effect of vitamin E at a dose of 200 mg/kg/day on the deformability of the young group, this treatment resulted in a statistically significant enhancement in RBC deformability of aged rats compared with their own control.

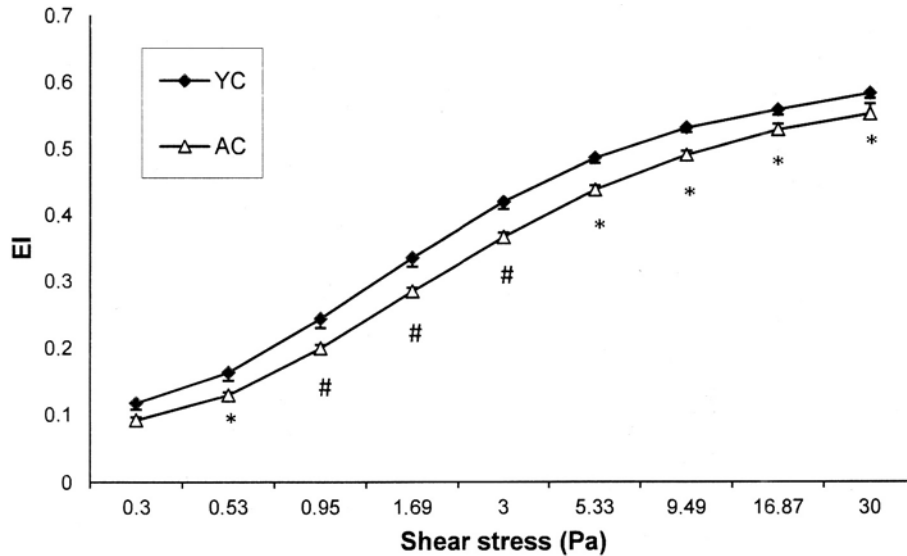


Fig 1. Elongation index (EI) vs. shear stress for RBCs. EI values for aged rats RBC were significantly lower at all shear stresses.

Values are expressed as means  $\pm$  SE; N = 10). \* $P < 0.05$ , # $P < 0.01$ , significantly different from the young group.

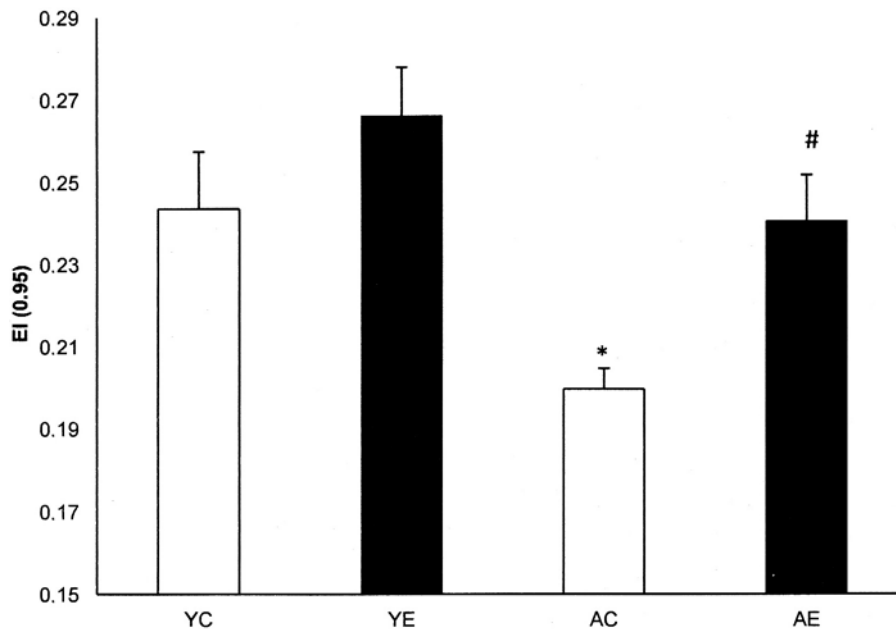


Fig 2. Effect of vitamin E on RBC deformability of young and aged rats measured at a shear stress of 0.95 Pa.

Values are expressed as means  $\pm$  SE; N = 10. \* $P < 0.01$  difference from YC and YE groups, # $P < 0.05$  difference from AC group.

Table 1. Effect of vitamin E on erythrocyte aggregation values of young and aged rats

Groups	AI	AMP (au)	$t_{1/2}$ (s)
YC	46.17 ± 6.51	11.73 ± 1.4	7.97 ± 2.78
YE	52.07 ± 1.97	10.56 ± 0.44	3.74 ± 0.42
AC	61.93 ± 1.44*	16.76 ± 0.71*	2.17 ± 0.15*
AE	59.96 ± 4.26*	15.64 ± 1.77*#	2.77 ± 0.76*

AI – aggregation index, AMP – amplitude of aggregation,

$t_{1/2}$  – aggregation half time, au – arbitrary units.

Values are expressed as means ± SE; N = 10. \*P < 0.01 difference from YC group, #P < 0.01 difference from YE group.

### RBC aggregation

The aggregation index (AI), amplitude of aggregation (AMP) and aggregation half time ( $t_{1/2}$ ) values of experimental groups are shown in Table 1. While AI and AMP of aged groups were significantly increased,  $t_{1/2}$  significantly decreased in the aged groups compared to young groups. Taken together, these three parameters have show increased RBC aggregation in the aged group. There was no effect of vitamin E treatment on aggregation parameters in both young and aged groups.

### Plasma viscosity

PV of experimental groups is shown in Fig. 3. A statistically significant increment in PV was measured in aged rats. Vitamin E did not change the PV level in both young and aged treatment groups compared to their own controls.

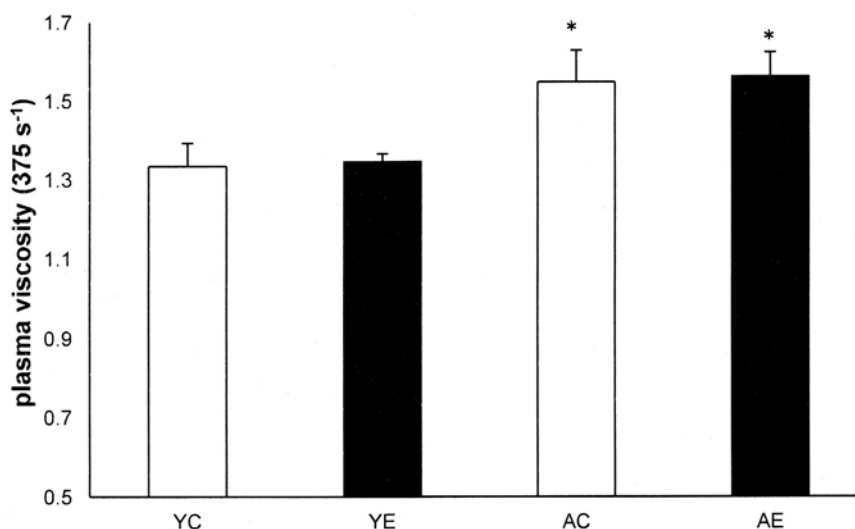


Fig 3. Effect of vitamin E on plasma viscosity values of young and aged rats measured at a shear rate of 375/s<sup>-1</sup>. Values are expressed as means ± SE; N = 10. \*P < 0.01 difference from YC and YE groups.

Table 2. Effect of vitamin E on oxidant/antioxidant parameters of young and aged rats

	YC	YE	AC	AE
TOS (μmol H <sub>2</sub> O <sub>2</sub> Eq/l)	6.93 ± 0.28	6.13 ± 1.43	8.53 ± 0.40*	7.04 ± 0.14#
TAS (mmol Trolox Eq/l)	0.52 ± 0.02	0.60 ± 0.04	0.49 ± 0.04	0.56 ± 0.02
OSI (au)	1.29 ± 0.08	0.94 ± 0.16	1.78 ± 0.13*	1.31 ± 0.07#

Values are expressed as means ± SE; N = 10. \*P < 0.01 difference from YC and YE groups, #P < 0.01 difference from AC group.

### Total oxidant status and total antioxidant status

Plasma TOS and TAS levels of young and aged experimental groups are shown in Table 2. Plasma levels of TOS were significantly increased in the aged control group as compared with the young groups. This increase was significantly decreased by vitamin E treatment in the aged group compared to its own control group. As seen in Table 2, there was no statistically significant difference between the TAS results of the experimental groups.

### Oxidative stress index

Table 2 also shows alterations in the oxidative stress index for the study groups. In parallel to TOS and TAS results, OSI levels were increased in aged rats when compared to young rats. Vitamin E treatment significantly reduced this index in the aged treatment group (AE group) in comparison with the aged control (AC group).

### Plasma vitamin E status

The retention time of vitamin E in rat plasma was 8.2 min with a total run time of less than 10 min. No endogenous interfering peak was observed in individual blank plasma at the vitamin E retention time, thereby confirming the specificity of the analytical method. In our study, we estimated that endogenous vitamin E of rat plasma sample had approximately 4.06 μg/ml concentration by the standard curve according to the procedure of the multiple point standard addition interpolation type method.

Table 3. Effect of vitamin E treatment on plasma vitamin E values of young and aged rats

Groups	YC	YE	AC	AE
Mean concentrations of vitamin E ( $\mu\text{g/ml}$ )	$3.78 \pm 0.12$	$8.43 \pm 0.51^*$	$3.01 \pm 0.16^*$	$4.42 \pm 0.28^{\#}$

Values are expressed as means  $\pm$  SE; N = 10. \*P < 0.01 difference from YC group,  $^{\#}$ P < 0.05 difference from AC group.

With this developed method, vitamin E concentration in the rat plasma samples obtained from YC, YE, AC and AE groups (total 40 subjects) was analysed. Table 3 represents the status of plasma vitamin E of the experimental groups. Decreased plasma levels of vitamin E were observed in aged control rats when compared with young control rats. As expected, vitamin E supplementation induced a statistically significant increment in the vitamin E plasma level in both young and aged rats compared to their own control groups. This increase was more obvious in the young group than in the aged group.

## Discussion

In general, the tissues in which the vasculature has sufficient regulatory ability can compensate their blood needs by an appropriate change of vascular geometry. With age, potentially harmful structural and functional changes in the vascular structure have been seen (Jin et al., 2009; Wray et al., 2009; Seals et al., 2011). This effect of aging is known to cause deterioration in many tissue perfusions (Delp et al., 1998; Donato et al., 2006; Wray et al., 2009). Additionally, recent studies have shown that blood rheological parameters must be within the physiological limits for good tissue perfusion (Baskurt and Meiselman, 2003). As noted in introduction, aging is accompanied by impaired haemorheological parameters such as blood viscosity, RBC deformability, and RBC aggregation. Because oxidative damage has long been implicated in the aging process, dietary antioxidant supplementation may be an effective treatment for the correction of these impaired haemorheological parameters. Therefore, the aim this study was to investigate the effect of dietary vitamin E supplementation to improve blood rheological parameters in aged rats.

The results of the current study indicate that aging caused a decrease in RBC deformability. As compatible with our results, age-related impairments in RBC deformability have been previously reported in many studies. For example, increased shear elastic modulus, which is associated with enhanced membrane rigidity and impaired RBC deformability, has been found in aged rats when compared to young rats (Rifkind et al., 1999). Additionally, it is known that RBC susceptibility to centrifugal packing at 200 g increases with age, suggesting an age-related decrease in RBC deformability (Judkiewicz et al., 1988). Goi et al. (2005) reported significant decreases in RBC  $\beta$ -D glucuronidase and neutral sialidase activities in elderly subjects compared with young adults. They suggested that decreased RBC membrane fluidity observed in elderly subjects could account for the diminished expression of these enzymes. Simi-

larly, a significant reduction in RBC membrane  $\text{Na}^+/\text{K}^+$  and  $\text{Ca}^{2+}$ -ATP-ase activities was observed in older subjects in comparison with the younger ones (Rabini et al., 1997). These ATPases are known to play a very important role in the maintenance of RBC deformability through its regulation of cell cation and water content and hence cytoplasmic viscosity (Mohandas and Shohet, 1981). Decreased activities of the enzymes may explain the decrease in RBC deformability seen in aged rats. In summary, especially considering the capillary bed, RBC deformability should be the most important factor affecting the flow of blood, since RBCs have to enter and pass by deforming through capillaries whose diameters are smaller than their resting diameter (Secomb and Hsu, 1997). Hence, the decreased RBC deformability in aging may contribute to impaired tissue perfusion in aging.

In addition to the above-mentioned reasons for decreased deformability in aging, free radical-induced cell membrane damage might also play an important role in the observed deterioration in RBC deformability. In accordance with Harman's free radical theory, we found significantly higher TOS levels and impaired RBC deformability in aged rats, suggesting enhanced oxidative damage to RBCs. This finding was consistent with previous studies, which showed a relationship between oxidative stress and decreased RBC deformability (Baskurt et al., 1998; Sentürk et al., 2001; Aydogan et al., 2008; Kim et al., 2008). In the present study, we found that the antioxidant treatment (vitamin E) reversed the reduced deformability and increased the oxidative stress level in aged rats. This is in agreement with the report by Yerer and Aydogan (2004), who found that vitamin E improves impaired red cell deformability due to oxidative stress caused by sodium nitroprusside (SNP). Therefore, it can be suggested that the effect of aging on RBC deformability seems to be associated with oxidative stress.

It is well known that organisms are protected against oxidative stress via enzymatic and non-enzymatic antioxidative mechanisms. Under normal conditions, a delicate balance exists between the rates of free radical formation and their removal by antioxidant enzymes and molecules (Kucukatay et al., 2007). Measuring only one of the oxidant or antioxidant parameters usually does not give proper information about the oxidative status in the organism. Hence, we measured both TOS and TAS in the present study. The plasma TAS levels of aged rats were lower than in young rats, but the difference was not statistically significant. Although the TAS level tended to increase after administration of vitamin E in the aged group, this increase was not statistically significant, either. Additionally, we used OSI as another indi-

cator of oxidative stress. OSI is the ratio of TOS to TAS and it has been suggested that it may reflect the state of oxidative status more accurately than TOS (Harma et al., 2005). In the present study, OSI was significantly higher in aged rats than in young controls. Vitamin E treatment declined the OSI level to control values in aged rats similarly to TOS values.

Another haemorheological parameter determined in this study was the RBC aggregation, which is the reversible adhesion of adjacent RBCs (Shiga et al., 1990). This study has shown that aging has an increasing effect on AI and AMP while decreasing the effect on  $t_{1/2}$ . Taken together, the increment in AI and AMP and the decrement in  $t_{1/2}$  indicate increased RBC aggregation. In contrast to the results of RBC deformability, these results of RBC aggregation observed in aged rats were not reversed by vitamin E treatment. Increased RBC aggregation in aged rats may be explained in terms of fibrinogen, which is a soluble plasma glycoprotein, synthesized by the liver (Ajmani and Rifkind, 1998). Several reports indicate aging-induced enhancements in plasma fibrinogen concentration, which is one of the major determinants of RBC aggregation (Sharp et al., 1996; Ajmani and Rifkind, 1998; Kovács et al., 2006). Although we did not measure the plasma fibrinogen level in our experiments, the enhanced RBC aggregation in aged rats may be attributed to its increment in these rats. We also found increased PV in the aged rats. It is a well-known fact that increased PV is strongly correlated with increased fibrinogen in the plasma, with a lesser contribution by globulins, cholesterol and triglycerides (Ajmani and Rifkind, 1998; Fu and Nair, 1998). This possible age-dependent increment in fibrinogen may be the reason of increased RBC aggregation and PV in aged rats. We could not find any improving effect of vitamin E on the increased PV and RBC aggregation in the aged rats.

In our study, a reduced plasma vitamin E level was found in the aged rats when compared to young rats. Decreased levels of vitamin E in aged rats of our study were consistent with previous studies (Kumaran et al., 2008, 2009). Vitamin E protects cells from oxidative damage by its powerful antioxidant effects, thus maintaining structural integrity of virtually all cells in the body (Ingold et al., 1987). It can be speculated that a decreased vitamin E level in an organism may be one of the reasons for increased oxidative stress in old age. Indeed, Terasawa et al. (2000) showed that reduced plasma and tissue level of vitamin E was associated with increased lesions in the proximal aorta and increased rates of lipid peroxidation. The reduced plasma vitamin E level observed in aged rats may be due to reductions in GSH with aging, since regeneration of  $\alpha$ -tocopherol from its toco-heroxyl radical is dependent on ascorbic acid and GSH (Meister, 1992). Ascorbic acid is regenerated in the presence of reduced glutathione (Wefers and Sies, 1988). It was shown that when GSH was depleted by buthionine sulphoximine (BSO) treatment in rats, the levels of antioxidants, glutathione, ascorbic acid and vitamin E were significantly decreased in the liver of the

treated animals (Rajasekaran et al., 2002). Another reason for reduction of the plasma vitamin E level in aged rats may be the decreased activity or expression of  $\alpha$ -tocopherol transfer protein ( $\alpha$ -TTP). It has been shown that  $\alpha$ -TTP has a fundamental role in determining the level of vitamin E in plasma and tissues (Rimbach et al., 2002). For example, a mutation of  $\alpha$ -TTP gene has been shown to cause decreased plasma and tissue levels of vitamin E (Ben Hamida et al., 1993). There are very few studies in the literature investigating the level of this protein in old age. In one of these studies,  $\alpha$ -TTP and its mRNA levels were found to be decreased in lung tissue of aged mice (Valacchi et al., 2007).

In conclusion, the results of the present study demonstrate that aging produces haemorheological changes. This is partly due to increased oxidative stress, since only RBC deformability could be corrected with vitamin E treatment; RBC aggregation did not change in aged rats with this treatment. Our findings provide supporting evidence that oxidative stress markedly increases during aging and vitamin E, at least partially, can improve oxidative stress-induced alterations in haemorheological parameters in aging.

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