ERYTHROCYTE MEMBRANE STABILITY IS ASSOCIATED WITH THE RED CELLS DISTRIBUTION WIDTH, UNDER THE ANTAGONISTIC INFLUENCES OF THE LEVELS OF LDL- AND HDL-CHOLESTEROL

A ESTABILIDADE DA MEMBRANA DO ERITRÓCITO ESTÁ ASSOCIADA À AMPLITUDE DE DISTRIBUIÇÃO DOS ERITRÓCITOS, SOB AS INFLUÊNCIAS ANTAGÔNICAS DOS NÍVEIS DE COLESTEROL *LDL* E COLESTEROL *HDL*

Wener Barbosa-Resende[1]
Vanessa Custódio Afonso Rocha[2]
Leticia Ramos de Arvelos[1]
Lucas Moreira Cunha[1]
Alice Vieira da Costa[1]
Elmiro Santos Resende[2]
Ubirajara Coutinho Filho[3]
Nilson Penha-Silva[1*]

RESUMO: A obesidade causa muitas alterações bioquímicas que podem afetar a composição e o comportamento de muitas células do corpo, como os eritrócitos. **Objetivo:** Compreender como a estabilidade da membrana do eritrócito é afetada pelo tipo de cirurgia bariátrica, conhecida como *bypass* gástrico em Y-de-*Roux* (*RYGB*), utilizando análise de componentes principais (*PCA*) e máquina de vetores de suporte (*VSM*). **Métodos:** Uma população de 24 mulheres obesas (36,46 ± 9,8 anos) foi avaliada antes e aos 14, 28, 42 e 56 dias após a cirurgia**.** **Resultados:** As relações entre a estabilidade da membrana eritrocitária e os índices antropométricos, hematimétricos e bioquímicos foram analisadas por *PCA* e *SVM*. A *PCA* mostrou que 1) a perda de peso dos voluntários do estudo foi associada à diminuição da contagem de glóbulos vermelhos e ao aumento dos índices hematológicos volume corpuscular médio (*MCV*), hemoglobina corpuscular média (*MCH*) e concentração de hemoglobina corpuscular média (*MCHC*); 2) menor colesterol total (*t-C*) e colesterol de lipoproteína de baixa densidade (*LDL-C*) foram associados a valores mais elevados de hemoglobina (*Hb*) e hematócrito (*Ht*); 3) níveis mais baixos de triglicerídeos (*TG*) e colesterol de lipoproteína de muito baixa densidade (*VLDL-C*) e níveis mais elevados de colesterol de lipoproteína de alta densidade (*HDL-C*) foram associados a valores mais baixos da amplitude de distribuição dos eritrócitos (*RDW*). O *SVM* mostrou que as variáveis de estabilidade eritrocitária apresentam relações não lineares com o *RDW*, sob a influência antagônica dos níveis de *LDL-C* em relação às concentrações de *HDL-C*. **Conclusão:** A estabilidade da membrana eritrocitária está associada à distribuição de volume das células vermelhas do sangue, sob influência antagônica dos níveis de colesterol *LDL* e colesterol *HDL*.

Palavras-chave: Análise de componentes principais; Máquina de vetores de suporte; Cirurgia bariátrica; Estabilidade da membrana eritrocitária.

[1]Biophysicochemistry Laboratory, Institute of Biotechnology, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.
[2]Clinical Hospital, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.
[3]Chemical Engineering Faculty, Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil.
[*]Corresponding author: nspenha@gmail.com

ABSTRACT: Obesity causes many biochemical changes that can affect the composition and behavior of many cells in the body, such as erythrocytes. **Objective:** To understand how the erythrocyte membrane stability is affected by the type of bariatric surgery known as Roux-en-Y gastric bypass (RYGB) using principal component analysis (PCA) and support vector machine (VSM). **Methods**: A population of 24 obese women (36.46 ± 9.8 years) was assessed before and at 14, 28, 42 and 56 days after surgery. **Results**: The relations between erythrocyte membrane stability and anthropometric, hematimetric and biochemical indices were analyzed by PCA and SVM. PCA showed that 1) the weight loss of study volunteers was associated with a decrease in red blood cell counts and increase in the hematologic indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC); 2) lower total (t-C) and low density lipoprotein cholesterol (LDL-C) were associated with higher hemoglobin (Hb) and hematocrit (Ht) values; 3) lower levels of triglycerides (TG) and very low density lipoprotein cholesterol (VLDL-C) and higher levels of high density lipoprotein cholesterol (HDL-C) were associated with lower values of red cell distribution width (RDW). SVM showed that the erythrocyte stability variables have nonlinear relations with RDW, under the antagonistic influence of the levels of LDL-C in relation to the HDL-C concentrations. **Conclusion**: Erythrocyte membrane stability is associated with the red cells distribution width, under the antagonistic influences of the levels of LDL- and HDL-cholesterol.

Keywords: Principal component analysis; Support vector machine; Bariatric surgery; Erythrocyte membrane stability.

1 INTRODUCTION

Obesity is a disorder caused by excess energy intake, which results in accumulation of fat (MISRA; KHURANA, 2008) and tendency to elevation in the blood levels of triglycerides (TG), total (t-C) and low density lipoprotein cholesterol (LDL-C), which in turn predisposes to the development of various other diseases, such as atherosclerosis, hypertension, insulin resistance and type 2 diabetes mellitus (KOGAI; LUTOV; SELYATITSKAYA, 2008).

Elevated cholesterol levels may alter the composition and various properties of the erythrocyte membrane, such as permeability, deformability and osmotic stability (COOPER, R. A., 1977; QIAN; RAI; HELLER, 2014; RODUIT; VAN DER GOOT; DE LOS RIOS; YERSIN *et al.*, 2008). Both LDL as the high density lipoprotein (HDL) may affect erythrocyte membrane composition, by the addition and removal of cholesterol, respectively (EJIMA; IJICHI; OHNISHI; MARUYAMA *et al.*, 2000; MEURS; HOEKSTRA; VAN WANROOIJ; HILDEBRAND *et al.*, 2005).

The osmotic stability of the erythrocyte membrane can be evaluated by subjecting these cells to hypotonicity gradient, and quantitating hemoglobin released during hemolysis by spectrophotometry (PENHA-SILVA; ARVELOS; CUNHA; AVERSI-FERREIRA *et al.*, 2008; PENHA-SILVA; FIRMINO; DE FREITAS REIS; DA COSTA HUSS *et al.*, 2007).

The curve of absorbance as a function of NaCl concentration can be adjusted by sigmoidal regression, with basis in the Boltzmann equation, which comprises two constants, H_{50} and dX, the first with an inverse relation and the second with a direct relation with the stability of red cell membrane. In many studies, it has been used the inverse of the constant H_{50} ($1/H_{50}$ or $invH_{50}$) in order to both constants (dX and $invH_{50}$) have direct relations with the erythrocyte membrane stability. These constants are highly dependent on blood internal milieu, especially with lipid profile variables, and have enough interindividual variability to make them relevant variables in the study of dyslipidemias (DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013; DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO *et al.*, 2014; NETO; DE AVELAR; ARANTES; JORDAO *et al.*, 2013).

Furthermore, the variables dX and H_{50} present significant associations with the hematologic variable red blood cell distribution width (RDW) (DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013; DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO *et al.*, 2014; NETO; DE AVELAR; ARANTES; JORDAO *et al.*, 2013), which has been associated with cardiovascular disorders (LIPPI; SANCHIS-GOMAR; DANESE; MONTAGNANA, 2013; MONTAGNANA; CERVELLIN; MESCHI; LIPPI, 2012). Several studies show that the RDW can be used as a predictor of mortality in these (HUNZIKER; STEVENS; HOWELL, 2012; LIPPI; SANCHIS-GOMAR; DANESE; MONTAGNANA, 2013) and many other diseases.

Erythrocyte membrane stability has complex, simultaneous and non-simultaneous associations with a wide range of blood biochemical variables and the set of the erythrogram variables. Understanding the nature of these reactions is a complicated task that requires the use of various statistical tools, such as multiple linear regression, canonical correlation and path analysis in populations subject to greater variation in the set of those variables (DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013; DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO *et al.*, 2014; NETO; DE AVELAR; ARANTES; JORDAO *et al.*, 2013; NETTO; FABBRI; DE FREITAS; NETO *et al.*, 2014), as is the case of patients undergoing bariatric surgery (CUSTODIO AFONSO ROCHA; RAMOS DE ARVELOS; PEREIRA FELIX; NOGUEIRA PRADO DE SOUZA *et al.*, 2012; DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013).

The aim of this study was to better understand how the erythrocyte membrane stability is affected by the type of bariatric surgery known as Roux-en-Y gastric bypass (RYGB) using principal component analysis (PCA) and support vector machine (VSM), tools that have been successfully applied in health sciences, particularly in studies on AIDS (BHAKAT; MARTIN;

SOLIMAN, 2014; GERNS STOREY; RICHARDSON; SINGA; NAULIKHA *et al.*, 2014; WEI; LI; CHEN; WANG *et al.*, 2015), cancer (GOSTEK; AWSIUK; PABIJAN; RYSZ *et al.*, 2015; GUYON; WESTON; BARNHILL; VAPNIK, 2002; PARK; CHOI; CHUNG; KIM *et al.*, 2014) and obesity (CABALLERO; FERNANDEZ; GARRIGA; ABREU *et al.*, 2007; HE; LIU; XIAO; ZHANG *et al.*, 2008; LOPEZ-MEYER; SCHUCKERS; MAKEYEV; SAZONOV, 2010; PANAZZOLO; SICURO; CLAPAUCH; MARANHAO *et al.*, 2012).

2 MATERIAL AND METHODS

2.1 POPULATION AND PROCEDURES

The study population consisted of 8 women classified as having class II obesity (BMI between 35.00 and 39.99 kg/m^2) and comorbidities and 16 women with class III obesity (BMI $\geq$ 40.00/m^2) (WHO, 2005), with average age of 36.46 ± 9.8 years, recruited among the candidates for bariatric surgery from the Obesity Center of Uberlândia (CENTROBESO, Uberlândia, MG, Brazil). Patients who participated in the study were not insulin-dependent diabetics and also did not fit the general exclusion criteria adopted by the institution, which included: anesthetic risk classified by the American Society of Anesthesiologists (ASA) as ASA IV; esophagogastric varices with portal hypertension; significant intellectual limitations in patients without adequate family support; uncontrolled current psychiatric disorder, including alcohol and illicit drugs abuse.

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

The study population was analyzed before and 14, 28, 42 and 56 days after surgery. Additional details about blood sample collection, determination of hematologic, biochemical and erythrocyte stability variables are described in detail in previous publications (CUSTODIO AFONSO ROCHA; RAMOS DE ARVELOS; PEREIRA FELIX; NOGUEIRA PRADO DE SOUZA *et al.*, 2012; DE ARVELOS; ROCHA; FELIX; DA CUNHA *et al.*, 2013).

2.2 PRINCIPAL COMPONENT ANALYSIS (PCA)

The PCA is a method used when there are a very large number of variables. It facilitates the visualization of data and identification of association patterns. PCA is a type of multivariate statistics which enables condensation of the original variables information into new variables called principal components (PCs). These new variables explain the maximum variability of the data and are generated by specific linear combinations of the original variables.

This requires that each PC be orthogonal to the others and that all PCs do not show covariance between them.

PCs relate to the original variables without the variability of one PC to be associated with variability of the other PC, because there is no correlation between PCs, which eliminates the multicollinearity. This allows describing the original data, evaluating relations between the different variables by the covariance matrix of the loads (F) and identifying relationships between different observations by the grouping of data in the main components (LATTIN; CARROLL; GREEN, 2011).

If X is the matrix of original variables and Z is the matrix of the main components, then Z = XuD - 1/2 and F = uD 1/2, where u and D are respectively the autovectors and the matrix of the autovalues (λ) that are generated by solving the equation Ru = λu, where R is the matrix of correlations of X (DE LA HOZ; DE LA HOZ; ORTIZ; ORTEGA *et al.*, 2015; LATTIN; CARROLL; GREEN, 2011; SAAVEDRA; CÓRDOVA; GÁLVEZ; QUEZADA *et al.*, 2013).

PCA was applied as an unsupervised classification method, using Stats, Psych, Hmisc and FactoMiner routines in R language, with prcomp function, for analysis of the experimental points of the variables: 1) inverse of the NaCl concentration causing 50% hemolysis (invH50) and 2) variation in the NaCl concentration required to promote 100% hemolysis (dX), which are erythrocyte stability variables; 3) time; 4) body weight; 5) body mass index (BMI); 6) red blood cells (RBC); 7) red cell distribution width (RDW); 8) hemoglobin (Hb); 9) hematocrit (Ht); 10) mean corpuscular volume (MCV); 11) mean corpuscular hemoglobin (MCH); 12) mean corpuscular hemoglobin concentration (MCHC); 13) triglycerides (TG); 14) total (t-)C, 15) high density lipoprotein (HDL-C), 16) low density lipoprotein (LDL-C) and 17) very-low-density lipoprotein cholesterol (VLDL-C); and 18) glucose (Glu).

2.3 SUPPORT VECTOR MACHINE (SVM)

In essence, SVM is a tool to maximize a particular mathematical function of a set of data and must learn to tell the difference between two groups (NOBLE, 2006). This is done based on the theory of statistical learning and the principle of minimization of structural risk (ZHANG; CHEN; WANG; CHEN, 2015).

SVM is a learning technique that uses the statistical theory of the error, also known as theory of Vapnik-Chervonenkis, in the solution of classification problems, prediction of values and analysis of failures. It is an algorithm that learns by example to assign labels to objects. In the method of the SVM, the data group or input vectors, also called support vectors, are selected and used to obtain hyperplanes (or borders) capable of separating the remaining data.

This is done in such a way that for each group of separated data, the use of adjusted linear or non-linear functions is able to map and to correctly predict the class and values of new data from the same learning domain with the lowest possible error (KARATZOGLOU; MEYER; HORNIK, 2006; LIN; TSAI, 2014; WEI; LI; CHEN; WANG *et al.*, 2015).

In the SVM, the original data are mapped in a space of large dimension H, which is defined from a kernel function k that can be linear, polynomial, a Gaussian radial basis function (RBF) or sigmoid. The choice must be made in order to favor the acquisition of minor errors.

In this case $k(x,x') = [\Phi(x), \Phi(x')]$, with x' being the representation of data in the high-dimension space. The mapping can be described as $\Phi: X \rightarrow H$, where Φ represents the mapping, H is the high-dimension space and X is the original space. The case of the RFB, used in this study to be more robust than the others, $k(x,x') = \exp(\gamma|x-x'|^2)$, where γ represents the parameter of deviation from the core k(x,x') and that must be adjusted together with the parameter of regularization C, which represents a weighting between the training error and the error of generalization.

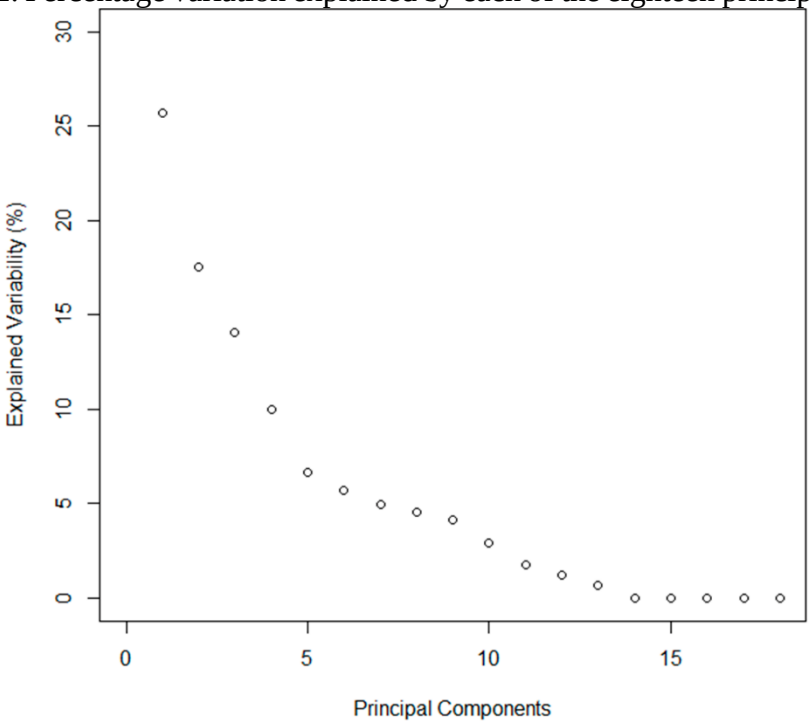
In obtaining the values of the parameter γ, the parameter of regularization C and the weights associated with the method, one can use the grid search method with k-fold validation. This method consists of exploring the adjustments of the values of the parameter γ and the parameter of regularization C throughout the sample space, which consists in different combinations of values of the selected parameters. The best result is obtained by the k-fold validation technique. In this technique, for each grid point, the experimental points used in the adjustment are further subdivided in mutually exclusive k subsets and, successively, each of the k subsets is used to evaluate the fit quality and obtaining the best model at the end of k data subdivision steps and successive improvement of adjustments (KARATZOGLOU; MEYER; HORNIK, 2006). In this study, the SVM models were obtained using 10-fold cross-validation with simple grid search to find the best fit model (DELEN; WALKER; KADAM, 2005).

The SVM was used in R language with e1071 and MASS routines (KARATZOGLOU; MEYER; HORNIK, 2006) and RBF kernel function to evaluate the membrane stability variables ($invH_{50}$ and dX) of human erythrocytes. The 120 experimental points of the membrane stability variables and the variables time, body weight, BMI, RBC, RDW, Hb, Ht, MCH, MCV, MCHC, TG, t-C, HDL-C, VLDL-C, LDL-C and Glu, have been used to obtain the descriptive models of membrane stability, to evaluate models and to generalize the behavior descriptions of the variables $invH_{50}$ and dX.

3 RESULTS AND DISCUSSION

In the PCA, 96.3% of the variability in the data (y axis) is explained by the ten first PCs (x-axis), as shown in Figure 1.

Figure 1. Percentage variation explained by each of the eighteen principal components.



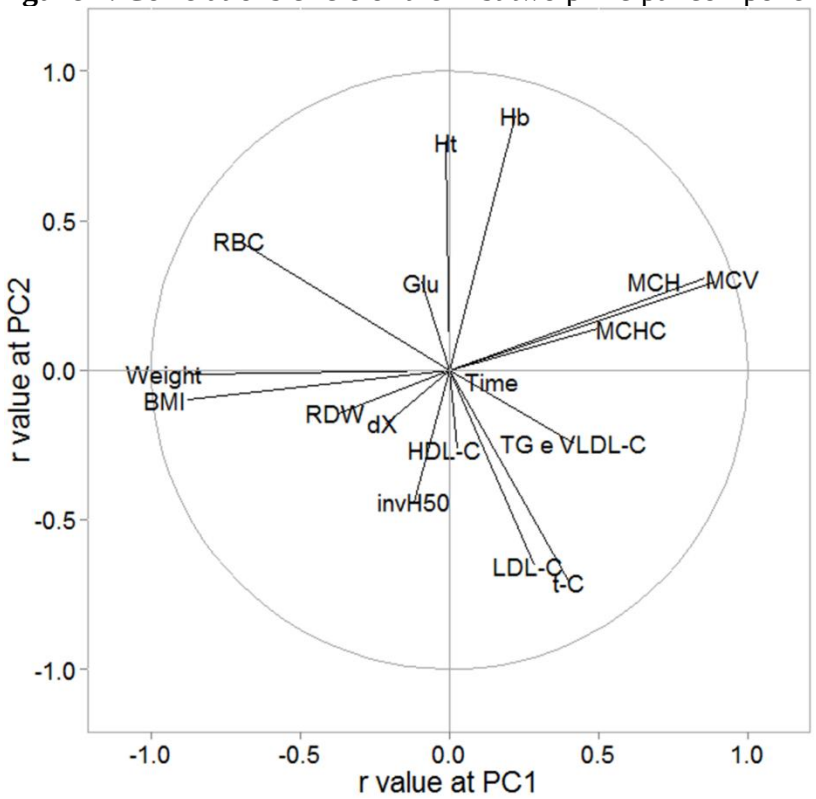
Source: Prepared by the authors.

As more than 95% of variability in the data of the 18 original variables is explained by the first ten components, this means that the original set of variables contains some variables that are conceptually associated with each other. This can be observed in the correlations circle of the first two PCs shown in Figure 2, where each axis shows the variation in the values of r (between -1 and +1) for each original variable.

The existence of such associations can be seen by the overlapping or close proximity between variables, such as occurs between the hematologic variables MCV and MCH, between the pairs of biochemical variables TG and VLDL-C (overlapping) and t-C and LDL-C (proximity), and between the anthropometric variables weight and BMI (proximity). These associations are caused by the existence of conceptual relations between those pairs of variables, since MCH = Hb/RBC and MCV = Ht/RBC (GEORGE-GAY; PARKER, 2003); t-C = HDL-C + LDL-C + VLDL-C; VLDL-C = TG/5 (FRIEDDEWALD; LEVY; FREDRICKSON, 1972); and BMI = weight/height^2 (GARROW; WEBSTER, 1985).

The correlation coefficients (r) of each of the original variables with the first ten principal components are shown in Table 1.

Figure 2. Correlations circle of the first two principal components (PC).



BMI, body mass index; $invH_50$, inverse of the NaCl concentration that causes 50% hemolysis; dX, variation in NaCl concentration necessary to promote 100% hemolysis; RBC, red blood cell; RDW, red cell distribution width; Hb, hemoglobin; Ht, hematocrit; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; TG, triglyceride; t-C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Glu, glucose. **Source:** Prepared by the authors.

Larger r values mean stronger and more relevant correlations with the respective main component, but not with the other, because one component does not influence the other, since they are orthogonal to each other. Smaller values of r mean weaker and less significant correlations with the main component, even when they are statistically significant ($p < 0.05$, two-way ANOVA). This study considered relevant the correlations associated with a value of $r > 0.5$ or < -0.5.

In the first principal component (PC1), which explains 25.7% of the data variability, the most significant positive correlations were obtained with the variables MCH, MCV and MCHC, while the most significant negative correlations were obtained with the variables BMI, body weight and RBC. As the first principal component is one that has the highest percentage of explained variability (Fig 1), it certainly presents the most significant changes related to the set of variables.

Indeed, what is most significant in the data for this study is the dramatic decrease in weight and, consequently, in the BMI, which is the goal of the surgical intervention.

Table 1. Values of r for the correlations of the first ten principal components with the original data

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
AEV (%)	25.7	43.2	57.3	67.3	74.0	79.7	84.7	89.2	93.4	96.3
Time	0.00	-0.04	0.10	0.01	-0.43	-0.77*	0.15	0.40*	0.15	-0.05
Weight	-0.86*	-0.01	-0.17	0.19*	0.02	0.00	0.22*	-0.06	-0.02	-0.03
BMI	-0.88*	-0.10	-0.15	-0.05	-0.14	0.07	0.14	-0.05	-0.12	0.11
invH50	-0.12	-0.44*	0.36*	-0.02	-0.42*	-0.20*	-0.08	-0.56*	0.11	0.24*
dX	-0.21	-0.18	0.34*	0.12	-0.55*	0.17	-0.18	0.13	-0.62*	-0.16
RBC	-0.70*	0.43*	0.07	-0.49*	-0.04	0.01	0.05	-0.07	0.09	-0.22*
RDW	-0.37*	-0.14	-0.64*	-0.14	-0.30*	0.01	-0.37*	-0.19*	0.16	0.07
Hb	0.22*	0.85*	0.10	-0.32*	-0.24*	0.06	0.07	-0.16	0.05	-0.15
Ht	-0.01	0.76*	0.22*	-0.58*	-0.15	0.06	0.04	-0.02	0.00	0.05
MCH	0.89*	0.30*	0.02	0.21	-0.17	0.04	0.02	-0.09	-0.03	0.06
MCV	0.86*	0.31*	0.15	-0.03	-0.11	0.05	-0.01	0.04	-0.10	0.30*
MCHC	0.50*	0.14	-0.27*	0.57*	-0.18	-0.02	0.07	-0.31*	0.11	-0.43*
TG	0.42*	-0.25*	-0.71*	-0.41*	0.03	-0.14	0.11	-0.07	-0.23*	-0.03
tC	0.40*	-0.71*	0.24*	-0.47*	0.00	0.08	-0.02	0.01	0.08	-0.17
HDL-C	0.03	-0.26*	0.62*	-0.12	0.29*	-0.22*	0.42*	-0.33*	-0.21*	-0.05
VLDL-C	0.42*	-0.25*	-0.71*	-0.41*	0.03	-0.14	0.11	-0.07	-0.23*	-0.03
LDL-C	0.29*	-0.65*	0.40*	-0.34*	-0.09	0.21*	-0.18	0.13	0.24*	-0.17
Glu	-0.09	0.29*	0.22*	-0.04	0.42*	-0.45*	-0.62*	-0.13	-0.19*	-0.10

PC (1-10), Principal Component; AEV, accumulated explained variability; BMI, body mass index; $invH_50$, inverse of the NaCl concentration that causes 50% hemolysis; dX, variation in NaCl concentration necessary to promote 100% hemolysis; RBC, red blood cell; RDW, red cell distribution width; Hb, hemoglobin; Ht, hematocrit; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; TG, triglyceride; t-C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Glu, glucose. *p < 0.05 (two-way ANOVA) indicates statistically significant correlations. **Source:** Prepared by the authors.

However, associated with this desirable change there is a significant tendency of decrease in the RBC count, certainly due to the mechanism that links this trend to the increase observed in the MCV variable. Indeed, individuals who underwent bariatric surgery tend to have deficiencies of the vitamin cobalamin, which is a known cause of elevation in MCV and generation of the called macrocytic anemia (ALVES DE REZENDE; COELHO; OLIVEIRA; PENHA SILVA, 2009). Deficiency of this vitamin causes a decrease in the rate of division of the precursors of mature red blood cells, releasing into the bloodstream cells (reticulocytes) with greater volume and with greater concentration of hemoglobin, which is certainly the origin of the trend of increase in the values of MCV and MCHC associated with decreased body weight.

In the second principal component (PC2), which explains 17.5% of the data variability, significant positive correlations were obtained with Hb and Ht, while significant negative correlations were obtained with t-C and LDL-C. As one PC does not interfere with other, these

correlations have meanings that are independent of the associations with body weight changes revealed by the analysis of the first PC. Analysis of this second PC reveals that in the studied population the lower levels of tC and LDL-C were associated with higher values of Hb and Ht. This makes sense, since LDL is involved in the transfer of cholesterol to the erythrocyte membrane. High levels of LDL-C are associated with transference of such a so large amount of cholesterol to the membrane of this cell that it will suffer the morphological changes that characterize the so-called spur-cell anemia (COOPER, 1969; COOPER; ARNER; WILEY; SHATTIL, 1975; COOPER; LESLIE; KNIGHT; DETWEILER, 1980).

In the case of the third principal component (PC3), which explains 14.1% of the data variability, the variables that stood out were TG, VLDL-C and RDW, with negative correlations, and HDL-C, with a positive correlation. These correlations shall mean that in the studied population lower levels of TG and VLDL-C and increased HDL-C levels are associated with lower values of RDW. Since high values of RDW were associated with higher cholesterol contents in the erythrocyte membrane (TZIAKAS; CHALIKIAS; GRAPSA; GIOKA *et al.*, 2012) and HDL is a lipoprotein that removes excess of cholesterol from the membrane of that cell, it makes sense that an increase in HDL-C is associated with a decrease in RDW. The fourth principal component (PC4), which explains 10% of the data variability, showed a significant positive correlation with MCHC and negative correlations with Ht and RBC, which has an obvious meaning, because higher MCHC and lower RBC counts are recognizably associated with lower values of Ht.

The PCs of numbers 5 to 9 do not present significant correlations with more than a single variable and the PC10 did not show even a single significant correlation (Table 1).

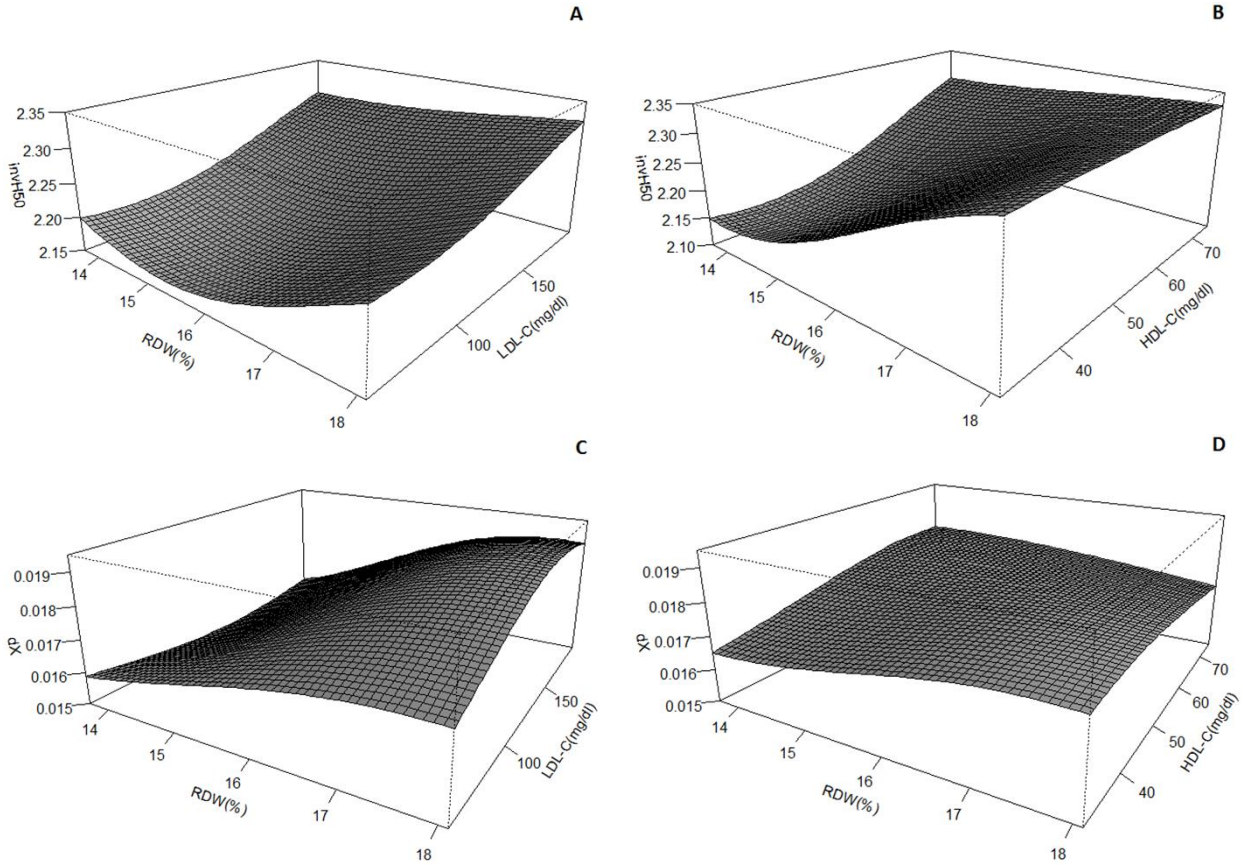
The existence of weak correlations, but statistically significant (Table 1) may be a result of less importance, greater heterogeneity of the data, or even inability of the model to detect relationships that escape most expressively from linearity.

Indeed, analysis using SVM reveal the existence of non-linearity between some variables evaluated in this study. Figure 3 shows three-dimensional graphics of some combinations of invH50, dX, LDL-C, HDL-C and RDW, considering all other variables with fixed values described by their respective averages. The x-axis for all graphs is variable RDW, the y axis represents erythrocyte stability variables (invH50 or dX) and the z axis is the lipid variable (LDL-C or HDL-C).

This figure illustrates that the membrane stability variables invH50 (Figs 3A and 3B) and dX (Figs 3C and 3D) exhibit non-linear relations with RDW, influenced in an antagonistic manner by the cholesterol concentrations of LDL (Figs 3A and 3C) and HDL (Figs 3B and

3D). This analysis based on the use of SVM shows that the correlations between those stability variables and RDW are not linear, but predominantly inverse. This is perfectly consistent with the non-relevant but significant inverse correlations observed between those stability variables and RDW in the third principal component analysis (Table 1). This inverse correlation between erythrocytes stability and RDW is also consistent with other studies reported in the literature (DE FREITAS; MARQUEZ-BERNARDES; DE ARVELOS; PARAISO *et al.*, 2014; NETO; DE AVELAR; ARANTES; JORDAO *et al.*, 2013).

Figure 3. Influence of the levels of LDL- and HDL-cholesterol on the relations between the variables of erythrocyte membrane stability (invH50 and dX) and the hematologic variable red cell distribution width (RDW), according to analyses done with use of Support Vector Machine (SVM) (r^2> 0.72).



Source: Prepared by the authors.

Furthermore, this certainly makes sense in light of the fact that LDL is capable of transferring cholesterol to the erythrocyte membrane (EJIMA; IJICHI; OHNISHI; MARUYAMA *et al.*, 2000; MEURS; HOEKSTRA; VAN WANROOIJ; HILDEBRAND *et al.*, 2005) and that high RDW values are associated with high contents of membrane cholesterol in these cells (TZIAKAS; CHALIKIAS; GRAPSA; GIOKA *et al.*, 2012), probably due to

impaired deformability of red blood cells (VAYA; RIVERA; DE LA ESPRIELLA; SANCHEZ *et al.*, 2015).

On the other hand, the antagonistic influences of LDL-C and HDL-C on the correlations of invH50 and dX with RDW also make a lot of sense, because these lipoproteins have antagonistic actions on the erythrocyte membrane, with LDL providing and HDL removing cholesterol.

The content of cholesterol in erythrocyte membrane is essential for the maintenance of fluidity and various rheological properties, such as aggregability, deformability and adhesiveness (ARBUSTINI, 2007; KOLODGIE; BURKE; NAKAZAWA; CHENG *et al.*, 2007). An increase in membrane cholesterol content occurs in hypercholesterolemic conditions (COOPER; ARNER; WILEY; SHATTIL, 1975; SCHICK; SCHICK, 1985), in which part of the cholesterol excess of LDL is directed to the erythrocyte membrane (MARTINEZ; VAYA; MARTI; GIL *et al.*, 1996), reducing its fluidity (COOPER, R.A., 1977; DWIGHT; MENDES RIBEIRO; HENDRY, 1996; MULLER; ZIEGLER; DONNER; DROUIN *et al.*, 1990), increasing blood viscosity and hindering the delivery of oxygen to tissues (CHABANEL; FLAMM; SUNG; LEE *et al.*, 1983; KOTER; BRONCEL; CHOJNOWSKA-JEZIERSKA; KLIKCZYNSKA *et al.*, 2002). That is why the influence of plasma lipids in the blood rheology has been the subject of many studies (ALOULOU; VARLET-MARIE; MERCIER; BRUN, 2006; HERNANDEZ; BOLLINI; MENGARELLI; RASIA *et al.*, 2015; IRACE; CARALLO; SCAVELLI; ESPOSITO *et al.*, 2014; MARTINEZ; VAYA; MARTI; GIL *et al.*, 1996; SEKI; SUMINO; NARA; ISHIYAMA *et al.*, 2006; VAYA; MARTINEZ TRIGUERO; RICART; PLUME *et al.*, 2009; VELCHEVA; ANTONOVA; DIMITROVA; DIMITROV *et al.*, 2006).

4 CONCLUSIONS

The principal component analysis revealed that 1) the weight loss of the study volunteers was associated with a decrease in red blood cell counts and increase in the hematologic indices MCV, MCH and MCHC; 2) lower levels of t-C and LDL-C were associated with higher values of Hb and Ht; 3) lower levels of TG and VLDL-C and increased HDL-C levels were associated with lower values of RDW. Analyses based on support vector machine showed that the erythrocyte membrane stability variables (invH50 and dX) present nonlinear relations with RDW, under the antagonistic influence of the LDL-C levels in relation to HDL-C concentrations. This finding suggests these stability variables may also have the ability to predict the health status worsening that was originally attributed to higher values of RDW.

AKNOWLEDGMENTS

We would like to thank CAPES (PE-PNPD AUX 2718/2011) and CNPq (307705/2012-9) for the financial supports that have enable the development of this study.

REFERENCES

ALOULOU, I.; VARLET-MARIE, E.; MERCIER, J.; BRUN, J. F. Hemorheological disturbances correlate with the lipid profile but not with the NCEP-ATPIII score of the metabolic syndrome. **Clin Hemorheol Microcirc**, 35, n. 1-2, p. 207-212, 2006.

ALVES DE REZENDE, C. H.; COELHO, L. M.; OLIVEIRA, L. M.; PENHA SILVA, N. Dependence of the geriatric depression scores on age, nutritional status, and haematologic variables in elderly institutionalized patients. **J Nutr Health Aging**, 13, n. 7, p. 617-621, Aug 2009. doi: https://doi.org/10.1007/s12603-009-0172-0.

ARBUSTINI, E. Total erythrocyte membrane cholesterol: an innocent new marker or an active player in acute coronary syndromes? **J Am Coll Cardiol**, 49, n. 21, p. 2090-2092, May 29 2007. doi: https://doi.org/10.1016/j.jacc.2007.03.014.

BHAKAT, S.; MARTIN, A. J.; SOLIMAN, M. E. An integrated molecular dynamics, principal component analysis and residue interaction network approach reveals the impact of M184V mutation on HIV reverse transcriptase resistance to lamivudine. **Mol Biosyst**, 10, n. 8, p. 2215-2228, Aug 2014. doi: https://doi.org/10.1039/C4MB00253A.

CABALLERO, J.; FERNANDEZ, L.; GARRIGA, M.; ABREU, J. I. *et al.* Proteometric study of ghrelin receptor function variations upon mutations using amino acid sequence autocorrelation vectors and genetic algorithm-based least square support vector machines. **J Mol Graph Model**, 26, n. 1, p. 166-178, Jul 2007. doi: https://doi.org/10.1016/j.jmgm.2006.11.002.

CHABANEL, A.; FLAMM, M.; SUNG, K. L.; LEE, M. M. *et al.* Influence of cholesterol content on red cell membrane viscoelasticity and fluidity. **Biophys J**, 44, n. 2, p. 171-176, Nov 1983. doi: https://doi.org/10.1016/S0006-3495(83)84288-X.

COOPER, R. A. Anemia with spur cells: a red cell defect acquired in serum and modified in the circulation. **J Clin Invest**, 48, n. 10, p. 1820-1831, Oct 1969. doi: https://doi.org/10.1172/JCI106148.

COOPER, R. A. Abnormalities of cell-membrane fluidity in the pathogenesis of disease. **N Engl J Med**, 297, n. 7, p. 371-377, Aug 18 1977. doi: https://doi.org/10.1056/NEJM197708182970707.

COOPER, R. A. Abnormalities of cell-membrane fluidity in the pathogenesis of disease. . **Seminars in Medicine of the Beth Israel Hospital**, 297, n. 7, p. 371-377, 1977. doi: https://doi.org/10.1056/NEJM197708182970707.

COOPER, R. A.; ARNER, E. C.; WILEY, J. S.; SHATTIL, S. J. Modification of Red-Cell Membrane Structure by Cholesterol-Rich Lipid Dispersions - Model for Primary Spur Cell

Defect. **Journal of Clinical Investigation**, 55, n. 1, p. 115-126, 1975. doi: https://doi.org/10.1172/JCI107901.

COOPER, R. A.; LESLIE, M. H.; KNIGHT, D.; DETWEILER, D. K. Red cell cholesterol enrichment and spur cell anemia in dogs fed a cholesterol-enriched atherogenic diet. **J Lipid Res**, 21, n. 8, p. 1082-1089, Nov 1980. doi: https://doi.org/10.1016/S0022-2275(20)34769-6.

CUSTODIO AFONSO ROCHA, V.; RAMOS DE ARVELOS, L.; PEREIRA FELIX, G.; NOGUEIRA PRADO DE SOUZA, D. *et al.* Evolution of nutritional, hematologic and biochemical changes in obese women during 8 weeks after Roux-en-Y gastric bypasss. **Nutr Hosp**, 27, n. 4, p. 1134-1140, Jul-Aug 2012. Available from: http://www.nutricionhospitalaria.com/pdf/5812.pdf.

DE ARVELOS, L. R.; ROCHA, V. C.; FELIX, G. P.; DA CUNHA, C. C. *et al.* 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. **J Membr Biol**, 246, n. 3, p. 231-242, Mar 2013. doi: https://doi.org/10.1007/s00232-013-9524-0.

DE FREITAS, M. V.; MARQUEZ-BERNARDES, L. F.; DE ARVELOS, L. R.; PARAISO, L. F. *et al.* Influence of age on the correlations of hematological and biochemical variables with the stability of erythrocyte membrane in relation to sodium dodecyl sulfate. **Hematology**, 19, n. 7, p. 424-430, Oct 2014. doi: https://doi.org/10.1179/1607845413Y.0000000145.

DE LA HOZ, E.; DE LA HOZ, E.; ORTIZ, A.; ORTEGA, J. *et al.* PCA filtering and probabilistic SOM for network intrusion detection. **Neurocomputing**, 164, p. 71-81, 2015. doi: https://doi.org/10.1016/j.neucom.2014.09.083.

DELEN, D.; WALKER, G.; KADAM, A. Predicting breast cancer survivability: a comparison of three data mining methods. **Artificial Intelligence in Medicine**, 34, n. 2, p. 113-127, Jun 2005. doi: https://doi.org/10.1016/j.artmed.2004.07.002.

DWIGHT, J. F.; MENDES RIBEIRO, A. C.; HENDRY, B. M. Effects of HMG-CoA reductase inhibition on erythrocyte membrane cholesterol and acyl chain composition. **Clin Chim Acta**, 256, n. 1, p. 53-63, Dec 9 1996. doi: https://doi.org/10.1016/S0009-8981(96)06412-1.

EJIMA, J.; IJICHI, T.; OHNISHI, Y.; MARUYAMA, T. *et al.* Relationship of high-density lipoprotein cholesterol and red blood cell filterability: cross-sectional study of healthy subjects. **Clin Hemorheol Microcirc**, 22, n. 1, p. 1-7, 2000.

FRIEDDEWALD, W. T.; LEVY, R. I.; FREDRICKSON, D. S. Estimation of the concentration of low-density lipoproteins cholesterol in plasma, without use of the preparative ultracentrifuge. **Clinical Chemistry**, 18, n. 6, p. 499-502, 1972. doi: https://doi.org/10.1093/clinchem/18.6.499.

GARROW, J. S.; WEBSTER, J. Quetelet's index (W/H2): as a measure of fatness. **Int J Obesity**, 9, p. 147-153, 1985.

GEORGE-GAY, B.; PARKER, K. Understanding the complete blood count with differential. **J Perianesth Nurs**, 18, n. 2, p. 96-114; quiz 115-117, Apr 2003. doi: https://doi.org/10.1053/jpan.2003.50013.

GERNS STOREY, H. L.; RICHARDSON, B. A.; SINGA, B.; NAULIKHA, J. *et al.* Use of principal components analysis and protein microarray to explore the association of HIV-1-specific IgG responses with disease progression. **AIDS Res Hum Retroviruses**, 30, n. 1, p. 37-44, Jan 2014. doi: https://doi.org/10.1089/aid.2013.0088.

GOSTEK, J.; AWSIUK, K.; PABIJAN, J.; RYSZ, J. *et al.* Differentiation between single bladder cancer cells using principal component analysis of time-of-flight secondary ion mass spectrometry. **Anal Chem**, 87, n. 6, p. 3195-3201, Mar 17 2015. doi: https://doi.org/10.1021/ac504684n.

GUYON, I.; WESTON, J.; BARNHILL, S.; VAPNIK, V. Gene selection for cancer classification using support vector machines. **Machine Learning**, 46, n. 1-3, p. 389-422, 2002. doi: https://doi.org/10.1023/A:1012487302797.

HE, L. N.; LIU, Y. J.; XIAO, P.; ZHANG, L. *et al.* Genomewide linkage scan for combined obesity phenotypes using principal component analysis. **Ann Hum Genet**, 72, n. Pt 3, p. 319-326, May 2008. doi: https://doi.org/10.1111/j.1469-1809.2007.00423.x.

HERNANDEZ, G.; BOLLINI, A.; MENGARELLI, G.; RASIA, M. *et al.* Protective effect of quercetin against in vitro erythrocyte rheology alterations produced by arsenic. **Clin Hemorheol Microcirc**, 59, n. 4, p. 355-364, May 11 2015. doi: https://doi.org/10.3233/CH-141849.

HUNZIKER, S.; STEVENS, J.; HOWELL, M. D. Red cell distribution width and mortality in newly hospitalized patients. **Am J Med**, 125, n. 3, p. 283-291, Mar 2012. doi: https://doi.org/10.1016/j.amjmed.2011.08.021.

IRACE, C.; CARALLO, C.; SCAVELLI, F.; ESPOSITO, T. *et al.* Influence of blood lipids on plasma and blood viscosity. **Clin Hemorheol Microcirc**, 57, n. 3, p. 267-274, 2014. doi: https://doi.org/10.3233/CH-131705.

KARATZOGLOU, A.; MEYER, D.; HORNIK, K. Support Vector Machines in R. **Journal of Statistical Software**, 15, n. 9, Apr 2006. doi: https://doi.org/10.18637/jss.v015.i09.

KOGAI, M. A.; LUTOV, U. V.; SELYATITSKAYA, V. G. Hormonal and biochemical parameters of metabolic syndrome in male patients with body weight excess and obesity. **Bull Exp Biol Med**, 146, n. 6, p. 806-808, Dec 2008. doi: https://doi.org/10.1007/s10517-009-0415-4.

KOLODGIE, F. D.; BURKE, A. P.; NAKAZAWA, G.; CHENG, Q. *et al.* Free cholesterol in atherosclerotic plaques: where does it come from? **Curr Opin Lipidol**, 18, n. 5, p. 500-507, Oct 2007. doi: https://doi.org/10.1097/MOL.0b013e3282efa35b.

KOTER, M.; BRONCEL, M.; CHOJNOWSKA-JEZIERSKA, J.; KLIKCZYNSKA, K. *et al.* The effect of atorvastatin on erythrocyte membranes and serum lipids in patients with type-2

hypercholesterolemia. **Eur J Clin Pharmacol**, 58, n. 8, p. 501-506, Nov 2002. doi: https://doi.org/10.1007/s00228-002-0507-9

LATTIN, J.; CARROLL, J. D.; GREEN, P. E. Análise de componentes principais. *In*: **Análise de dados multivaridados**. São Paulo: Cengage Learning, 2011. cap. 4, p. 67-101.

LIN, T. H.; TSAI, T. L. Constructing a linear QSAR for some metabolizable drugs by human or pig flavin-containing monooxygenases using some molecular features selected by a genetic algorithm trained SVM. **J Theor Biol**, 356, p. 85-97, Sep 7 2014. doi: https://doi.org/10.1016/j.jtbi.2014.04.021

LIPPI, G.; SANCHIS-GOMAR, F.; DANESE, E.; MONTAGNANA, M. Association of red blood cell distribution width with plasma lipids in a general population of unselected outpatients. **Kardiol Pol**, 71, n. 9, p. 931-936, 2013. doi: https://doi.org/10.5603/KP.2013.0228.

LOPEZ-MEYER, P.; SCHUCKERS, S.; MAKEYEV, O.; SAZONOV, E. Detection of Periods of Food Intake using Support Vector Machines. **2010 Annual International Conference of the Ieee Engineering in Medicine and Biology Society (Embc)**, p. 1004-1007, 2010. doi: https://doi.org/10.1109/IEMBS.2010.5627796.

MARTINEZ, M.; VAYA, A.; MARTI, R.; GIL, L. *et al.* Erythrocyte membrane cholesterol/phospholipid changes and hemorheological modifications in familial hypercholesterolemia treated with lovastatin. **Thromb Res**, 83, n. 5, p. 375-388, Sep 1 1996. doi: https://doi.org/10.1016/0049-3848(96)00147-8.

MEURS, I.; HOEKSTRA, M.; VAN WANROOIJ, E. J.; HILDEBRAND, R. B. *et al.* HDL cholesterol levels are an important factor for determining the lifespan of erythrocytes. **Exp Hematol**, 33, n. 11, p. 1309-1319, Nov 2005. doi: https://doi.org/10.1016/j.exphem.2005.07.004.

MISRA, A.; KHURANA, L. Obesity and the metabolic syndrome in developing countries. **J Clin Endocrinol Metab**, 93, n. 11 Suppl 1, p. S9-30, Nov 2008. doi: https://doi.org/10.1210/jc.2008-1595.

MONTAGNANA, M.; CERVELLIN, G.; MESCHI, T.; LIPPI, G. The role of red blood cell distribution width in cardiovascular and thrombotic disorders. **Clin Chem Lab Med**, 50, n. 4, p. 635-641, Apr 2012. doi: https://doi.org/10.1515/cclm.2011.831

MULLER, S.; ZIEGLER, O.; DONNER, M.; DROUIN, P. *et al.* Rheological properties and membrane fluidity of red blood cells and platelets in primary hyperlipoproteinemia. **Atherosclerosis**, 83, n. 2-3, p. 231-237, Aug 1990. doi: https://doi.org/10.1016/0021-9150(90)90168-I

NETO, M. B.; DE AVELAR, E. B.; ARANTES, T. S.; JORDAO, I. A. *et al.* Bivariate and multivariate analyses of the correlations between stability of the erythrocyte membrane, serum lipids and hematological variables. **Biorheology**, 50, n. 5-6, p. 305-320, 2013. doi: https://doi.org/10.3233/BIR-130641.

NETTO, R. D. M.; FABBRI, C.; DE FREITAS, M. V.; NETO, M. B. *et al.* Influence of Plasmodium vivax malaria on the relations between the osmotic stability of human erythrocyte membrane and hematological and biochemical variables. **Parasitology Research**, 113, n. 3, p. 863-874, Mar 2014. doi: https://doi.org/10.1007/s00436-013-3717-4.

NOBLE, W. S. What is a support vector machine? **Nature Biotechnology**, 24, n. 12, p. 1565-1567, Dec 2006. doi: https://doi.org/10.1038/nbt1206-1565.

PANAZZOLO, D. G.; SICURO, F. L.; CLAPAUCH, R.; MARANHAO, P. A. *et al.* Obesity, metabolic syndrome, impaired fasting glucose, and microvascular dysfunction: a principal component analysis approach. **BMC Cardiovasc Disord**, 12, p. 102, 2012. doi: https://doi.org/10.1186/1471-2261-12-102.

PARK, J. S.; CHOI, S. B.; CHUNG, J. W.; KIM, S. W. *et al.* Classification of Serous Ovarian Tumors Based on Microarray Data Using Multicategory Support Vector Machines. **2014 36th Annual International Conference of the Ieee Engineering in Medicine and Biology Society (Embc)**, p. 3430-3433, 2014. doi: 10.1109/EMBC.2014.6944360.

PENHA-SILVA, N.; ARVELOS, L. R.; CUNHA, C. C.; AVERSI-FERREIRA, T. A. *et al.* Effects of glycerol and sorbitol on the thermal dependence of the lysis of human erythrocytes by ethanol. **Bioelectrochemistry**, 73, n. 1, p. 23-29, Jun 2008. doi: https://doi.org/10.1016/j.bioelechem.2008.04.002.

PENHA-SILVA, N.; FIRMINO, C. B.; DE FREITAS REIS, F. G.; DA COSTA HUSS, J. C. *et al.* Influence of age on the stability of human erythrocyte membranes. **Mech Ageing Dev**, 128, n. 7-8, p. 444-449, Jul-Aug 2007. doi: https://doi.org/10.1016/j.mad.2007.06.007.

QIAN, S.; RAI, D.; HELLER, W. T. Alamethicin disrupts the cholesterol distribution in dimyristoyl phosphatidylcholine-cholesterol lipid bilayers. **J Phys Chem B**, 118, n. 38, p. 11200-11208, Sep 25 2014. doi: https://doi.org/10.1021/jp504886u.

RODUIT, C.; VAN DER GOOT, F. G.; DE LOS RIOS, P.; YERSIN, A. *et al.* Elastic membrane heterogeneity of living cells revealed by stiff nanoscale membrane domains. **Biophys J**, 94, n. 4, p. 1521-1532, Feb 15 2008. doi: https://doi.org/10.1529/biophysj.107.112862.

SAAVEDRA, J.; CÓRDOVA, A.; GÁLVEZ, L.; QUEZADA, C. *et al.* Principal Component Analysis as an exploration tool for kinetic modeling of food quality: A case study of a dried apple cluster snack. **Journal of Food Engineering**, 119, n. 2, p. 229-235, 2013. doi: https://doi.org/10.1016/j.jfoodeng.2013.05.036.

SCHICK, B. P.; SCHICK, P. K. Cholesterol exchange in platelets, erythrocytes and megakaryocytes. **Biochim Biophys Acta**, 833, n. 2, p. 281-290, Feb 8 1985. doi: https://doi.org/10.1016/0005-2760(85)90200-0.

SEKI, K.; SUMINO, H.; NARA, M.; ISHIYAMA, N. *et al.* Relationships between blood rheology and age, body mass index, blood cell count, fibrinogen, and lipids in healthy subjects. **Clinical Hemorheology and Microcirculation**, 34, n. 3, p. 401-410, 2006.

TZIAKAS, D.; CHALIKIAS, G.; GRAPSA, A.; GIOKA, T. *et al.* Red blood cell distribution width: a strong prognostic marker in cardiovascular disease: is associated with cholesterol content of erythrocyte membrane. **Clin Hemorheol Microcirc**, 51, n. 4, p. 243-254, 2012. doi: https://doi.org/10.3233/CH-2012-1530

VAYA, A.; MARTINEZ TRIGUERO, M.; RICART, A.; PLUME, G. *et al.* Erythrocyte aggregability and AB0 blood groups. **Clin Hemorheol Microcirc**, 41, n. 1, p. 67-72, 2009. doi: https://doi.org/10.3233/CH-2009-1163.

VAYA, A.; RIVERA, L.; DE LA ESPRIELLA, R.; SANCHEZ, F. *et al.* Red blood cell distribution width and erythrocyte deformability in patients with acute myocardial infarction. **Clin Hemorheol Microcirc**, 59, n. 2, p. 107-114, 2015. doi: https://doi.org/10.3233/CH-131751.

VELCHEVA, I.; ANTONOVA, N.; DIMITROVA, V.; DIMITROV, N. *et al.* Plasma lipids and blood viscosity in patients with cerebrovascular disease. **Clin Hemorheol Microcirc**, 35, n. 1-2, p. 155-157, 2006.

WEI, Y.; LI, J.; CHEN, Z.; WANG, F. *et al.* Multistage virtual screening and identification of novel HIV-1 protease inhibitors by integrating SVM, shape, pharmacophore and docking methods. **Eur J Med Chem**, 101, p. 409-418, Aug 28 2015. doi: https://doi.org/10.1016/j.ejmech.2015.06.054.

WHO. **BMI Classification**. 2005. Available from: http://www.assessmentpsychology.com/icbmi.htm.

ZHANG, X. L.; CHEN, W.; WANG, B. J.; CHEN, X. F. Intelligent fault diagnosis of rotating machinery using support vector machine with ant colony algorithm for synchronous feature selection and parameter optimization. **Neurocomputing**, 167, p. 260-279, Nov 1 2015. doi: https://doi.org/10.1016/j.neucom.2015.04.069.