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Bivariate and Multivariate Analyses of the Influence of Blood Variables of Patients Submitted to Roux-en-Y Gastric Bypass on the Stability of Erythrocyte Membrane against the Chaotropic Action of Ethanol

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Abstract The stability of the erythrocyte membrane, which is essential for the maintenance of cell functions, occurs in a critical region of fluidity, which depends largely on its composition and the composition and characteristics of the medium. As the composition of the erythrocyte membrane is influenced by several blood variables, the stability of the erythrocyte membrane must have relations with them. The present study aimed to evaluate, by bivariate and multivariate statistical analyses, the correlations and causal relationships between hematologic and biochemical variables and the stability of the erythrocyte membrane against the chaotropic action of ethanol. The validity of this type of analysis depends on the homogeneity of the population and on the variability of the studied parameters, conditions that can be filled by patients who undergo bariatric surgery by the technique of Roux-en-Y gastric bypass since they will suffer feeding restrictions that have great impact on their blood composition. Pathway analysis revealed that an increase in hemoglobin leads to decreased stability of the cell, probably through a process mediated by an increase in mean corpuscular volume. Furthermore, an increase in the mean corpuscular hemoglobin (MCH) leads to an increase in erythrocyte membrane stability, probably because higher values of MCH are associated with smaller quantities of red blood cells and a larger contact area between the cell membrane and ethanol present in the medium.

Keywords Red blood cell · Membrane stability · Ethanol · Chaotropic action · Bariatric surgery · Feeding restriction

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Introduction

Membrane stability is essential for the maintenance of cell functions, and compromising its integrity can not only affect these functions but also lead to cell death (McNeil and Steinhardt 1997). In theory, the stability of a membrane can be changed by the same factors that affect protein stability, such as pH, temperature, drugs and stabilizing and destabilizing solutes. Furthermore, other factors, more specifically related to the composition of the membrane, such as the content of phospholipids and cholesterol, may also compromise the structural organization of the amphiphilic system of the membrane as well as its fluidity (Singer and Nicolson 1972; Cribier et al. 1993; Murray and Granner 2012). For the cell to remain physiologically active, it is essential that the membrane fluidity is within ideal limits (Garcia et al. 2005).

Membrane fluidity is essential for determining the shape, the deformability and, therefore, the rheological properties of the cell (Aloulou et al. 2006; Maeda et al. 2006; Martínez et al. 1996; Seki et al. 2006; Velcheva et al. 2006; Zilberman-Kravits et al. 2006; Uydu et al. 2012).

Studies both in vitro and in vivo have shown that the cholesterol content in the erythrocyte membrane depends on the plasma lipoproteins (Cooper et al. 1972, 1975; Cooper 1977), and the fluidity of these cells is markedly reduced with an increasing mole fraction of cholesterol/phospholipids (Kroes et al. 1972; Vanderkooi et al. 1974; Shattil and Cooper 1976; Lemmich et al. 1997; Cazzola et al. 2004). Notably, low-density lipoprotein cholesterol (LDL), which is responsible for the exchange of cholesterol with the erythrocyte membrane (Nikolic et al. 2007), affects the rheology of the blood fluid and, therefore, predisposes to atherosclerosis more intensely than the levels of total cholesterol (t-C) (Hoefner et al. 2001; Okada et al. 2004).

In spur-cell anemia, erythrocytes have a higher cholesterol content in their membranes (Cooper 1969), which leads to the development of a phenotype of spurs. Clinical observations show that when healthy red blood cells (RBCs) are transfused into an individual affected with this disease, they take the form of spur cells due to the transfer of cholesterol from plasma lipoproteins to their membranes (Cooper 1969). Indeed, the relationship between membrane cholesterol and the shape of the erythrocyte is evidenced by the correlation existing between the content of cholesterol in this cell membrane and the variable red cell distribution width (RDW). This finding links the RDW not only to the pathogenesis of atherosclerosis but also to its pathophysiological complications (Tziakas et al. 2007, 2008, 2011, 2012; Yu et al. 2010; Nishizaki et al. 2012).

The environment in which the RBC is found, therefore, is a decisive factor in determining the composition and fluidity of the cell membrane; and it can be changed by diet, physical activity and many diseases. Thus, the composition and degree of fluidity of the erythrocyte membrane should reflect these changes in blood (Schick and Schick 1985; Martínez et al. 1996; Ozdemirler et al. 2001). Imbalances in the diet, a sedentary lifestyle and hereditary factors are conditions that may, alone or in combination, lead to disruption of energy homeostasis and to the development of dyslipidemia, which will affect the molar ratio of cholesterol/phospholipids of the erythrocyte membrane (Martínez et al. 1998; Michalska-Malecka et al. 2008; Spengler et al. 2008). Therefore, the stability of the erythrocyte membrane must have relations with the blood lipid levels.

The RBC is a suitable model for evaluation of the stability of the membrane because it is a biological material which is easily obtainable through a minimally invasive procedure and without harm to the patient. Also, hemolysis can be followed by spectrophotometric quantification of hemoglobin released in the lysis (Cunha et al. 2007; Penha-Silva et al. 2008; de Freitas et al. 2010; Fonseca et al. 2010; Mansur et al. 2010; Lemos et al. 2011).

This study assessed, through bivariate and multivariate statistical analyses, the correlations and causal relationships between the stability of erythrocytes against the chaotropic action of ethanol and blood variables (hematologic and biochemical) in a population of patients undergoing Roux-en-Y gastric bypass (RYGB). After surgery, these patients suffer a drastic food restriction that has a huge impact on the blood variables (Custódio Afonso Rocha et al. 2012). The validity and effectiveness of a correlation analysis depends largely on the existence of a lower interindividual variability but with greater variability in the variables, conditions that can best be offered in the population considered in this study in relation to the general population.

Materials and Methods

Population

This study was approved by the Ethics Committee in Research of the Federal University of Uberlândia (023/08), and all 24 volunteers who participated in the study signed informed consent forms.

The subjects (8 women with class II obesity and comorbidities and 16 morbidly obese women, mean age 36.46 ± 9.8 years) were recruited among the candidates for bariatric surgery of the Obesity Center of Uberlândia (CENTROBESO) who were not diabetic on insulin and did not fulfill the general exclusion criteria adopted by the institution (anesthetic risk classified as ASA IV; esophagogastric varices with portal hypertension; significant intellectual limitations in patients without adequate family support; current uncontrolled psychiatric disorder, including abuse of alcohol and illicit drugs).

Collection of Blood Samples

Blood samples were collected by venipuncture in evacuated tubes (Vacutainer; Becton Dickinson, Juiz de Fora, Brazil) containing EDTA as an anticoagulant for the determination of erythrogram and evaluation of membrane stability and without anticoagulant for the biochemical determinations. Blood collection occurred after a 12 h overnight fast before surgery and on days 14, 28, 42 and 56 after surgery.

Determination of Hematologic and Biochemical Variables

The erythrogram (automated system Cell-Dyn 3700; Abbott Diagnostics, Abbott Park, IL) and biochemical assays (automated analyzer Architect C 8000, Abbott

Diagnostics) were done in the Laboratory of Clinical Analyses of the Clinical Hospital of the Federal University of Uberlândia.

Reagents and Equipment

The NaCl and ethanol used (Labsynth, Diadema, Brazil) had a purity of 99.5 %, which was duly corrected in the preparation of solutions. Mass measurements were made on a digital analytical balance (model 870; A&D Company, Tokyo, Japan). Volume measurements were made with automatic pipettes (Labsystems, Helsinki, Finland). Incubations were carried out in a thermostatic bath (model MA 184; Marconi, Piracicaba, Brazil). Absorbance readings were performed in a digital spectrophotometer (model UV-1650; Shimadzu, Tokyo, Japan). Centrifugations were performed in a temperature-controlled centrifuge (model CF15RX II; Hitachi Koki, Hitachinaka, Japan).

Determination of Human Erythrocyte Membrane Stability against the Chaotropic Action of Ethanol

Duplicate sets of microtubules (Eppendorf, Hamburg, Germany) containing 1.5 ml of NaCl 0.9 g dl^{-1} and increasing concentrations of 0–20 % ethanol were prepared and preincubated at 37 °C for 10 min. After preincubation, the microtubes received aliquots of 10 µl of whole blood and were carefully homogenized. After incubation for 30 min at 37 °C, the tubes were centrifuged for 10 min at 1,600×g and the supernatants removed for reading of absorbance at 540 nm (A_{540}) (Cunha et al. 2007; Penha-Silva et al. 2007, 2008; de Freitas et al. 2008, 2010; Mansur et al. 2010; Lemos et al. 2011).

Determination of the Transition Curves of Lysis

The dependence of A_{540} on the ethanol concentration was adjusted to a line of sigmoidal regression according to the Boltzmann equation

$$A_{540} = \frac{A_1 - A_2}{1 + e^{(X - D_{50})/dX_e}} + A_2 \quad (1)$$

in which A_1 and A_2 represent, respectively, the mean values of A_{540} at the minimum and maximum plateaus, D_{50} is the concentration of ethanol capable of promoting 50 % lysis and dX_e is the variation in the chaotropic concentration responsible for converting erythrocytes from a completely integer (A_1) to a completely lysed state (A_2).

Statistical Analysis of Data

Relations between variables of erythrocyte stability against the chaotropic action of ethanol and the hematologic and biochemical variables considered in this study were treated statistically by bivariate and multivariate analyses.

In the bivariate correlations, each of the variables for evaluating stability (dX_e, D_{50} and $dX_e \cdot D_{50}$) was fitted by linear regression as a function of each of the different biochemical and hematologic variables. From these correlations we obtained the matrices of correlation and significance that were used to build the groups of hematologic and biochemical variables of the multivariate treatment. These analyses were performed using the application Origin 8.0 (Microcal, Northampton, MA).

Multivariate analyses were performed using the application of free distribution GENES (Federal University of Viçosa, Viçosa, Brazil) and divided into two stages: (1) search for correlation and (2) search for cause and effect.

Canonical correlations were used to search for correlations between the mathematical parameters of stability and the hematologic and biochemical variables. The group formed by the variables dX_e and $dX_e \cdot D_{50}$ constituted the dependent variable and the groups of hematologic or biochemical variables constituted the statistical explanatory variable. These analyses used the Pearson correlation matrices.

The study of the relations of cause and effect was developed through pathway analysis (Wright 1923; Li 1975; Khatri et al. 2012) and considered as the dependent variable the product $dX_e \cdot D_{50}$ and as explanatory variables the same groups of biochemical and hematologic parameters used in the canonical analysis.

Results and Discussion

In this study, blood samples from volunteers were analyzed before (day 0) and 14, 28, 42 and 56 days after bariatric surgery. A detailed description of the study population and the evolution of nutritional, hematologic and blood biochemical variables during the 8 weeks of the study was previously published (Custódio Afonso Rocha et al. 2012). Table 1 presents the baseline characteristics of the study population. After surgery, there was a decrease in all biochemical parameters (glucose, t-C, LDL, very low–density lipoprotein cholesterol [VLDL] and triglycerides [TG]), with a tendency to stabilize, although high-density lipoprotein cholesterol (HDL) also declined. After surgery, there was also a progressive decrease in the values of most hematologic variables (RBC, hemoglobin [Hb], hematocrit [Ht], mean corpuscular volume [MCV], mean corpuscular Hb [MCH] and mean corpuscular Hb concentration [MCHC]) and an increase in the RDW (Supplementary Table 1).

The study used patients submitted to bariatric surgery by RYGB due to the large influence of this procedure on the

Table 1 Baseline characteristics of the study population

Characteristic	
Number of subjects	24
Gender (*n*, %)	
Female	24 (100.0)
Age (years, mean ± SD)	36.5 ± 9.8
BMI (kg/m^2, mean ± SD)	44.3 ± 5.3
Hematologic profile	
Red blood cells (million/mm^3)	4.67 ± 0.3
Hemoglobin (g/dl)	13.7 ± 0.8
Hematocrit (%)	40.7 ± 2.5
Mean corpuscular volume (fl)	87.2 ± 4.9
Mean corpuscular hemoglobin (pg)	29.4 ± 2.1
Mean corpuscular hemoglobin concentration (g/dl)	33.7 ± 1.0
Red cell distribution width (%)	15.4 ± 1.0
Lipid profile	
Total cholesterol (mg/dl)	197.54 ± 39.3
High-density lipoprotein cholesterol	45.7 ± 9.3
Low-density lipoprotein cholesterol	120.6 ± 33.7
Very low-density lipoprotein cholesterol	31.3 ± 14.2
Triglycerides (mg/dl)	156.3 ± 70.9
Medical history (*n*, %)[a]	
Depression	1 (4.2)
Diabetes mellitus	1 (4.2)
Disc herniation	1 (4.2)
Hiatal hernia	1 (4.2)
Hypertension	11 (45.8)
Hypothyroidism	2 (8.3)
Impaired fasting glucose	7 (29.2)
Osteoarthritis	2 (8.3)
Tachycardia	1 (4.2)
Medication (*n*, %)[a]	
Amlodipine	1 (4.2)
Atenolol	4 (16.7)
Atorvastatin	1 (4.2)
Captopril	1 (4.2)
Ciprofibrate	1 (4.2)
Cyclobenzaprine hydrochloride	1 (4.2)
Enalapril	4 (16.7)
Escitalopram	1 (4.2)
Fluoxetine	2 (8.3)
Hydrochlorothiazide	3 (12.5)
Indapamide	2 (8.3)
Levothyroxine sodium	3 (12.5)
Metformin	4 (16.7)
Omeprazole	2 (8.3)
Potassium losartan	2 (8.3)
Sertraline	2 (8.3)
Simvastatin	2 (8.3)
Without medication	10 (41.7)

[a] Some patients had multiple medical conditions and used different types of drugs

values of hematologic and biochemical variables. Greater variation in these variables is desirable in the search for correlations between them and the behavior of the erythrocyte membrane. The use of lipid and glucose-lowering drugs by some of the volunteers (Table 1) decreases the variability in blood levels of lipids and glucose. Similarly, supplementation with vitamins and minerals, recommended as a routine practice for patients after 15 days of surgery (Custódio Afonso Rocha et al. 2012), interferes with the variability of the erythrogram. Even with these limitations in the study population, obtaining the same variance in a group of people in the general population would require a significantly greater number of individuals, which would increase the interindividual variations of various kinds, making it difficult to draw conclusions.

The multivariate statistical analysis was based on the matrix of bivariate correlations (Table 2) between the stability parameters and biochemical and hematologic variables, along with the respective matrix of significance (Table 3). The organization of the groups used in multivariate analyses (Table 4) was based on the nature of the variables (hematologic or biochemical) and the nature (direct or reverse) and significance ($P < 0.05$) of the correlations presented in the correlation matrix.

The search for relationships between groups was performed by analysis of canonical correlations. The canonical correlations are a logical extension of multiple linear regressions, which differ by correlate groups of dependent and independent variables. The base of such statistical analysis is the construction of a linear combination of variables for each group in order to maximize the correlation between the two groups. The attribution of weights for the independent and dependent variables allows us to obtain the maximum correlation (canonical correlation) between the two sets of variables (dependent and independent) (Hair et al. 2006). In these analyses the previously organized groups of variables (Table 4) were the explanatory variable, and the stability parameters dX_e and $dX_e \cdot D_{50}$ constituted the dependent variable.

The combined variable of stability, $dX_e \cdot D_{50}$, was defined as a product of variables dX_e and D_{50} to preserve the direct relationships that both individual variables have with stability. These individual variables have different meanings. While dX_e represents the change in concentration of chaotrope (ethanol) necessary and sufficient to bring the erythrocyte population from an integer to a completely lysed state, D_{50} is the concentration of chaotrope necessary to promote the lysis of half the population of erythrocytes. A higher value of dX_e means that the transition of lysis is less abrupt and the erythrocyte membrane is more stable. Moreover, the higher the value of D_{50}, the greater is the stability of the RBC population. The combined variable $dX_e \cdot D_{50}$ preserves the direct relations of both individual

Table 2 Matrix of correlations (R^2) of biochemical and hematological variables with the parameters of stability against the chaotropic action of ethanol (dX_e, D_{50} and $dX_e \cdot D_{50}$)

	Glu	TG	t-C	HDL	LDL	RBC	Ht	Hb	MCV	MCH	MCHC	RDW	D_{50}	dX_e	$dX_e \cdot D_{50}$
Glu	1.000														
TG	0.004	1.000													
t-C	0.039[a]	0.259[a]	1.000												
HDL-C	0.065[a]	0.000	0.039[a]	1.000											
LDL-C	0.089[a]	0.088[a]	0.908[a]	0.000	1.000										
RBC	0.018	0.008	0.007	0.024	0.011	1.000									
Ht	0.002	0.000	0.004	0.001	0.007	0.472[a]	1.000								
Hb	0.005	0.003	0.010	0.001	0.018	0.378[a]	0.912[a]	1.000							
MCV	0.008	0.020	0.001	0.017	0.001	0.089[a]	0.239[a]	0.267[a]	1.000						
MCH	0.004	0.028	0.000	0.012	0.001	0.109[a]	0.166[a]	0.292[a]	0.872[a]	1.000					
MCHC	0.006	0.018	0.011	0.001	0.026	0.033	0.006	0.048[a]	0.015	0.217[a]	1.000				
RDW	0.001	0.000	0.001	0.000	0.002	0.006	0.153[a]	0.161[a]	0.384[a]	0.327[a]	0.002	1.000			
D_{50}	0.067[a]	0.072[a]	0.016	0.004	0.042[a]	0.001	0.001	0.001	0.000	0.000	0.001	0.005	1.000		
dX_e	0.062[a]	0.011	0.002	0.001	0.007	0.003	0.021	0.021	0.060[a]	0.053[a]	0.001	0.032	0.052	1.000	
$dX_e \cdot D_{50}$	0.069[a]	0.014	0.003	0.001	0.010	0.003	0.020	0.021	0.056[a]	0.051[a]	0.001	0.032	0.089	0.994	1.000

[a] R^2 values associated with significant correlations ($P < 0.05$)

Glu blood glucose, *TG* triglyceride, *t-C* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *RBC* red blood cell, *Ht* hematocrit, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red cell distribution width

Table 3 Matrix of significances of biochemical and hematological variables with the parameters of stability against the chaotropic action of ethanol (dX_e, D_{50} and $dX_e \cdot D_{50}$)

	Glu	TG	t-C	HDL-C	LDL-C	RBC	Ht	Hb	MCV	MCH	MCHC	RDW	D_{50}	dX_e	$dX_e \cdot D_{50}$
Glu															
TG	0.510														
t-C	0.042*	0.000*													
HDL-C	0.008*	0.968	0.041*												
LDL-C	0.002*	0.002*	0.000*	0.919											
RBC	0.169	0.375	0.379	0.111	0.271										
Ht	0.621	0.853	0.526	0.705	0.391	0.000*									
Hb	0.458	0.590	0.317	0.704	0.168	0.000*	0.000*								
MCV	0.347	0.152	0.750	0.174	0.797	0.002*	0.000*	0.000*							
MCH	0.538	0.087	0.930	0.258	0.740	0.001*	0.000*	0.000*	0.000*						
MCHC	0.421	0.165	0.287	0.797	0.095	0.061	0.431	0.023*	0.215	0.000*					
RDW	0.810	0.893	0.701	0.979	0.633	0.435	0.000*	0.000*	0.000*	0.000*	0.623				
D_{50}	0.007*	0.005*	0.196	0.513	0.033*	0.696	0.761	0.708	0.976	0.916	0.791	0.458			
dX_e	0.010*	0.284	0.676	0.742	0.391	0.586	0.135	0.133	0.011*	0.017*	0.775	0.063	0.019		
$dX_e \cdot D_{50}$	0.006*	0.222	0.599	0.811	0.315	0.610	0.143	0.136	0.014*	0.019*	0.735	0.064	0.002	0.000	

* $P < 0.05$, indicating statistically significant correlations

Glu blood glucose, *TG* triglyceride, *t-C* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *RBC* red blood cell, *Ht* hematocrit, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red cell distribution width

variables with the stability and may represent in a unique manner what the individual variables represent in isolation.

A canonical correlation analysis does not allow one to obtain findings in terms of cause and effect, so the existence of canonical correlations between the groups of variables only means the existence of associations which are measurable but not necessarily with a causal relationship between them. Thus, further analyses are necessary to

Table 4 Organized groups for the multivariate statistical analyses (canonical correlations and path analyses)

Group	Variables	Characteristics
1	Hb, Ht, MCV, MCH and MCHC	Hematologic variables whose simple correlations are positive with dX_e and/or $dX_e \cdot D_{50}$
2	t-C, LDL-C	Biochemical variables whose simple correlations are positive with dX_e and/or $dX_e \cdot D_{50}$
3	RBC and RDW	Hematologic variables whose simple correlations are negative with dX_e and/or $dX_e \cdot D_{50}$
4	Glu, TG, HDL-C	Biochemical variables whose simple correlations are negative with dX_e and/or $dX_e \cdot D_{50}$

Glu blood glucose, *TG* triglyceride, *t-C* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *RBC* red blood cell, *Ht* hematocrit, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red cell distribution width

better understand the relationships among the variables that define or influence a certain characteristic of interest. It was in this sense that Li (1975) adapted and popularized the analysis of path coefficients, already used by other authors. The importance of path analysis lies in the fact that it decomposes the effects observed in the correlation analysis in direct and indirect (or mediated) effects, which gives us greater security at the time of choosing the variables that are responsible for a particular effect of interest. It is through path analysis that we can state with certainty that between our findings there were no false-positive or false-negative correlations caused by the interaction of the multiple variables involved in the system. In a biological universe where the variables are involved in a network of multiple influences, this possibility cannot be disregarded (Wright 1923; Li 1975; Khatri et al. 2012).

Cause and effect was investigated using path analysis in order to determine the existence of direct or indirect relationships of causes between $dX_e \cdot D_{50}$ and the variables of groups shown in Table 4.

The canonical correlations between the parameters of stability and the groups formed by hematologic variables (groups 1 and 3 of Table 4) showed no significant relationship with the first canonical pair (Tables 5, 6) and therefore will not be considered in this discussion.

The behavior observed for the hemolysis by ethanol seems to depend on that observed for hypotonic hemolysis. The osmotic stability of erythrocytes showed a strong correlation with RDW (Bernardino Neto 2011), differently from the stability in ethanol. The difference in the mechanism of action would be the cause of the difference between osmotic lysis and lysis by ethanol. Lysis induced by ethanol is dependent on the direct contact of this chaotrope with the membrane, where it will promote opening of pores (Chi and Wu 1991). However, hypotonic lysis is closely related to the osmolarity of the medium and the cell volume, so the stability of RBCs is very sensitive to these two factors and relates inversely to them. In this sense, it is possible that the osmotic stability could reflect better the pathological conditions that have been associated with RDW, including vascular complications in diabetes (Malandrino et al. 2012) and the risk of mortality from cardiovascular diseases (Patel et al. 2010; Nishizaki et al. 2012; Tziakas et al. 2012).

Table 5 Canonical correlations and canonical pairs estimated between the stability parameters (dX_e and $dX_e \cdot D_{50}$) and the variables of group 1

	Canonical pairs	
	1st	2nd
r	0.3284	0.1230
Significance	0.2130	0.8169
Variables	First canonical pair	
dX_e	0.9956	−0.0943
$dX_e \cdot D_{50}$	0.9850	−0.1723
Ht	0.4494	0.1350
Hb	0.4462	−0.0253
MCV	0.7612	0.4436
MCH	0.7113	0.1823
MCHC	0.0662	−0.5494

Ht hematocrit, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration

Table 6 Canonical correlations and canonical pairs estimated between the stability parameters (dX_e and $dX_e \cdot D_{50}$) and the variables of group 3

	Canonical pairs	
	1st	2nd
r	0.1847	0.0387
Significance	0.4408	0.6936
Variables	First canonical pair	
dX_e	0.9985	−0.0542
$dX_e \cdot D_{50}$	0.9912	−0.1325
RBC	−0.2991	−0.9542
RDW	−0.9742	0.2255

RBC red blood cell, *RDW* red cell distribution width

The biochemical parameters arranged in groups 2 and 4 (Table 4) showed significant or borderline correlations with the first canonical pair (Tables 7, 8).

Table 7 Canonical correlations and canonical pairs estimated between the stability parameters (dX_e and $dX_e \cdot D_{50}$) and the variables of group 2

	Canonical pairs	
	1st	2nd
r	0.2692	0.0470
Significance	0.0911	0.6322
Variable	First canonical pair	
dX_e	−0.5501	0.8351
$dX_e \cdot D_{50}$	−0.6141	0.7893
t-C	−0.5051	−0.8631
LDL-C	−0.7436	−0.6686

t-C total cholesterol, *LDL-C* low-density lipoprotein cholesterol

Table 8 Canonical correlations and canonical pairs estimated between the stability parameters (dX_e and $dX_e \cdot D_{50}$) and the variables of group 4

	Canonical pairs	
	1st	2nd
r	0.3871	0.1149
Significance	0.0060*	0.5043
Variables	First canonical pair	
dX_e	−0.6485	0.7612
$dX_e \cdot D_{50}$	−0.7064	0.7078
TG	0.5446	0.3680
HDL-C	−0.1635	−0.8373
Glu	0.7884	−0.5816

* $P < 0.05$, indicating statistically significant correlations

TG triglyceride, *HDL-C* high-density lipoprotein cholesterol, *Glu* blood glucose

The variables from group 2 had a borderline correlation ($P = 0.0911$) with the group constituted by dX_e and $dX_e \cdot D_{50}$, in which the stability parameters had canonical loads close together, which means that they contributed in an approximately equal manner to the correlations between these groups, with a slight predominance of the parameter $dX_e \cdot D_{50}$ in relation to dX_e. Among the biochemical variables, LDL was the variable that showed the greatest weight in group 2.

LDL is the lipoprotein involved in the exchange of cholesterol with the membrane of the erythrocytes by simple diffusion, directed to the structure with a lower molar fraction of cholesterol/phospholipids in order to search for mass balance (Nikolic et al. 2007). This explains why LDL was the most impactful variable in group 2. The direct correlation observed between the variables in group 2 and the stability of erythrocytes can be explained in light of the role of cholesterol in the membrane of the cell. The rigid steroid ring of cholesterol reduces the freedom of lateral movement of lipids and increases the intensity of the intermolecular forces of van der Waals, giving greater rigidity to the membrane of these cells (Hubbell and McConnell 1971; Shinitzky and Inbar 1976; Raffy and Teissié 1999). The higher the plasma levels of LDL, the greater the flow of cholesterol to the membrane, which becomes more rigid and stable as long as there is no impairment of fluidity and deformability of the membrane. Stability must not be a property that varies linearly with increasing cholesterol concentration since an excessive increase in cholesterol would reduce drastically the membrane fluidity, affecting the deformability of the cell and increasing its susceptibility to lysis.

The combined parameter $dX_e \cdot D_{50}$ was more impactful than the single parameter dX_e in the canonical correlation with the variables of group 2.

The variables of group 4 were those that had most significant correlations with the parameters dX_e and $dX_e \cdot D_{50}$ ($P = 0.0060$). These parameters again showed values of canonical loads close to one another, with $dX_e \cdot D_{50}$ contributing more to the correlation between the two groups, which corroborates the assumption of greater coverage of the combined parameter. Blood glucose was the variable that gave greater weight in determining this correlation, followed by blood levels of TG. HDL presented a negative canonical load, contrary to what we saw in the bivariate regression, however without greater significance, given the small size of the absolute value of the load in relation to those of the other variables in its group.

The reverse direction found for the relation between stability and the values of the variable TG is justified since VLDL = TG/5 (Friedewald et al. 1972).

The correlations between the parameters dX_e and $dX_e \cdot D_{50}$ and group 4 variables had a stronger influence of glucose, both characterized by decreased stability with increased blood glucose levels. It is likely that this loss in cell stability determined by glucose is related to the glycation of membrane proteins, altering the structure and, in consequence, the function of biological molecules that play essential roles in maintaining the structural organization of the membrane amphiphilic system (Shin et al. 2008)

The path analyses to search for cause-and-effect relationships between stability and blood variables were conducted using the combined parameter of stability ($dX_e \cdot D_{50}$), based on the fact that it was associated with larger canonical loads than the single parameter dX_e.

The path analyses did not reveal the existence of direct or mediated effects with values above that of the residual variable for relations in groups 2, 3 and 4 with the parameter $dX_e \cdot D_{50}$ (Tables 9, 10, 11). However, several values of direct and mediated effects were greater than the residual variable in the relations of group 1 variables and $dX_e \cdot D_{50}$. The variable MCHC presented indirect effects

Table 9 Direct, indirect and total effects of the correlations between the variables of group 2 and $dX_e \cdot D_{50}$

Variable	Effect	Via	$dX_e \cdot D_{50}$	Variable	Effect	Via	$dX_e \cdot D_{50}$
LDL-C	Direct		0.5308	t-C	Direct		−0.4542
	Indirect	t-C	−0.4327		Indirect	LDL-C	0.5056
	Total		0.0981		Total		0.0515

Effect of the residual variable, 0.9855; coefficient of determination, 0.0287

LDL-C low-density lipoprotein cholesterol, *t-C* total cholesterol

Table 10 Direct, indirect and total effects for the correlations between the variables of group 3 and $dX_e \cdot D_{50}$

Variable	Effect	Via	$dX_e \cdot D_{50}$	Variable	Effect	Via	$dX_e \cdot D_{50}$
RBC	Direct		−0.0364	RDW	Direct		−0.1768
	Indirect	RDW	−0.0135		Indirect	RBC	−0.0028
	Total		−0.0499		Total		−0.1796

Effect of the residual variable, 0.9830; coefficient of determination, 0.0335

RBC red blood cell, *RDW* red cell distribution width

Table 11 Direct, indirect and total effects for the correlations between the variables of group 4 and $dX_e \cdot D_{50}$

Variable	Effect	Via	$dX_e \cdot D_{50}$	Variable	Effect	Via	$dX_e \cdot D_{50}$
Glu	Direct		−0.2675	TG	Direct		−0.1016
	Indirect	TG	−0.0065		Indirect	Glu	−0.0172
		HDL-C	0.0112			HDL-C	−0.0002
	Total		−0.2629		Total		−0.1190
HDL-C	Direct		0.0440				
	Indirect	Glu	−0.0678				
		TG	0.0003				
	Total		−0.0234				

Effect of the residual variable, 0.9584; coefficient of determination, 0.0814

Glu blood glucose, *HDL-C* high-density lipoprotein cholesterol, *TG* triglyceride

through Hb and MCH that were greater than the value of the residual variable (Table 12). The variables Ht, Hb, MCV and MCH had direct and indirect effects greater than the value of the residual variable, except for the indirect effects through MCHC and the total effect.

The coefficient of determination obtained for group 1 in the pathway analysis was 0.1051, and the magnitude of the residual variable effect was 0.946 (Table 12). The variable Ht showed direct and positive effects on the stability 5.62 times greater than the magnitude of the value of the residual variable. This indicates that there must be a direct causal link between the membrane stability of erythrocytes and Ht values. This relationship was statistically evident in the calculations of the path analysis. However, the strong and direct correlation between stability and Ht observed in the path analysis was not found in bivariate correlation and did not appear in the value of the total effect in path analysis. This happened because this effect was masked by variables such as MCV and Hb, as well as other variables that were not involved in this study. This indicates that the use of simple linear correlation to study the relationship between the stability of erythrocytes and Ht is not appropriate. There are external factors that are strong enough to prevent the correlation between these variables from appearing in a bivariate study (Bernardino Neto 2011).

The direct effects of MCV and Hb on erythrocyte stability were negative and, respectively, 5.72 and 5.69 times greater than the magnitude of the residual variable. This means that the direct effects of Hb and MCV contributed to decrease the values of $dX_e \cdot D_{50}$ or, in other words, contributed to reducing the stability of erythrocytes. A higher content of Hb in the erythrocyte induces a higher difference in concentration between the internal and external environment of that cell, which must then hold more water and have a higher volume (MCV). The bulkier erythrocytes are less stable, while those less bulky, which have their membrane lipids organized with more approximation and intensification of the molecular attractive forces of van der Waals, are more stable (Cunha et al. 2007; Penha-Silva et al. 2008).

The variable MCH presented a direct effect on the stability 6.67 times greater than the residual variable with the

Table 12 Direct, indirect and total effects of the correlations between the variables of group 1 and the parameter $dX_e \cdot D_{50}$

Variable	Effect	Via	$dX_e \cdot D_{50}$	Variable	Effect	Via	$dX_e \cdot D_{50}$
Ht	Direct		5.3176	MCH	Direct		6.3137
	Indirect	Hb	−5.1720		Indirect	Ht	2.1694
		MCV	−2.6294			Hb	−2.9216
		MCH	2.5758			MCV	−5.0297
		CHCM	0.0505			MCHC	−0.3057
	Total		0.1425		Total		0.2262
Hb	Direct		−5.4152	MCHC	Direct		−0.6565
	Indirect	Ht	5.0787		Indirect	Ht	−0.4092
		MCV	−2.7807			Hb	−1.1900
		MCH	3.4064			MCV	−0.6509
		MCHC	−0.1443			MCH	2.9396
	Total		0.1449		Total		0.0331
MCV	Direct		−5.3831				
	Indirect	Ht	2.5974				
		Hb	−2.7973				
		MCH	5.8992				
		MCHC	−0.0794				
	Total		0.2368				

Effect of the residual variable, 0.9460; coefficient of determination, 0.1051

Ht hematocrit, *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration

positive direction, which means that the greater the value of MCH, the higher is the stability of the erythrocyte membrane. This finding appears to conflict with the inverse relationship observed between the stability of erythrocyte membrane and Hb concentration. How could MCH and Hb have opposite correlations with the stability being conceptually so close? What is the role of the chaotrope (ethanol) in the relationship between MCH and stability since this inverse relationship does not occur in erythrocyte lysis by hypotonic shock (Bernardino Neto 2011) but only in lysis by ethanol?

The solution to this apparent paradox can be understood based on a simple analysis. From two different populations of cells with the same content of Hb, the population with higher MCH should have fewer cells (lower RBC count) and, thus, lower contact surface between cells and medium. If lysis by ethanol occurs by leakage of the membrane phospholipids by direct action of the chaotrope on the cell surface, a lower surface of contact between cell and medium should decrease the effectiveness of this mechanism (Fig. 1), requiring a higher concentration of ethanol to promote lysis, i.e., higher values of D_{50} and $dX_e \cdot D_{50}$. This would explain the direct relationship between MCH and stability of erythrocytes against the chaotropic action of ethanol. Nevertheless, hypotonic lysis must be solely dependent on the salt concentration difference between the internal and external media of the cell but not on the amount of cells. As the concentration of water in the external environment is substantially greater than the concentration of ethanol, the variation in surface contact between the cell membrane and the medium caused by changes in cell count (RBC) does not affect the mechanism of lysis by hypotonic shock with the same intensity as it affects the mechanism of lysis by the chaotropic action of ethanol.

LDL is correlated closely with the fluidity and, therefore, the stability of the membrane. Since LDL is the more important lipoprotein in assessing the risk of

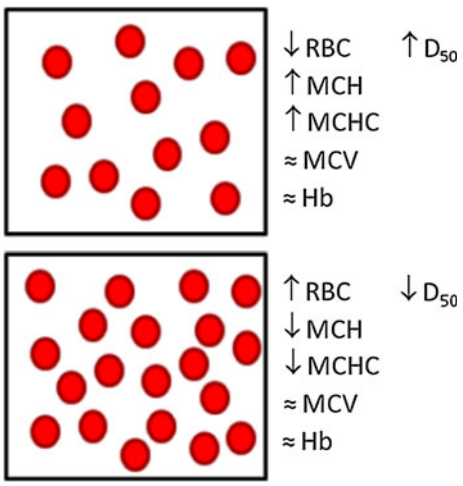

Fig. 1 Analogy used to explain why the MCH has a positive effect on the stability of erythrocytes against the chaotropic action of ethanol. The higher the value of RBC, the greater the contact surface between the cell membrane and medium (ethanol). This makes more efficient the lysis induced by the chaotrope so that lower concentrations of ethanol are required, i.e., the lower the stability (and the lower the D_{50}) of the erythrocyte. *Hb* hemoglobin, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, RBC red blood cell

atherosclerosis, this lipoprotein must be a link between the stability of the erythrocyte and the risk of developing this disease. This should indicate that the stability of the erythrocyte may be a further indirect indicator of the risk of developing atherosclerosis.

As $dX_e \cdot D_{50}$ was more sensitive to changes in blood variables that affect the stability of the membrane in response to the chaotropic action of ethanol, it should be the elective indicator of the stability of erythrocyte.

The search for causal relations for the stability of the erythrocyte membrane by multivariate analysis revealed that bivariate regression can produce some questionable results due to the variable being involved in a complex system under the influence of a wide range of factors. This was evident in the analysis of correlation between the stability of the erythrocyte and Hb, in which the direct relationship between these variables could only be verified in pathway analysis after excluding the influence of other variables in the same group. Hb contributes to reducing the stability of the cell, probably through a process mediated by an increase in MCV. For the specific mechanism of lysis by the action of ethanol, the stability of the erythrocyte membrane varies directly with MCH, probably because higher values of MCH are related to lower values of RBC and greater contact surface between the membrane and the chaotropic agent in the solution.

Many other variables of the complex system that is the human blood also influence the stability of the erythrocyte membrane. These variables include plasma concentrations of electrolytes and albumin (Fonseca et al. 2010) and the proper composition of the cell membrane. Besides these, there are the variables that influence the erythrogram, such as blood levels of iron, folate and cyanocobalamin (Alves de Rezende et al. 2009; Toh et al. 2009). Certainly, the inclusion of all these variables in the analysis should allow an even more accurate picture of the interrelationship between the stability of the red cell membrane against the chaotropic action of ethanol and its natural environment in human blood.

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References

Aloulou I, Varlet-Marie E, Mercier J, Brun JF (2006) Hemorheological disturbances correlate with the lipid profile but not with the NCEP-ATPIII score of the metabolic syndrome. Clin Hemorheol Microcirc 35:207–212

Alves de Rezende CH, Coelho LM, Oliveira LM, Penha-Silva N (2009) Dependence of the geriatric depression scores on age, nutritional status, and haematologic variables in elderly institutionalized patients. J Nutr Health Aging 13:617–621

Bernardino Neto M (2011) Analysis of correlations between stability of erythrocyte membrane, serum lipids and hematologic variables. Federal University of Uberlândia, Dissertation

Cazzola R, Rondanelli M, Russo-Volpe S, Ferrari E, Cestaro B (2004) Decreased membrane fluidity and altered susceptibility to peroxidation and lipid composition in overweight and obese female erythrocytes. J Lipid Res 45:1846–1851

Chi LM, Wu WG (1991) Mechanism of hemolysis of red blood cell mediated by ethanol. Biochim Biophys Acta 1062:46–50

Cooper RA (1969) Anemia with spur-cells: a red cell defect acquired in serum and modified in the circulation. J Clin Invest 48:1820–1831

Cooper RA (1977) Abnormalities of cell-membrane fluidity in the pathogenesis of disease. N Engl J Med 297:371–377

Cooper RA, Diloy-Puray M, Lando P, Greenberg MS (1972) An analysis of lipoproteins, bile acids and red cell membrane associated with target cell and spur-cell in patients with liver disease. J Clin Invest 51:3182–3192

Cooper RA, Arner EC, Wiley JS, Shattil SJ (1975) Modification of red cell membrane structure by cholesterol-rich lipid dispersions: a model of primary spur-cell defect. J Clin Invest 55:115–126

Cribier S, Morrot G, Zachowski A (1993) Dynamics of the membrane lipid phase. Prostaglandins Leukot Essent Fatty Acids 48:24–32

Cunha CC, Arvelos LR, Costa JO, Penha-Silva N (2007) Effects of glycerol on the thermal dependence of the stability of human erythrocytes. J Bioenerg Biomembr 39:341–347

Custódio Afonso Rocha V, Ramos de Arvelos L, Pereira Felix G, Prado N, de Souza D, Bernardino Neto M, Santos Resende E, Penha-Silva N (2012) Evolution of nutritional, hematologic and biochemical changes in obese women during 8 weeks after Roux-en-Y gastric bypasss. Nutr Hosp 27:1134–1140

de Freitas MV, Netto RCM, Huss JCC, De Souza TMT, Costa JO, Firmino CB, Penha-Silva N (2008) Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. Toxicol In Vitro 22:219–224

de Freitas MV, Oliveira MR, dos Santos DF, de Cássia Mascarenhas Netto R, Fenelon SB, Penha-Silva N (2010) Influence of the use of statin on the stability of erythrocyte membranes in multiple sclerosis. J Membr Biol 233:127–134

Fonseca LC, Arvelos LR, Netto RCM, Lins AB, Garrote-Filho MS, Penha-Silva N (2010) Influence of the albumin concentration and temperature on the lysis of human erythrocytes by sodium dodecyl sulfate. J Bioenerg Biomembr 42:413–418

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502

Garcia JJ, Martínez-Ballarín E, Millán-Plano S, Allué JL, Albendea C, Fuentes L, Escanero JF (2005) Effects of trace elements on membrane fluidity. J Trace Elem Med Biol 19:19–22

Hair JF, Black B, Babin B, Anderson RE, Tatham RL (2006) Multivariate data analysis. Prentice Hall, Upper Saddle River, NJ

Hoefner DM, Hodel SD, O'Brien JF, Branum EL, Sun D, Meissner I, McConnell JP (2001) Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL system. Clin Chem 47:266–274

Hubbell WL, McConnell HM (1971) Molecular motion in spin-labeled phospholipids and membranes. J Am Chem Soc 93:314–326

Khatri P, Sirota M, Butte AJ (2012) Ten years of pathway analysis: current approaches and outstanding challenges. PLoS Comput Biol 8:1–10

Kroes J, Ostwald R, Keith A (1972) Erythocyte membrane: compression of lipid phases by increased cholesterol content. Biochim Biophys Acta 274:71–74

Lemmich J, Mortensen K, Ipsen JH, Honger T, Bauer R, Mouritsen OG (1997) The effect of cholesterol in small amounts on lipid bilayer softness in the region on the main phase transition. Eur Biophys J 25:293–304

Lemos GSD, Márquez-Bernardes LF, Arvelos LR, Paraiso LF, Penha-Silva N (2011) Influence of glucose concentration on the membrane stability of human erythrocytes. Cell Biochem Biophys 61:531–537

Li CC (1975) Path analysis: a primer. Boxwood, Pacific Grove, CA

Maeda N, Cicha I, Tateishi N, Suzuki Y (2006) Triglyceride in plasma: prospective effects on microcirculatory functions. Clin Hemorheol Microcirc 34:341–346

Malandrino N, Wu WC, Taveira TH, Whitlatch HB, Smith RJ (2012) Association between red blood cell distribution width and macrovascular and microvascular complications in diabetes. Diabetologia 55:226–235

Mansur PHG, Cury LKP, Leite JOB, Pereira AA, Penha-Silva N, Andrade AO (2010) The approximate entropy of the electromyographic signals of tremor correlates with the osmotic fragility of human erythrocytes. Biomed Eng Online 9:29

Martínez M, Vayá A, Martí R, Gil L, Lluch I, Carmena R, Aznar J (1996) Erythrocyte membrane cholesterol/phospholipid changes and haemorheological modifications in familial hypercholesterolemia treated with lovastatin. Thromb Res 83:375–388

Martínez M, Vayá A, Gil L, Martí R, Dalmau J, Aznar J (1998) The cholesterol/phospholipid ratio of the erythrocyte membrane in children with familial hypercholesterolemia. Its relationship with plasma lipids and red blood cell aggregability. Clin Hemorheol Microcirc 18:259–263

McNeil PL, Steinhardt RA (1997) Loss, restoration and maintenance of plasma membrane integrity. J Cell Biol 137:1–4

Michalska-Malecka K, Slowinska L, Dorecka M, Romaniuk W (2008) Correlations in some pathogenetic factors and values of hemorheological parameters in age-related macular degeneration. Clin Hemorheol Microcirc 38:209–216

Murray RK, Granner DK (2012) Membranes: structure, assembly and function. In: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA (eds) Harper's illustrated biochemistry, 29th edn. McGraw-Hill, New York

Nikolić M, Stanić D, Baricević I, Jones DR, Nedić O, Niketić V (2007) Efflux of cholesterol and phospholipids derived from the haemoglobin-lipid adduct in human red blood cells into plasma. Clin Biochem 40:305–309

Nishizaki Y, Yamagami S, Suzuki H, Joki Y, Takahashi S, Sesoko M, Yamashita H, Kuremoto K, Shinozaki T, Daida H (2012) Red blood cell distribution width as an effective tool for detecting fatal heart failure in super-elderly patients. Intern Med 51:2271–2276

Okada M, Matsuto T, Miida T, Obayashi K, Zhu Y, Fueki Y (2004) Lipid analyses for the management of vascular diseases. J Atheroscler Thromb 11:190–199

Ozdemirler G, Küçük S, Orhan Y, Aykaç-Toker G, Uysal M (2001) Lipid and protein oxidation in erythrocyte membranes of hypercholesterolemic subjects. Clin Biochem 34:335–339

Patel KV, Semba RD, Ferrucci L, Newman AB, Fried LP, Wallace RB, Bandinelli S, Phillips CS, Yu B, Connelly S, Shlipak MG, Chaves PH, Launer LJ, Ershler WB, Harris TB, Longo DL, Guralnik JM (2010) Red cell distribution width and mortality in older adults: a meta-analysis. J Gerontol A Biol Sci Med Sci 65:258–265

Penha-Silva N, Firmino CB, De Freitas Reis FG, Huss JCC, De Souza TMT, De Freitas MV, Netto RCM (2007) Influence of age on the stability of human erythrocyte membranes. Mech Ageing Dev 128:444–449

Penha-Silva N, Arvelos LR, Cunha CC, Aversi-Ferreira TA, Gouvêa-e-Silva LF, Garrote-Filho MS, Finotti CJ, Bernardino-Neto M, de Freitas Reis FG (2008) Effects of glycerol and sorbitol on the thermal dependence of the lysis of human erythrocytes by ethanol. Bioelectrochemistry 73:23–29

Raffy S, Teissié J (1999) Control of lipid membrane stability by cholesterol content. Biophys J 76:2072–2080

Schick BP, Schick PK (1985) Cholesterol exchange in platelets, erythrocytes and megakaryocytes. Biochim Biophys Acta 833:281–290

Seki K, Sumino H, Nara M, Ishiyama N, Nishino M, Murakami M (2006) Relationships between blood rheology and age, body mass index, blood cell count, fibrinogen, and lipids in healthy subjects. Clin Hemorheol Microcirc 34:401–410

Shattil SJ, Cooper RA (1976) Membrane microviscosity and human platelet function. Biochemistry 15:4832–4837

Shin S, Ku Y-H, Suh J-S, Singh M (2008) Rheological characteristics of erythrocytes incubated in glucose media. Clin Hemorheol Microcirc 38:153–161

Shinitzky M, Inbar M (1976) Microviscosity parameters and protein mobility in biological membranes. Biochim Biophys Acta 433:133–149

Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of the cell membranes. Science 175:720–731

Spengler MI, Bertoluzzo SM, Catalani G, Rasia ML (2008) Study on membrane fluidity and erythrocyte aggregation in equine, bovine and human species. Clin Hemorheol Microcirc 38:171–176

Toh SY, Zarshenas N, Jorgensen J (2009) Prevalence of nutrient deficiencies in bariatric patients. Nutrition 25:1150–1156

Tziakas DN, Kaski JC, Chalikias GK, Romero C, Fredericks S, Tentes IK, Kortsaris AX, Hatseras DI, Holt DW (2007) Total cholesterol content of erythrocyte membranes is increased in patients with acute coronary syndrome: a new marker of clinical instability? J Am Coll Cardiol 49:2081–2089

Tziakas DN, Chalikias GK, Stakos D, Tentes IK, Chatzikyriakou SV, Mitrousi K, Kortsaris AX, Boudoulas H, Kaski JC (2008) Cholesterol composition of erythrocyte membranes and its association with clinical presentation of coronary artery disease. Coron Artery Dis 19:583–590

Tziakas DN, Chalikias GK, Stakos D, Tentes IK, Papazoglou D, Thomaidi A, Grapsa A, Gioka G, Kaski JC, Boudoulas H (2011) Independent and additive predictive value of total cholesterol content of erythrocyte membranes with regard to coronary artery disease clinical presentation. Int J Cardiol 150:22–27

Tziakas D, Chalikias G, Grapsa A, Gioka T, Tentes I, Konstantinides S (2012) Red blood cell distribution width: a strong prognostic marker in cardiovascular disease is associated with cholesterol content of erythrocyte membrane. Clin Hemorheol Microcirc 51:243–254

Uydu HA, Yıldırmış S, Orem C, Calapoglu M, Alver A, Kural B, Orem A (2012) The effects of atorvastatin therapy on rheological characteristics of erythrocyte membrane, serum lipid profile and oxidative status in patients with dyslipidemia. J Membr Biol 245:697–705

Vanderkooi J, Fischkoff S, Chance B, Cooper RA (1974) Fluorescent probe analysis of the lipid architecture of natural and experimental cholesterol-rich membranes. Biochemistry 13:1589–1595

Velcheva I, Antonova N, Dimitrova V, Dimitrov N, Ivanov I (2006) Plasma lipids and blood viscosity in patients with cerebrovascular disease. Clin Hemorheol Microcirc 35:155–157

Wright S (1923) The theory of path coefficients—a reply to Nile′s criticism. Genetics 8:239–255

Yu MM, Xu Y, Zhang JH, Wang CH, Wang XC, Cheng ZP, Xu BL (2010) Total cholesterol content of erythrocyte membranes levels are associated with the presence of acute coronary syndrome and high sensitivity C-reactive protein. Int J Cardiol 145:57–58

Zilberman-Kravits D, Harman-Boehm I, Shuster T, Meyerstein N (2006) Increased red cell aggregation is correlated with HbA1C and lipid levels in type 1 but not type 2 diabetes. Clin Hemorheol Microcirc 35:463–471