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Associação de obesidade e subfrações de LDL avaliadas por índice de massa corpórea, circunferência abdominal e diabetes: o estudo Elsa – Brasil

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Cardiologia

Orientador: Prof. Dr. Márcio Sommer Bittercourt

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Association of obesity and LDL subfractions evaluated by body mass index, waist circumference, and diabetes status: The ELSA-Brasil study

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CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BMI	Body mass index
CHD	Coronary heart disease
CETP	Cholesteryl ester transfer protein
CT	Computed tomography
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ELSA-Brasil	Brazilian Longitudinal Study for Adult Health
FFAs	Free fat acids
HDL	High-density lipoprotein
HDL-c	High-density lipoprotein-cholesterol
HF	Hypercholesterolemia
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HOMA-IR	Homeostasis model assessment-estimated insulin resistance
HSL	Hormone-sensitive lipase
IBGE	*Instituto Brasileiro de Geografia e Estatística*
lbLDL	large and buoyant low-density lipoprotein
lbLDL-c	large buoyant low-density lipoprotein-cholesterol
IQR	Interquartile range
IR	Insuline resistance
LDL	Low-density lipoprotein

LDL-c	Low-density lipoprotein-cholesterol
LPL	Lipoprotein lipase
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
sdLDL	small and dense low-density lipoprotein
sdLDL-c	small dense low-density lipoprotein-cholesterol
SD	Standard deviation
T2D	Type 2 diabetes
TG	Triglycerides
UC	Ultracentrifugation
VAP	Vertical auto profile
VAT	Visceral adipose tissue
VLDL	Very-low density lipoprotein
WC	Waist circumference

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RESUMO

Moraes EG. *Associação de obesidade e subfrações de LDL avaliadas por índice de massa corpórea, circunferência abdominal e diabetes: o estudo Elsa – Brasil* [Tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo, 2023.

Introdução: As lipoproteínas de baixa densidade (LDL) compreendem um pool de partículas com diferentes densidades. Estudos sugerem que indivíduos obesos com índice de massa corpórea (IMC) e circunferência abdominal (CA) elevados apresentam subfrações pequenas e densas de LDL (sdLDL-c) aumentadas. Não está claro se o diabetes (DM2) e a resistência à insulina (RI) podem modificar essa associação. **Objetivos:** Procuramos esclarecer a relação entre obesidade e sdLDL-c, bem como a interferência do diabetes e da resistência insulínica nessa relação. **Métodos:** Foram incluídos 4.111 (50,4±8,6 anos de idade, 45,5% homens) indivíduos sem doença cardiovascular prévia nem uso de medicamentos hipolipemiantes. O LDL-c total e suas subfrações (LDL1-c, LDL2-c, LDL3-c e LDL4-c) foram medidos por ultracentrifugação zonal vertical. Consideramos as subfrações LDL1-c e LDL2-c como LDL grande flutuante (lbLDL-c) e as subfrações LDL3-c e LDL4-c como sdLDL-c. Analisamos a associação entre as subclasses de LDL-c, IMC e CA por meio de análise de regressão linear e estratificada pela presença de DM2 e RI. **Resultados:** Para sdLDL-c, observou-se associação direta com hipertensão, DM2, glicemia de jejum, colesterol total, LDL-c e triglicerídeos. Na análise multivariada, após ajuste para idade, sexo, raça e triglicerídeos, a forte associação de sdLDL-c com IMC (β 95% CI 0,16 (0,13 – 0,19)) e CA (β 95% CI 0,22 (0,19) - 0,26)) persistiu. Após estratificação, a associação de sdLDL-c e CA esteve presente apenas naqueles com resistência à insulina ou diabetes. O IMC teve um impacto menor do que a CA nessa associação. **Conclusões:** CA e IMC foram fortemente associados com subfrações de sdLDL-c. Além disso, essa associação foi modificada pelo diabetes e pelo estado de resistência à insulina.

Descritores: Lipoproteínas LDL**;** Obesidade; Índice de massa corporal,; Circunderência da cintura.

ABSTRACT

Moraes EG. *Association of obesity and LDL subfractions evaluated by body mass index, waist circumference, and diabetes status: The ELSA-Brasil study* [Thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo", 2023.

Introduction: Low-density lipoproteins (LDL) comprise a pool of particles with different densities. Studies suggest that obese individuals with elevated body mass index (BMI) and waist circumference (WC) have increased small and dense LDL subfractions (sdLDL-c). It is unclear if diabetes (T2D) and insulin resistance (IR) may modify this association. **Objectives:** We aimed to clarify the relationship between obesity and sdLDL-c, as well as the interference of diabetes and insulin resistance in this relationship. **Methods:** We included 4,111 (50.4±8.6 years of age, 45.5% men) individuals with neither prior cardiovascular disease nor use of lipid-lowering medications. Total LDL-c and its subfractions (LDL_1-c, LDL_2-c, LDL_3-c and LDL_4-c) were measured by vertical zonal ultracentrifugation. We considered the subfractions LDL_1-c and LDL_2-c as large buoyant LDL (lbLDL-c) and the subfractions LDL_3-c and LDL_4-c as sdLDL-c. We analyzed the association between LDL-c subclasses, BMI and WC using linear regression analysis and stratified by the presence of T2D and IR. **Results:** For sdLDL-c, a direct association with hypertension, T2D, fasting plasma glucose, total cholesterol, LDL-c and triglycerides was observed. In multivariate analysis, after adjustment for age, sex, race and triglycerides, the strong association of sdLDL-c with BMI (β 95% CI 0.16 (0,13 – 0,19)) and WC (β 95% CI 0.22 (0.19 - 0.26)) persisted. After stratification the association of sdLDL-c and WC was present only in those with insulin resistance or diabetes. BMI showed a smaller impact than WC on this association. **Conclusions:** WC and BMI were strongly associated with sdLDL-c subfractions. Further, this association was modified by diabetes and insulin resistance status.

Descriptors: Lipoproteins LDL; Obesity; Body mass index; Waist circumference.

1 INTRODUCTION

1 INTRODUCTION

Cardiovascular disease is the leading cause of death in the world. Despite a rate decline recently, they still are responsible for about one in every three deaths in the United States of America. According to DATASUS there is a similar scenario in Brazil. The most common clinical presentations are in the form of atherosclerotic coronary artery disease, ischemic stroke, and peripheral artery disease. In 2019, Brazilian data demonstrated about 330,000 deaths related to atherosclerotic events.

These clinical manifestations have atherosclerosis as a common pathophysiological mechanism. This is a chronic inflammatory disease with a pathway of irritative aggressions resulting in endothelial dysfunction and structural changes. These abnormalities allow the low-density lipoproteins particles to the subendothelial space and LDL-cholesterol to deposit in the arterial wall, followed by immune system cells infiltration, macrophages, and smooth muscle cells activation, inflammation and, thus, development of the atherosclerotic plaque.

This association between atherosclerosis and elevated plasma cholesterol levels is also well known for scenarios such as in patients with familial hypercholesterolemia (HF), who have high LDL cholesterol levels and early coronary heart disease (CHD)[1]. In particular, LDL cholesterol has been shown to be an independent predictor of premature CHD in many studies[2] and statin therapy can safely reduce the 5-year incidence of major coronary events, coronary revascularization, and stroke by about one fifth per mmol/L reduction in LDL cholesterol, largely irrespective of the initial lipid profile or other presenting characteristics[3].

Plasma cholesterol is distributed in five different classes of lipoprotein classes: 1) high - density lipoprotein (HDL); 2) low - density lipoprotein (LDL); 3) very - low density

lipoprotein (VLDL); 4) intermediate - density lipoprotein (IDL) and 5) lipoprotein(a) (Lp(a)). Recent studies have shown that LDL cholesterol is not a single lipoprotein, but a pool of cholesterol particles of different densities. LDL lipoproteins are heterogeneous in their density, size, chemical composition, and atherogenic potential. These different subtypes can be grouped into several subfractions by different methods. There are some standardized methods for identifying LDL subfractions. They are: I) gradient gel electrophoresis using non-denaturing conditions. It is commonly used to characterize the LDL distribution by particle size; II) high-performance gel filtration chromatography determines LDL subfractions by particle size; III) magnetic resonance spectroscopy and IV) vertical ultracentrifugation technique[4]. Despite the variable number of subfractions according to the methodology used, several studies have stratified them in two major fractions: large and buoyant (lbLDL) and small and dense (sdLDL)[5].

Obesity, defined as an excess of body fat and commonly measured by body mass index (BMI), is a prevalent condition worldwide. It is linked to increased cardiovascular disease risk, mainly due to its comorbidities, such as hypertension, insulin resistance, type 2 diabetes (T2D), and dyslipidemia[6,7].

Dyslipidemia in obesity, especially visceral obesity, is characterized by elevated triglycerides (TG) levels, low high-density lipoprotein (HDL) and predominance of the small and dense low lipoprotein (sdLDL) distribution pattern, forming the so-called atherogenic lipid triad[8,9] which is known as metabolic dyslipidemia[8].

Obesity is related to increased sdLDL[10]. Many epidemiological studies have demonstrated a strong association of indices of obesity, such as visceral adipose tissue (VAT)[11], subcutaneous adipose tissue (SAT), waist circumference (WC) and body mass index (BMI), with coronary heart disease (CHD) and its risk factors[12-14]. Evidence suggests that body fat distribution, such as SAT and VAT, are more strongly associated

with CHD when compared to BMI or WC. Finally, abdominal fat volume measured by computed tomography (CT) scanning is positively associated with sdLDL-c but inversely associated with large buoyant LDL-c (lbLDL-c)[15].

Evidence suggests that insulin resistance is the most probable link between obesity and obesity-associated metabolic dyslipidemia[8]. Insulin resistance and metabolic dyslipidemia are associated with adiposopathy[16].

It is believed that the most important molecular mediators of obesity-related insulin resistance are adipokines, produced by adipocytes and accumulated macrophages in adiposopathy[17] which are insulin-resistant and increases lipolysis and release of free fatty acids (FFAs) into the circulation. Increased FFAs concentration provokes lipotoxicity, as another mechanism of obesity related insulin resistance in non-adipose tissue[18].

Insulin suppresses lipolysis in adipose tissue by hormone-sensitive lipase (HSL) inhibition, thereby controlling the release of FFAs into the circulation[19]. It also suppresses very low-density lipoproteins (VLDL) secretion from the liver[20]. In the circulation, lipoprotein lipase (LPL)-driven hydrolysis of TG from VLDL particles is stimulated by insulin, as well as the activity of hepatic lipase (HL), so overall, insulin stimulates TG-rich lipoprotein degradation. In the liver, insulin promotes dephosphorylation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, activating the enzyme and stimulating the rate of cholesterol synthesis[19]. In the state of insulin resistance, plasma clearance of TG-rich lipoproteins is delayed, resulting in hypertriglyceridemia. Under these circumstances, cholesteryl ester transfer protein (CETP) activity promotes the exchange of TG with cholesteryl esters between lipoprotein particles. As a result, LDL and HDL particles become enriched with TG and, after subsequent hydrolysis by plasma lipases, smaller and denser. These structural changes are accompanied by functional

consequences, resulting in the accumulation of small, dense (sdLDL) and dysfunctional HDL particles[21].

The sdLDL-c levels are also elevated in diabetic dyslipidemia, leading to increased cardiovascular disease (CVD) risk in this population[22,23]. It is also known that this association is related to BMI and WC, with the latter being a better predictor[24]. Due to known metabolic pathways involving lipoprotein lipase inhibition, increased hepatic lipase activity on HDL and LDL, T2D and IR play an important role in the potential lipid profile changes in obese individuals[8,9,25].

2 OBJECTIVE

2 OBJECTIVE

In the present study we aimed to analyze the association between LDL-c subfractions, and obesity assessed by BMI and WC and how this could be modified by the presence or absence of T2D and IR in a multiethnic Brazilian cohort.

3 METHODS

3 METHODS

3.1 Sample

Between August 2008 and December 2010, 15,015 men and women were enrolled in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), a prospective longitudinal cohort composed of civil servants aged 35 to 74 years from six Brazilian cities. The study excluded servants with severe cognitive impairment or communication difficulties; pregnancy or recent childbirth (less than four months before the first interview); and, if retired, living outside of a study center's metropolitan area. The volunteers went to the study centers for evaluation and tests. They were also evaluated on in their respective workplaces for collection of sociodemographic reports, medical history, occupational exposure, familial medical history, reproductive health, medical assistance, psychosocial factors, body weight history, food and alcohol consumption, smoking status, physical activity, medicine use, cognitive function and mental health. In all centers, the participants were attended by trained professionals under strict quality control[26,27]. We included all participants from the São Paulo center (n = 5,061) who underwent LDL-c measurement using the vertical auto profile (VAP) method. Exclusion criteria for the present analysis were the lack of serum measurement of any component of the lipid profile, previous history of cardiovascular disease (myocardial infarction, stroke, heart failure and coronary revascularization), and participants using any lipid lowering drug at baseline. After applying these exclusion criteria, the total sample size was n = 4,111 **(Figure 1)**.

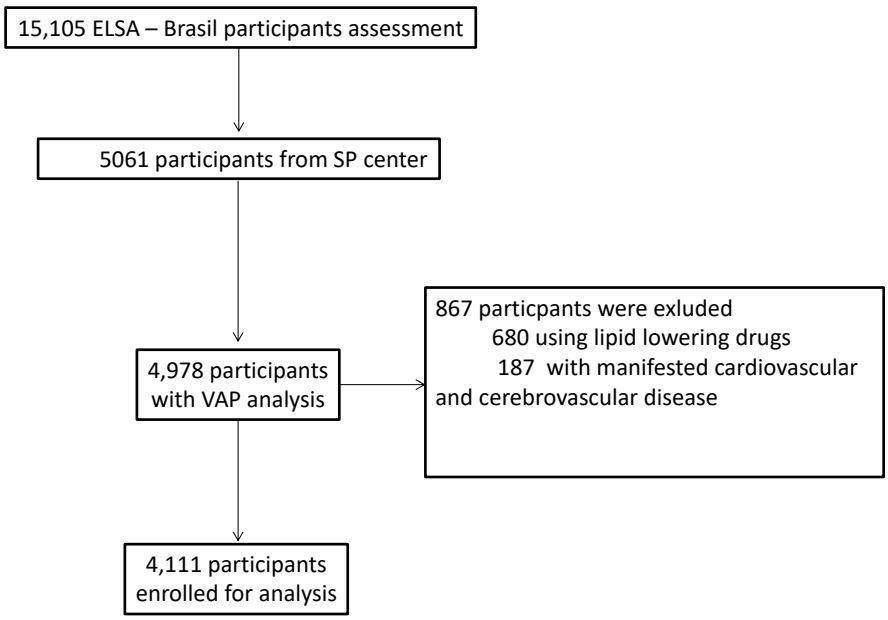


Figure 1 – Sample study flowchart
Source: Personal archive

3.2 Race

Race was defined in a self-declared answer according to the terms used in the IBGE (*Instituto Brasileiro de Geografia e Estatistica*) census "black", "brown", "white", "yellow" and "indigenous".

3.3 Blood analysis including LDL-c and subfractions

Blood collection of the participants was performed by venipuncture following nocturnal fasting. Samples were then centrifuged in each research center and stored in tubes at -80^{o}C to be transported to the São Paulo center. After blood collection during nocturnal fasting, the samples were centrifuged at the sites and stored in tubes at - 80°C.

LDL-cand its subfractions LDL_1-c, LDL_2-c, LDL_3-c and LDL_4-c were measured by the VAP method (Atherotech®), a gradient ultracentrifugation (UC) method with inverted rate zonal, single vertical spin, that simultaneously measures cholesterol concentrations after fraction separation and has a close correlation to the conventional method[5]. After the UC step, cholesterol of each subfraction was measured by enzymatic methods. We considered the subfractions LDL_1-c and LDL_2-c as large buoyant LDL (lbLDL-c) and the subfractions LDL_3-c and LDL_4-c as small and dense LDL-c (sdLDL-c). In addition, we also used the sdLDL-c/ total LDL-c ratio, as a log-transformed variable due to its normal distribution. It represents the amount of sdLDL-c in LDL-c. the CRP level was measured using a high-sensitivity assay by immunochemistry–nephelometry (BN II; Siemens).

3.4 Hypertension

Defined as systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg or verified treatment with anti-hypertensive medication during the last 2 weeks.

3.5 Diabetes

T2D diagnosis was defined as a reported history of diabetes mellitus, insulin use or any oral antidiabetic drugs. Also, l laboratory criteria included fasting plasma glucose ≥ 126 mg/dl; HbA1c levels ≥ 6.5% or a 2-hour oral glucose tolerance test ≥ 200 mg/dl.

3.6 Insulin resistance

The homeostasis model assessment-estimated insulin resistance (HOMA-IR) value was obtained by fasting blood glucose X 0.0555 X fast blood insulin/22.5[28].

3.7 Hypercholesterolemia

Hypercholesterolemia was defined as a low-density lipoprotein cholesterol (LDL-C) level ≥130 mg/dL.

3.8 Obesity measurements

3.8.1 Body Mass Index

Based on measured height and weight obtained on ELSA-Brasil, calculated by dividing the weight by the square of the height.

3.8.2 Waist circumference

WC was measured with a measuring tape equidistant from the lower margin of the rib and the iliac crest[29].

3.9 Other variables

3.9.1 Smoking

Participants were classified as never smokers, current smokers and former smokers, that is if the participant stopped smoking, but his/her tobacco exposure was more than 100 cigarettes throughout life.

3.10 Statistical analysis

Continuous variables are presented as mean and standard deviation (±SD) if normally distributed or as median (interquartile range, IQR) if non-normal distributed, while categorical variables were presented as absolute and relative frequencies. Data are displayed by four BMI strata (< 18.5; 18.5-25; 25-30 and > 30 kg/m^2), sdLDL-c upper (above p50=53.4) and lower (below p50=53.4) than the 50^{th} percentile and lbLDL-c upper (above p50=41.2) and lower (below p50=41.2) the 50^{th} percentile.

We performed comparisons of quantitative variables across groups using analysis of variance (ANOVA) or Kruskal-Wallis test according to their distributions. Categorical variables were analyzed by the Chi square test ($\chi 2$). As a correlation measurement, we used the Spearman test in bivariate analysis and, to assess the association between LDL-c subfractions and other variables, we constructed bivariate and multivariate linear regression models. For these models, we standardized LDL-C, LDL_1-c, LDL_2-c, LDL_3-c, LDL_4-c and LDL-c log ratio. Multiple linear regression models were adjusted for race, sex, age and triglycerides. Further, we constructed a sensitivity analysis stratifying by T2D status and IR.

Statistical significance was defined as $p < 0.05$. All analyses were performed with Stata version 14.0 (StataCorp,USA).

3.11 Ethical aspects and funding

The ELSA-Brasil protocol complied with the main Resolution 196/96, Resolution CNS 346/05 (multicentric projects) and Resolution CNS 347/05 (biologic material storage). In addition, the protocol was approved by each institution's committee of ethical research involved and by the National Committee of Ethics in Research of the National Health Council (CONEP).

The Ethical Committee, Recruitment and Social Communication helps in the coordination of accomplishments of ethical aspects and communication with the study involved institutions and with their participants.

All the volunteers were oriented about the longitudinal study design during Phase 1 of the cohort and subscribed to the consent form. The consent also allows access to their medical records, in both health institutions and under their doctor's control. A decision to refuse to remain in the study is always sovereign and respected.

The ELSA-Brasil study was funded, by the *Ministério da Saúde, Departamento de Ciência e Tecnologia (DECIT), Ministério de Ciência e Tecnologia, Financiadora de Estudos e Projetos (Finep*) and by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)*, under the process 01 06 0010.00 RS, 01 06 0212.00 BA, 01 060300.00 ES, 01 06 0278.00 MG, 01 06 0115.00 SP e 01 06 0071.00 RJ.

4 RESULTS

4 RESULTS

We included 4,111 participants with a mean age of 50.4 (±8.6) years-old, and 1,871 (45%) were males. As expected, we observed a positive association between higher body mass index (BMI) and waist circumference (WC), triglycerides, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting glucose, LDL-C and total cholesterol and hypertension, while there was an inverse association between BMI and HDL-c **(Table 1)**.

Table 1 – Baseline demographic characteristics of the studied sample

Clinical Profile	**n = 4.111**	**Body Mass Index**				
Mean (±SD) or n (%)	Total	< 18.5	18.5 - 25	25 - 30	> 30	P Value
Age (years)	50.4 (±8.6)	48.8 (±8.7)	49.98 (±8.7)	50.7 (±8.5)	50.57 (±8.5)	0.0473
Male (n, %)	1871 (45.5)	20 (51.2)	639 (43.1)	835 (50.8)	377 (39.8)	
Race (n, %)						
White	2,382 (57.9)	29 (74.3)	905 (61.0)	944 (57.4)	504 (53.2)	
Black	580 (14.1)	2 (5.1)	165 (11.1)	237 (14.4)	176 (18.5)	
Brown	897 (21.8)	4 (10.2)	292 (19.7)	371 (22.5)	230 (24.2)	
Other	203 (4.9)	4 (10.2)	106 (7.1)	68 (4.1)	25 (2.6)	
Hypertension (n, %)	1,099 (26.75)	2 (5.13)	249 (16.80)	469 (28.55)	379 (40.11)	
SBP (mmHg)	119.1 (±16.48)	111.9 (±16.7)	115.1 (±15.8)	120.2 (±16.07)	123.7 (±16.2)	< 0.0001
DBP (mmHg)	75.1 (±10.8)	69.89 (±11.7)	71.8 (±10.4)	75.4 (±10.0)	80.0 (±10.6)	
T2D (%)	688 (16.7)	4 (10.2)	143 (9.6)	261 (15.9)	280 (29.5)	
FPG (mg/dL)	109.5 (±27.4)	100.2 (±8.0)	104.0 (±18.9)	109.4 (±25.0)	118.9 (±38.4)	
HbA1c (%)	5.42 (±0.91)	5.39 (±0.56)	5.30 (±0.73)	5.39 (±0.87)	5.68 (±1.17)	
Smoking (n, %)						
Current	687 (16.7)	14 (35.9)	290 (19.6)	268 (16.3)	115 (12.1)	
Former	1215 (29.5)	3 (7.7)	364 (24.5)	549 (33.4)	299 (31.5)	
Total Cholesterol (mg/dL)	215.0 (±41.7)	194.6(±33.3)	210.7 (±40.0)	216.8 (±40.3)	219.5 (±45.9)	

Continue

Continued

LDL-c (mg/dL)	132.40(±34)	112.2 (±24.1)	128.7 (±32.7)	134.5 (±33.9)	135.3 (±36.5)	
HDL-c (mg/dL)	56.44 (±14.55)	64.82 (±16.71)	60.16 (±15.3)	55.03 (±14.1)	52.74 (±12.45)	
Triglycerides (mg/dl)	110 (79 - 159)	84 (65 - 105)	92 (69 - 127)	117 (84 - 169)	136 (99 - 183)	
LDL1-c (mg/dL)	22.6 (±9.5)	20.5 (± 8.9)	22.3 (±9.8)	22.8 (±9.4)	22.7 (±9.1)	0.2266
LDL2-c (mg/dL)	32.8 (±17.9)	36.5 (±12.6)	35.4 (±17.2)	31.6 (±18.1)	30.7 (±18.3)	
LDL3-c(mg/dL)	47.1 (±20.2)	33.8 (±12.6)	43.6 (±19.6)	48.7 (±20.1)	50.3 (±20.6)	
LDL4-c(mg/dL)	10.4 (±12.1)	5.3 (± 5.1)	8.7 (±9.1)	11.3 (±11.1)	11.98 (±12.1)	< 0.0001
lbLDL-c (mg/dL)	55.4 (±24.1)	57.0 (±18.9)	57.76 (±23.8)	54.41 (±24.2)	53.4 (±24.2)	
sdLDL-c (mg/dL)	57.6 (± 26.6)	39.1 (±14.3)	52.3 (±25.2)	60.0 (±26.8)	62.3 (±27.1)	
WC (cm)	89.26 (±12.4)	67.8 (±6.0)	78.8 (±7.1)	90.6 (7.1)	104.08 (±9.7)	

SD: standard deviation; % percentage; BMI: body mass index; FPG: fasting plasma glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1C: glycated hemoglobin WC: waist circumference; lbLDL-c: large buoyant low-density lipoprotein-cholesterol; sdLDL-c: small dense low-density lipoprotein-cholesterol. Total cholesterol, LDL-c, HDL-c, and TG were determined by conventional analysis (not VAP).

For sdLDL-c, we observed a worse profile for all traditional risk factors for cardiovascular disease, including SBP, DBP, T2D, fasting plasma glucose, total cholesterol, LDL-c, triglycerides, and hypertension, in individuals above the 50^{th} percentile for sdLDL-c (p50 = 53.4). Also, there was an association between male sex and lower HDL-c levels **(Table 2)**. These findings contrasted with the large buoyant LDL (lbLDL-c), where values above the 50^{th} percentile were associated with a lower prevalence of traditional risk factors and higher HDL-c levels. Additionally, individuals with higher levels of lbLDL-c were more likely to be females **(Table 3)**. For lbLDL-c we found an inverse association with both BMI and WC whereas the opposite was found concerning sdLDL-c.

Table 2 – Sample characteristics according to small dense LDL-c percentiles

Clinical Profile	Small-dense LDL-c		P-value
	p<50th	p>50th	
Age (years)	50.0 (±8.6)	50.8 (±8.5)	< 0.0001
Male (%)	663 (32.5)	1208 (58.2)	
Race			
White (%)	1197 (59.4)	1185 (57.9)	
Black (%)	302 (15.0)	278 (13.6)	0.104
Brown (%)	431 (21.4)	466 (22.8)	
Others (%)	85 (4.2)	118 (5.7)	
Total Cholesterol (mg/dL)	199.5 (±38.4)	230.3 (± 39.1)	0.0025
LDL-c(mg/dL)	117.6 (±28.7)	147.5 (±32.5)	
HDL-c (mg/dL)	61.1 (±15.3)	51.9 (±12.1)	
Triglycerides (mg/dl)	88 (67 - 119)	140 (102 - 192)	< 0.0001
T2D (%)	272 (13.35)	416 (20.07)	
FPG (mg/dL)	105.5 (±21.0)	113.5 (±32.0)	
HbA1c (%)	5.37 (±0.83)	5.47 (±0.98)	0.0006
Hypertension (%)	449 (22.0)	650 (31.3)	
SBP (mmHg)	116.6 (±16.3)	121.6 (±16.1)	
DBP (mmHg)	73.3 (±10.6)	76.89 (±10.6)	< 0.0001
Smoking			
Current (%)	309 (15.2)	378 (18.2)	
Former (%)	566 (27.8)	649 (31.3)	
WC (cm)	85.92 (±12.42)	92.54 (±11.60)	

SD: standard deviation; % percentage; BMI: body mass index; DLP: Dyslipidemia; SBP: systolic blood pressure; DBP: diastolic blood pressure; glucose: fasting glucose; HbA1C: glycated hemoglobin WC: waist circumference. Total cholesterol, LDL-c, HDL-c and TG were determined by conventional analysis (not VAP).

Table 3 – Sample characteristics according to large and buoyant LDL-c percentiles

Clinical Profile	Large buoyant LDL-C		P value
	p<50th	p>50th	
Age	49.5 (±8.6)	51.1 (±8.5)	< 0.0001
Male (n, %)	1,120 (59)	751 (33.9)	
Race (n, %)			
White (%)	1,036 (54.58)	1346 (60.82)	0.001
Black (%)	285 (15.02)	295 (13.33)	
Brown (%)	457 (24.08)	440 (19.88)	
Others (%)	100 (5.27)	103(4.65)	
Total Cholesterol (mg/dL)	191.1 (±41.2)	228.65 (± 37.0)	
LDL-c(mg/dL)	117.0 (±29.9)	145.6 (±32.1)	
HDL-c (mg/dL)	50.2 (±12.2)	61.7 (±14.2)	< 0.0001
Triglycerides (mg/dl)	103 (70 - 160)	116 (86 - 158)	
T2D (n, %)	387 (20.4)	301 (13.6)	
FPG (mg/dL)	110.87 (±32.0)	108.39 (±22.4)	0.003
HbA1c (%)	5.417 (±1.02)	5.43 (±0.81)	0.55
Hypertension (n, %)	562 (29.6)	537 (24.2)	
SBP (mmHg)	121.1 (±16.6)	117.3 (±15.9)	< 0.0001
DBP (mmHg)	76.44 (±10.9)	73.9 (±10.5)	
Smoking (n, %)			
Current (%)	344 (18.1)	343 (15.5)	0.002
Former (%)	590 (31.1)	625 (28.2)	
WC (cm)	90.8 (±12.4)	87.8 (±12.2)	< 0.0001

SD: standard deviation; % percentage; BMI: body mass index; DLP: Dyslipidemia; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1C: glycated hemoglobin WC: waist circumference. Total cholesterol, LDL-c, HDL-c and TG were determined by conventional analysis (not VAP).

When evaluating each LDL-C subfraction, we noted that LDL_2-c level decreases as BMI increases, while the LDL_1-c concentration was similar across BMI groups. The LDL_2-c levels drove the overall inverse association between lbLDL-c and BMI. On the other hand, both LDL_3-c and LDL_4-c increase as BMI rises.

In the linear regression models, we observe a strong correlation of LDL-c with BMI and WC. For sdLDL-c, the association with BMI and WC was also strong, but not between lbLDL-c and BMI or WC. Also, there was a robust association of the LDL-c log ratio with BMI and WC. In multivariate analysis, these findings were consistent even after adjustment for age, sex, race, and triglycerides **(Table 4, Figures 2 and 3)**.

Table 4 – Univariate and Multivariate linear regression for the association of LDL-c, lbLDL, sdLDL and LDL-c log ratio with BMI and WC

Lipid profile (per 1SD)	**BMI**		**WC**	
	Bivariate	**Multivariate**	**Bivariate**	**Multivariate**
	β (95% CI)		**β (95% CI)**	
LDL-c (mg/dl)	0.08 (0.05; 0.11)	0.06 (0.03; 0.09)	0.12 (0.09; 0.15)	0.09 (0.05 -; 0.12)
lbLDL-c (mg/dl)	0.03 (0.004; 0.064)	0.001 (- 0.02; 0.03)	0.0006 (-0.03; 0.03)	0.03 (-0.03; 0.03)
sdLDL-c (mg/dl)	0.15 (0.12; 0.18)	0.05 (0.02; 0.08)	0.28 (0.25; 0.31)	0.09 (0.06; 0.12)
LDL-c log ratio (sdLDL-c/LDL-c)	0.088 (0.05; 0.11)	0.04 (0.01; 0.07)	0.20 (0.17; 0.23)	0.07 (0.04; 0.10)

LDL -C: LDL cholesterol; lbLDL: large buoyant LDL subfractions; sdLDL: small and dense LDL subfractions. The multivariate analyses were adjusted for sex, age, race, and triglycerides.

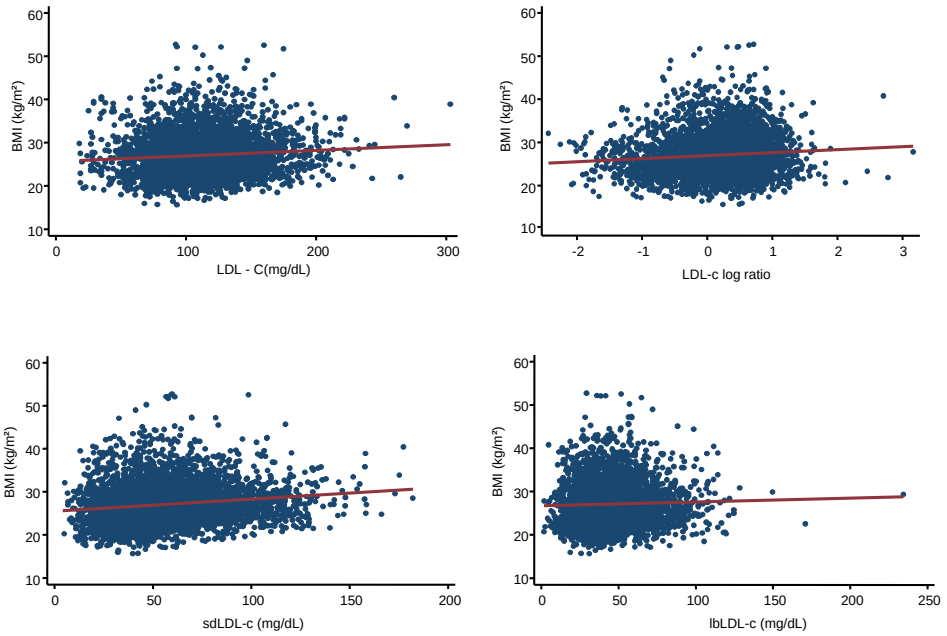


Figure 2 – Correlation between BMI and LDL-c, LDL-c log ratio, sdLDL-c and lbLDL-c.
Source: Personal archive

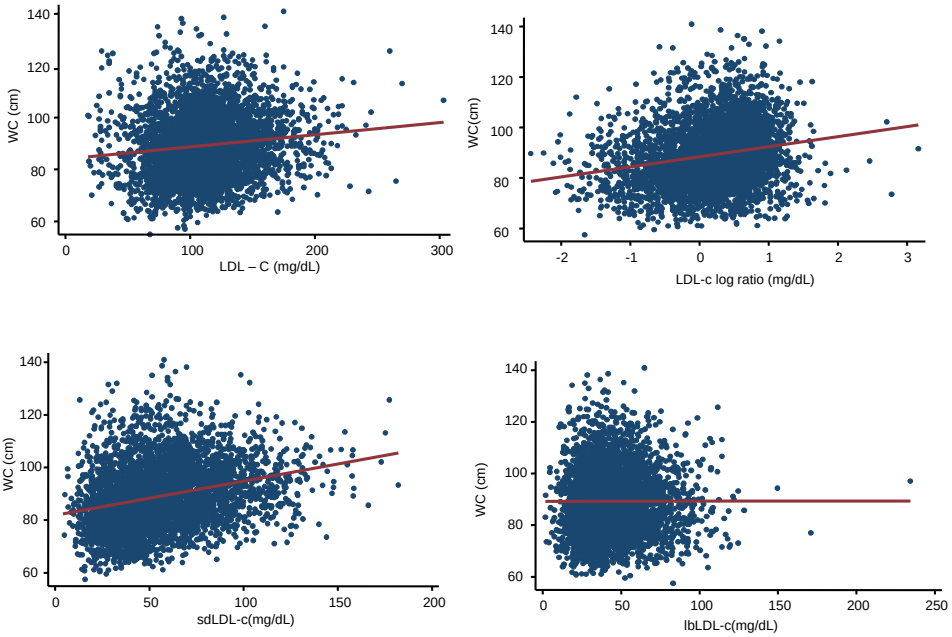
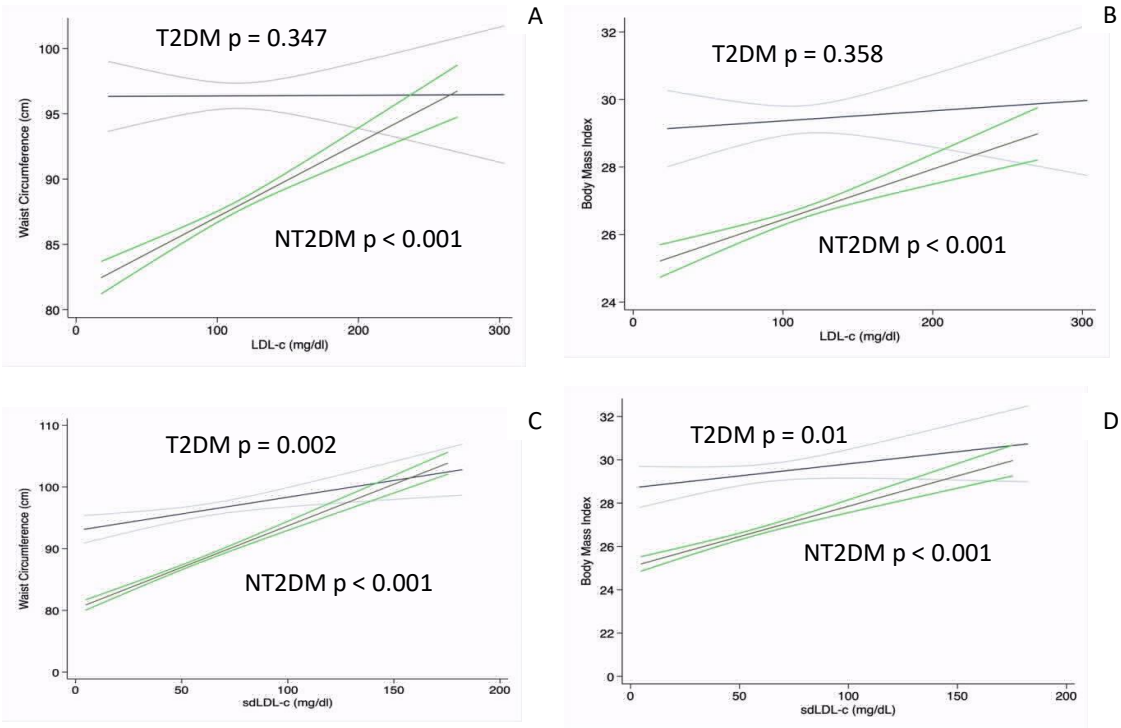


Figure 3 – Correlation between WC and LDL-c, LDL-c log ratio, sdLDL-c and lbLDL-c.
Source: Personal archive

Further, we observed that the T2D modifies the association between sdLDL-c and both WC and BMI **(Figure 4)**, since this association was especially stronger in non-T2D diabetes even after adjustment for age, sex, race, and triglycerides **(Table 5)**. For the lbLDL-c interaction was positive with WC but negative with BMI. We also constructed multiple regression models stratified by IR ($p<50^{th}$ and $p\geq50^{th}$) that corroborated the findings **(Tables 6 and 7)**.



T2DM – type 2 diabetes; NT2DM – nondiabetic

Figure 4 – Univariate association of the LDL-c and sdLDL-c with BMI and WC according to T2D diagnosis
Source: Personal archive

Table 5 – Multivariate logistic regression for the association of lbLDL-c, sdLDL-c with BMI and WC

sdLDL-c (per 1SD increase)	**Non-T2D**	**T2D**	**Interaction p-value**
WC	0.18 (0.12; 0.25)	0.008 (-0.12; -0.14)	< 0.001
BMI	0.31 (0.15; 0.47)	0.003 (-0.31; 0.31)	0.038
lbLDL-c (per 1SD increase)	**Non-T2D**	**T2D**	**interaction p-value**
WC	0.001 (-0.05; 0.05)	-0.09 (-0.18; -0.001)	0.009
BMI	0.015 (-0.11; 0.14)	-0.17 (-0.39; 0.04)	0.11

Results are presented in β (95% CI). lbLDL: large buoyant LDL subfractions; sdLDL: small and dense LDL subfractions; WC: waist circumference; BMI: body mass index.

Table 6 – Univariate linear regression for the association of sdLDL-c and lbLDL-c with WC and BMI

	HOMA IR		
sdLDL-c (per 1 SD increase)	**p<50**	**p>50**	**interaction p-value**
	β (-/SD)	**β (-/SD)**	
WC	.27 (.05)	.08 (.05)	< .001
BMI	.58 (.13)	.04 (.11)	.001
lbLDL-c (per 1 SD increase)	**p<50**	**p>50**	**interaction p-value**
	β (-/SD)	**β (-/SD)**	
WC	.05 (.04)	.04 (.03)	.019
BMI	.08 (.08)	.08 (.08)	.038

lbLDL-c: large and buoyant LDL-c; WC: waist circumference; BMI: body mass index; SD: standard deviation

Table 7 – Multivariate linear regression for the association of sdLDL-c and lbLDL-c with WC and BMI

HOMA IR			
sdLDL-c (per 1 SD increase)	**p<50**	**p>50**	**interaction p-value**
	β (-/SD)	**β (-/SD)**	
WC	0.27 (0.17; 0.38)	0.08 (-0.01; 0.18)	< .001
BMI	0.58 (0.32; 0.84)	0.04 (-0.18; 0.27)	0.001
lbLDL-c (per 1 SD increase)	**p<50**	**p>50**	**interaction p-value**
	β (-/SD)	**β (-/SD)**	
WC	0.05 (-0.03; 0.14)	0.04 (-0.03; 0.11)	.019
BMI	0.17 (-0.04; 0.40)	0.08 (-0.08; 0.24)	.038

lbLDL-c: large and buoyant LDL-c; WC: waist circumference; BMI: body mass index; SD: standard deviation

5 DISCUSSION

5 DISCUSSION

The main findings of this cross-sectional study were that WC and BMI were strongly associated with small and dense LDL-c subfractions, but only weakly associated with large buoyant LDL-c subfractions. After stratification for T2D, the association of the sdLDL-c with WC and BMI was more pronounced in participants without diabetes. These data suggest that part of the effect of obesity on LDL-c is related to a specific profile of LDL-C subfractions and that T2D and insulin resistance may play a role in the obese population, directly influencing LDL subfractions distribution. It is well known that insulin resistance and diabetes influence LDL subfraction distribution (i.e., IR promotes formation of small, dense LDL particles in the setting of lipoprotein lipase inhibition, and increased activity of hepatic lipase and cholesteryl ester transfer protein (CETP)[30, 31].

Dyslipidemia is the most common comorbidity associated with obesity, followed by hypertension and T2D[6]. This study performed in a multi-ethnic population confirms previous observations[9,32,33] of the predominance of small dense LDL pattern and its association with obesity related risk factors. The association between BMI and sdLDL-c has been addressed in the past. In some studies, only normoinsulinemic men[34] were included, in other normoinsulinemic men and normoinsulinemic women[10], in different ethnic groups and with different sdLDL analysis methodologies[35,36]. Our data corroborate previous findings in which the higher the BMI the higher the percentage of LDL-C that is small and dense. Unlike other studies however, this used the VAP methodology and not the sdLDL particle analysis. In comparison with previous studies our data refers to a broader sample, including both genders, different ethnicities, and people with and without T2D. Finally, we also found a more intense association between sdLDL and WC than with BMI, probably because the former has a greater association with visceral fat while the latter with subcutaneous fat. Visceral fat is known to correlate better with metabolic abnormalities than subcutaneous fat[11,37-41].

Studies show that sdLDL-c is increased in metabolic syndrome (MS)[42], it increases with the number of MS traits[43], and was a predictive marker of future cardiovascular and cerebrovascular events[44]. It was observed that the sdLDL-c / LDL-c ratio (reflecting total

percentage of LDL that is sdLDL) correlates as well or better with MS presence than LDL-c and sdLDL-c alone.

There is a clear association between abdominal obesity and insulin resistance/hyperinsulinemia, which is the main driving force for the development of dyslipidemia in the obese[7] as well as in people with T2D and IR[45-47]. Dyslipidemia in individuals with T2D also manifests with the atherogenic pattern[22,30,48,49]. The Insulin Resistance Atherosclerosis Study showed that WC was a strong predictor of peripheral insulin resistance in lean individuals[50]. Wang et al.[24] confirmed WC as a better predictor of T2D than BMI and the waist to hip relationship (WHR). In a large international study involving 168,000 people, Balkau et al.[51] showed that within any BMI ("normal", overweight, obese), there was a progressive increase in the prevalence of T2D in quintiles of waist circumference. Thus, the association of T2D with WC, which is a marker of visceral obesity, is evident. For this reason, the association of WC and sdLDL-c must have remained intense even after stratification for T2D, while there was a loss of the strength of association with BMI, which reflects subcutaneous fat. Relevant studies have found an association between the concentration of sdLDL particles with atherosclerosis, such as the Québec Cardiovascular Study[52] which demonstrated an association between sdLDL and ischemic coronary disease in men, and the ARIC study, in which sdLDL-c had association with future coronary events[53]. The combination of our findings might allow stratifying risk in the obese population with atherogenic dyslipidemia[54], with or without T2D and IR, using, in particular, waist circumference as a predictor.

5.1 Limitations

Our study has some limitations. As a cross-sectional study, the described associations do not assess temporality. We enrolled only participants from the Sao Paulo center, since the VAP method was applied only to this research center. Our sample is more heterogenous than previous studies, because it includes a wide range of ages, different ethnicities, persons with T2D and smokers, for example. On the other hand, this is the largest cross-sectional study to date that investigates the relationship between

obesity, sdLDL-c, WC, and BMI using the VAP method in a multiethnic sample of individuals with or without T2D.

6 CONCLUSION

6 CONCLUSION

In conclusion, sdLDL-c is more associated with visceral adiposity, which was better evaluated by WC than BMI in our data. This association was even more evident in the individuals without T2D.

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