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Article

Hypertriglyceridemia promotes dysfunctions in high-density lipoprotein increasing the cardiovascular risk

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Hypertriglyceridemia is associated with several metabolic diseases. The triglycerides (TG) disrupt the cholesterol reverse transport and contribute to increased levels of low-density lipoprotein (LDL). High-density lipoprotein (HDL) acts in cholesterol reverse transport as an anti-inflammatory and antioxidant. This study aims to investigate the role of hypertriglyceridemia in the functionality of HDL. Individuals were divided into 4 groups based on high or low HDL-c and triglycerides levels. Biochemical and anthropometric analysis were performed. This study demonstrated that triglycerides promote dysfunctions on HDL, increasing the cardiovascular risk. Blood pressure was higher in subjects with low HDL. Women presented higher levels of HDL-c and low percentage of fat mass. The highest levels of triglycerides were observed in older age. In addition, high levels of triglycerides were associated with higher total cholesterol and LDL-c levels, non-HDL-c, non-esterified fatty acids, and blood glucose, increasing in the ratio of non-HDL-c/HDL-c and ApoB/ApoA-I. The increase of triglycerides levels progressively impairs the antioxidant capacity of HDL, probably due to a higher occurrence of fatty acid peroxidation in individuals with hypertriglyceridemia. Patients with high HDL and low TG levels increased the Lag Time. Furthermore, a positive correlation was found between TG versus HDL particle size, variables that depend on age and anthropometric parameters.

Keywords: Triglycerides. Lipoproteins. Apolipoprotein A-I. Dyslipidemia.

INTRODUCTION

Cardiovascular risk markers are used to identify and monitor patients with high risk for coronary events such as hypertension, diabetes mellitus, chronic kidney disease, and dyslipidemias (Xavier *et al.*, 2013). Most of these diseases can be avoided by non-pharmacological strategies such as proper diet, smoking cessation and exercise. The Westernization lifestyle, especially modern diet, has contributed negatively to prevent and control modifiable cardiovascular risk factors. This pattern of behavior is the main factor associated with hypertriglyceridemia, accepted as an important marker for cardiovascular risk (Backes *et al.*, 2016; Pereira, Francischi, Lancha, 2003).

Hypertriglyceridemia is one of the main causes of metabolic syndrome, directly associated with insulin resistance, obesity, diabetes mellitus and hepatic disease (Cifuentes-Goches *et al.*, 2012; Feng *et al.*, 2016; Schild, Santos, Alves, 2013; Venturini *et al.*, 2013). At metabolic

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level, hypertriglyceridemia exerts potent effect on cholesterol ester transfer protein (CETP) activity, disrupting the cholesterol reverse transport promoted by high-density lipoprotein (HDL) and contributing to increased levels of low-density lipoprotein (LDL) (Von Eckardstein, Nofer, Assmann, 2001).

HDL represents a heterogeneous group of lipoproteins composed by small discoid and spherical particles which density is determined by a precise balance between proteins (structural apolipoproteins, enzymes, lipid transfer proteins and other minor proteins) and lipids content (esterified and non-esterified cholesterol, fatty acids and triglycerides) (Lima, Ricardo, Couto, 2006). Together, these components determine changes in size and functionality of HDL (Kontush *et al*., 2015).

In addition to the very well-established role of HDL in cholesterol reverse transport (Lima, Ricardo, Couto, 2006), other relevant functions have been identified. Thus, f HDL functionality was related to anti-inflammatory, anti-platelet aggregation and antioxidant capacity, which is attributed to the apolipoprotein AI (ApoA-I) and enzymes like lipoproteins associated phospholipase A_2 (Lp-PLA_2), and Paraoxonase 1 (PON1) (Barter *et al.*, 2004). Though the role of hypertriglyceridemia in cholesterol reverse transport is accepted, its impact on these additional functions remains an exciting area for new investigations.

Similarly, for hypertriglyceridemia, low HDL levels have been considered an independent biomarker for cardiovascular disease (Natarajan, Ray, Cannon, 2010). Different based-population studies had shown that plasma concentrations of HDL-cholesterol (HDL-c) and ApoA-I were independent and inverse predictors for the risk of occurrence of cardiovascular events (Besler *et al.*, 2011; Machado-Lima *et al.*, 2013; Valiyaveettil *et al.*, 2008). However, for other parameters of HDL functionality, complementary studies are needed (Kontush, Chantepie, Chapman, 2003; Mendivil *et al.*, 2016).

Therefore, regarding the increased prevalence of hypertriglyceridemia and reduced level of HDL-c observed in individuals with high cardiovascular risk and in general population, this study aims to investigate the role of hypertriglyceridemia in the functionality of HDL related with size, composition and antioxidant capacity in Brazilian subjects.

MATERIAL AND METHODS

Study design and population

This is a cross-sectional, analytical and non-probabilistic study. The subjects were selected from the data center of the Laboratory of Clinical and Toxicological Analysis (LACT) linked to the Federal University of Ceará (Brazil). From that, 260 Brazilian subjects attended to the invitation to participate in this trial, and after applying inclusion and exclusion criteria, 130 were selected. In order to investigate the role of triglyceride levels in different HDL-c status, the subjects were divided in 4 groups: [1] low HDL-c (<40 mg/dL) and low TG (<150 mg/dL) (HDL_LTG_L); [2] low HDL-c and high TG (>200 mg/dL) (HDL_LTG_H); [3] high HDL-c (>60 mg/dL) and low TG (HDL_HTG_L); and [4] high HDL-c and high TG (HDL_HTG_H). Patients with HDL-c levels between 40-60 mg/dL and TG between 150-250 mg/dL were considered as intermediates. The cut-off points for HDL-c and triglycerides were based in Brazilian Guideline on Dyslipidemias and Prevention of Atherosclerosis (Xavier et al., 2013).

The inclusion criteria were both sexes, aged between 20 and 75 years old. For exclusion criteria were considered malnourished individuals, pregnant or nursing, alcoholics, illicit drug users, subjects under lipid-lowering therapy and patients with previous cardiovascular events. Potential risk of occult cardiovascular disease was not considered in recruiting patients. This study was approved by the Research Ethic Committee of Federal University of Ceara (N° 44550015.0.0000.5054) and the data collection was performed only after signature of the free consent form by the patients.

Demographic and clinical evaluation

By direct interview, the socioeconomic profile was investigated using a structured form, reporting characteristics regarding sex, ethnicity, age and familiar income. The clinical evaluation investigated current clinical history, family history of chronic diseases (father and mother), regular use of medications and/or vitamins, and measurement of blood pressure. Habitual

physical activity was investigated by a questionnaire (Baecke, Burema, Frijters, 1982), validated to Brazilian population (Florindo *et al.*, 2004), from which a score was established based on physical activity at work, sport during leisure time and physical activity during leisure time excluding sport.

Anthropometric and body composition evaluation

Body weight was measured using a Balmak 111 professional mechanical scale (Balmak - SP) with a limit capacity of 150 kg and an accuracy of 100 g. Height measurements were performed by the stadiometer with limit of 2.1 m and accuracy of 1 mm. These data were used to calculate the body mass index (BMI), defined as body mass divided by height in square meters (kg/m^2). The BMI value was classified according to the WHO (WHO, 1995).

Waist circumference (WC) was measured using an inelastic and flexible tape with 1 mm accuracy. WC values were classified considering the cutoff point proposed by International Diabetes Federation (SBD 2017). The evaluation of the body fat mass (FM) percentage was performed using the Biodynamics 450® bioimpedance equipment (TBW/ACT Medical - SP). FM percentage was evaluated according previously described protocols (Pollock, Wilmore, 1993).

Biochemical evaluations

For biochemical analysis, after 12h of fasting, blood samples were collected in tubes without anticoagulant and with separating gel. Aiming to obtain serum, samples were centrifuged (3000 rpm, 15 min, 4°C) and aliquots were stored at -80°C. Using enzymatic reagents (Bioclin®, Labtest® and Randox®) and automated spectrophotometer (Mindray BS 200® and Cobas Mira®), the levels of glucose, total cholesterol, triglycerides, HDL-c, ApoA-I, ApoB and non-esterified fatty acids (NEFA) were analyzed. The LDL-c was determined by the Friedewald formula: LDL-c = TC – HDL-c – TG/5; this formula was only applied for individuals with TG < 400 mg/dL (Friedewald, Levy, Fredrickson, 1972). In order to evaluate the cardiovascular risk, the non-HDL-c (non-HDL-c = TC – HDL-c), non-HDL-c/HDL-c and Apo B/Apo A-I ratio were calculated. For the classification of lipid profile, the reference values proposed by the Brazilian Guideline on Dyslipidemias and Prevention of Atherosclerosis were used(Xavier *et al.*, 2013).

Subclasses of HDL lipoprotein

The subfractions of HDL were determined using the Lipoprint System (Quantrimetrix®). This system uses linear electrophoresis in non-denaturing polyacrylamide gel to separate and quantify lipoprotein fractions and subfractions. Ten subfractions of HDL (HDL-1 to HDL-10) were identified and grouped as following: large (HDL-1 to HDL-3), intermediate (HDL-4 to HDL-7) and small (HDL-8 to HDL-10) diameter. Results were presented in percentage and adjusted by HDL-c concentration (mg/dL). The functionality of HDL was estimated by the HDL_{1-2}/HDL_{9-10} ratio.

Antioxidant capacity of HDL

The antioxidant capacity of HDL was evaluated by the Lag Time assay proposed by Ziouzenkova (Ziouzenkova *et al.*, 1998). The patients' HDL was obtained by precipitation and used as an antioxidant substrate. The principle of analysis is the measure of conjugated dienes produced through LDL oxidation by copper ions. LDL was isolated by ultracentrifugation from a pool of normolipidemic human plasma donors, the dialyzed and adjusted into a concentration of 40 mg of protein in 500 µL of deionized H2O.

Adding HDL to this blend, it is expected that the oxidation of LDL will be reduced. Resistance to LDL oxidation (Lag Time) was calculated and expressed as the time between the start of the reaction and the intersection of time with the extrapolated line of the propagation phase at 234 nm on the spectrophotometer. The maximum rate of conjugated diene formation was determined by maximum absorbance/min.

From this kinetic profile were calculated: Vmax (maximum accumulation rate of absorbing products, indicating rate of lipid oxidation), Tmax (time for the

maximum rate of lipid oxidation), and ODmax (maximum intensity of oxidized products) as shown previously (Pinchuk, Lichtenberg, 2014).

The relationship in functionality of HDL and TG was reinforced by correlation between TG *versus* large HDL, TG *versus* small HDL, TG *versus* HDL_{1-2}/HDL_{9-10} and TG versus Lag Time.

Statistical analysis

To compare qualitative variables, chi-square test (Q^2) was used and the results were presented in absolute values followed by respective percentage. For the determination of the tests, the quantitative variables distribution was considered normal according to the *Kolmogorov-Smirnov test*. Quantitative variables were presented as mean and standard deviation. For parametric variables, ANOVA test with Tukey post-hoc test were used, and for non-parametric variables Kruskal-Wallis test was applied. The relationship between variables was expressed quantitatively using Pearson's correlation.

Multivariate linear regression model was used to identify the effect of the increase of each 50 mg/dL of triglycerides concentration on the size, composition and antioxidant capacity of HDL, making the adjustment according to five models; Model 1: adjusted for age; Model 2: adjusted for age and BMI; Model 3: adjusted for age, BMI and WC; Model 4: adjusted for age, BMI, WC, SBP and DBP; Model 5: adjusted for age, BMI, WC, SBP, DBP and LDL-c. Multivariate linear regression allows the incorporation of many predictors in the estimation of the effects of a predictor in the presence of other isolated or in association covariates. The estimated coefficients depend on the predictors and can be quite variable when the predictors are correlated (Krzywinski, Altman, 2015).

All statistical analysis were performed using the *Statistical Package for the Social Sciences*® (SPSS), version 16.0 (SPSS Incorporation. 2007). The significance level considered was $p < 0.05$.

RESULTS

The demographic and clinical data of the subjects are summarized in Table I. In this study, subjects were divided in four groups, according to serum concentration of triglycerides and HDL-c. Thus, it was possible to infer some observations. Individuals with the highest levels of triglycerides were those of older age. In addition, among men, there was a higher frequency of individuals with lower levels of HDL-c, unlike women, who had a higher frequency of higher levels of HDL-c. Familiar income, family history and habitual physical activity did not present any difference between the groups.

TABLE I - Demographic and clinical profile of subjects

		HDL_LTG_L (n = 37)	HDL_LTG_H (n = 32)	HDL_HTG_L (n = 50)	HDL_HTG_H (n = 11)	*P*
Age		**37 ± 14**	49 ± 11*	34 ± 12	50 ± 19*	**<0.001**
Sex	Male	22 ± 59.5*	20 ± 62.5*	8 ± 16.0	3 ± 27.3	**<0.001**
	Female	15 ± 40.5	12 ± 37.5	42 ± 84.0*	8 ± 72.7*	
Ethnicity	White	12 ± 27.9	12 ± 27.9	14 ± 32.6	5 ± 11.6	0.307
	Black	2 ± 22.2	5 ± 55.6	2 ± 22.2	0 ± 0.0	
	Other[a]	22 ± 28.6	15 ± 19.5	34 ± 44.2	6 ± 7.7	
Family history	Yes	16 ± 29.6	17 ± 31.5	14 ± 25.9	7 ± 13.0	0.050
	No	21 ± 27.6	15 ± 19.7	36 ± 47.4	4 ± 5.3	

TABLE I - Demographic and clinical profile of subjects

Age		**HDL_LTG_L (n = 37)**	**HDL_LTG_H (n = 32)**	**HDL_HTG_L (n = 50)**	**HDL_HTG_H (n = 11)**	*P*
		37 ± 14	49 ± 11*	34 ± 12	50 ± 19*	**<0.001**
Therapeutic drugs	Yes	14 ± 29.2	19 ± 39.6*	11 ± 22.9	4 ± 8.3	**0.008**
	No	23 ± 28.0	13 ± 15.9	39 ± 47.6*	7 ± 8.5	
HPA		7.7 ± 1.7	7.4± 1.2	7.5 ± 1.5	7.0 ± 2.3	0.716

The variable age is expressed in mean ± standard deviation;
Qualitative variables are presented in absolute values followed by the respective percentage (in parentheses);
Family history of chronic diseases were self-reported;
HDLLTGL = low HDL-c and low TG;
HDLLTGH = low HDL-c and high TG;
HDLHTGL = high HDL-c and low TG;
HDLHTGH = high HDL-c and high TG.;
MW = minimum wage;
HPA = habitual physical activity.
(a) Brown, yellow and indigenous;
(*) Significant difference between groups ($p < 0.05$).

Anthropometric parameters and body composition of the subjects are shown in Table II. The results show that the group that presented low HDL-c and high triglycerides levels (HDL_LTG_H) was associated with higher weight, BMI and WC. High percentage of fat mass was observed in the men of the groups with high values of triglycerides, but there is not a difference between the women.

TABLE II - Anthropometric parameters and body composition of subjects

		HDL_LTG_L (n = 37)	**HDL_LTG_H (n = 32)**	**HDL_HTG_L (n = 50)**	**HDL_HTG_H (n = 11)**	*P*
Weight (Kg)		72.0 ± 19.9	83.3 ± 17.1*	63.8 ± 13.5	63.6 ± 13.7	**<0.001**
BMI (Kg/m^2)		27.7 ± 6.1	31.2 ± 4.4*	24.7 ± 4.3	25.1 ± 3.5	**<0.001**
WC (cm)	Men	86.1 ± 22.6	102.9 ± 9.4*	87.3 ± 12.8	96.2 ± 13.0	**<0.001**
	Women	84.4 ± 10.3	92.5 ± 8.9*	76.8 ± 9.5	80.4 ± 6.0	**0.002**
FM (%)	Men	25.8 ± 5.9	29.6 ± 5.3*	20.7 ± 6.6	29.0 ± 6.0*	**0.001**
	Women	32.3 ± 4.9	34.8 ± 6.0	30.7 ± 5.2	34.4 ± 5.2	0.062

The variables were expressed as mean ± (standard deviation);
HDL_LTG_L = low HDL-c and low triglycerides;
HDL_LTG_H = low HDL-c and high triglycerides;
HDL_HTG_L = high HDL-c and low triglycerides;
HDL_HTG_H = high HDL-c and high triglycerides;
BMI = body mass index;
WC = waist circumference;
FM = fat mass.
(*) Significant difference between other groups ($p < 0.05$).

The representation of the groups' biochemical parameters and blood pressure are displayed in Table III. The simultaneous occurrence of low HDL and high triglycerides (HDL_LTG_H) levels were associated with an increase in plasma glucose's values. In addition, high levels of triglycerides were more connected to higher total cholesterol and LDL-c. There was an increase in the concentration of non-HDL-c and non-esterified fatty acids (NEFA) in the groups with hypertriglyceridemia. Similarly, the HDL_LTG_H group had a significant increase in the ratio of non-HDL-c/HDL-c. ApoA-I was higher in the groups with higher HDL-c levels, as it was expected; furthermore, the ApoB/ApoA-I ratio was higher in the HDL_LTG_H. Systolic and diastolic blood pressure values were higher in subjects with low HDL, regardless of the TG levels.

TABLE III - Biochemical profile and blood pressure

	HDL_LTG_L (n = 37)	HDL_LTG_H (n = 32)	HDL_HTG_L (n = 50)	HDL_HTG_H (n = 11)	*P*
Glucose (mg/dL)	96 ± 38	130 ± 58*	87± 8	96 ± 14	**<0.001**
Total cholesterol (mg/dL)	161 ± 35	206 ± 40	204 ± 38	247 ± 39*	**<0.001**
HDL-c (mg/dL)	37 ± 4	36 ± 5	69 ± 10*	64 ± 6*	**<0.001**
non-HDL-c (mg/dL)	125 ± 34	170 ± 39*	136 ± 38	183 ± 38*	**<0.001**
non-HDL-c/HDL-c	3.40 ± 0.89	4.75 ± 1.29*	2.02 ± 0.65	2.88 ± 0.65	**<0.001**
LDL-c (mg/dL)	105 ± 31	105 ± 32	119 ± 37	138 ± 42*	**0.021**
Triglycerides (mg/dL)	100 ± 30	347 ± 182*	86 ± 32	243 ± 39*	**<0.001**
Apo A-I (mg/dL)	109 ± 11	109 ± 20	158 ± 46*	167 ± 24*	**<0.001**
Apo B (mg/dL)	97 ± 25	111 ± 25	100 ± 26	133 ± 29*	**<0.001**
Apo B/Apo A-I	0.89 ± 0.23	1.06 ± 0.37*	0.67 ± 0.24	0.81 ± 0.20	**<0.001**
NEFA (mmol/L)	0.83 ± 0.16	1.25 ± 0.43*	0.99 ± 0.33	1.14 ± 0.32*	**<0.001**
SBP (mmHg)	123 ± 15	131 ± 17*	118 ± 13	127 ± 16	**0.005**
DBP (mmHg)	102 ± 11*	88 ± 11	78 ± 11	82 ± 10	**0.004**

The variables were expressed as mean ± (standard deviation);
HDL_LTG_L = low HDL-c and low triglycerides;
HDL_LTG_H = low HDL-c and high triglycerides;
HDL_HTG_L = high HDL-c and low triglycerides;
HDL_HTG_H = high HDL-c and high triglycerides;
HDL-c = cholesterol in high density lipoprotein;
LDL-c = cholesterol in low density lipoprotein;
VLDL-c = cholesterol in very low density lipoprotein;
NEFA = non-esterified fatty acids;
SBP = systolic blood pressure;
DBP = diastolic blood pressure.
(*) Significant difference between groups ($p < 0.05$).

Analysis of the distribution of HDL lipoprotein subfractions is shown in Table IV. Subfractions HDL_1 to $HDL_{4\ presented\ a\ higher}$ percentage of subjects with high HDL-c; furthermore, association with high TG promoted a significant decrease in these subfractions. In addition, HDL_{10} was similar when these groups were compared (HDL_HTG_H = 14.4% and HDL_HTG_L = 10.1%). Besides that, subjects with low HDL-c levels were more frequent among HDL_{10}. Finally, the negative impact of high TG was confirmed by $HDL_{1\text{-}2}/HDL_{9\text{-}10}$ ratio (HDL_HTG_H = 0.97 and HDL_HTG_L = 2.17; $p < 0.001$).

TABLE IV - Distribution of HDL subfractions of subjects

	HDL_LTG_L (n = 37)	HDL_LTG_H (n = 32)	HDL_HTG_L (n = 50)	HDL_HTG_H (n = 11)	*P*
Percentage (%)					
HDL_1	5.9 ± 4.9	5.4 ± 3.5	10.5 ± 4.8*	6.6 ± 3.6	**0.002**
HDL_2	7.8 ± 4.2	5.2 ± 2.7	13.2 ± 5.1*	9.8 ± 4.4	**<0.001**
HDL_3	6.2 ± 2.4	4.4 ± 1.9	10.2 ± 2.8*	7.9 ± 3.5	**<0.001**
HDL_4	8.4 ± 2.7	7.4 ± 2.2	10.7 ± 2.1*	8.8 ± 2.8	**0.001**
HDL_5	9.1 ± 2.8	9.7 ± 2.4	8.5 ± 1.4	8.6 ± 1.2	0.321
HDL_6	16.7 ± 4.3	19.5 ± 4.2*	14.8 ± 2.6	17.2 ± 2.8	**0.002**
HDL_7	7.4 ± 1.1	8.9 ± 2.4*	6.4 ± 1.4	8.1 ± 3.4*	**0.001**
HDL_8	9.8 ± 1.8	10.9 ± 3.1	8.7 ± 2.2	9.7 ± 4.7	0.141
HDL_9	8.1 ± 2.1	8.8 ± 2.0	7.0 ± 2.8	8.1 ± 2.3	0.092
HDL_{10}	20.6 ± 12.2*	19.6 ± 8.4*	10.1 ± 7.6	14.4 ± 7.2	**0.002**
$HDL_{1\text{-}2}/HDL_{9\text{-}10}$	0.74 ± 0.78	0.43 ± 0.27	2.17 ± 1.73*	0.97 ± 0.78	**<0.001**

The variables were expressed as mean ± (standard deviation);
HDL_LTG_L = low HDL-c and low triglycerides;
HDL_LTG_H = low HDL-c and high triglycerides;
HDL_HTG_L = high HDL-c and low triglycerides;
HDL_HTG_H = high HDL-c and high triglycerides;
HDL= high density lipoprotein.
(*) Significant difference between other groups ($p < 0.05$).

Table V describes results of antioxidant capacity of HDL. The Lag Time in the group with high HDL-c and low TG (HDL_HTG_L) levels was significantly higher in comparison to the other groups (86 min versus 80 min, 76 min and 72 min). It is important to empathize that subjects with higher HDL-c levels who had high concentrations of triglycerides did not present a Lag Time increase.

TABLE V - Antioxidant capacity of HDL of subjects

Variables	HDL_LTG_L (n = 37)	HDL_LTG_H (n = 32)	HDL_HTG_L (n = 50)	HDL_HTG_H (n = 11)	*p*
Lag Time (min)	80 ± 14	77 ± 18	86 ± 16*	72 ± 8	**0.001**
Vmax (mU/min)	0.90 ± 0.44	0.91 ± 0.40	0.90 ± 0.49	0.80 ± 0.31	0.934
Tmax (min)	166 ± 23	163 ± 28	170 ± 26	158 ± 16	0.163
ODmax (OD)	0.39 ± 0.09	0.37 ± 0.09	0.37 ± 0.10	0.38 ± 0.04	0.301

Variable were expressed as mean ± (standard deviation);
Lag Time = time without lipid oxidation;
Vmax = maximum rate of lipid oxidation;
Tmax = time for the maximum rate of lipid oxidation;
ODmax = maximum intensity of oxidized products.
(*) Significant difference between groups ($p < 0.05$).

In order to correlate triglycerides concentrations with HDL'S functionality, these parameters were analyzed according toPearson's correlation. A negative correlation of weak to moderate intensity was found in TG *versus* large HDL (r = -0.415; $p < 0.001$), TG *versus* HDL_{1-2}/HDL_{9-10} (r = -0.347; $p = 0.003$) and in TG *versus* Lag Time (r = -0.180; $p = 0.043$). Moreover, a positive correlation was found in TG *versus* small HDL (r = 0.358; $p = 0.002$). These data indicate that HDL'S functionality is negatively influenced by the TG levels and that this influence is more perceptible when the subject's HDL particles have a smaller diameter.

To better identify the influence of TG level on functionality of HDL, some regression models were tested, as shown in table VI. Many regressions analyze involve more than one regressor variable. In this way, when it is intended to eliminate the interference of the mean values of one or more regressor variables in the analysis, multivariate linear regression is performed. This analysis takes place by calculating the r^2 coefficient, which indicates the percentage of total variability exerted by the proposed model. The association established in the regression model is calculated using the β coefficient, which represents the slope of the line generated in a scatter diagram.

TABLE VI - Influence of triglycerides concentration (per each 50 mg/dL) on the percentage of large and small HDL, and HDL_{1-2}/HDL_{9-10} ratio

		Large HDL (%)	Small HDL (%)	HDL_{1-2}/HDL_{9-10} (%)
Crude	ß	-1.579	1.461	-0.135
	r^2	-0.415	0.358	-0.347
	P	**<0.001**	**0.002**	**0.003**
Model 1	ß	-1.27	1.097	-0.096
	r^2	-0.333	0.269	-0.248
	P	**0.005**	**0.024**	**0.036**
Model 2	ß	-1.133	1.001	-0.084
	r^2	-0.298	0.245	-0.216
	P	**0.007**	**0.035**	0.054
Model 3	ß	-1.006	0.863	-0.07
	r^2	-0.268	0.214	-0.182
	p	**0.016**	0.072	0.111
Model 4	ß	-	0.91	-0.071
	r^2	-	0.227	-0.187
	p	-	0.061	0.106
Model 5	ß	-	0.438	-0.178
	r^2	-	0.056	-0.222
	p	-	0.681	0.077

Model 1: adjusted for age;
Model 2: adjusted for age and BMI;
Model 3: adjusted for age, BMI and WC;
Model 4: adjusted for age, BMI, WC, SBP and DBP;
Model 5: adjusted for age, BMI, WC, SBP, DBP and LDL-c.
(*) - Significant difference ($p < 0.05$);

Results showed that the increase of each 50 mg/dL of triglycerides (crude) culminates in the reduction of 1.58% of large HDL, which is associated to a reduction of 0.14% of the HDL_{1-2}/HDL_{9-10} ratio and an increase of 1.46% in small HDL. The rise in TG levels influenced on the percentage of large HDL, and this influence remained present even after adjustment with variables of age, BMI and WC (Models 2 and 3). However this did not happen with the percentage of the small HDL and the HDL_{1-2}/HDL_{9-10} ratio. Furthermore, an increase of each 50 mg/dL of TG levels was associated with a reduction on the antioxidant capacity of HDL (B = -0.18; $p = 0.043$).

DISCUSSION

The relationship between serum TG and cardiovascular risk is controversial. Among the raised questions, the relationship between risk and circadian variability of TG levels, the inverse correlation of TG and HDL-c concentrations, and the association with a pro-oxidant and pro-inflammatory state in individuals with hypertriglyceridemia stand out. It is known that several genetic mutations, as in the APOC2 or APOA2 apoprotein genes, manifest in the laboratory by increasing TG levels and the HDL dysfunction.To improve the understanding around this topic, several research groups have sought to associate the determination of serum TG with cholesterol transport pathways, mainly the reverse transport pathway, orchestrated by HDL, an anti-atherogenic and antioxidant particle (Moura, 2019).

This study demonstrated that the triglycerides levels play a role in HDL's functionality, which contributes to the cardiovascular risk, since systolic and diastolic blood pressure values were higher in subjects with low HDL. Among the selected population, women presented higher levels of HDL-c and low percentage of fat mass more frequently. Moreover, the highest levels of triglycerides were observed in older people. Furthermore, high levels of triglycerides were associated with higher total cholesterol and LDL-c levels, non-HDL-c, non-esterified fatty acids, and blood glucose, which increases the ratio of non-HDL-c/HDL-c and ApoB/ApoA-I. Our results showed that the increase of triglycerides levels progressively implies in the reduction of HDL's size and antioxidant capacity. These variables depend on age, BMI and WC.

These discoveries are relevant, because it is important to understand biochemical markers and their functionality to identify cardiovascular risk. The results expand the perspective about the relationship between TG and HDL, demonstrating that antioxidant capacity and size of HDL are modulated by TG levels. Previous studies have suggested this correlation (Cifuentes-Goches *et al*., 2012; Er *et al*., 2016; Kontush, Chantepie, Chapman, 2003; Lima, Ricardo, Couto, 2006; C. Pereira *et al*., 2014; Walldius *et al*., 2001) and described different mechanisms to explain endogenous and exogenous factors responsible for this association (Cifuentes-Goches *et al*., 2012; Lima, Carvalho, Sousa, 2007; Qin *et al*., 2015; Walldius *et al*., 2001; Xavier *et al*., 2013). These factors collectively stimulate more synergic pathways than independent actions that contribute to high TG and low HDL. In general, obesity induced by "modern" diet patterns is the most relevant link between this lipid profile, as it has been broadly described (Kim *et al*., 2006; Oliveira *et al*., 2010; Roever *et al*., 2016; Da Silva, De Souza, Damasceno, 2013; Tereshina, Ivanenko, 2014).

Our results showed that individuals with hypertriglyceridemia, regardless of HDL-c level, have higher prevalence of diabetes, hypertension and excess of weight. Kim (Kim *et al*., 2006) previously showed that higher BMI is associated with lower levels of HDL-c, as well as stating that overweight is strongly related to concentrations of HDL cholesterol and triglycerides and weakly related to concentrations of total and LDL cholesterol. Although Oliveira (Oliveira *et al*., 2010) did not identify a correlation between HDL-c and BMI, the author observed that TC and LDL-c were more frequently correlated to BMI.

In addition to the negative impact of weight and BMI in the relationship of TG and HDL, it was also observed a significant increase in WC and FM in the individuals included in the group that presented low HDL and high triglycerides ($HDL_L TG_H$). Recently, the Sardinha (Sardinha *et al*., 2016) study reinforced the association of WC and BMI in individuals with simultaneous increased TG and reduced HDL-c. Previously, Oliveira (Oliveira *et al*., 2010) demonstrated that TG and HDL-c maintain relations with FM. This profile was associated with

glucose unbalanced metabolism and increased NEFA levels observed in our study, suggesting the presence of insulin resistance in these individuals, as shown by Ying-Mei Feng (Feng *et al*., 2016). Therefore, as demonstrated by the Mendivil study (Mendivil *et al*., 2016) the connection of hypertriglyceridemia, overweight or obesity, and lower HDL-c can be explained by impaired reverse cholesterol transport mediated by HDL.

Our results expand this relationship in terms of functionality of HDL particles, rather than to only confirm that TG and HDL-c are associated and modulated by other factors, such as obesity and insulin resistance. In addition, we suggest that the antiatherogenic role of HDL is not only limited due to the ability of this lipoprotein with cholesterol transport.

In agreement with this additional role of HDL, our results showed that individuals with lower concentrations of HDL-c, regardless of TG levels, had a higher amount of small HDL particles. Kontushi (Kontush, Chantepie, Chapman 2003) proposed that small particles of HDL are more functional and play an anti-inflammatory and antioxidant role. Despite that, our results did not identify improvement in the antioxidant capacity of HDL in both groups with low levels of HDL. This is because the antioxidant function of HDL is dependent of ApoA-I and enzymes such as phospholipase A2 associated with lipoprotein (Lp-PLA_2) and Paraoxonase 1 (PON1), and possibly other intrinsic proteins of HDL (Kontush, Chantepie, Chapman 2003; Lima, Ricardo, Couto, 2006; Da Silva, De Souza, Damasceno, 2013).

The ApoA-I levels of individuals with low HDL-c was lower than the values observed in groups with high $HDL\text{-}c_{,\ regardless}$ of TG level. Plus, non-HDL-c/HDL-c and ApoB/ApoA-I ratios were higher in the $HDL_L TG_H$ group. These data are supported by Quin (Qin *et al*., 2015), who described that ApoB/apoAI and non-HDL-c/HDL-c are useful in predicting cardiovascular events among Chinese individuals, more prominently than age, blood pressure and triglycerides.

So, it is important to analyze and discuss the functionality of HDL beyond its concentrations. In the study of Shuhei *et al.* (2010), the size and composition of HDL were compared among two groups with different concentrations of HDL-c; it was observed that individuals with higher HDL-c level had a higher percentage of large HDL (HDL_2). Complementally, our study constructed more specific groups and information about subfractions of HDL (HDL_1 - HDL_{10}), and suggested that hypertriglyceridemia may influence in additional functions as size and antioxidant capacity of HDL more than HDL-c level. It is important to emphasize that $HDL_{9\text{-}10}$ subfractions are closely linked to increased cardiovascular risk, as small HDL particles are associated with lower cardiovascular risk, highlighting the beneficial potential of the $HDL_{1\text{-}2}$ subfraction (Li *et al.,* 2016).

Previous studies showed that the relationship between HDL-c and cardiovascular disease is contestable (Haase *et al*., 2012; Voight *et al*., 2012), especially when the research shows that individuals with high HDL-c have a higher risk of death from disease in general. Due to this fact, many other studies are emerging to clarify the cardioprotective relationship of other parameters of quantity and quality related to the HDL particle (Sviridov *et al*., 2008), mainly with wide knowledge about the various biological functions of HDL described, and that it cannot be evaluated only by plasma HDL-c concentrations.

The biological functions of high-density lipoprotein contribute to explain the cardioprotective role of lipoprotein in addition to the quantitative levels of HDL cholesterol. A randomized study showed that the diet enriched with extra virgin olive oil and nuts is able to decrease triglyceride levels, as well as to improve HDL function in individuals with high cardiovascular risk. The diet promoted an increase in the cholesterol efflux of the cells and a decrease in the activity of the cholesteryl ester transfer protein. Additionally, there was an increase in the percentage of large HDL particles, in HDL's ability to esterify cholesterol, as well as in paraoxonase-1 activity, preventing the formation of oxidized lipids. Thus, it is possible that there is a correlation between decreased risk of cardiovascular disease and HDL particle function (Hernáez *et al.,* 2017).

Although pharmacological treatments of dyslipidemia are focused in the reduction of cholesterol, and here we highlight the unquestionable benefits of statins and fibrates for hypertriglyceridemia; the drug modulation of HDL cholesterol did not show similar success (Barter, 2009; Barter *et al*., 2007; Lincoff *et al*., 2017; Nicholls *et al*.,

2015). Therefore, our results confirm adjuvant interventions such reduced caloric intake, food containing unsaturated fatty acids, especially those of the omega type, flavonoids and other phenolic compounds, as well as the practice of physical activity are able to decrease triglyceride levels, improving the functionality of HDL associated with size and antioxidant capacity of this lipoprotein.

Altogether, the data presented in this study consolidates the need for further studies aiming at a better understanding of the influence of triglyceridemia on HDL functions, since markers of cardiovascular disease are usually analyzed basically by their concentration, which does not consider that the energy and the biochemical metabolism of macromolecules is highly interrelated.

The present study concludes that hypertriglyceridemia promotes changes in functionality of HDL, influencing negatively in its size and antioxidant capacity, and possibly decreasing its anti-atherogenic potential, contributing to the cardiovascular risk.

CONFLICT OF INTEREST

The authors declared that they have nothing to disclose about conflict of interest regarding this manuscript.

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ETHICAL APPROVAL (INCLUDING REFERENCE NUMBER)

This study was approved by the Research Ethic Committee of Federal University of Ceara (N° 44550015.0.0000.5054) and the data collection was performed only after signature of the free consent form by the patients.

CONTRIBUTORSHIP

RRCM and TLS: wrote the manuscript; RRCM, NRTD, SMMLV, JMF and MGRQ: Project planning; RRCM, LGL, HZQL, MBD, CAV, PMVS, TLS and ESS: Data collect; RRCM, PAL, FCC, GAV and ELHS: performed the experiments; RRCM, GCM, NRTD, MES, TLS and MGRQ: analyzed, interpreted data and reviewed critically the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript

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