

Dependency of Whole Blood Viscosity and Plasma Viscosity on Electrolytes and Lipids - An in Vitro Study

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Abstract-- Blood viscosity is a crucial hemodynamic biomarker because it determines the friction against blood vessels, the quantity of oxygen that is delivered to organs and tissues, and the degree of exertion by the heart. Many factors affect blood viscosity, either directly or inversely. e.g., fibrinogen and hematocrit. The effect of other factors (e.g., cholesterol) on blood viscosity is still under investigation. The aim of this study is to evaluate the effect of both electrolytes [sodium (Na⁺), calcium (Ca⁺⁺), and potassium (K⁺)] and lipids [triglycerides (TGs), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)] on both whole blood viscosity (WBV) and plasma viscosity (PV). WBV was measured by rotational viscosity at high and low shear rates. PV was measured using a rotational viscometer at a single shear rate. A strong correlation of WBV and PV with Ca⁺⁺ was found. TGs had an effect on WBV at a low shear rate. LDL and HDL were correlated directly with both WBV and PV. The observed relationships can be explained as a direct effect of both electrolytes and lipids on PV. On the other hand, the relationship between WBV and both electrolytes and lipids can be explained indirectly, namely through the effect of lipids and electrolytes on red blood cell aggregation and deformation.

Index Term-- Blood Viscosity, Plasma viscosity, Electrolytes, Lipids

INTRODUCTION

Blood flow reflects the interplay between the physical principles of hydraulics, pressure, flow, and resistance, whereby the velocity or rate of flow depends highly on vessel diameter and the characteristics of the fluid (i.e., blood viscosity). Altered hemorheological parameters play a key role in atherogenesis. Many cardiovascular risk factors, including aging, obesity and carotid intima-media thickness, are linked with changes of rheological parameters [1-4]. The risk of acute myocardial infarction and cardiovascular death increases with elevations in whole blood viscosity (WBV)[4, 5]. Furthermore, the blood rheological properties serve as an important indicator for the early detection of many diseases[6]. A recent study confirmed that WBV is a predictor of cardiovascular events [7]. In addition, marked hemorheological impairments were observed in diabetic patients, and a high WBV is associated with increased insulin resistance [8-10].

The water and salt balances of the body are closely interrelated; therefore, blood viscosity may be related to the ionic balance of the body. In addition, metal ions (zinc, iron, magnesium, etc.) are involved in the control of enzymatic

activities in cells and affect the degrees of colloid compaction and hydration and colloid solubility in the cytosol. Changes in these parameters may affect the rheological characteristics of erythrocytes [11].

Hyperlipidemia is one of the most important cardiovascular risk factors. High levels of cholesterol and triglycerides, as well as reduced levels of HDL cholesterol, can cause damage to the arterial wall through different mechanisms. Among these, it has been hypothesized that blood lipids can result in alteration of blood or plasma viscosity. In fact, many studies have reported the association between serum lipid levels and the rheological properties of the blood. Nevertheless, the data for the relationships between blood or plasma viscosity and lipids are not unequivocal and are sometimes even difficult to interpret[12, 13].

In light of the contradictions in the data reported in the previous studies and of the non-Newtonian behavior of whole blood, the present work aims to evaluate the effect of lipids and electrolytes on whole blood viscosity (WBV) at two levels of shear rate in order to extrapolate the relationships between them. As plasma viscosity plays a great role in whole blood viscosity, the present work also evaluates the relation between plasma viscosity (PV) and both lipids and electrolytes.

MATERIALS AND METHODS

Sample collection and preparation

Twenty blood samples were collected from donors of the same age and gender. All blood samples were collected after overnight fasting in a quiet environment at normal ambient temperature. Blood was withdrawn after a 10-min resting period and in a seated position. The blood was collected from the antecubital vein 90 s after the application of a tourniquet and without removing it to avoid the effect of pressure on the blood samples.

Biochemical analysis

Five milliliters of blood from each donor was withdrawn into the blind evacuated tube to obtain serum for lipid profiling and for electrolyte measurements. The blood samples were sent to a clinical lab to measure the lipid profile, which includes high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TGs). Additionally,

sodium (Na^+), calcium (Ca^{++}), and potassium (K^+) were measured as blood electrolytes. All of the values measured were in the normal reference ranges for all biochemical parameters.

Whole blood and plasma viscosity measurements

Whole blood viscosity (WBV) and plasma viscosity (PV) were measured at 37°C using a rotational viscometer fitted with a small sample cup and a temperature controller (Brookfield Digital LVTD viscometer). WBV was measured at two shear rates, 22.5, and 225.0 s^{-1} , at a constant hematocrit, 25%. PV was measured at a single shear rate of 225 s^{-1} . A volume of 0.5 ml of whole blood sample or plasma was added to the viscometer.

Statistical analysis

The values of WBV and PV were presented as the mean \pm SD in all graphs. A correlation analysis of the results was performed. A scatter diagram of the values obtained was drawn, and the Pearson's correlation coefficient (R^2) was determined.

Results

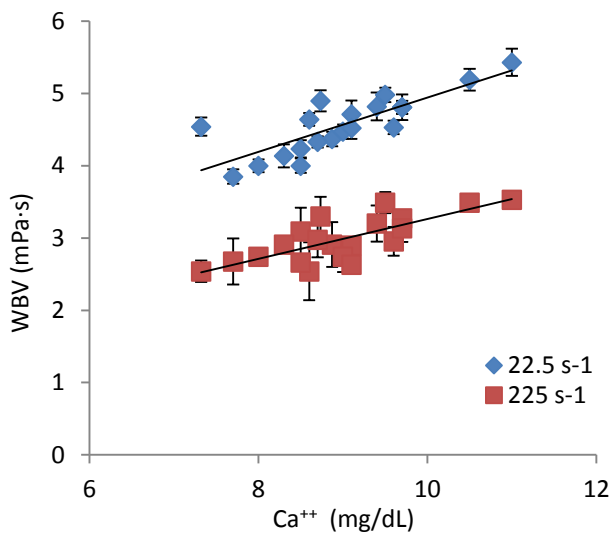
The value of WBV obtained was $4.55 \pm 0.39\text{ mPa}\cdot\text{s}$ at a shear rate of 22.5 s^{-1} and $2.97 \pm 0.31\text{ mPa}\cdot\text{s}$ at a shear rate 225.5 s^{-1} . PV was $1.26 \pm 0.04\text{ mPa}\cdot\text{s}$. WBV showed shear-thinning behavior due to a decrease in WBV as the shear rate increased. A strong linear correlation between WBV and Ca was observed at both high and low shear rates: for shear rates

of 22.5 s^{-1} and 225 s^{-1} , R^2 was 0.7 and 0.6, respectively. The correlation between WBV and Ca^{++} was linearly positive. Na^+ and K^+ were weakly correlated to WBV ($R^2 < 0.5$) under high and low shear rates. All three of the cations showed a positive correlation to WBV. Figure 1(a-c) shows the relationship between WBV as dependent parameter and electrolyte as independent parameter. Linear equations were used to describe the relationship between WBV and electrolyte concentration.

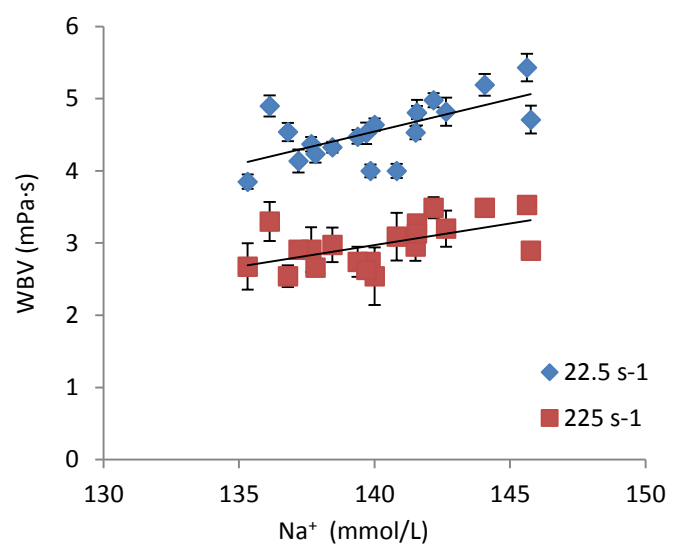
PV was strongly correlated to both Ca^{++} and K^+ ($R^2 = 0.7$ and 0.6 , respectively). For both Ca^{++} and K^+ , a linear proportionality was evident. A moderately positive correlation between PV and Na^+ was observed as well [$R^2 = 0.5$, Figure 2 (a-c)].

WBV strongly correlated to TG levels at 22.5 s^{-1} . In contrast, there was only a weak correlation between WBV and TGs at 225 s^{-1} ($R^2 = 0.4$). WBV was strongly correlated to LDL at 22.5 s^{-1} ($R^2=0.6$) and moderately correlated to LDL at 225 s^{-1} ($R^2 = 0.5$). WBV was correlated strongly to HDL at 22.5 and 225 s^{-1} ($R^2= 0.6$). Figure 3 (a-c) shows the positive correlation between WBV and each of TGs, LDL, and HDL at 22.5 and 225 s^{-1} . These relationships between WBV and the lipid parameters were all linear.

PV was weakly correlated to each of cholesterol and TGs ($R^2 = 0.4$ for each relationship). A strong correlation between PV and each of LDL and HDL was indicated ($R^2 = 0.8, 0.7$, respectively). These correlations are shown in Figure 4 (a-c).



(a)



(b)

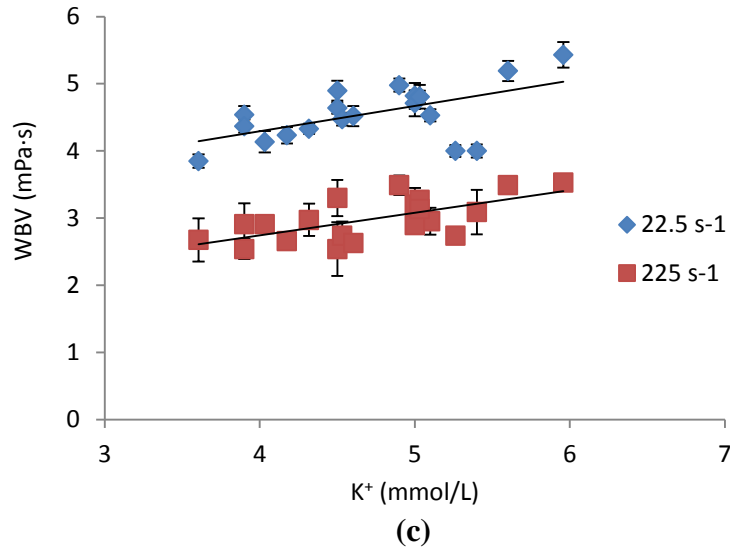
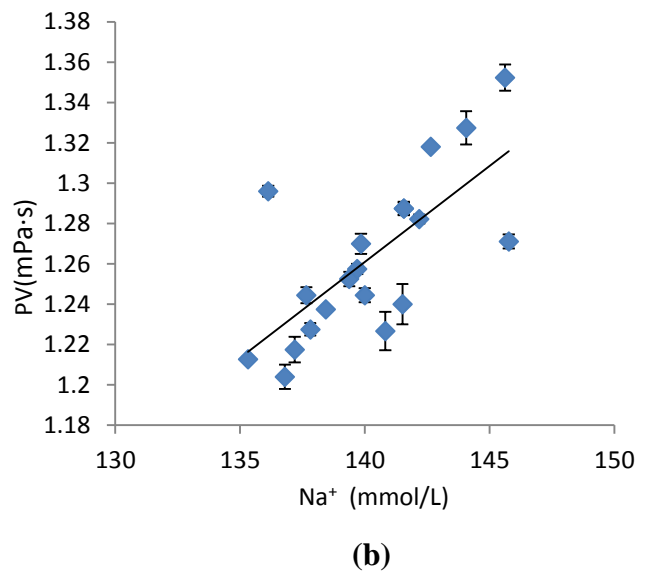
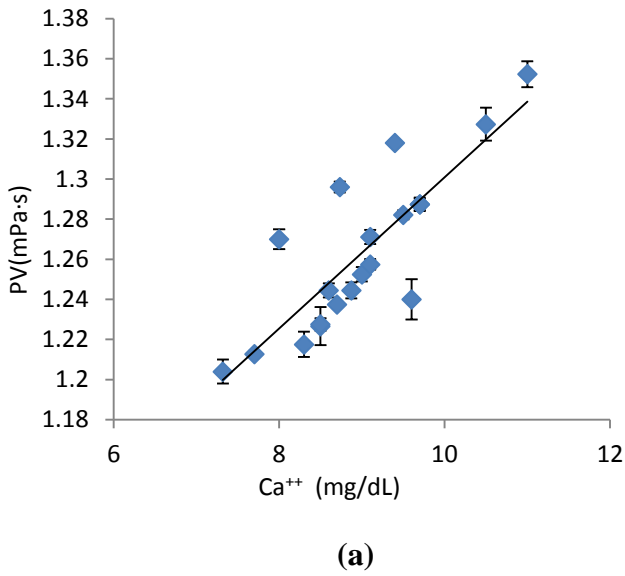


Fig. 1. Relationships between WBV and electrolyte concentrations (a) WBV is correlated strongly to Ca^{++} at both values of shear rate ($R^2 = 0.7$ at $22.5\ s^{-1}$ and $R^2 = 0.6$ at $225\ s^{-1}$). (b) WBV is weakly correlated to Na^+ at both values of shear rate ($R^2 = 0.4$ at $22.5\ s^{-1}$ and $R^2 = 0.3$ at $225\ s^{-1}$). (c) WBV is weakly correlated to K^+ at both values of shear rate ($R^2 = 0.4$ at $22.5\ s^{-1}$ and $R^2 = 0.4$ at $225\ s^{-1}$).



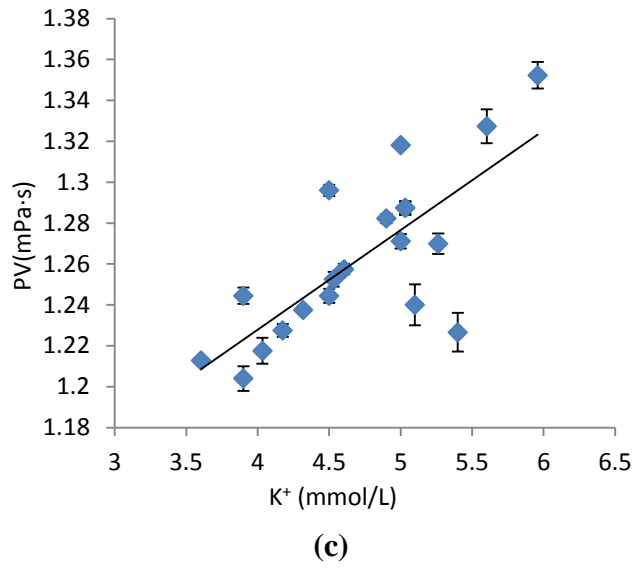
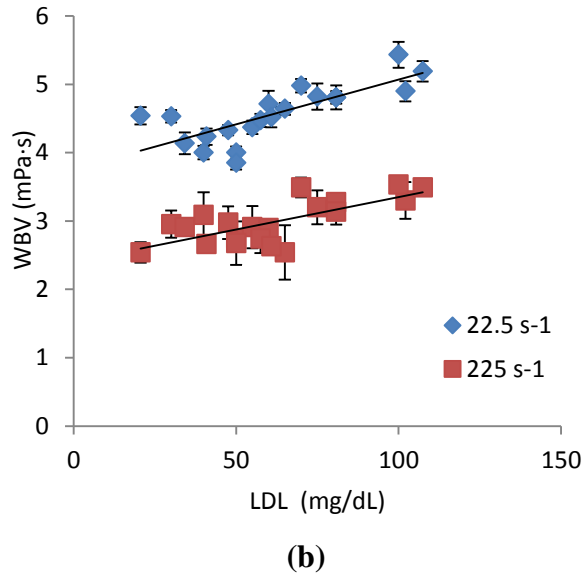
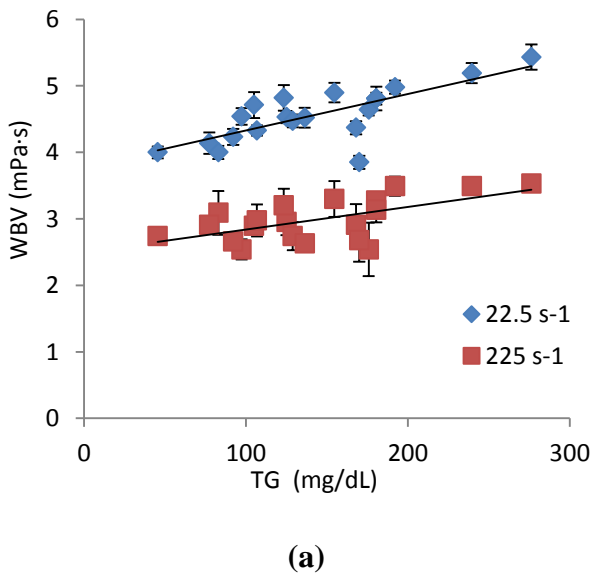
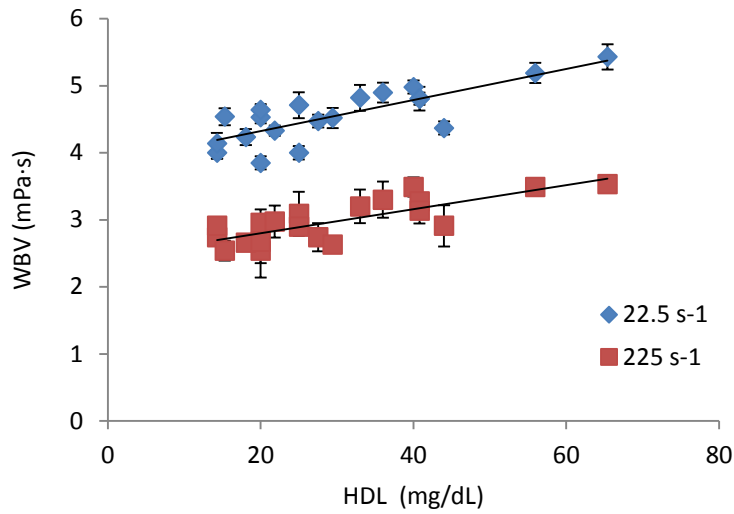


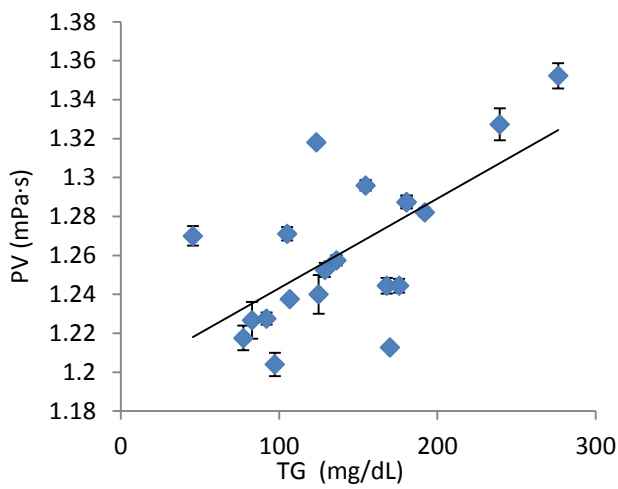
Fig. 2. Relationships between PV and electrolyte concentration at a constant shear rate 225 s^{-1} (a) PV is correlated strongly to Ca^{++} ($R^2 = 0.7$). (b) PV is correlated moderately to Na^+ ($R^2 = 0.5$). (c) PV is strongly correlated to K^+ ($R^2 = 0.6$).



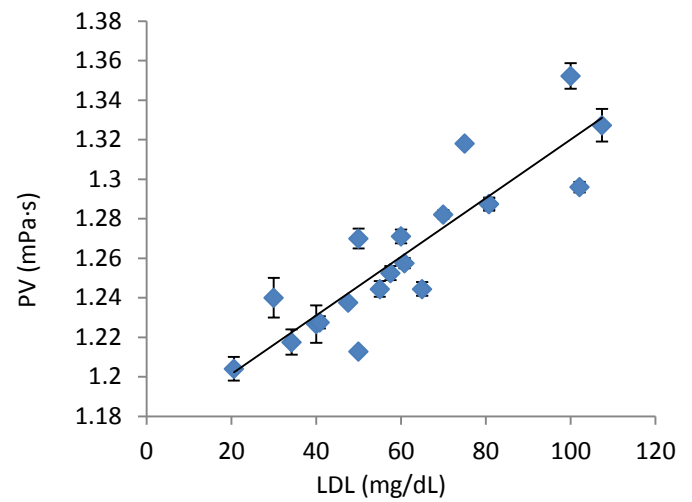


(c)

Fig. 3. Relationships between WBV and lipid profile parameters (a) WBV is strongly correlated to TGs at shear rate 22.5 s^{-1} ($R^2 = 0.6$). (b) WBV is strongly correlated to LDL at 22.5 s^{-1} ($R^2 = 0.6$). (c) WBV is strongly correlated to HDL at both values of shear rate ($R^2 = 0.6$ at 22.5 s^{-1} and $R^2 = 0.6$ at 225 s^{-1}).



(a)



(b)

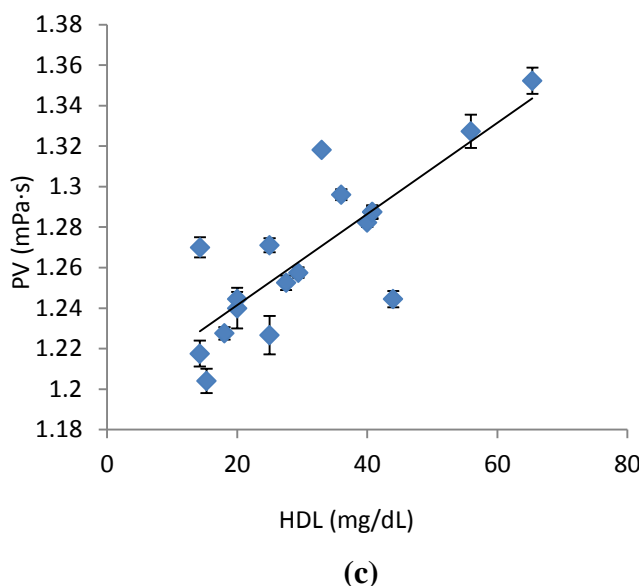


Fig. 4. Relationships between PV and lipid profile parameters (a) PV is correlated weakly to cholesterol ($R^2 = 0.4$) (b) PV is weakly correlated to TGs ($R^2 = 0.4$). (c) PV is strongly correlated to LDL at 22.5 s^{-1} ($R^2 = 0.8$). (d) PV is strongly correlated to HDL ($R^2 = 0.7$).

DISCUSSION

Blood is a two-phase suspension of formed elements, i.e., of red blood cells (RBCs), white blood cells (WBCs), and platelets, suspended in an aqueous solution of organic molecules, proteins, and salts that is called plasma. Because blood behaves as a non-Newtonian fluid, the apparent viscosity of blood depends on the existing shear forces and is determined by the hematocrit, plasma viscosity, RBC aggregation, and the mechanical properties of RBCs[14]. Yann Lamarre *et al.* found that the viscosity of normal blood is equal to $5.86 \pm 0.8 \text{ mPa}\cdot\text{s}$ [15]. B. Y. Vazquez reported that the value of blood viscosity and plasma viscosity for 91 samples taken from men was $6.1 \pm 1.0 \text{ cP}$ and $1.67 \pm 0.29 \text{ cP}$. He obtained his results using a cone and plate Brookfield viscometer (Model Dv-II; Brookfield Engineering Laboratories, Middleboro, MA) at a shear rate of 160 s^{-1} at 37°C [16]. Our study indicated that there is a reduction in viscosity values that is due to increased shear rate. We measured WBV at a lower hematocrit (25 %) than the previous studies, which is clearly the reason for obtaining lower WBV values in comparison with these studies. The PV values were in agreement with the previous studies.

Ca^{2+} is a universal and ubiquitous signaling molecule regulating the cell cycle and cell fate, metabolism, structural integrity, motility, and volume. Most of the Ca^{2+} in the cytosol is bound and buffered by numerous Ca^{2+} -binding proteins, by phospholipids, and by inorganic phosphate. When bound and buffered Ca^{2+} are included, the total intracellular Ca^{2+} in red blood cells (RBCs) reaches $5.7 \mu\text{M}$ [17]. Blood rheology may be influenced by parathyroid hormone and calcitonin, both of which are endocrine modulators of calcium homeostasis [18]. It was concluded that an increase in calcium in erythrocytes leads to severe echinocytosis and altered blood viscosity[18]. Many studies have shown a relationship between erythrocyte deformation and intracellular calcium concentration[19].

Chien *et al.* explored the link between erythrocyte deformation and blood viscosity. They indicated that the increase in erythrocyte stiffness hardness may cause an increase in blood viscosity[20]. Olatunji, L. A. *et al.* reported an increase in plasma viscosity due to an increase in calcium uptake[21]. It is clear that the effect of calcium concentration on erythrocyte deformation is one of the major factors affecting whole blood viscosity. Our results showed a strong correlation between WBV and Ca^{++} concentration (Figure 1a), which is in accord with the previous investigations. The Ca^{++} concentration may influence the WBV through the effect on erythrocyte deformation. Additionally, Ca^{++} could raise the PV, which is directly proportional to WBV. Figure 2a shows a direct relationship between PV and Ca^{++} concentration, which is in agreement with the previous findings.

Reinhart, W. H. *et al.* indicated that the optimum osmolality for the viscosity of an RBC suspension was shifted toward hyperosmolality, while lower osmolalities increased the suspension viscosity exponentially[22]. Their results were obtained after incubation of erythrocytes with different NaCl concentrations to obtain different osmotic conditions[22]. De Simone, G. *et al.* found that either WBV or PV are increased in males and that both are increased by obesity, high sodium intake, and aging, as well as by race[23]. The role of Na^+ as an osmolality moderator in blood is well known; hence, the effect of Na^+ on blood viscosity may be explained by the effect of Na^+ on osmolality. Sodium alginate may induce RBC aggregation in a dose-dependent manner. This finding was reported by Zhao, L. *et al.*[24]. RBC aggregation has a direct effect on blood circulation and blood and plasma viscosity. Our results (Figure 1b) showed a direct linear relation between WBV and Na^+ concentrations but the correlation between them was weak ($R^2 < 0.5$) at both shear rates. PV is also directly proportional to Na^+ (Figure 2b) but with an $R^2 < 0.5$.

Serum potassium is one of the main factors in regulating blood pressure. Pikilidou, M. I. *et al.* observed a reverse relation between serum potassium and blood pressure, supporting a close pathophysiological connection between serum potassium and essential hypertension[25]. Ozolua, R. I. *et al.* suggested that potassium adaptation may not affect hemorheology. The reduced ability of platelets to aggregate--by mechanisms not clearly understood--has implications for reducing thromboembolism[26]. Our results showed weak correlations between WBV and K⁺ concentration at both low and high shear rates (Figure 1c), which is in accordance with the previous studies. In contrast, a moderate correlation between PV and K⁺ concentration was obtained (Figure 2c).

Plasma triglycerides elevate plasma viscosity; however, the contribution of plasma triglycerides to blood viscosity after adjustment for other major covariates has not been reported. Robert S Rosensona *et al.* found that blood viscosity was the dependent variable at a shear rate of 100 s⁻¹ and that there were statistically significant associations with triglycerides, HDL cholesterol, and total serum protein. Blood viscosity at 1 s⁻¹ was associated in a significant way with triglycerides, fibrinogen, total serum protein, and with a biomarker for diabetes mellitus. Their findings had been obtained after correction the hematocrit to 45%[27]. It was found that atherogenic diets rich in saturated fat and cholesterol influence the blood viscosity and red blood cell (RBC) aggregability, which are parameters associated with increased risk of circulatory disorders[28]. Our results showed a strong correlation between WBV and TGs at a low shear rate and a weak correlation at a high shear rate (Figure 3a). TGs can influence RBC aggregation and hence WBV. This can explain our findings that there is an association between TG concentration and WBV.

Carallo, C. *et al.* analyzed the effect of LDL on blood and plasma viscosity in their study examining the influence of lipids on blood and plasma viscosity[29]. Rosensona R. S. *et al.* found that normalized blood viscosity values at each measured shear rate correlated inversely with HDL cholesterol and positively with fibrinogen. The mean plasma viscosity was 1.39 ± 0.08 mPa·s and the mean serum viscosity was 1.27 ± 0.06 mPa·s. Plasma viscosity correlated with fibrinogen (r = 0.51, P < 0.0001), total serum protein (r = 0.33, P < 0.0001), and triglyceride concentrations (r = 0.33, P < 0.0015)[30]. Irace, C. *et al.* found that hyperlipidemic subjects (n = 315) had higher values of plasma viscosity (1.44 ± 0.13 vs. 1.40 ± 0.12 cP, p = 0.007), and blood viscosity (4.51 ± 0.54 vs. 4.35 ± 0.55 cP, p = 0.013) compared to normolipidemic subjects (n = 95). They indicated that plasma viscosity was directly associated with LDL cholesterol, and inversely with erythrocyte rigidity and HDL cholesterol. In a multiple regression analysis, the association with LDL and HDL was strengthened, though these two variables as a whole accounted for only 5% (adjusted R²) of the variability of plasma viscosity[31].

The previous studies observed a correlation between PV and both LDL and HDL, which is in accordance with our results (Figure 4 b, c). The strong correlation between WBV

and both LDL and HDL at high and low shear rates is shown in Figure 3 (b, c). The effect of LDL and HDL on WBV could be indirect and may be due to the effect of LDL and HDL on the deformability and aggregability of RBCs, which can have an effect on BV.

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