Brazilian Journal of Physics
ISSN: 0103-9733
luizno.bjp@gmail.com
Sociedade Brasileira de Física
Brasil

Pruper de Freitas, Maria Camila; Martins Figueiredo Neto, Antonio; Giampaoli, Viviane; da Conceição Quintaneiro Aubin, Elisete; de Araújo Lima Barbosa, Milena Maria; Teixeira Damasceno, Nágila Raquel

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Brazilian Journal of Physics, vol. 46, núm. 2, abril, 2016, pp. 163-169
Sociedade Brasileira de Física
São Paulo, Brasil

Available in: http://www.redalyc.org/articulo.oa?id=46444888005

Braz J Phys (2016) 46:163–169
DOI 10.1007/s13538-015-0395-y

SOCIEDADE BRASILEIRA DE FÍSICA

GENERAL AND APPLIED PHYSICS

Z-Scan Analysis: a New Method to Determine the Oxidative State of Low-Density Lipoprotein and Its Association with Multiple Cardiometabolic Biomarkers

Maria Camila Pruper de Freitas[1] · **Antonio Martins Figueiredo Neto**[2] · **Viviane Giampaoli**[3] · **Elisete da Conceição Quintaneiro Aubin**[3] · **Milena Maria de Araújo Lima Barbosa**[1] · **Nágila Raquel Teixeira Damasceno**[1]

Received: 13 November 2015 / Published online: 6 January 2016

Abstract The great atherogenic potential of oxidized low-density lipoprotein has been widely described in the literature. The objective of this study was to investigate whether the state of oxidized low-density lipoprotein in human plasma measured by the Z-scan technique has an association with different cardiometabolic biomarkers. Total cholesterol, high-density lipoprotein cholesterol, triacylglycerols, apolipoprotein A–I and apolipoprotein B, paraoxonase-1, and glucose were analyzed using standard commercial kits, and low-density lipoprotein cholesterol was estimated using the Friedewald equation. A sandwich enzyme-linked immunosorbent assay was used to detect electronegative low-density lipoprotein. Low-density lipoprotein and high-density lipoprotein sizes were determined by Lipoprint® system. The Z-scan technique was used to measure the non-linear optical response of low-density lipoprotein solution. Principal component analysis and correlations were used respectively to resize the data from the sample and test association between the θ parameter, measured with the Z-scan technique, and the principal component. A total of 63 individuals, from both sexes, with mean age 52 years (±11), being overweight and having high levels of total cholesterol and low levels of high-density lipoprotein cholesterol, were enrolled in this study. A positive correlation between the θ parameter and more anti-atherogenic pattern for cardiometabolic biomarkers together with a negative correlation for an atherogenic pattern was found. Regarding the parameters related with an atherogenic low-density lipoprotein profile, the θ parameter was negatively correlated with a more atherogenic pattern. By using Z-scan measurements, we were able to find an association between oxidized low-density lipoprotein state and multiple cardiometabolic biomarkers in samples from individuals with different cardiovascular risk factors.

Keywords Cardiovascular diseases · Lipoproteins · Low-density lipoprotein · Cardiometabolic biomarkers · Z-scan technique

✉ Maria Camila Pruper de Freitas
camilapruper@hotmail.com

✉ Nágila Raquel Teixeira Damasceno
nagila@usp.br

[1] Department of Nutrition, School of Public Health, University of Sao Paulo, Sao Paulo, Av. Dr. Arnaldo, 715, 01246-904 Sao Paulo, SP, Brazil

[2] Experimental Physics Department, Institute of Physics, University of Sao Paulo, Sao Paulo, Brazil

[3] Department of Statistics, Institute of Mathematics and Statistics, University of Sao Paulo, Sao Paulo, Brazil

1 Introduction

Cardiovascular disease (CVD) is the leading cause of premature morbidity and mortality worldwide. Statistical data from the World Health Organization (WHO) showed that CVD is responsible for 48 % of deaths in the world. In Brazil, CVD represents 33 % of all deaths and 78 % of deaths due to chronic diseases [1]. Although CVD includes complex mechanisms, atherosclerosis is the physiopathological basis of its primary and secondary clinical events [2].

Classical cardiovascular risk factors, such as age, hypertension, smoking, high levels of glucose, dyslipidemia, physical inactivity, overweight, and obesity, are the focus for primary prevention of CVD [3]. However, these factors fail to explain all types of cardiovascular events. According to Toshima et al., lack of association with hypertension, serum

cholesterol, smoking, and sex suggested that oxidized low-density lipoprotein (oxLDL) is an independent risk factor for CVD [4].

Regarding this point, new biomarkers, associated with atherogenic characteristics, such as oxLDL [4], electronegative low-density lipoprotein [LDL(−)] [5], and apolipoprotein B (APOB) [6], and our anti-atherogenic characteristics, such as apolipoprotein A–I (APOAI) [7], have been related to cardiovascular risk, emphasizing the greatest atherogenic potential and crucial role of modified low-density lipoprotein (LDL) in the atherosclerotic process [8–10].

Changes in low-density lipoprotein (LDL) composition generate LDL particles that contribute to the atherosclerotic process by (i) increasing the content of small and dense LDLs (LDL_{SMALL}) [8, 9], (ii) releasing oxidative products [11], (iii) retaining LDL by interaction with proteoglycans [12], (vi) increasing cross-reaction with glucose [13], and (v) enriching the content of non-esterified fatty acids (NEFAs), apolipoprotein C-III (APOCIII), and apolipoprotein E (APOE) in LDL(−) [13, 14].

In 1999, Gómez et al. [15] used the Z-scan technique as an experimental method to evaluate the non-linear refractive indices of micellar lyotropic liquid crystals. The physical principle of the Z-scan technique is based on the fact that some materials can absorb part of the high-intensity light (from a laser) incident on them and that light increases their temperature, resulting in a variation of their refractive index [16]. LDL particles have a hydrophilic shell and a hydrophobic core, which resembles a micellar aggregate of liquid crystals. The variation of the particle composition may contribute differently to the non-linear optical response depending on the particular state of the LDL and respond to optical fields in a way that depends on the structure and local ordering of the aggregates [17].

Regarding this possibility, Gómez et al. [18] used the Z-scan technique to investigate the non-linear optical response of native LDL and LDL oxidized in vitro by copper ions. This response varies according temperature and concentration of LDL particles. It was the pioneer study, and it showed that Z-scan signals increase linearly with concentration of native LDL and the oxLDL does not show non-linear optical response.

During the process of LDL oxidation particles in vitro, significant changes occurred in their structures as in the electrical density profile, in size polydispersity, and in the degree of flexibility of the APO-B protein on the particle [19].

Recently, Santos et al. [20] also measured the non-linear optical response of native and oxLDL solutions, from human plasma, by Z-scan technique, and showed that it depends on a balance involving anti-oxidants and oxidative products in the sample. The linear optical absorption decreases, as a function of the oxidation time, and it is related to the production of lipid hydroperoxides and consumption of LDL's carotenoids, which is a consequence of lipid peroxidation processes. In this sense, the Z-scan technique could be a complementary tool to estimate the level of oxLDL in human plasma and, consequently, evaluate the CVD risk of a patient.

Since the presence of modified LDL particles in the plasma is a key event in the progression of atherosclerosis, it is reasonable to expect that the results obtained with the Z-scan technique can be associated with other cardiometabolic biomarkers and give complementary information about the CVD risk.

Therefore, this study aimed to investigate whether the recently proposed Z-scan technique to measure the oxidative state of LDL is associated with multiple cardiometabolic biomarkers assessed by principal component analysis (PCA). The Z-scan uses a non-linear optical technique to study the response of LDL in solution from human plasma.

2 Methods

2.1 Patients and Study Design

This cross-sectional study included individuals from both sexes (ages 30–74 years), who have never had a cardiovascular event as evaluated by both electrocardiogram and clinical history. All participants signed an informed consent form. The study protocol was approved by the Ethics Committee from the University Hospital and Ethics Committee from the School of Public Health (University of Sao Paulo, SP, Brazil).

Individuals who were undernourished, pregnant or lactating, illicit drug users, or alcoholics and those who presented previous cardiovascular events, uncontrolled acute or severe chronic illness, or uncontrolled psychiatric disorders were excluded.

2.2 Demographic, Clinical, and Anthropometric Features

Demographic (sex and age) and clinical profiles were assessed using a structured questionnaire. Clinical evaluation consisted of current information on drugs, smoke, blood pressure and actual chronic diseases, and familial history of chronic disease.

For anthropometric evaluation, we considered the following items: weight (kg)—measured using a digital scale II® Control (Plenna; São Paulo, SP, Brazil) and height (m)—measured by a portable stadiometer Altura exata® (TBW; São Paulo, SP, Brazil). The waist circumference (WC) (cm) was measured by a 1.0-mm precision inelastic and flexible tape (TBW; São Paulo, SP, Brazil), using the anatomical midpoint between the lowest rib and the iliac crest as reference. The body mass index (BMI) (kg/m^2) was calculated from weight and height measurements obtained, and it is defined as body mass (kg) divided by height (m) squared.

2.3 Biochemical Analysis

Blood samples were collected after 12-h fasting. To analyze total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triacylglycerols (TAG), we used standard methods (Labtest, Lagoa Santa, MG, Brazil). The content of low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald equation [21].

The APOAI and APOB were determined by a standard protocol based on an immuno-turbidimetric method and using commercially available kits (Autokits APOAI® and APOB®; Randox Chemicals USA Inc.; Richmond, VA, USA).

Paraoxonase 1 (PON1) activity was determined according to Mackness et al. [22] and was expressed as nanomole per minute per milliliter.

Plasma glucose was determined using the commercial, enzymatic-colorimetric kit Glucose PAP Liquiform® (Labtest; Lagoa Santa, MG, Brazil).

The LDL(−) was detected by a sandwich enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies to LDL(−) [23]. Sizes of the LDL and high-density lipoprotein (HDL) subfractions were determined by using the Lipoprint® system (Quantimetrix®; Redondo Beach, CA, USA), which is based on the separation and quantitation of lipoprotein subfractions through a non-denaturing polyacrylamide gel. All analyses were performed in duplicate, and the intra- and inter-analyst variability coefficients were less than 10 %.

2.4 Z-Scan Technique

LDL particles ($1.019 \leq \text{density} < 1.063$ g/mL) were separated from plasma samples by ultracentrifugation (56.000 rpm; 4 °C; 18 h; fixed-angle rotor). The LDL particles were desalted, and their total protein content was determined by using the BCA Protein Assay method (Pierce® Kit; Thermo Scientific; Waltham, MA, USA) for later adjustment to 1.0 mg/mL.

The Z-scan setup is composed of a continuous-wave Nd:YVO_4 ($\lambda = 532$ nm) laser, with a Gaussian profile beam. The laser beam was chopped (frequency = 17 Hz) and focused by using a lens (diameter = 25.4 mm; focal distance, $f = 150$ mm). The Rayleigh length z_0 was 3.84 mm (0.20). The transmitted light was collected by a silicon photodetector placed at the far field. The distance between the beam waist and detector was about 150 cm. Samples were placed in sample holders with 200-μm optical path. The incident power of the laser on the samples varied in the range 121.9–153.1 mW (1.0). Additional details about the setup may be found in [20, 24].

The Z-scan technique is used to investigate characteristics of the native and modified LDL based on the sample's absorbing property to locally increase (slightly) its temperature when illuminated by a laser beam. It is then formed a thermal lens in the sample that induces a change in its refractive index. The typical shape of the light transmittance curve as a function of sample position along the beam focus axis has a peak-to-valley inflection. The peak-to-valley amplitude in the normalized light transmittance is a dimensionless parameter named θ [24]. It was previously established that in more oxidized (modified) LDL particles, the θ parameter is smaller than that obtained in native (non-modified) LDL.

2.5 Statistical Analysis

Results of descriptive analyzes were presented as mean and standard deviation (±) or median and interquartile range, depending on the variable distribution. The *t-Student* and *Mann-Whitney* tests [25, 26] were used to analyze the differences between genders.

The PCA was used to resize the data of samples and standardize it according to cardiometabolic biomarkers and parameters related with atherogenic LDL profile and results presented in loading coefficients. The PCA consists in rewriting the original variables into new ones called principal components (PC) obtained from a coordinate transformation, in order to simplify the variations existing in multivariate data. Thus, the multivariate nature of data can be projected on a reduced number of dimensions while preserving as much information as possible, by calculating linear combinations between the original variables and the results presented as loadings [27]. This study used the eigenvalue-greater-than-one rule, proposed by Kaiser [28] for extraction of the most significant PC, to explain the variance of the data and to consider the measurement of sampling adequacy.

First, a PCA was performed to standardize the sample data according to the cardiometabolic biomarkers. One array of data was constructed (63 individuals; 15 variables), in which the sample data were arranged in rows and the columns were ordinate by variables. In addition, other PCA was performed to standardize the sample data according to parameters related with atherogenic LDL profile (array of data 63 individuals; seven variables), in which the samples were arranged in rows and the columns were ordinate by variables.

Spearman correlations [25, 26] were used to analyze the association between the θ parameter from the Z-scan measurement and the PC projected by PCA.

All statistical tests were performed using the Statistical Package for the Social Sciences® (IBM SPSS Statistics to Windows, version 20.0, Armonk, NY: IBM Corp) [29], with a 5 % significance level.

3 Results

The total set analyzed consisted of 63 individuals, mean age 52 years (±11), and women represented 68 % of the sample

population. Self-reported chronic diseases show high prevalence of diseases (92 %). This status was confirmed by current drugs use (81 %) and family history of chronic disease (89 %). Nineteen percent of the individuals were smokers. These clinical parameters were similar for both sexes. The mean values for BMI showed that the individuals are overweight, and men had greater WC, as expected. Regarding the biochemical profile, high TC and low HDL-C plasma levels were observed. Women showed higher HDL-C and APOAI levels, and men showed higher percentage of LDL_{SMALL} and smaller LDL size. The values of the θ parameter were bigger in women, but in comparison to men, both groups did not show significant differences (Table 1).

The PCA was applied in order to summarize similarities or differences between individuals. Thus, the PCA allowed projecting of the data in a planar space with 15 dimensions (15 PC), and according to the eigenvalue-greater-than-one rule, proposed by Kaiser [28], the first five components were most significant to explain the 81.9 % of the total variance of original data. Table 2 shows the loading coefficients of the five most significant PC for cardiometabolic biomarkers by PCA.

The first principal component (PC1) contributes to 29.4 % of the data variance. This PC showed a more atherogenic pattern, concentrating positive loading coefficients, respectively, for TC, LDL-C, TAG, non-HDL-C, APOB, LDL_{SMALL}, and LDL(−), as well as a negative loading coefficient for LDL size.

The second principal component (PC2) contributes to 15.4 % of the data variance and also showed an atherogenic pattern, with negative loading coefficients for HDL-C and HDL_{LARGE} as well as positive loading coefficients for TAG, LDL_{SMALL}, and HDL_{SMALL}.

The third principal component (PC3) accounted for 13.3 % of the variance and showed an anti-atherogenic pattern. Positive loading coefficients for HDL-C, APOAI, and PON1 were also observed.

The fourth principal component (PC4) was responsible for 13 % of the variance and also showed an anti-atherogenic pattern, with negative loading coefficients for TAG and LDL_{SMALL} and positive loading coefficients for LDL_{LARGE} and LDL size.

The fifth and last principal component (PC5), with 10.8 % of the variance, showed a more atherogenic pattern with negative loading coefficients for HDL-C as well as opposite loading coefficients for glucose and TAG, despite the negative loading coefficients for electronegative LDL.

Table 3 shows loading coefficients of the two most significant PC for parameters related with atherogenic LDL profile. The PCA allowed projecting the data in a planar space with seven dimensions (seven PC), and according to the

Table 1 Demographic, anthropometric, and biochemical individual characteristics, according to gender

Variables	Total ($n = 63$)	Men ($n = 20$)	Women ($n = 43$)	p value
BMI (kg/m^2)	31(±6)	30(±5)	31(±6)	0.69
WC (cm)	99 (±12)	105 (±13)	97 (±11)	*0.02*
TC (mg/dL)	203 (±43)	199 (±36)	205 (±46)	0.68
HDL-C (mg/dL)	37 (±9)	32 (±8)	40 (±8)	*<0.001*
LDL-C (mg/dL)	138 (±38)	136 (±27)	139 (±42)	0.74
TAG (mg/dL)	117 (91; 175)	124 (99; 220)	116 (89; 160)	0.16
Glucose (mg/dL)	96 (86; 107)	99 (91; 110)	96 (86; 105)	0.48
Non-HDL-C (mg/dL)	166 (±43)	168 (±36)	166 (±46)	0.82
APOB (mg/dL)	105 (±26)	105 (±20)	106 (±28)	0.98
APOAI (mg/dL)	130 (±26)	120 (±19)	135 (±27)	*0.03*
PON1 (nmol min^{-1} mL^{-1})	54 (±30)	58 (±34)	52 (±28)	0.46
HDL_{LARGE} (%)	31 (±8)	28 (±8)	32 (±8)	0.15
HDL_{SMALL} (%)	19 (±8)	21 (±8)	19 (±8)	0.29
LDL_{LARGE} (%)	26 (±6)	27 (±4)	26 (±6)	0.47
LDL_{SMALL} (%)	2 (1; 4)	3 (1; 6)	1 (1; 3)	0.05
LDL size (Å)	270 (266; 272)	268 (264; 271)	271 (268; 273)	*0.04*
LDL(−) (U/L)	10 (3; 37)	5 (2; 13)	12 (4; 47)	0.08
Z-scan (θ parameter)	8.5E−04 (±5.2E−04)	7.5E−04 (±4.7E−04)	9.0E−04 (±5.3E−04)	0.29

Results reported as mean and standard deviation (±) our median and interquartile range. Tests: *t-Student* and *Mann-Whitney*. Statistical significance: $p < 0.05$ (significant results are in italics)

BMI body mass index, *WC* waist circumference, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TAG* triacylglycerols, *Non-HDL-C* non-high-density lipoprotein cholesterol, *APOB* apolipoprotein B, *APOAI* apolipoprotein AI, *PON1* paraoxonase 1, *LDL(−)* electronegative low-density lipoprotein

Table 2 Principal component analysis (PCA) for cardiometabolic biomarkers

Variables $n = 63$	Principal Component (PC)				
	PC1	PC2	PC3	PC4	PC5
TC (mg/dL)	*0.949*	0.098	0.208	−0.124	0.035
HDL-C (mg/dL)	−0.048	*−0.323*	*0.780*	0.003	*−0.410*
LDL-C (mg/dL)	*0.957*	0.036	0.065	0.056	−0.074
TAG (mg/dL)	*0.341*	*0.389*	−0.034	*−0.506*	*0.544*
Glucose (mg/dL)	0.147	−0.139	0.159	0.052	*0.863*
Non-HDL-C (mg/dL)	*0.956*	0.164	0.046	−0.123	0.121
APOAI (mg/dL)	0.065	−0.018	*0.868*	0.003	0.077
APOB (mg/dL)	*0.928*	0.211	0.012	−0.128	0.013
PON1 (nmol min^{-1} mL^{-1})	0.192	0.233	*0.598*	−0.021	0.128
HDL_{LARGE} (%)	−0.148	*−0.942*	0.138	0.003	−0.045
HDL_{SMALL} (%)	0.145	*0.890*	0.171	−0.151	−0.063
LDL_{LARGE} (%)	0.293	0.193	−0.260	*0.781*	0.106
LDL_{SMALL} (%)	*0.480*	*0.353*	−0.197	*−0.630*	0.184
LDL size (Å)	*−0.388*	−0.232	0.122	*0.761*	−0.150
LDL(−) (U/L)	*0.350*	−0.091	0.180	0.178	*−0.545*
% Variance	29.4	15.4	13.3	13.0	10.8
% Cumulative variance	29.4	44.8	58.1	71.1	81.9

Results reported as loadings and most significant are in italics. Eigenvalue-greater-than-one rule was used for extraction of most significant PC and to explain the variance (%)

TC total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TAG* triacylglycerols, *Non-HDL-C* non-high-density lipoprotein cholesterol, *APOB* apolipoprotein B, *APOAI* apolipoprotein A–I, *PON1* paraoxonase 1, *LDL(−)* electronegative low-density lipoprotein

Table 3 Principal component analysis (PCA) for parameters related with LDL atherogenic profile

Variables $n = 63$	Principal Component (PC)	
	PC 1	PC 2
TC (mg/dL)	*0.892*	*0.364*
LDL-C (mg/dL)	*0.939*	0.158
TAG (mg/dL)	0.136	*0.909*
APOB (mg/dL)	*0.872*	*0.403*
LDL_{SMALL} (mg/dL)	*0.353*	*0.830*
LDL_{LARGE} (mg/dL)	*0.851*	0.038
LDL(−) (U/L)	*0.518*	*−0.455*
% Variance	51.0	29.2
% Cumulative variance	51.0	80.2

Results reported as loadings and most significant are in italics. Eigenvalue-greater-than-one rule was used for extraction of most significant PC and to explain the variance (%)

TC total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *TAG* triacylglycerol, *APOB* apolipoprotein B, *LDL(−)* electronegative low-density lipoprotein

eigenvalue-greater-than-one rule, proposed by Kaiser [28], the first two components were most significant for the explained 80.2 % of the total variance of original data.

The first principal component (PC1) represented 51 % of the variance in sample, and the second component (PC2) explained 29 %; both presented atherogenic pattern. In the PC1, positive loading coefficients were observed for the majority atherogenic variables [TC, LDL-C, APOB, LDL_{SMALL}, LDL_{LARGE}, and LDL(−)] as well as PC2 (TC, TAG, APOB, and LDL_{SMALL}) and negative for LDL(−).

Table 4 shows correlations between PCA data and the θ parameter, as measured with the Z-scan technique. Positive correlations between θ parameter and cardiometabolic biomarkers were obtained for PC with more anti-atherogenic patterns (PC3: $r = 0.30$; $p = 0.02$ and PC4: $r = 0.41$; $p = < 0.001$) and a negative correlation between PCs with a more atherogenic pattern (PC5; $r = -0.40$; $p = < 0.001$). Regarding the parameters related with atherogenic LDL profile, the θ parameter was negatively correlated with the PC2 ($r = -0.40$; $p = < 0.001$).

4 Discussion

The Z-scan technique is an alternative tool, which measures a parameter associated to the presence of modified LDL particles in the plasma of individuals with multiple cardiometabolic risk factors. The θ parameter can be used as a measurement of the oxidative state of LDL in human plasma in experimental and clinical setting, to understand changes associated with increased CVD risk.

Table 4 Correlations between θ parameter, as measured with the Z-scan technique, and principal component (PC) by principal component analysis (PCA) for cardiometabolic biomarkers and for parameters related with atherogenic LDL profile

Z-scan measures *versus* Principal Components (PC)	*r*	*p* value
PCA for cardiometabolic biomarkers		
PC1—atherogenic pattern	0.03	0.81
PC2—atherogenic pattern	0.02	0.88
PC3—anti-atherogenic pattern	0.30	*0.02*
PC4—anti-atherogenic pattern	0.41	*<0.001*
PC5—atherogenic pattern	−0.40	*<0.001*
PCA for parameters related with atherogenic LDL profile		
PC1—atherogenic pattern	0.19	0.13
PC2—atherogenic pattern	−0.40	*<0.001*

Results reported as coefficient correlations. *Spearman* correlations. Statistical significance $p < 0.05$

The results from this study showed for the first time an association between cardiometabolic biomarkers and parameters related with atherogenic LDL profile, summarized by PCA, and θ parameter.

LDL deposited in the artery wall shows a pivotal role in the initiation and development of atherosclerosis, and its chemical and structural modification is essential for its atherogenic profile [8]. Therefore, modified LDL can exhibit changes in APOB, its major protein, lipids (phospholipids, non-esterified cholesterol, and free triglycerides), and minor components (phenolic substances and liposoluble anti-oxidants), which gives a heterogeneous three-dimensional shape for this lipoprotein [30]. Despite this complex composition, cholesterol content in LDL particle is the most common parameter monitored in clinical practice and used in most predictive equation to estimate cardiovascular risk [31].

In addition, high levels of TAG have been associated with increased risk of myocardial infarction, ischemic heart disease, and death [32] and can also stimulate the cholesterol-ester transfer protein (CETP) activity, which promotes cholesterol transfer from HDL to particles rich in APOB, contributing to the decrease of HDL-C and generation of LDL_{SMALL} [33]. The LDL_{SMALL} are associated with increased CVD risk, since it can easier migrate into the subendothelial space and binds to proteoglycans with a higher affinity; this favors the permanence of these lipoproteins in the arterial wall. It also presents low affinity to the LDL receptor (B/E receptor), making it more susceptible to oxidation and glycation [8, 9, 12]. According to Nikolic et al. [34], individuals with high levels of small and dense LDLs have three to seven times higher risk to develop CVD, independently of their levels of LDL-C.

Regarding the dynamic interaction between the atherogenic LDL profile and high levels of TC, TAG, APOB, and LDL_{SMALL} during the development of atherosclerosis, the association between these parameters and the Z-scan measurements acquires a stronger relevance, because this method is able to identify the oxidative state of the LDL particle.

As stressed before, the Z-scan optical signal (the θ parameter) was determined by measuring the amplitude of peak-to-valley optical response to light absorption by samples. Its amplitude depends on the sample capacity to conduct heat. Recently, Santos et al. [20] assessed the influence of anti-oxidants on the amplitude of θ parameter, which depends on the α-tocopherol amount in LDL. Furthermore, during the oxidation process, APOB is modified and oxidative products (such as lipid hydroperoxides) are formed. These results suggest that both anti-oxidants in LDL and the oxidative products influence the total capacity of this lipoprotein in solution to absorb and conduct heat. These authors also showed that, in vitro, completely oxidized LDL does not show non-linear optical signal (i.e., $\theta = 0$), suggesting that oxidation increases thermal diffusivity.

Regarding the atherosclerosis inflammatory and oxidative basis, modified LDL has been associated with other diseases (cancer, rheumatoid arthritis, and systemic lupus erythematosus) [35–37]. Monteiro et al. [38] found that patients with periodontitis showed reduced values of θ parameter, as measure of Z-scan uses a non-linear optical technique to study the response of LDL. This result reinforces the fact that periodontitis increases the risk of CVD. However, the intensity of Z-scan signal (θ parameter) showed significant increase after 12-week odontological treatment.

LDL(−) is a modified LDL particle that has been described in the literature as a potential biomarker for CVD, obesity, and diabetes [39]. Interestingly, in the present study, LDL(−) content showed a bimodal contribution to the PCA results, in which LDL(−), as an early oxidative biomarker, presented positive loading coefficient in a more atherogenic pattern (PC1 for parameters related with atherogenic LDL profile) despite negative loading coefficient in others (PC2 for atherogenic LDL profile and PC5 for cardiometabolic biomarkers). The result observed in PC2 for atherogenic LDL profile reinforced the importance of LDL(−) as oxidative biomarker and the sensitivity of Z-scan technique to detect distinct LDL oxidation state in human plasma.

The ability of the Z-scan technique to show association with atherogenic and anti-atherogenic patterns for multiple cardiometabolic biomarkers reinforces the sensitivity of this tool in identifying multiple changes in atherogenic components present in plasma. The θ parameter was positively correlated with PC3, which presented positive loading coefficient of PON1, an anti-oxidant enzyme present in HDL responsible for hydrolysis of oxidative products in LDL [40]. In addition, positive loading coefficient levels to HDL-C and APOAI represent the protective pattern of PC3 since the HDL hallmark is to efficiently transport cholesterol to the liver contributing to decrease the number of small and dense LDLs [41]. In summary, this profile shows characteristics in the functionality of HDL particles. The complex composition of HDL (lipids, enzymes, and proteins) directly influences the protective functions of this lipoprotein, such as reverse transport of cholesterol, and anti-inflammatory, anti-thrombotic, and anti-oxidative activities [42].

The influence of cholesterol content in the lipoproteins and its relation to atherosclerosis is widely accepted in the literature. To expand this discussion, in this study, we investigated the relevance of new and potential biomarkers, as the size of lipoproteins in the atherogenic and anti-atherogenic patterns analyzed by PCA. Regarding this, the PC4 for cardiometabolic biomarkers (anti-atherogenic pattern) showed positive loading coefficients for LDL_{SMALL}. On the other hand, this same PC showed negative loading coefficient for LDL_{LARGE} and positive loading coefficient for TAG. Moreover, PC4 was correlated positively with the θ parameter.

Furthermore, the negative association between the θ parameter and PC5 for cardiometabolic biomarkers (negative loading coefficients for HDL-C and positive loading coefficient for TAG and glucose) indicates the reliability of this technique to detect a more atherogenic pattern in a sample.

5 Conclusion

In conclusion, the Z-scan technique is a tool able to identify the atherogenic and anti-atherogenic patterns in plasma from individuals with cardiovascular risk factors. Hereafter, it is possible that the Z-scan technique can be used as a measurement of the oxidative state of LDL in human plasma. Therefore, the Z-scan technique can be used as a complementary tool able to summarize multiple changes in cardiometabolic biomarkers.

Acknowledgments Antonio Martins Figueiredo Neto received grant support from the National Institute of Science and Technology Complex Fluids (INCT-FCx, 2010–2014), Research Nucleus Support in Complex Fluids at the University of Sao Paulo (NAP-FCx, 2011), and Support Foundation Research of Sao Paulo State (FAPESP-2011/13616-4). Nagila Raquel Teixeira Damasceno received grant support from Support Foundation Research of Sao Paulo State (FAPESP-2011/12523-2). Maria Camila Pruper de Freitas received grant scholarship from Coordination for the Improvement of Higher Education Personnel (CAPES).

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