

**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***

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**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 ( $36,46 \pm 9,8$  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 triglicérides (*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.

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**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 (SVM). **Methods:** A population of 24 obese women ( $36.46 \pm 9.8$  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  $\text{inv}H_{50}$ ) in order to both constants ( $dX$  and  $\text{inv}H_{50}$ ) 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; GERNIS 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<sup>2</sup>) and comorbidities and 16 women with class III obesity (BMI  $\geq 40.00/m^2$ ) (WHO, 2005), with average age of  $36.46 \pm 9.8$  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 ( $\lambda$ ) that are generated by solving the equation  $Ru = \lambda u$ , 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  $\Phi$  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  $\gamma$  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.

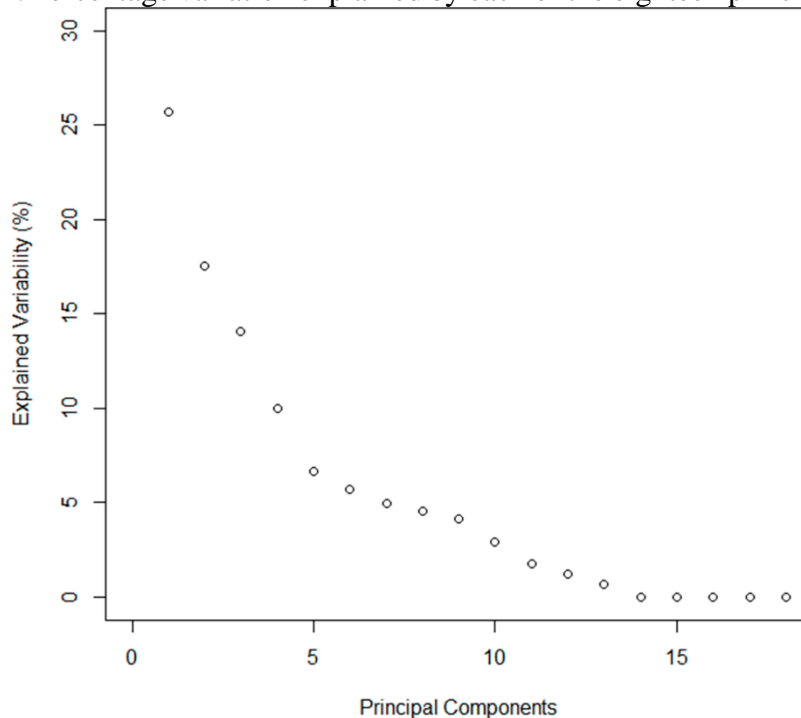
In obtaining the values of the parameter  $\gamma$ , the parameter of regularization  $C$  and the weights associated with the method, one can use the grid search method with  $k$ -fold validation. This method consists of exploring the adjustments of the values of the parameter  $\gamma$  and the parameter 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 ( $\text{invH}_{50}$  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  $\text{invH}_{50}$  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.

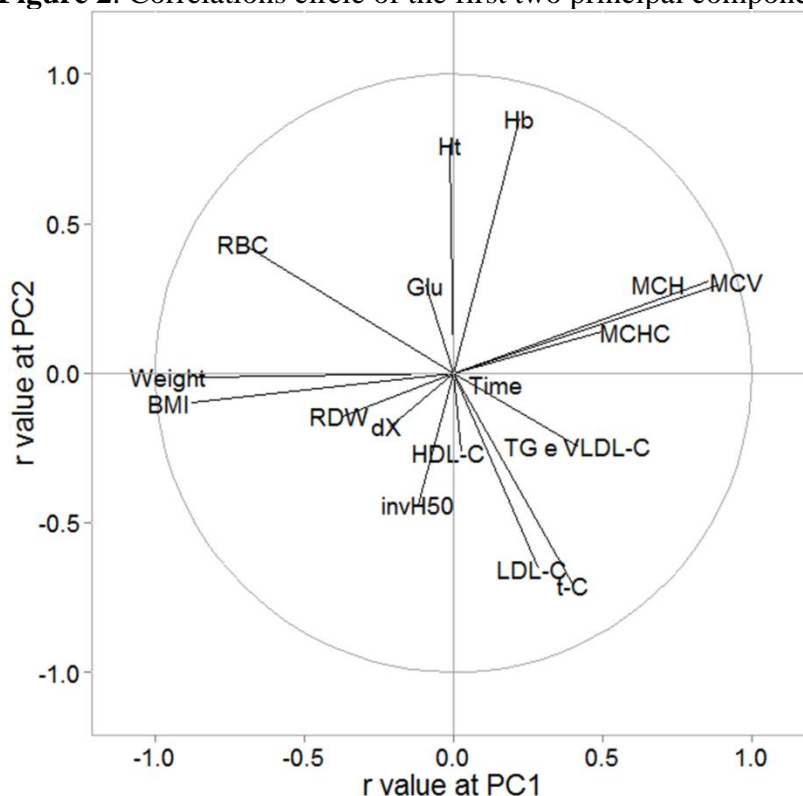


**Source:** Prepared by the authors.

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<sub>50</sub>, 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<sub>50</sub>, 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).

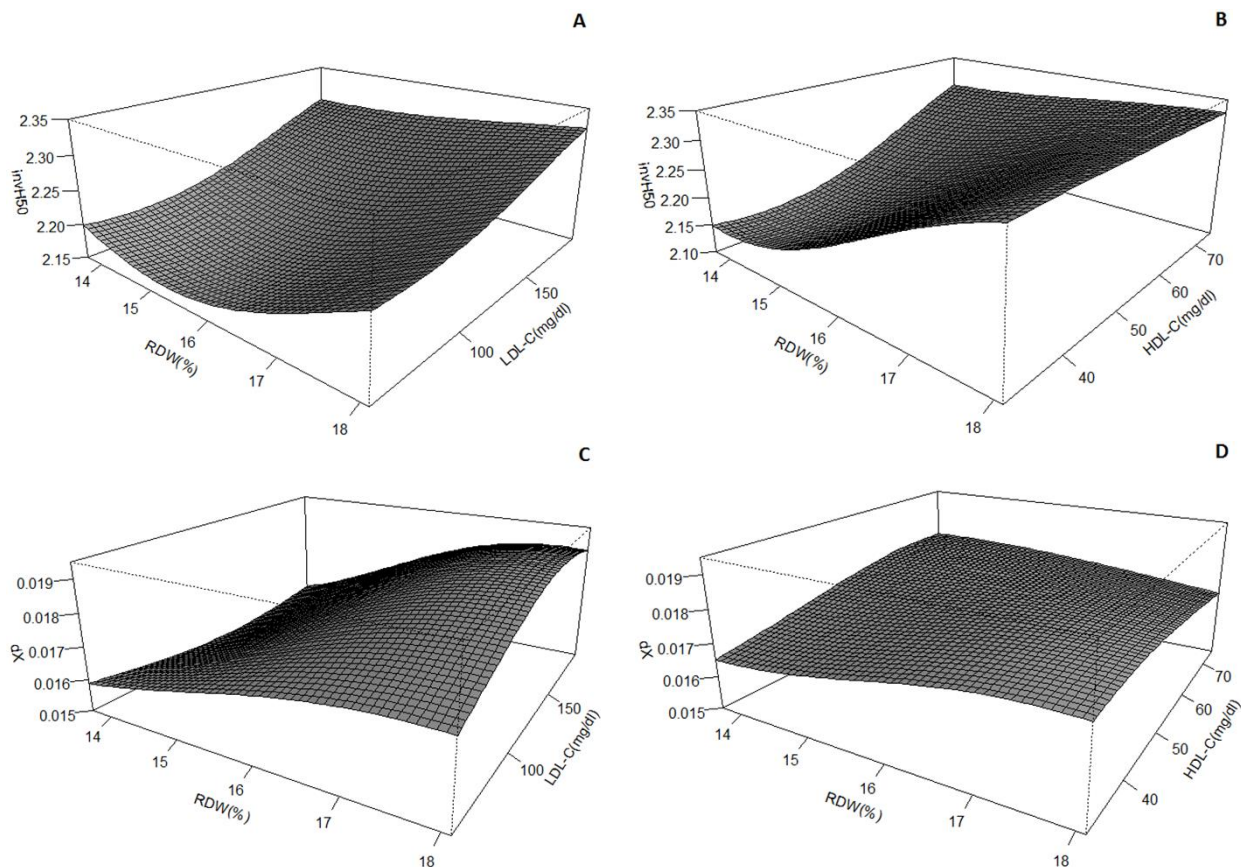
The existence of weak correlations, but statistically significant (Table 1) may be a result of less importance, greater heterogeneity of the data, or even inability of the model to detect relationships that escape most expressively from linearity.

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; KOLODZIE; 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; KLIKCYNSKA *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.

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