Increased plasma lipids in triple-negative breast cancer and impairment in HDL functionality in advanced stages of tumors

Maria Isabela Bloise Alves Caldas Sawada
Universidade Nove de Julho (UNINOVE)
Monique F. Mello Santana
Hospital das Clínicas (HCFMUSP) da Faculdade de Medicina da Universidade de São Paulo
Mozania Reis
Universidade Nove de Julho (UNINOVE)
Sayonara Assis
Hospital das Clínicas (HCFMUSP) da Faculdade de Medicina da Universidade de São Paulo
Lucas Alves Pereira
Universidade Nove de Julho (UNINOVE)
Daniele Ribeiro Santos
Hospital das Clínicas (HCFMUSP) da Faculdade de Medicina da Universidade de São Paulo
Valéria Sutti Nunes
Hospital das Clínicas (HCFMUSP) da Faculdade de Medicina da Universidade de São Paulo
Maria Lucia Cardillo Correa-Giannella
Hospital das Clínicas (HCFMUSP) da Faculdade de Medicina da Universidade de São Paulo
Luiz Henrique Gebrim
Centro de Referência da Saúde da Mulher (Hospital Pérola Byington)
Marisa Passarelli (m.passarelli@uni9.pro.br)
Universidade Nove de Julho (UNINOVE)

Research Article

Keywords: breast cancer, cholesterol, 27-hydroxycholesterol, oxysterols, HDL, cholesterol efflux, biomarkers.

Posted Date: May 31st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1663300/v1

Abstract

Background: the association between plasma lipids and breast cancer (BC) has been extensively explored although results are still conflicting especially regarding the relationship with high-density lipoprotein cholesterol (HDLc) levels. HDL mediates cholesterol and oxysterol removal from cells limiting sterols necessary for tumor growth, inflammation, and metastasis and this may not be reflected by measuring HDLc.

Methods: we addressed recently diagnosed, treatment-naïve BC women (n=163), classified according to molecular types of tumors and clinical stages of the disease, in comparison to control women (CTR; n=150) regarding plasma lipids and lipoproteins, HDL functionality and composition in lipids, oxysterols and apo A-I. HDL was isolated by plasma discontinuous density gradient ultracentrifugation. Lipids (total cholesterol, TC; triglycerides, TG; and phospholipids, PL) were determined by enzymatic assays, apo A-I by immunoturbidimetry, and oxysterols (27, 25, and 24-hydroxycholesterol), by gas chromatography coupled with mass spectrometry. HDL-mediated cell cholesterol removal was determined in macrophages previously overloaded with cholesterol and ^{14}C-cholesterol.

Results: lipid profile was similar between CTR and BC groups after adjustment per age. In the BC group, lower concentrations of TC (84%), TG (93%), PL (89%), and 27-hydroxicholesterol (61%) were observed in HDL, although the lipoprotein ability in removing cell cholesterol was similar to HDL from CRT. Triple-negative (TN) BC cases presented higher levels of TC, TG, apoB, and non-HDLc when compared to other molecular types. Impaired HDL functionality was observed in more advanced BC cases (stages III and IV), as cholesterol efflux was around 28% lower as compared to stages I and II.

Conclusios: the altered lipid profile in TN cases may contribute to channeling lipids to tumor development in a hystotype with a more aggressive clinical history. Moreover, findings reinforce the dissociation between plasma levels of HDLc and HDL functionality in determining BC outcomes.

Background

Female breast cancer (BC) is the most commonly diagnosed cancer worldwide (11.7%) and is the fifth leading cause of cancer death (6.9%) [1]. Being considered a heterogeneous disease, the molecular classification of breast tumors is widely utilized as a tool for therapeutic choice and prognosis. It is based on the expression of estrogen and progesterone receptors [(luminal A; LA); (luminal B; LB)], human epidermal growth factor receptor 2 (HER2), or the absence of them (triple-negative; TN) [2, 3].

Over the last decades, evidence has emerged linking plasma lipid levels to development and worse BC outcomes [4–7]. This relates to tumor cell reprogramming enabling more lipids for cell division and metastasis [8]. Nonetheless, clinical and epidemiological data demonstrated either positive, negative, or no impact of hyperlipidemia on BC incidence [9–13]. In the same manner, the association between statins and the risk of BC is controversial [14, 15].

Particularly, much attention has been paid to the role of cholesterol in the high-density lipoproteins (HDLc), with most of the results pointing to a protective role of plasma HDLc in BC risk prevention [4, 10, 11]. These results may relate to the beneficial HDL activities in removing excess cholesterol and oxysterols - necessary for replication and metastasis - from tumor cells [16]. Besides, HDL has antioxidant and anti-inflammatory activities and acts as a cargo lipoprotein for bioactive lipids, proteins, and microRNAs that may modulate tumor growth and evolution [17, 18]. Nonetheless, there is still controversy and some studies only show a weak association between HDLc and BC, and others, a positive relation or no association at all [9, 12, 13, 19].

In resemblance to HDL's role in atherosclerosis and other chronic non-communicable diseases, it is possible that the HDLc metric is not the best choice as a predictor variable for determining BC incidence and evolution [20]. In this sense, alterations in HDL functionality are considered, especially by taking into account the modulation of this lipoprotein composition and function by the tumor microenvironment [21].

HDL is well recognized for its ability in removing cell cholesterol allowing its uptake by the liver, secretion into bile, and excretion in feces, namely by the reverse cholesterol transport. Together with other HDL actions, reverse cholesterol transport allows the mechanistic association of HDL with the prevention of atherosclerosis. In analogy, in cell tumors, reverse cholesterol transport can be considered as a defensive mechanism that prevents cholesterol accumulation that supplies cell proliferation and metastasis. In this regard, the expression of HDL receptors, such as ABCA-1 and SR-BI are changed in tumors and related to epithelial-mesenchymal transition, tumor growth, and spreading [22–25].

HDL also mediates the transport of oxysterols produced intracellularly by the oxidation of the cholesterol molecule. Considering its higher hydrophobicity when compared to other oxysterols and cholesterol, 27HC diffuses across the plasma membrane [26]. Besides, its transference to HDL is facilitated by the ATP binding cassette transporters A-1 and G-1 (respectively, ABCA-1 and ABCG-1) located in the cell membrane of tumor cells and macrophages that infiltrate the tumor area [27]. Then, its flux outside cells is considered an additional route for reverse cholesterol transport, limiting sterol content that relates to cell inflammation, oxidative stress, and proliferation [18, 28]. On the other side, as a selective estrogen receptor modulator (SERM), 27HC potentiates tumor growth and metastasis in BC. In human BC samples, the expression of the enzymes involved in the production of 27HC (CYP27A1) and metabolism (CYP7B1) are, respectively, increased and diminished and associated with a poor tumor prognosis [29]. In addition, tumor metastasis is related to 27HC-dependent activation of the liver X receptor (LXR) [29–32].

HDL removes cholesterol and oxysterols from cells, making acceptable the idea that it limits the intracellular accumulation of sterols and their negative impact on BC. In this way, it was addressed in newly diagnosed women with BC categorized according to the clinical stage of the disease and molecular classification of the tumor, without pharmacological or surgical intervention, compared to control women (CTR): 1) plasma lipid profile [cholesterol total (TC), HDLc, apolipoprotein B (apoB) and triglycerides (TG)]; 2) the composition of isolated HDL in lipids, main species of oxysterols and apolipoprotein A-I (apoA-I); and 3) the ability of HDL to mediate the removal of cholesterol from macrophages.

Plasma lipids were similar between CTR and all women with BC, but when categorized according to tumor molecular type, triple-negative (TN) BC cases had higher plasma TC, TG, and apoB values compared to other molecular types. Despite having lower levels of CT, phospholipids (PL), and 27HC, HDL from women with BC maintained its ability to remove cellular cholesterol when compared to HDL from CTR cases. In advanced BC (covering clinical stages III and IV), despite similar composition in apoA-I and lipids, HDL had an impaired ability to remove cholesterol from macrophages.

Methods

Two hundred and one women newly diagnosed with BC between 18 and 80 years old in any clinical stage and with the molecular classification of the tumor were recruited at Hospital Pérola Byington. A hundred and fifty-seven women without any type of cancer were recruited at Universidade de São Paulo and at Unidade Básica de Saúde Dra. Ilza Weltman Hutzler (control group; CTR). Exclusion criteria were diabetes mellitus, chronic kidney disease (estimated glomerular filtration rate < 60mL/min/1.73m2), autoimmune diseases, smokers, alcoholics, use of contraceptives, and hormone replacement therapy, pregnancy, previous history of any cancer, and *in situ* breast disease. After excluding those who did not meet the eligibility criteria, 150 CTR and 163 women with BC remained in the study. All participants were informed about the study and signed an informed written consent previously approved by institutional Ethics Committees, in accordance with the Declaration of Helsinki, including approval for publication (Universidade Nove de Julho, # 3.139.460; Centro de Referência da Saúde da Mulher, Hospital Pérola Byington, #3.225.220; and Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, #3.317.909).

The molecular classification of tumors was obtained from medical records being accessed immunohistochemically after percutaneous biopsy or surgery, according to the American College of Pathologists [33]. Samples that were positive for hormone receptors (estrogen and progesterone) were those in which > 1% of the tumor cells showed positive nuclear staining of moderate to strong intensity on immunohistochemistry and were classified as Luminal A and B neoplasia if they presented the Ki67 index below or above 14%, respectively. Samples that exhibited > 10% of invasive tumor cells with strong staining in the plasma membrane were considered HER2 positive. In case of moderate staining in > 10% of the cells or strong in < 10% of the cells, the sample was re-evaluated by *in situ* hybridization and was considered positive if a HER2/centromere ratio > 2.0; or if a HER2/centromere ratio < 2.0 with mean HER2 > 6 signals per cell (greater than 120 signals in 20 nuclei). Tumor samples that did not express either hormonal or HER2 receptors were classified as triple-negative (TN) breast cancers [34].

The current investigation followed the STROBE Statement. The checklist is in the Supporting Information (S1 file). For sample calculation, the number of new cases of BC included for treatment at Hospital Pérola Byington during 2018 (2,985 cases) was taken into account; the study design, with the comparison of outcome variables between two main groups (CTR vs. BC); the main outcome variables; the effect size of the variables according to the main studies published in the area; and the probability of committing a

type 1 error (0.05) and type 2 error (0.20), with 80% power, resulted in 144 patients in each group (pairing 1:1).

Blood collection

Venous blood was drawn after 12h fasting and plasma immediately was isolated after centrifugation (3,000 rpm, 4°C, 15 min). Plasma TC, TG, and HDLc were determined by enzymatic techniques. Low-density lipoprotein cholesterol (LDLc) was determined by the Friedewald formula [35]. ApoB was quantified by immunoturbidimetry (Randox Lab. Ltd. Crumlin, UK).

Isolation of plasma lipoproteins

HDL (D = 1.063–1.21 g/mL) was isolated from BC and control women´s plasma by discontinuous density ultracentrifugation and immediately frozen at -80°C in a 5% saccharose solution. HDL composition in lipids (TC, TG, and PL) was determined by enzymatic techniques. ApoA-I was determined by the immunoturbidimetric method (Randox Lab. Ltd. Crumlin, UK).

Low-density lipoprotein isolation and acetylation

LDL (D = 1.019–1.063 g/mL) was obtained by sequential ultracentrifugation of plasma from healthy volunteers and was purified by discontinuous density ultracentrifugation. After protein quantification by the Lowry technique [36], LDL was incubated with acetic anhydride as previously described [37]. Acetylated LDL was dialyzed and utilized to load macrophages with cholesterol.

HDL-mediated cholesterol efflux from bone marrow-derived macrophages

The Institutional Animal Care and Research Advisory Committee (Universidade Nove de Julho # 7070120821, 08/23/2021) approved the study according to the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. C57BL/6 J mice were housed with free access to commercial chow (Nuvilab CR1, São Paulo, Brazil) and drinking water in a conventional animal facility at 22 ± 2°C under a 12h light/dark cycle. Undifferentiated bone marrows cells were obtained from C57BL/6 wild-type mouse´s tibia and femora, as previously described [38]. Cells were differentiated into macrophages by incubating with L929 cells-conditioned medium (ATCC, American Tissue Culture Collection) and plated in culture dishes for 5 days at 37°C, under 5% (v/v) CO_2. The medium was changed for a new one and after 6 days replaced by DMEM (low glucose, containing 1% penicillin/streptomycin and 10% heat-inactivated fetal calf serum). Macrophages were overloaded with acetylated LDL (50 µg/mL) and ^{14}C-cholesterol (0.3 µCi/mL) for 48 h, and after washing incubated with DMEM containing fatty acid-free albumin for equilibration of intracellular cholesterol pools. Cells were incubated for 6 h with HDL (50 µg/mL) from BC or control women; control incubations were performed in the absence of HDL (basal efflux). After incubation, the radioactivity in the medium was determined as well as in the cell lipid extract. The cholesterol efflux was determined as ^{14}C-cholesterol in the medium / ^{14}C-cholesterol in the medium + ^{14}C-cholesterol in cells x 100. Values from basal efflux were subtracted from those

obtained after incubation with HDL, in order to express the specific ability of HDL in removing cell cholesterol.

Oxysterols quantification in isolated HDL

Oxysterols (24-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol) were measured from 1 mL of extracted HDL as previously described [40]. Briefly, a mixture of 100 ng of oxysterol deuterium-labeled (7α-hydroxycholesterol-d7, 7β-hydroxycholesterol-d7, 25-hydroxycholesterol-d7, 27-hydroxycholesterol-d7) (Avanti Polar Lipids, Alabaster, USA) was added as internal standard. Alkaline saponification was done by adding 10 mL of ethanolic KOH (0.4 M) solution for 2h, at room temperature with adjustment to pH 7 with phosphoric acid, followed by the addition of 20 mL of chloroform and 6 mL of water. After vigorous shaking and centrifugation at 4°C, the aqueous phase was removed and the organic phase evaporated. The lipid extract was dissolved in toluene (1 mL). Oxysterols were isolated from cholesterol by solid-phase extraction (Sigma- Aldrich Supelclean LC-Si SPE Tubes SUPELCO, Bellefonte, USA). The sample (1mL in toluene) was applied to the column previously conditioned with 2 mL of hexane, following washing with 1 mL of hexane. Sterols were eluted with 1.5% isopropanol in hexane (8mL), and oxysterols were further eluted with 30% isopropanol in hexane (6mL). The solvent was evaporated and samples were derivatized (100 µL of pyridine and 100 µL of N, O-bis (trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (BSTFA; Sigma- Aldrich, St. Louis, USA), for 1 h at 60◦C). One microliter of the derivatized sample (1µL) was injected into a gas chromatograph coupled to a mass spectrometer (Shimadzu GCMS-QP2010, Kyoto, Japan) by an automatic injector and analyzed in selected ion monitoring. The separation was performed on a Restek capillary column (100% dimethyl polysiloxane —RxiR − 1 ms. Cat. #13323), 30 m, internal diameter 0.25 mm, for 30 min, using helium as mobile phase, with a constant linear velocity of 44.1 cm/sec. The oven started at 240◦C with an increment of 5◦C/min, for 7 min up to 290◦C. The mass spectrometer operated in impact electron mode at an ionization voltage of 70 eV with the temperature of the ion source at 300◦C. The quantification was performed by comparing the peak areas of the standard curve and correcting for internal standards.

Statistical analysis

The Shapiro-Wilk test was used to analyze normality; parametric data were represented by the mean and standard deviation of the sample and compared by Student's t-test, with or without Welch correction, depending on the performance of Levene's Test regarding sample sphericity. When evaluating more than two samples, analysis of variance of non-repeated measures was used with Tukey or Games-Howell post-test, according to the analysis of variance (homo or heteroscedasticity, respectively) and with bootstrap. Covariate analyzes were performed to adjust the outcome variables with Sidak's post-test. Non-parametric data were represented by the median of lower and upper quartiles and compared with each other using the Mann-Whitney test for two samples, and when comparing more than two samples, the Kruskal-Wallis test was used. When normalization was necessary, the variables were log-transformed. Frequencies were compared using the Chi-square test. A value of $P < 0.05$ was considered statistically significant. IBM® SPSS Statistics (version 27.0), GraphPad Prism (version 5.04) for Windows, and Microsoft® Excel for Mac (version 16.52) software were used for data tabulation and analysis.

Results

In the BC group, the median age and the frequency of postmenopausal status, dyslipidemia and hypertension were higher in comparison to the CTR, although BMI, overweight (BMI $\geq$ 25 kg/m^2) and reported statin use were similar between groups. Among tumors, the frequency (%) of histological types was: ductal (87.7), lobular (7.4), mucinous (4.3), and metaplastic (0.6). As expected, a higher frequency of LA and LB tumors was observed. 70.8% of BC women were categorized in clinical stages I and II, and 29.2% in advanced stages of disease (stages III and IV) (Table 1). The postmenopausal status was similar among clinical stages (stage I= 66.7%; stage II= 59.6%; stage III= 74.2%; and stage IV = 68.8%; χ^2 = 2.011; *P* = 0.570).

Table 1
Age, anthropometric and clinical data of control and breast cancer women

	CTR		BC		*P*
n	150		163		
Age (years)*	51 (38–59)		55 (49–63)		**< 0.001**
BMI (kg/m^2)*	28 (25–31)		27 (24–31)		0.644
	n	**%**	**n**	**%**	
BMI $\geq$ 25 kg/m^2	108	73.5	106	68.4	0.179
Premenopausal	75	50.7	56	34.4	**< 0.001**
Postmenopausal	73	49.3	107	65.6	**< 0.001**
Dyslipidemia	18	12	35	21.9	**0.021**
Statin use	9	6	19	11.8	0.074
Hypertension	33	22.4	61	37.9	**0.003**
Histological type of BC					
Ductal			143	87.7	
Lobular			12	7.4	
Mucinous			7	4.3	
Metaplastic			1	0.6	
Molecular type of BC					
LA			46	28.2	
LB			65	39.8	
HER2			26	16	
TN			26	16	
Clinical stage of disease					
I			57	35.4	
II			57	35.4	

*Values in median and interquartile ranges 25%-75%. CTR = control women; BC = breast cancer women; BMI = body mass index; LA = luminal A; LB = luminal B; TN = triple-negative. Comparisons were done by the χ^2 test.

	CTR	BC	*P*
III	31	19.3	
IV	16	9.9	

*Values in median and interquartile ranges 25%-75%. CTR = control women; BC = breast cancer women; BMI = body mass index; LA = luminal A; LB = luminal B; TN = triple-negative. Comparisons were done by the χ^2 test.

Table 1.

Plasma lipid profile adjusted per age and the ratios TC/apoB and TG/HDLc were similar between CTR and BC groups (Table 2). In isolated HDL, the concentrations of TC, TG, and PL were lower in the BC group, while apoA-I was similar in both groups. 27HC content in HDL was lower in BC as compared to CTR even after adjustment for age, but the concentrations of 24HC and 25HC in HDL were similar between groups. Despite some alterations in composition, HDL particles from BC and CTR presented similar abilities in mediating macrophage cholesterol removal (Table 2).

Table 2
Plasma lipids, HDL composition, and HDL-mediated cholesterol efflux in women with BC and CTR

	CTR	BC	*P*
n*	132	126	
Age (years)	**48 (37–58)**	**55 (49–63)**	**< 0.001**
BMI (kg/m^2)	28 (25–31)	27 (24–31)	0.391
TC (mg/dL)	172 (151–199)	178 (155–206)	0.423
TG (mg/dL)	84 (59–122)	92 (68–116)	0.224
apoB (mg/dL)	111 (89–139)	107 (82–141)	0.428
HDLc (mg/dL)	41 (35–51)	40 (32–48)	0.109
LDLc (mg/dL)	111 (95–131)	115 (95–142)	0.522
non-HDLc (mg/dL)	127 (109–156)	136 (113–163)	0.252
TC/apoB (mg/dL)	1,5 (1,3–1,9)	1,7 (1,4–2,0)	0.065
TG/HDL (mg/dL)	1,9 (1,3–3,1)	2,3 (1,5–3,5)	0.091
HDL-TC (mg/dL)	**44 (34–55)**	**37 (30–46)**	**0.001**
HDL-TG (mg/dL)	**14 (10–19)**	**13 (10–16)**	**0.039**
HDL-PL (mg/dL)	**98 (80–131)**	**87 (73 – 116)**	**0.006**
HDL-apoA-I (mg/dL)	104 (82–141)	112 (82 – 142)	0.700
24HC (ng/mL)	38.9 (25.9–99.0)	34.9 (24.1–65.4)	0.203
25HC (ng/mL)	8.1 (5.1–10.7)	6.9 (5.4–9.3)	0.111
27HC (ng/mL)	**12.6 (7.4–19.4)**	**7.7 (4.6–13.1)**	**0.002**
^{14}C-cholesterol efflux (%)	12 (9–18)	13 (9–19)	0.501

Plasma lipids were determined by enzymatic colorimetric methods and apoB by immunoturbidimetry. HDLc was determined after precipitation of lipoproteins containing apoB; non-HDLc was calculated as TC-HDLc. Oxysterols were quantified in isolated HDL by gas chromatography-mass spectrometry (GC-MS). The cholesterol efflux was determined in BMDM overloaded with ^{14}C-cholesterol and acetylated LDL, utilizing 50 µg/mL of HDL as a cholesterol acceptor.

BMI = body mass index; TC = total cholesterol; TG = triglycerides; apoB = apolipoprotein B; HDLc = high-density lipoprotein cholesterol; LDLc = low density lipoprotein cholesterol; PL = phospholipids; apoA-I = apolipoprotein A-I; 24HC = 24-hydroxycholesterol (CTR, n = 82; BC, n = 82); 25HC = 25-hydroxycholesterol (CTR, n = 82; BC, n = 82); 27HC = 27-hydroxycholesterol (CTR, n = 81; BC, n = 80). Comparisons were made using the Mann-Whitney test; data shown in median and interquartile ranges (25%-75%). *P* values were age-adjusted.

Table 2.

When comparing the molecular types of BC, age and BMI were similar, but TC was higher in TN as compared to LA, LB, and HER2 tumors. Besides, plasma levels of TG, apoB, and non-HDLc were greater in TN as compared to LB and HER2 (Table 3). Although plasma lipids were different in TN tumors, the ratios TC/apoB ($P = 0.065$) and TG/HDLc ($P = 0.091$) did not reach statistical difference. Oxysterols in HDL were similar among LA, LB, HER2, and TN as well as the ability of cell cholesterol removal (Table 3).

Table 3
Plasma lipids, HDL composition, and HDL-mediated cholesterol efflux according to the molecular type of BC.

	LA	LB	HER2	TN	*P*
n	46	65	26	26	
Age (years)	60 (52–64)	54 (48–63)	55 (49–61)	53 (41–61)	0.107
BMI (kg/m^2)	27 (24–31)	28 (25–31)	27 (24–31)	27 (24–30)	0.793
TC (mg/dL)	193 (162–228)	173 (155–205)	173 (151–193)	198 (173–214)	**0.022**$^{\#}$
TG (mg/dL)	103 (66–139)	84 (67–110)	82 (63–105)	102 (91–134)	**0.026**$^{\$}$
apoB (mg/dL)	129 (90–151)	98 (81–123)	100 (81–127)	134 (102–158)	**0.005**$^{\$}$
HDLc (mg/dL)	41 (34–49)	42 (33–53)	42 (32–52)	42 (36–50)	0.997
LDLc (mg/dL)	123 (99–155)	111 (90–138)	117 (85–134)	127 (98–148)	0.116
non-HDLc (mg/dL)	147 (121–184)	129 (104–162)	135 (105–151)	155 (130–174)	**0.013**$^{\$}$
TC/apoB	1.7 (1.4–2.0)	1.8 (1.5–2.1)	1.8 (1.5–2.0)	1.5 (1.3–2.0)	0.313

Plasma lipids were determined by enzymatic colorimetric methods and apoB by immunoturbidimetry. HDLc was determined after precipitation of lipoproteins containing apoB; non-HDLc was calculated as TC-HDLc. Oxysterols were quantified in isolated HDL by gas chromatography-mass spectrometry (GC-MS). The cholesterol efflux was determined in BMDM overloaded with ^{14}C-cholesterol and acetylated LDL, utilizing 50 µg/mL of HDL as a cholesterol acceptor.

CTR = control women; BC = breast cancer; BMI = body mass index; TC = total cholesterol; TG = triglycerides; apoB = apolipoprotein B; HDLc = high-density lipoprotein cholesterol; LDLc = low density lipoprotein cholesterol; PL = phospholipids; apoA-I = apolipoprotein A-I; 24HC = 24-hydroxycholesterol (CTR, n = 82; BC, n = 82); 25HC = 25-hydroxycholesterol (CTR, n = 82; BC, n = 82); 27HC = 27-hydroxycholesterol (CTR, n = 81; BC, n = 80). Comparisons were made using the Kruskall-Wallis test with a significance of 0.05. Data shown in median and interquartile ranges (25%-75%). LA = luminal A; LB = luminal B; TN = triple negative. $^{\#}$ compared to LA, LB, and HER2; $^{\$}$ compared to LB and HER2.

	LA	LB	HER2	TN	*P*
TG/HDLc	2.3 (1.3–3.4)	1.9 (1.3–3.1)	1.7 (1.3–3.2)	2.5 (2.0–3.4)	0.358
HDL-TC (mg/dL)	39 (30–49)	40 (27–52)	41 (29–49)	45 (23–58)	0.886
HDL-TG (mg/dL)	15 (12–34)	17 (11–62)	13 (11–18)	24 (14–91)	0.055
HDL-PL (mg/dL)	90 (71–119)	90 (77–121)	104 (82–124)	96 (77–117)	0.435
HDL-apoA-I (mg/dL)	114 (83–142)	108 (76–150)	108 (86–139)	107 (90–123)	0.894
24HC (ng/mL)	28 (22–49)	36 (23–78)	35 (28–58)	35 (24–88)	0.681
25HC (ng/mL)	7.3 (5.6–9.2)	8.3 (6.4–9.8)	6.6 (4.6–9.4)	6.0 (4.5–7.6)	0.110
27HC (ng/mL)	5.8 (4.5–10.6)	10.9 (4.4–13.8)	7.2 (4.7–14.6)	5.7 (4.1–14.2)	0.580
^{14}C-cholesterol efflux (%)	14 (10–17)	12 (9–19)	13 (8–15)	11 (8–18)	0.921

Plasma lipids were determined by enzymatic colorimetric methods and apoB by immunoturbidimetry. HDLc was determined after precipitation of lipoproteins containing apoB; non-HDLc was calculated as TC-HDLc. Oxysterols were quantified in isolated HDL by gas chromatography-mass spectrometry (GC-MS). The cholesterol efflux was determined in BMDM overloaded with ^{14}C-cholesterol and acetylated LDL, utilizing 50 µg/mL of HDL as a cholesterol acceptor.

CTR = control women; BC = breast cancer; BMI = body mass index; TC = total cholesterol; TG = triglycerides; apoB = apolipoprotein B; HDLc = high-density lipoprotein cholesterol; LDLc = low density lipoprotein cholesterol; PL = phospholipids; apoA-I = apolipoprotein A-I; 24HC = 24-hydroxycholesterol (CTR, n = 82; BC, n = 82); 25HC = 25-hydroxycholesterol (CTR, n = 82; BC, n = 82); 27HC = 27-hydroxycholesterol (CTR, n = 81; BC, n = 80). Comparisons were made using the Kruskall-Wallis test with a significance of 0.05. Data shown in median and interquartile ranges (25%-75%). LA = luminal A; LB = luminal B; TN = triple negative. $^{\#}$ compared to LA, LB, and HER2; $^{\$}$ compared to LB and HER2.

Table 3.

When subjects were categorized according to the stage of the disease reflected by increased levels of Ki67, it was observed that plasma lipids and their ratios were similar among groups. The composition of HDL in TC, PL, apoA-I, and oxysterols was similar among stages. Only HDL-TG was increased on stage III as compared to I, II, and IV. Reduced cholesterol efflux was observed with HDL isolated from BC women in more advanced stages of the disease (III and IV) as compared to stages I and II. (Table 4).

Table 4
Plasma lipids, HDL composition, and HDL-mediated cholesterol efflux according to clinical stages of BC.

	I	II	III	IV	*P*
n	57	57	31	16	
Age (years)	60 (49–64)	53 (46–61)	56 (51–61)	55 (48–66)	0.287
BMI (kg/m^2)	27 (24–31)	28 (25–31)	27 (25–31)	25 (23–30)	0.291
Ki67	20 (10–30)	20 (10–30)	30 (20–70)	35 (30–72)	**< 0.001** **I-III < 0.001** **I-IV < 0.001** **II-III 0.015** **II-IV 0.005** **III-IV 0.416** **I-II 0.122**
TC (mg/dL)	187 (162–218)	179 (155–203)	189 (167–208)	183 (154–241)	0.600
TG (mg/dL)	94 (64–117)	92 (73–122)	92 (73–115)	84 (60–111)	0.760
apoB (mg/dL)	105 (81–138)	107 (87–137)	109 (84–141)	105 (84–150)	0.972
HDLc (mg/dL)	42 (33–51)	42 (33–50)	46 (39–55)	41 (33–45)	0.376

Plasma lipids were determined by enzymatic colorimetric methods and apoB by immunoturbidimetry. HDLc was determined after precipitation of lipoproteins containing apoB; non-HDLc was calculated as TC-HDLc. Oxysterols were quantified in isolated HDL by gas chromatography-mass spectrometry (GC-MS). The cholesterol efflux was determined in BMDM overloaded with ^{14}C-cholesterol and acetylated LDL, utilizing 50 µg/mL of HDL as a cholesterol acceptor.

CTR = control women; BC = breast cancer; BMI = body mass index; TC = total cholesterol; TG = triglycerides; apoB = apolipoprotein B; HDLc = high-density lipoprotein cholesterol; LDLc = low density lipoprotein cholesterol; PL = phospholipids; apoA-I = apolipoprotein A-I; 24HC = 24-hydroxycholesterol (CTR, n = 82; BC, n = 82); 25HC = 25-hydroxycholesterol (CTR, n = 82; BC, n = 82); 27HC = 27-hydroxycholesterol (CTR, n = 81; BC, n = 80). Comparisons were made using the Kruskall-Wallis test with a significance of 0.05. Data shown in median and interquartile ranges (25%-75%).

	I	II	III	IV	*P*
LDLc (mg/dL)	121 (98–146)	109 (92–137)	118 (97–139)	117 (98–172)	0.476
non-HDLc (mg/dL)	140 (118–169)	132 (105–159)	139 (116–163)	132 (108–196)	0.640
TC/apoB	1.8 (1.4–2.1)	1.7 (1.4–2.0)	1.7 (1.4–2.0)	1.6 (1.2–2.0)	0.685
TG/HDLc	1.9 (1.5–3.2)	2.3 (1.4–3.3)	2.1 (1.0–3.3)	2.4 (1.6–2.7)	0.875
HDL-TC (mg/dL)	44 (30–51)	38 (28–49)	45 (26–67)	38 (31–51)	0.336
HDL-TG (mg/dL)	15 (12–44)	15 (11–39)	38 (14–92)	18 (10–56)	**0.032** I-IV 0.831 II-IV 0.809 **III-IV 0.035** I-II 0.968 **I-III 0.009** **II-III 0.009**
HDL-PL (mg/dL)	90 (75–119)	89 (76–114)	117 (87–124)	87 (79–113)	0.116
HDL-apoA-I (mg/dL)	116 (83–144)	104 (78–135)	114 (85–141)	109 (94–149)	0.733

Plasma lipids were determined by enzymatic colorimetric methods and apoB by immunoturbidimetry. HDLc was determined after precipitation of lipoproteins containing apoB; non-HDLc was calculated as TC-HDLc. Oxysterols were quantified in isolated HDL by gas chromatography-mass spectrometry (GC-MS). The cholesterol efflux was determined in BMDM overloaded with ^{14}C-cholesterol and acetylated LDL, utilizing 50 µg/mL of HDL as a cholesterol acceptor.

CTR = control women; BC = breast cancer; BMI = body mass index; TC = total cholesterol; TG = triglycerides; apoB = apolipoprotein B; HDLc = high-density lipoprotein cholesterol; LDLc = low density lipoprotein cholesterol; PL = phospholipids; apoA-I = apolipoprotein A-I; 24HC = 24-hydroxycholesterol (CTR, n = 82; BC, n = 82); 25HC = 25-hydroxycholesterol (CTR, n = 82; BC, n = 82); 27HC = 27-hydroxycholesterol (CTR, n = 81; BC, n = 80). Comparisons were made using the Kruskall-Wallis test with a significance of 0.05. Data shown in median and interquartile ranges (25%-75%).

	I	II	III	IV	*P*
24HC (ng/mL)	31 (19–48)	35 (26–75)	28 (25–50)	56 (34–128)	0.276
25HC (ng/mL)	6.5 (5.1–8.5)	7.1 (5.3–8.7)	9.7 (6.6–10.6)	6.0 (4.3–8.9)	0.247
27HC (ng/mL)	8.8 (4.6–15.1)	9.7 (4.6–14.4)	5.0 (3.9–9.1)	5.7 (3.4–10.1)	0.267
^{14}C-cholesterol efflux (%)	14 (10–20)	13 (10–18)	9 (7–15)	9 (7–12)	**0.011** III-IV 0.424 **II-IV 0.014** **I-IV 0.004** **II-III 0.088** **I-III 0.033** I-II 0.578
Plasma lipids were determined by enzymatic colorimetric methods and apoB by immunoturbidimetry. HDLc was determined after precipitation of lipoproteins containing apoB; non-HDLc was calculated as TC-HDLc. Oxysterols were quantified in isolated HDL by gas chromatography-mass spectrometry (GC-MS). The cholesterol efflux was determined in BMDM overloaded with ^{14}C-cholesterol and acetylated LDL, utilizing 50 µg/mL of HDL as a cholesterol acceptor.					
CTR = control women; BC = breast cancer; BMI = body mass index; TC = total cholesterol; TG = triglycerides; apoB = apolipoprotein B; HDLc = high-density lipoprotein cholesterol; LDLc = low density lipoprotein cholesterol; PL = phospholipids; apoA-I = apolipoprotein A-I; 24HC = 24-hydroxycholesterol (CTR, n = 82; BC, n = 82); 25HC = 25-hydroxycholesterol (CTR, n = 82; BC, n = 82); 27HC = 27-hydroxycholesterol (CTR, n = 81; BC, n = 80). Comparisons were made using the Kruskall-Wallis test with a significance of 0.05. Data shown in median and interquartile ranges (25%-75%).					

Table 4.

Discussion

In the present investigation, it was addressed plasma lipid levels and HDL composition and functionality in newly diagnosed women with BC in comparison to CTR women, and with respect to the molecular classification of the tumor and the clinical stage of the disease. In comparison to CRT, it was demonstrated that BC cases had 1) similar concentrations of plasma lipids adjusted per age, and 2) HDL particles less enriched in TC, PL, and TG. Divergent results have been published concerning the association of plasma lipids and lipoproteins in the development and prognosis of BC. Total cholesterol is considered a contributor factor for many types of cancer including BC, although the presence of

confounding factors such as heterogeneity of BC, disease duration and staging, disparity of ethnic population, age, menopausal state, lifestyle, and treatments may bias the interpretation and comparison among studies. Moreover, diabetes mellitus, insulin resistance, and obesity that change lipoprotein profiles are also recognized as potential contributors to BC.

Particularly, the majority of studies show increased levels of TC, LDL, and TG and reduced HDLc as strong predictors for BC [41–45]. In our case-control study, HDL plasma levels did not differ between CTR and BC cases. Body mass index was similar between groups and results adjusted per age showed similar levels of plasma TG. Conceivably, triglycerides metabolism by the lipoprotein lipase dictates HDL formation in circulation and ultimately HDLc plasma levels. Noteworthy to mention that diabetes mellitus and smoking which are conditions that reduce HDL were not included in the study and there was no interference of therapeutics since only newly diagnosed subjects were included.

Although the ability of HDL in removing cell cholesterol was preserved in all women with BC included in our casuistic as compared to CTR, the diminished content of TC, PL, and 27HC in the HDL particle from BC cases may be a consequence of alterations in cell tumors that mitigate sterol exportation to HDL. In addition to the intrinsic ability of HDL to receive cholesterol, it is important to consider that lipid efflux is modulated by cellular components, such as the bioavailability of free or unesterified cholesterol and the content and functionality of ABCA-1, ABCG-1, and SR-BI [46]. Characteristically, ABCA-1 is expressed in the mammary gland and its content is indicated as reduced in BC and associated with positive lymph nodes [47]. Its expression negatively affects the therapeutic efficiency of chemotherapy [48] and is referred to by some authors as a marker of TN tumors [49]. On the other hand, its deficiency contributes to an increase in cellular and mitochondrial cholesterol content, mitigating cell death processes mediated by this organelle, which favors tumor cell survival [24].

Solid tumors accumulate large amounts of cholesterol [50, 51] through increased synthesis and uptake of lipoproteins [51, 52]. SR-B1 mediates the efflux of free cholesterol down a concentration gradient for HDL. However, it can also promote the uptake of modified lipoproteins, favoring, on the contrary, the supply of cholesterol to cells and tumor progression [53]. Higher expression of SR-B1 is linked to greater aggressiveness and worse tumor prognosis [23, 54, 55], while mutations in the *Scarb1* are related to the inhibition of tumor proliferation [56].

Furthermore, the lower content of lipids in HDL could be linked to the lower detachment of surface components of TG-rich lipoproteins during lipolysis mediated by lipoprotein lipase, in a process recently referred to as reverse transport of remnant cholesterol [57]. However, it is not possible to say which mechanisms act on the composition of HDL in view of the present results but these events cannot be evidenced by the simplistic determination of HDLc in plasma, explaining some of the controversies regarding the association of HDLc with BC.

As expected, most tumors in our casuistic were of the luminal type (68%), with a predominance of stage I and II disease (70.8%). The frequency of advanced disease (stages III and IV) was highest in women with TN tumors (66.5%) and lowest in those with LA tumors (16%). Taking into account the molecular

classification of the tumor it was observed that: 1) TN cases had higher levels of TC, apo B, nonHDLc, and TG in comparison to LA, LB, and HER-2, but 2) HDLc and composition and functionality of the HDL particle were similar among all types. Changes observed were irrespective of BMI and may represent a distinct feature of TN tumors, channeling lipids to tumor development in a hystotype with a more aggressive clinical history, with a relatively low-survival rate and a high rate of metastasis.

Findings agree with previous studies that evidenced increased levels of VLDL-TG in TN BC, although changes in CT/apoB and TG/HDLc did not reach statistical significance. In this regard, it was demonstrated in a retrospective cohort that a high pretreatment TG/HDLc ratio was an independent predictor of the overall survival rate in TN tumors [58]. The enhanced expression of the LDL receptor and LDLR-related proteins 5 and 6 were found in TN tumors and associated with a higher ability of tumor growth and invasion; while the knockdown of LDLR-5 and 6 decreased tumorigenesis [51, 59–61].

A model of syngeneic tumor graft evidenced that tumor growth is accompanied by changes in the host lipid metabolism by stimulating and inhibiting, respectively, the synthesis and metabolism of very-low-density lipoproteins (VLDL). This ultimately seems to provide more energy to the tumor [62]. In addition, by using a targeted plasma liquid chromatography-tandem mass spectrometry (LC-MS/MS), potential lipid biomarkers were selected in TN cases of BC (including ceramides, phosphatidylcholine, lysophosphatidylcholine, and diacylglycerol), reaffirming the modification of the lipid profile in this molecular type [63].

Unlike other studies, we did not find changes in plasma lipids in HER2 positive BC cases [44, 64, 65]. In subjects with HER2-positive breast cancer, the analysis of lipoprotein profile by nuclear magnetic resonance demonstrated an enhanced level of specific VLDL subfractions as a marker of plasma lipid alteration in comparison to control women, even in the presence of similar BMI between groups. Reductions in HDL subfractions were surrogate markers for the response to neoadjuvant chemotherapy follow-up [66].

According to the clinical stage of the disease, a reduced intrinsic ability of the HDL particle in removing cell cholesterol was observed in stages III and IV as compared to stages I and II. This was independent of changes in HDL composition and plasma lipids reinforcing the dissociation between plasma levels of HDLc and HDL functionality as a whole particle. Increasing levels of intracellular cholesterol favor the formation of oxysterols allowing us to infer an increase in the concentration of 27HC in the tumor microenvironment that negatively drives BC evolution. The pathophysiological basis of reduced HDL functionality in advanced stages of breast cancer was not investigated in the present study. The inflammatory, oxidative, and immunogenic insults prevalent in advanced disease may favor the modification of HDL in the tumor microenvironment, compromising its function. In this sense, HDL may even facilitate tumor propagation as previously reported in experimental studies where modified HDL was oncogenic [41, 67]. Again, this supports the idea that the tumor energy demand adapts systemic metabolism in its favor.

The concept of HDL modulation by the tumor by reverse causation may unlink HDL, per se, as a predictor of tumor risk. In this sense, HDL would be applied more as a marker of tumor evolution than as a protector or inducer of its genesis.

To the best of our knowledge, this is the first demonstration of loss of HDL function according to disease burden, independently of plasma lipids. Study limitations include the absence of more detailed clinical data, including components of metabolic syndrome and visceral adiposity, and lifestyle information. However, BMI was similar between the groups, and comorbidities frankly associated with changes in lipid metabolism were excluded. It was not possible to determine which components of the HDL structure, independently of lipids and apo A-I, were responsible for the impairment of cholesterol efflux. A more detailed analysis of HDL proteomics, lipidomics, and microRNAs may add new information.

Conclusions

The results of the present investigation confirmed an increased level of plasma lipids in TN tumors probably driving the more aggressive type of BC. Moreover, findings contributed to a better understanding of the role played by HDL in BC, especially related to patients with a worse prognosis where the functionality of HDL in the removal of excess cellular cholesterol is impaired. As compared to controls only the composition of HDL was discriminative in BC subjects. A prospective cohort will help determine the prognostic value of HDL composition and functionality in predicting BC outcomes.

Abbreviations

ABCA-1	ATP binding cassette transporter A-1
ABCG-1	ATP binding cassette transporter G-1
apoA-I	Apolipoprotein A-I
apoB	Apolipoprotein B
TC	Total cholesterol
CYP7B1	P450 citochrom, family 7, subfamily B, member 1, 25-hydroxicholesterol 7-alfa-hydroxilase
CYP27A1	P450 citochrom, family 27, subfamily A, member 1, sterol 27-hydroxilase
PL	Phospholipids
HDL	High density lipoprotein
HDLc	High density lipoprotein cholesterol
HER2	Human epidermal growth factor receptor 2
BMI	Body mass index
Ki67	Antigen identified by Ki67 monoclonal antibody
LA	Luminal A
LB	Luminal B
LDL	Low density lipoporotein
LDLc	Low density lipoporotein cholesterol
LDLr	LDL receptor
LXR	Liver X receptor
SERM	Selective estrogen receptor modulator
TG	Triglycerides
TN	Triple negative
VLDL	Very low lipoprotein
VLDLc	Very low lipoprotein cholesterol
24HC	24-hydroxicholesterol
25HC	25-hydroxicholesterol
27HC	27-hydroxicholesterol

Declarations

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Universidade Nove de Julho (# 3.139.460; February, 2019); Centro de Referência da Saúde da Mulher (Hospital Pérola Byington; #3.225.220; March, 2019) and Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (#3.317.909; March, 2019). Informed consent was obtained from all subjects and/or their legal guardian(s).

Consent of publication: Authors consent to the journal's publication rules.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: This research was funded by Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP (grants # 2019/18431-4 to MP). MP is recipient of a research award from Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brazil.

Authors´ Contributions: Conceptualization, MP, LHG.; casuistic selection: MIBACS, MR; methodology, MFSM, SA, LAP, DRS, VSN; formal analysis, MIBACS, MP; investigation, MLCCG; data curation, MIBACS, MP; writing—original draft preparation, MIBACS, MP.; writing—review and editing, MP; resources, MP; project administration, MP; funding acquisition, MP. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors are thankful to Rafaela V Coutinho, Karina CLB Lima, Jessica AV Siqueira, Julia HC Dezotti, Raiana C Novaes, Jessica Mosello, Karina Cezar, and Jacira X Carvalho for helping in blood collection and obtaining clinical data; to Fernanda Faccioli Schober and Nurya Bustamante de Araújo, from Universidade Nove de Julho Animal Facility Unity for animal caring.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209–249.
2. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol [Internet]. 2020;38(12):1346–66.
3. Hamilton E, Shastry M, Shiller SM, Ren R. Targeting HER2 heterogeneity in breast cancer. Cancer Treat Rev. 2021;100: 102286.

4. Munir MT, Ponce C, Powell CA, Tarafdar K, Yanagita T, Choudhury M, et al. The contribution of cholesterol and epigenetic changes to the pathophysiology of breast cancer. J Steroid Biochem Mol Biol. 2018; 183:1–9.
5. McDonnell DP, Park S, Goulet MT, Jasper J, Wardell SE, Chang CY, et al. Obesity, cholesterol metabolism, and breast cancer pathogenesis. Cancer Res. 2014;74(18):4976–82.
6. Wei Y, Huang Y, Yang W, Huang K, Chen Y, Zeng Ket al. The significances and clinical implications of cholesterol components in human breast cancer. Science Progress. July 2021.
7. Guan X, Liu Z, Zhao Z, Zhang X, Tao S, Yuan B, et al. Emerging roles of low-density lipoprotein in the development and treatment of breast cancer. Lipids Health Dis. 2019, 18, 137.
8. Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. Oncogenesis. 2016;5(1): e189. Published 2016 Jan 25.
9. Nowak C, Ärnlöv J. A Mendelian randomization study of the effects of blood lipids on breast cancer risk. Nat Commun. 2018 Sep 27;9(1):3957.
10. Li X, Tang H, Wang J, Xie X, Liu P, Kong Y, et al. The effect of preoperative serum triglycerides and high-density lipoprotein-cholesterol levels on the prognosis of breast cancer. Breast. 2017 Apr;32:1–6. Epub 2016 Dec 8.
11. Fan Y, Ding X, Wang J, Ma F, Yuan P, Li Q, et al. Decreased serum HDL at initial diagnosis correlates with worse outcomes for triple-negative breast cancer but not non-TNBCs. Int J Biol Markers. 2015;30(2): e200-7.
12. His M, Dartois L, Fagherazzi G, Boutten A, Dupré T, Mesrine S, et al. Associations between serum lipids and breast cancer incidence and survival in the E3N prospective cohort study. Cancer Causes Control. 2017; 28; 77–88.
13. Chandler PD, Song Y, Lin J, Zhang S, Sesso HD, Mora S, et al. Lipid biomarkers and long-term risk of cancer in the Women's Health Study. Am. J. Clin. Nutr. 2016; 103:1397–1407.
14. Undela K, Srikanth V, Bansal D. Statin use and risk of breast cancer: a meta-analysis of observational studies. Breast Cancer Res Treat 2012; 135(1): 261–269.
15. Mansourian M, Haghjooy-Javanmard S, Eshraghi A, Vaseghi G, Hayatshahi A, Thomas J. Statins use and risk of breast cancer recurrence and death: a systematic review and meta-analysis of observational studies. J Pharm Pharm Sci 2016; 19(1): 72–81.
16. Huang X, He D, Ming J, He Y, Zhou C, Ren H, et al. High-density lipoprotein of patients with breast cancer complicated with type 2 diabetes mellitus promotes cancer cells adhesion to vascular endothelium via ICAM-1 and VCAM-1 upregulation. Breast Cancer Res Treat. 2016;155(3):441–55.
17. Rohatgi A, Westerterp M, von Eckardstein A, Remaley A, Rye KA. HDL in the 21st Century: A Multifunctional Roadmap for Future HDL Research. Circulation. 2021;143(23):2293–309.
18. Yamauchi Y, Rogers MA. Sterol Metabolism and Transport in Atherosclerosis and Cancer. Front Endocrinol (Lausanne). 2018 Sep 19; 9:509.

19. Kucharska-Newton AM, Rosamond WD, Mink PJ, Alberg AJ, Shahar E, Folsom AR. HDL-cholesterol and incidence of breast cancer in the ARIC cohort study. Annals of Epidemiol. 2008; 18:671–7.
20. Samadi S, Ghayour-Mobarhan M, Mohammadpour A, Farjami Z, Tabadkani M, Hosseinnia M, et al. High-density lipoprotein functionality and breast cancer: A potential therapeutic target. J Cell Biochem. 2019;120(4): 5756–5765.
21. Kopecka J, Godel M, Riganti C. Cholesterol metabolism: At the cross road between cancer cells and immune environment. Int J Biochem Cell Biol. 2020; 129:105876.
22. Pussinen PJ, Karten B, Wintersperger A, Reicher H, Mc Lean M, Malle E, et al. The human breast carcinoma cell line HBL-100 acquires exogenous cholesterol from high-density lipoprotein via CLA-1 (CD-36 and LIMPII analogous 1)-mediated selective cholesteryl ester uptake. Biochem J 2000; 349(Pt 2): 559–566.
23. Danilo C, Gutierrez-Pajares JL, Mainieri MA, Mercier I, Lisanti MP, Frank PG. Scavenger receptor class B type I regulates cellular cholesterol metabolism and cell signaling associated with breast cancer development. Breast Cancer Res 2013; 15(5): R87.
24. Smith B, Land H. Anticancer activity of the cholesterol exporter ABCA1 gene. Cell Rep 2012; 2(3): 580–590.
25. Sag D, Cekic C, Wu R, Linden J, Hedrick CC. The cholesterol transporter ABCG1 links cholesterol homeostasis and tumour immunity. Nat Commun 2015; 6: 6354.
26. Meaney S, Bodin K, Diczfalusy U, Björkhem I. J Lipid Res. 2002;43:2130–2135.
27. Sag D, Cekic C, Wu R, Linden J, Hedrick CC. The cholesterol transporter ABCG1 links cholesterol homeostasis and tumour immunity. Nat Commun. 2015 Feb 27;6:6354.
28. Nunes VS, Panzoldo NB, Leança CC, Parra ES, Zago VS, da Silva EJ, et al. Increased 27-hydroxycholesterol plasma level in men with low high density lipoprotein-cholesterol may circumvent their reduced cell cholesterol efflux rate. Clin Chim Acta. 2014; 433:169–73.
29. Wu Q, Ishikawa T, Sirianni R, Tang H, McDonald JG, Yuhanna IS, et al. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. Cell Rep. 2013;5(3):637–45.
30. Sharma B, Agnihotri N. Role of cholesterol homeostasis and its efflux pathways in cancer progression. J. Steroid Biochem. Mol. Biol. 2019;191:105377.
31. El Roz A, Bard JM, Huvelin JM, Nazih H. LXR agonists and ABCG1-dependent cholesterol efflux in MCF-7 breast cancer cells: relation to proliferation and apoptosis. Anticancer Res. 2012;32(7):3007–13.
32. Dalenc F, Iuliano L, Filleron T, Zerbinati C, Voisin M, Arellano C, et al. Circulating oxysterol metabolites as potential new surrogate markers in patients with hormone receptor-positive breast cancer: Results of the OXYTAM study. J Steroid Biochem Mol Biol. 2017; 169:210–218.
33. Hammond ME, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al; American Society of Clinical Oncology; College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen

and progesterone receptors in breast cancer (unabridged version). Arch Pathol Lab Med. 2010;134(7): e48-72.

34. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ; Panel members. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011;22(8):1736–47.
35. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499–502.
36. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein Measurement with the Folin-Phenol Reagent. J Biol Chem. 193:265–75, 1951.
37. Basu SK, Goldstein JL, Anderson RW, Brown MS. Degradation of cationized low-density lipoprotein and regulation of cho-lesterol metabolism in homozygous familial hypercholesterolemia fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 73, 3178-318210, 1976.
38. Minanni CA, Machado-Lima A, Iborra RT, Okuda LS, de Souza Pinto R, Santana MFM, et al. Persistent Effect of Advanced Glycated Albumin Driving Inflammation and Disturbances in Cholesterol Efflux in Macrophages. Nutrients. 2021 Oct 17;13(10): 3633.
39. Pinto RS, Ferreira GS, Silvestre GCR, Santana MFM, Nunes VS, Ledesma L, et al. Plasma advanced glycation end products and soluble receptor for advanced glycation end products as indicators of sterol content in human carotid atherosclerotic plaques. Diab Vasc Dis Res. 2022 Jan-Feb;19(1):14791641221085269.
40. Ferreira GS, Pinto PR, Iborra RT, Del Bianco V, Santana MFM, Nakandakare ER, et al. Aerobic Exercise Training Selectively Changes Oxysterol Levels and Metabolism Reducing Cholesterol Accumulation in the Aorta of Dyslipidemic Mice. Front Physiol. 2017 Sep 5;8:644.
41. Cedo L, Reddy ST, Mato E, Blanco-Vaca F, Escolà-Gil, JC. HDL and LDL: potential new players in breast cancer development. J Clin Med 2019; 8(6): 853.
42. Ghahremanfard F, Mirmohammadkhani M, Shahnazari B, Gholami G, Mehdizadeh J. The Valuable Role of Measuring Serum Lipid Profile in Cancer Progression. Oman Med. J. 2015, 30, 353–357.
43. Kumie G, Melak T, Baynes HW. The Association of Serum Lipid Levels with Breast Cancer Risks Among Women with Breast Cancer at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia. Breast Cancer Targets Ther. 2020, 12, 279–287.
44. Lu CW, Lo YH, Chen CH, Lin CY, Tsai CH, Chen PJ, Yang YF, et al. VLDL and LDL, but not HDL, promote breast cancer cell proliferation, metastasis and angiogenesis. Cancer Lett. 2017, 388, 130–138.
45. Michalaki V, Koutroulis G, Syrigos K, Piperi C, Kalofoutis A. Evaluation of serum lipids and high-density lipoprotein subfractions (HDL2, HDL3) in postmenopausal patients with breast cancer. Mol. Cell. Biochem. 2005, 268, 19–24.
46. Adorni MP, Ronda N, Bernini F, Zimetti F. High Density Lipoprotein Cholesterol Efflux Capacity and Atherosclerosis in Cardiovascular Disease: Pathophysiological Aspects and Pharmacological

Perspectives. Cells. 2021;10(3):574.

47. Schimanski S, Wild PJ, Treeck O, Horn F, Sigruener A, Rudolph C, et al. Expression of the lipid transporters ABCA3 and ABCA1 is diminished in human breast cancer tissue. Horm Metab Res. 2010;42(2):102–9.
48. Wang W, Lokman NA, Noye TM, Macpherson AM, Oehler MK, Ricciardelli C. ABCA1 is associated with the development of acquired chemotherapy resistance and predicts poor ovarian cancer outcome. Cancer Drug Resist. 2021; 4:485–502.
49. Pan H, Zheng Y, Pan Q, Chen H, Chen F, Wu J, et al. Expression of LXR–β, ABCA1 and ABCG1 in human triple–negative breast cancer tissues. Oncol Rep. 2019;42(5):1869–77.
50. Freeman MR, Solomon KR. Cholesterol and prostate cancer. J Cell Biochem. 2004;91(1):54–69; Krycer JR, Brown AJ. Cholesterol accumulation in prostate cancer: a classic observation from a modern perspective. Biochim Biophys Acta. 2013;1835(2):219 – 29.
51. De Gonzalo-Calvo D, López-Vilaró L, Nasarre L, Perez-Olabarria M, Vázquez T, Escuin D, et al. Intratumor cholesteryl ester accumulation is associated with human breast cancer proliferation and aggressive potential: a molecular and clinicopathological study. BMC Cancer. 2015; 15:460.
52. Murai T. Cholesterol lowering: role in cancer prevention and treatment. Biol Chem. 2015;396(1):1–11.
53. Gutierrez-Pajares JL, Ben Hassen C, Chevalier S, Frank PG. SR-BI: Linking Cholesterol and Lipoprotein Metabolism with Breast and Prostate Cancer. Front Pharmacol. 2016; 7:338.
54. Yuan B, Wu C, Wang X, Wang D, Liu H, Guo L, et al. High scavenger receptor class B type I expression is related to tumor aggressiveness and poor prognosis in breast cancer. Tumour Biol. 2016;37, 3581–3588.
55. Llaverias G, Danilo C, Mercier I, Daumer K, Capozza F, Williams T M. Role of cholesterol in the development and progression of breast cancer. Am. J. Pathol. 2011;178, 402–12.
56. Cao WM, Murao K, Imachi H, Yu X, Abe H, Yamauchi A, et al. A mutant high-density lipoprotein receptor inhibits proliferation of human breast cancer cells. Cancer Res. 2004; 64, 1515–21.
57. Kontush, A. HDL and Reverse Remnant-Cholesterol Transport (RRT): Relevance to Cardiovascular Disease. Trends Mol. Med. 2020; 26, 1086–1100.
58. Dai D, Chen B, Wang B, Tang H, Li X, Zhao Z, et al. Pretreatment TG/HDL-C Ratio Is Superior to Triacylglycerol Level as an Independent Prognostic Factor for the Survival of Triple Negative Breast Cancer Patients. J Cancer. 2016 Aug 7;7(12):1747–1754.
59. Catasus L, Gallardo A, Llorente-Cortes V, Escuin D, Muñoz J, Tibau A, et al. Low-Density Lipoprotein Receptor-Related Protein 1 Is Associated with Proliferation and Invasiveness in Her-2/Neu and Triple-Negative Breast Carcinomas. Hum. Pathol. 2011, 42, 1581–1588.
60. Gallagher EJ, Zelenko Z, Neel BA, Antoniou IM, Rajan L, Kase N, et al. Elevated Tumor LDLR Expression Accelerates LDL Cholesterol-Mediated Breast Cancer Growth in Mouse Models of Hyperlipidemia. Oncogene 2017, 36, 6462–6471.

61. Maubant S, Tahtouh T, Brisson A, Maire V, Némati F, Tesson B, et al. LRP5 Regulates the Expression of STK40, a New Potential Target in Triple-Negative Breast Cancers. Oncotarget 2018, 9, 22586–22604.
62. Huang J, Li L, Lian J, Schauer S, Vesely PW, Kratky D, et al. Tumor-Induced Hyperlipidemia Contributes to Tumor Growth. Cell Rep. 2016 Apr 12;15(2):336 – 48.
63. Eghlimi R, Shi X, Hrovat J, Xi B, Gu H. Triple Negative Breast Cancer Detection Using LC–MS/MS Lipidomic Profiling. Journal of Proteome Research 2020 19 (6), 2367–2378.
64. Flote VG, Vettukattil R, Bathen TF, Egeland T, McTiernan A, Frydenberg H, et al. Lipoprotein subfractions by nuclear magnetic resonance are associated with tumor characteristics in breast cancer. Lipids Health Dis. 2016, 15, 1–12.
65. Jung SM, Kang D, Guallar E, Yu J, Lee JE, Kim SW, et al. Impact of Serum Lipid on Breast Cancer Recurrence. J. Clin. Med. 2020, 9, 2846.
66. Corona G, Di Gregorio E, Vignoli A, Muraro E, Steffan A, Miolo G. 1H-NMR Plasma Lipoproteins Profile Analysis Reveals Lipid Metabolism Alterations in HER2-Positive Breast Cancer Patients. Cancers (Basel). 2021 Nov 21;13(22):5845.
67. Sawada MIBACS. Reduced high-density lipoprotein (HDL)-mediated cell cholesterol efflux and alteration in the composition of this lipoprotein in women with breast cancer. [dissertation]. São Paulo: Universidade Nove de Julho; 2021. 115 p.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupportinginformationSAWADA.docx