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**Clinical Research Article**

# Association of Cholesterol and Oxysterols in Adipose Tissue With Obesity and Metabolic Syndrome Traits

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## Abstract

**Objective:** Adipose tissue stores a substantial amount of body cholesterol in humans. Obesity is associated with decreased concentrations of serum cholesterol. During weight gain, adipose tissue dysfunction might be one of the causes of metabolic syndrome. The aim of this study is to evaluate cholesterol storage and oxidized metabolites in adipose tissue and their relationship with metabolic clinical characteristics.

**Methods:** Concentrations of cholesterol and oxysterols (27-hydroxycholesterol and 24S-hydroxycholesterol) in subcutaneous and visceral adipose tissue were determined by high-performance liquid chromatography with tandem mass spectrometry in 19 adult women with body mass index between 23 and 40 kg/m$^2$ from the FAT expandability (FATe) study. Tissue concentration values were correlated with biochemical and clinical characteristics using nonparametric statistics.

**Results:** Insulin correlated directly with 24S-hydroxycholesterol in both adipose tissues and with 27-hydroxycholesterol in visceral tissue. Leptin correlated directly with 24S-hydroxycholesterol in subcutaneous adipose tissue. Tissue cholesterol correlated directly with 27-hydroxycholesterol in both adipose tissues and with 24S-hydroxycholesterol in visceral tissue, where cholesterol correlation with 24S-hydroxycholesterol was higher than with 27-hydroxycholesterol. In addition, some tendencies were observed: serum high-density lipoprotein cholesterol tended to be inversely correlated with visceral adipose tissue cholesterol; high-sensitivity C-reactive protein tended to be correlated directly with subcutaneous adipose 24S-hydroxycholesterol and inversely with visceral 27-hydroxycholesterol.

**Conclusions:** Adipose tissue oxysterols are associated with blood insulin and insulin resistance. Tissue cholesterol correlated more with 27-hydroxycholesterol in subcutaneous adipose tissue and with 24S-hydroxycholesterol in visceral adipose tissue. Levels of adipose 24S-hydroxycholesterol seem to be correlated with some metabolic syndrome symptoms and inflammation while adipose 27-hydroxycholesterol could represent some protection against them.

**Key Words:** adipose tissue, 24S-hydroxycholesterol, 27-hydroxycholesterol, adipose tissue dysfunction, metabolic syndrome

**Abbreviations:** BMI, body mass index; FATe, FAT expandability; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HPLC-MS/MS, high-performance liquid chromatography coupled to tandem mass spectrometry; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostatic model assessment insulin resistance index; LDL, low-density lipoprotein.

Obesity is defined as an excessive accumulation of adipose tissue (1). Obesity, along with other factors such as high blood pressure, hyperglycemia, hypertriglyceridemia, or low levels of high-density lipoprotein (HDL) cholesterol, constitutes the metabolic syndrome (2). The metabolic syndrome is a risk factor for the development of type 2 diabetes mellitus, cardiovascular disease, and other adverse pathological conditions, and it has an increasing prevalence worldwide (2, 3). Obesity arises primarily as an interaction between the individual's genetic makeup and a positive energy balance resulting when caloric intake exceeds energy expenditure over time. This condition induces a complex remodeling of adipose tissue, which expands to adapt to the energy surplus and noticeably changes its structure and cellular composition (4-6). The distribution of adipose tissue in obesity has important implications because most of the metabolic abnormalities that have been found linked with obesity are related to abdominal visceral obesity (7). Subcutaneous and visceral adipose

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tissues show structural and functional differences, and therefore, they also behave differently when a deregulation of the adipose tissue occurs (8, 9). Most complications associated with obesity seem to depend on an excess of accumulation of visceral fat, acting as a marker of dysfunction in adipose tissue (10), which associates with insulin resistance and has a profound effect on the cardiometabolic risk profile, including derangement of plasma lipids (7).

A key element associated with cardiovascular disease risk in the population, including obese patients, is the concentration of low-density lipoprotein (LDL) cholesterol. Attending to the dose-response relationship between body mass index (BMI) and LDL cholesterol, subjects with obesity have lower plasma cholesterol than that expected from the relation between BMI and LDL cholesterol observed in subjects with normal weight (11). Cholesterol tends to decrease in blood precisely when the metabolic complications of obesity begin to appear. Adipose tissue plays a fundamental role in damping oscillations of lipids, including cholesterol (12). Cholesterol concentration in adipocytes is proportional to their size, mainly driven by their triglyceride content but independent of plasma concentration (13). Given that obesity increases cholesterol production (14) and given that adipocytes do not have synthesis capacity (15), cholesterol accumulation in the adipose tissue must be dependent on an influx of cholesterol. Hence, we hypothesized that such accumulation could explain the observed decrease in total and LDL cholesterol among obese subjects, and more important, it could favor the development of the adipose tissue dysfunction described in the metabolic syndrome.

Liver metabolism of cholesterol in obesity has been studied (15) but in other tissues is still poorly documented. This is the case of storage and catabolism of cholesterol, which includes formation of oxidized cholesterol derivatives, better known as oxysterols. Oxysterols can be formed through enzymatic reactions, mainly involving the CYP 450 enzymes superfamily, or they can be formed through nonenzymatic pathways (16). The involvement of these bioactive lipids in apoptosis or inflammatory processes (17-20), as well as in conditions such as atherosclerosis, has been demonstrated (21). Furthermore, the central role played by several oxysterols as regulators of cholesterol metabolism, upon liver X receptors binding, is now well characterized (22, 23). Thus, oxysterols are essential to explain the intracellular metabolism of cholesterol, so they may play a role in adipose tissue dysfunction associated with obesity, as there is evidence that they are produced intracellularly in adipocytes (24). Cholesterol is uptaken by adipocytes via LDL receptors SR-B1, LRP1, and VLDLR (25) where it is oxidized enzymatically and nonenzymatically. Oxidation into 27-hydroxycholesterol via CYP27A1 is the pathway that has been more often studied (24).

There are currently several methods described for the measurement of oxysterols in serum (26, 27). Most use gas chromatography coupled to mass spectrometry and high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) (28). Determination of cholesterol and oxysterols in adipose tissue is more challenging methodologically due to the complexity of the tissue and the lack of homogeneity of the sample. However, some researchers have achieved successful measurements of oxysterols by HPLC-MS/MS in different types of tissues, such as the hypothalamus, liver, and adipose tissue (29, 30).

Thus, to investigate the changes in adipose tissue cholesterol metabolism associated with obesity and metabolic syndrome–related changes, we measured cholesterol and oxysterols by HPLC-MS/MS in subcutaneous and visceral adipose tissue in several individuals at different stages of body weight and analyzed the correlations of adipose tissue sterols with various clinical and metabolic variables.

## Material and Methods

### Subjects

Adipose tissue specimens from 19 women aged between 30 and 60 years belonging to the FAT expandability (FATe) study (31) were selected. FATe is a study that collected specimens during surgery, and it was designed to identify factors that limit the expansion of subcutaneous adipose tissue in Caucasian individuals. None of the patients was diabetic or undergoing treatment to lower lipid levels. All patients signed the informed consent to participate in the protocol, approved by the reference medical ethics committee (Clinical Research Ethics Committee of Aragon, Spain).

### Sample Collection

Samples of subcutaneous adipose tissue from abdomen and visceral adipose tissue from omentum were collected from women undergoing a scheduled surgery: cholecystectomy, hernia repair, or bariatric surgery (31). Biopsies were washed, devoid of vascular and connective tissue, and subsequently stored at −80°C in cryopreservation vials (32).

### Lipid Extraction From Adipose Tissue

Each extraction process used approximately 100 mg of tissue, which was homogenized for 20 minutes in chloroform/ methanol (2:1, 1.2 mL) using an Ultra-Turrax disperser (IKA-Werke GmbH & Co. KG, Staufen, Germany). To ensure adequate rupture of cell membranes, samples were sonicated for 15 minutes. After this, chloroform-water (1:1, 200 µL of each) was added, and the samples were centrifuged in glass vials with screw cap. Three phases were separated correctly: an aqueous phase in the upper layer, a protein interface, and an organic phase containing the lipids in the lower layer. The lipid extract was transferred to a glass vial, dried under a nitrogen atmosphere, and subsequently reconstituted in 200 µL of methanol. Lipid extracts were stored at −80°C until posterior extraction of cholesterol and oxysterols.

### Cholesterol and Oxysterols Extraction and HPLC-MS/MS Measurement From Lipid Extract

The total lipid extract collected in the first extraction was transferred to a glass vial with screw cap. Concentrations of oxysterols and cholesterol were quantified using HPLC-MS/MS according to the method previously described (26). Briefly, 100 µL of lipid extract was transferred to a screw-capped vial, and deuterium-labeled internal standard ($^2$H6) cholesterol-26,26,26,27,27,27 (7.9 mM) was added to determine oxysterols. Another 100 µL of extract was transferred to a screw-capped vial, and deuterium-labeled internal standard ($^2$H7) cholesterol-25,26,26,26,27,27,27 was added to determine cholesterol. Alkaline hydrolysis was performed for 20 minutes at 60°C in an ultrasound bath, and lipids were extracted twice with 3 mL of hexane. The extracts were loaded onto the SPE cartridge (1 mg, Discovery DSC-18, Supelco,

Spain), which was preconditioned with 400 µL of methanol and gravity eluted. Sterols were desorbed with 1.4 mL of 2-propanol by gravity and 40 µL of the final mixtures were injected into the HPLC-MS/MS system. Due to an accidental mishappening during measurement, 1 cholesterol reading in subcutaneous adipose tissue could not be obtained.

### Serum Laboratory Parameters

Blood samples were drawn by venipuncture after a 12-hour fast. The levels of total cholesterol, triglycerides, and HDL cholesterol were measured in serum with standard enzymatic methods, all of them on a Beckman Coulter AU analyzer (Beckman Coulter, USA). Total cholesterol was quantified by the esterase-oxidase-4-aminoantipyrine method. Triglycerides were determined by the lipase peroxidase method. HDL cholesterol was determined by direct method (non-apoB lipoproteins). LDL cholesterol levels were estimated with the Friedewald formula when serum triglycerides were <400 mg/dL. Apolipoprotein A1, apolipoprotein B, lipoprotein (a), insulin, leptin, glucose, glycated hemoglobin (HbA1c), and high-sensitivity C-reactive protein (hs-CRP) were also measured in the FATe study (31). The homeostatic model assessment insulin resistance index (HOMA-IR) was calculated.

### Clinical Data

Systolic and diastolic blood pressure and physician diagnosis of hypertension were collected from electronic clinical records.

### Statistical Analysis

Data are expressed as median (percentile 25, percentile 75). Comparisons are performed with Mann-Whitney U tests for unrelated variables and Wilcoxon signed-rank tests for paired variables. The sample was split in 2 equal sized groups for description of the sample (cutoff value at 31 kg/m$^2$). Using the obesity threshold was considered but turned down because, given the groups sizes, it would have reduced statistical power without benefits and it would not provide results robust enough to support that the differences occur at that specific threshold. Associations were studied by calculating Spearman's correlation coefficient. Coefficients above 0.4 were considered as hypothesis-generating and enumerated. Fisher's transformation of the correlation coefficients was used to identify which coefficients differed most between the territories. *P*-values < 0.05 were considered significant. Statistical analysis was performed using R software version 3.4.4.

## Results

### Biochemical and Clinical Characteristics of the Sample

BMI of women in our sample spanned a range between 23 and 40 kg/m$^2$ (Table 1). Women with higher BMI had lower HDL cholesterol ($P = 0.033$) and apolipoprotein A1 ($P = 0.043$) and higher insulin ($P = 0.037$), HOMA-IR ($P = 0.008$), and HbA1c ($P = 0.024$).

**Table 1.** Biochemical (blood samples), clinical characteristics, and measurements on the sample of women

| | Overall (n = 19) | Lower-half BMI group (n = 9) | Upper-half BMI group (n = 10) | *P*-value |
|---|---|---|---|---|
| Age, years | 49.4 (38.3, 52.0) | 49.8 (41.4, 52.3) | 44.4 (37.4, 51.6) | 0.720 |
| BMI, kg/m$^2$ | 33.5 (29.0, 38.6) | 28.2 (25.7, 30.4) | 38.6 (37.4, 39.6) | — |
| Systolic blood pressure, mmHg | 120.0 (110.0, 128.0) | 124.0 (110.0, 127.0) | 120.0 (112.5, 128.0) | 0.711 |
| Diastolic blood pressure, mmHg | 75.0 (67.5, 81.5) | 74.0 (70.0, 86.0) | 75.5 (66.2, 80.0) | 0.743 |
| Serum total cholesterol, mg/dL | 197.0 (176.5, 224.0) | 222.0 (180.0, 234.0) | 190.0 (169.2, 207.5) | 0.141 |
| Serum triglycerides, mg/dL | 91.4 (75.7, 101.5) | 92.6 (83.2, 101.5) | 87.0 (74.8, 101.4) | 1.000 |
| Serum HDL cholesterol, mg/dL | 59.0 (51.5, 61.5) | 61.0 (59.0, 62.0) | 52.5 (49.0, 58.5) | 0.033 |
| Serum LDL cholesterol, mg/dL | 108.0 (102.0, 131.5) | 111.0 (106.0, 153.0) | 105.5 (98.5, 117.5) | 0.182 |
| Apolipoprotein A1, mg/dL | 154.0 (146.0, 177.0) | 166.0 (154.0, 188.0) | 147.5 (136.8, 155.5) | 0.043 |
| Apolipoprotein B, mg/dL | 102.0 (90.4, 116.5) | 101.0 (88.1, 121.0) | 104.0 (93.9, 111.5) | 0.870 |
| Lipoprotein (a), mg/dL | 20.4 (11.2, 45.4) | 22.9 (16.5, 76.4) | 17.5 (9.5, 28.7) | 0.236 |
| Insulin, µU/mL | 4.8 (3.3, 7.0) | 3.3 (2.4, 6.2) | 5.9 (4.8, 12.0) | 0.037 |
| Leptin, ng/mL | 25.3 (21.2, 38.5) | 27.5 (13.5, 37.4) | 25.1 (23.9, 36.3) | 0.780 |
| Glucose, mg/dL | 84.0 (76.5, 96.5) | 79.0 (76.0, 81.0) | 92.0 (84.8, 106.0) | 0.060 |
| HOMA-IR | 1.2 (0.6, 1.8) | 0.7 (0.5, 1.1) | 1.4 (1.2, 2.5) | 0.008 |
| HbA1c, % | 5.5 (5.2, 5.8) | 5.3 (5.1, 5.5) | 5.7 (5.5, 6.0) | 0.024 |
| hs-CRP, mg/dL | 0.41 (0.20, 0.82) | 0.41 (0.19, 0.92) | 0.40 (0.24, 0.71) | 0.870 |
| SCAT Cholesterol, mg/g | 1.090 (0.927, 1.344) | 1.098 (0.976, 1.357) | 0.999 (0.776, 1.282) | 0.436 |
| SCAT 27-hydroxcholesterol, µg/g | 0.059 (0.044, 0.089) | 0.061 (0.044, 0.097) | 0.058 (0.040, 0.076) | 0.780 |
| SCAT 24S-hydroxycholesterol, µg/g | 0.121 (0.093, 0.151) | 0.101 (0.096, 0.142) | 0.135 (0.098, 0.187) | 0.243 |
| VAT Cholesterol, mg/g | 0.987 (0.878, 1.235) | 0.934 (0.859, 1.066) | 1.107 (0.969, 1.350) | 0.133 |
| VAT 27-hydroxcholesterol, µg/g | 0.077 (0.057, 0.094) | 0.064 (0.044, 0.077) | 0.084 (0.063, 0.114) | 0.278 |
| VAT 24S-hydroxycholesterol, µg/g | 0.114 (0.087, 0.144) | 0.089 (0.074, 0.123) | 0.134 (0.095, 0.149) | 0.156 |

Data are expressed as median (interquartile range). *P*-values were calculated with Mann-Whitney U tests.
Abbreviations: BMI, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SCAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

## Cholesterol and Oxysterols in Adipose Tissue

We did not find within-subject differences (pairwise statistical tests) in the concentration of cholesterol or oxysterols between subcutaneous and visceral adipose tissue (Table 2). Surprisingly, the sterols concentrations in subcutaneous adipose tissue did not correlate within subjects with their concentrations in the visceral adipose tissue. Within each kind of adipose tissue, in both territories, cholesterol concentration significantly correlated directly with 27-hydroxycholesterol, and both oxysterols were significantly directly correlated between them. However, cholesterol directly correlated with 24s-hydroxycholesterol significantly only in visceral adipose tissue, where it showed higher correlation than with 27-hydroxycholesterol, in contrast with subcutaneous adipose tissue, where 27-hydroxycholesterol was more correlated (Table 3).

## Correlations Between Clinical Data and Lipids in Adipose Tissue

Only insulin and leptin had statistically significant correlations with oxysterols concentrations, and they were direct. No correlation between clinical variables and cholesterol concentration in adipose tissue reached statistical significance. Insulin and HOMA-IR were directly significantly correlated with 24S-hydroxycholesterol in both territories. They were also directly significantly correlated with 27-hydroxycholesterol in visceral adipose tissue. Leptin was directly significantly correlated with 24S-hydroxycholesterol in subcutaneous adipose tissue (Table 4).

Descriptively, we also enumerate correlations that were stronger than 0.4, disregarding statistical significance. Visceral adipose tissue cholesterol was inversely correlated with serum HDL cholesterol. Visceral adipose tissue 27-hydroxycholesterol was inversely correlated with serum triglycerides and with hs-CRP. 24S-hydroxycholesterol only showed 2 additional correlations above 0.4, which were both direct. It correlated with hs-CRP in subcutaneous adipose tissue and with diastolic blood pressure in visceral adipose tissue (Table 4).

In relation to the initial hypothesis of cholesterol storage, there was a weak negative correlation of cholesterol concentration in both territories with total and LDL serum cholesterol, although no statistical significance was reached. Correlation of BMI with adipose cholesterol concentration was weak and not statistically significant, but interestingly, correlations were in opposite directions: it was inverse in subcutaneous adipose tissue and direct in visceral adipose tissue (Table 4), as it was anticipated in the initial descriptive table (Table 1).

## Comparison of Subcutaneous and Visceral Adipose Tissue Lipids Concentration Associations With Clinical Variables

There were no differences in lipid concentrations between territories (Table 2), but here we describe differences between the correlation coefficients reported in the previous section (Table 4) that differed most using the Fisher's transformation of the correlation coefficients. The correlation of BMI with cholesterol concentration tended to be different and in opposite direction between the territories: –0.385 vs 0.230 in the subcutaneous and visceral adipose tissues, respectively. Other associations that differed in direction substantially were those of adipose lipid concentrations with serum HDL cholesterol, apolipoprotein A1, insulin, and HOMA-IR (Fig. 1).

Figure 2 shows the tendency mentioned in the previous results section for an inverse correlation between visceral adipose tissue cholesterol concentration and serum total cholesterol (Spearman's coefficient –0.221) and that women in the group with higher BMI tend to have both higher visceral

**Table 2.** Cholesterol and oxysterols concentration in subcutaneous and visceral adipose tissue

| | n | Subcutaneous adipose tissue | Visceral adipose tissue | *P*-value |
|---|---|---|---|---|
| Cholesterol, mg/g | 18 | 1.090 (0.927, 1.344) | 0.984 (0.869, 1.231) | 0.167 |
| 27-hydroxcholesterol, µg/g | 19 | 0.059 (0.044, 0.089) | 0.077 (0.057, 0.094) | 0.275 |
| 24S-hydroxycholesterol, µg/g | 19 | 0.121 (0.093, 0.151) | 0.114 (0.087, 0.144) | 0.395 |

Data are expressed as median (interquartile range). *P*-values were calculated with Wilcoxon signed-rank tests.
Abbreviation: BMI, body mass index.

**Table 3.** Inter- and Intratissue Spearman's rank correlation of lipid concentrations (n = 19)

| | Subcutaneous adipose tissue | | | Visceral adipose tissue | | |
|---|---|---|---|---|---|---|
| | Cholesterol[a] (n = 18) | 27-hydroxy-cholesterol | 24S-hydroxy-cholesterol | Cholesterol | 27-hydroxy-cholesterol | 24S-hydroxy-cholesterol |
| **Visceral adipose tissue** | **Intertissue correlations** | | | **Intratissue (visceral) correlations** | | |
| Cholesterol | 0.232 | –0.072 | 0.012 | — | **0.475*** | **0.705*** |
| 27-hydroxycholesterol | –0.197 | 0.070 | 0.151 | | — | **0.684*** |
| 24S-hydroxycholesterol | 0.187 | –0.018 | 0.232 | | | |
| **Subcutaneous adipose tissue** | **Intratissue (subcutaneous) correlations** | | | | | |
| Cholesterol (n = 18) | — | **0.552*** | 0.298 | | | |
| 27-hydroxycholesterol | | — | **0.567*** | | | |

Cell contents: Spearman's rank correlations. Boldfaced correlations with absolute value above 0.4. *$P < 0.05$.
[a]**Analysis involving subcutaneous adipose tissue cholesterol concentration are performed in 18 subjects.**

adipose tissue cholesterol and lower serum total cholesterol, although these observations are merely descriptive.

## Discussion

In this study, we explored whether cholesterol and oxidative metabolites concentrations in adipose tissue are related to the presence of metabolic syndrome traits and whether they are related to cholesterol deposition on adipose tissue as BMI increases. Those relationships could explain the paradoxical lower-than-expected serum total and LDL cholesterol concentration in obese subjects, and, more important, characterize the presence of adipose tissue dysfunction. To support our hypothesis, we measured cholesterol and oxysterols concentrations in subcutaneous and visceral adipose tissue using HPLC-MS/MS. We found several statistically confirmed associations: insulin correlated directly with 24S-hydroxycholesterol in both visceral and subcutaneous adipose tissues and with 27-hydroxycholesterol in the visceral tissue, and leptin correlated with 24S-hydroxycholesterol in subcutaneous adipose tissue. Tissue cholesterol correlated with 27-hydroxycholesterol in both adipose tissues and with 24S-hydroxycholesterol in the visceral tissue, and both oxysterols were highly correlated between them.

We also found several trends that can help focus future research: serum HDL cholesterol tended to be inversely correlated with visceral adipose tissue cholesterol and serum triglycerides with 27-hydroxycholesterol; hs-CRP tended to be correlated directly with subcutaneous adipose 24S-hydroxycholesterol and inversely with visceral 27-hydroxycholesterol. Diastolic blood pressure and visceral adipose 24S-hydroxycholesterol also tended to be directly correlated. Serum cholesterol tended to be inversely associated with adipose tissue cholesterol. In addition, several associations were in opposite directions in each of the territories: the association of BMI with adipose cholesterol (inverse in subcutaneous tissue and direct in visceral tissue), serum HDL cholesterol, and insulin. Altogether, our data support the concept that cholesterol is differentially accumulated in subcutaneous and visceral adipose tissue, and cholesterol metabolism within visceral adipose tissue may play a role in the adipose tissue dysfunction observed in obesity. These differences add to those previously described, such as a more important role of visceral adipose tissue in systemic inflammation (33).

Adipocyte and adipose tissue dysfunction may link obesity to several health problems (34) such as type 2 diabetes, dyslipidemia, hypertension, fatty liver disease, and atherosclerosis (1). Visceral adipose tissue expands in advanced phases of obesity, and when it reaches high volumes, in what is called visceral obesity, the metabolic syndrome appears. We observe a direct correlation of adipose 24S-hydroxycholesterol and insulin and insulin resistance. Insulin has a trophic action on adipose tissues, and the higher insulin levels occurring during the progression of the metabolic syndrome might reflect a last effort of the organism to store as much surplus energy as possible (34). The phasic recruitment of tissues for energy storage, first subcutaneous and then visceral, may imply that in late stages when insulin is already raising, visceral adipose tissue metabolic activity is also higher, which could explain that insulin also correlates directly with 27-hydroxycholesterol in visceral adipose tissue.

In general, with the exception just mentioned, we observe 24S-hydroxycholesterol directly associated with characteristics related to the metabolic syndrome such as a higher

**Table 4.** Spearman's rank correlation of lipid concentrations with clinical parameters (n = 19)

| | Subcutaneous adipose tissue | | | Visceral adipose tissue | | |
|---|---|---|---|---|---|---|
| | Cholesterol[a] | 27-hydroxy-cholesterol | 24S-hydroxy-cholesterol | Cholesterol | 27-hydroxy-cholesterol | 24S-hydroxy-cholesterol |
| Age | −0.267 | −0.046 | −0.202 | −0.353 | −0.339 | −0.311 |
| BMI | −0.385 | −0.100 | 0.284 | 0.230 | 0.246 | 0.163 |
| Systolic blood pressure | 0.243 | 0.184 | −0.001 | −0.090 | 0.028 | 0.112 |
| Diastolic blood pressure | 0.226 | 0.048 | −0.019 | 0.086 | 0.126 | **0.400** |
| Serum total cholesterol | −0.282 | −0.002 | 0.020 | −0.221 | 0.004 | 0.017 |
| Serum triglycerides | 0.017 | −0.186 | −0.290 | 0.166 | **−0.409** | −0.095 |
| Serum HDL cholesterol | 0.114 | 0.351 | 0.346 | **−0.410** | −0.128 | −0.133 |
| Serum LDL cholesterol | −0.115 | −0.198 | −0.016 | −0.209 | −0.358 | −0.146 |
| Apolipoprotein A1 | 0.106 | 0.312 | 0.167 | −0.361 | −0.184 | −0.160 |
| Apolipoprotein B | −0.078 | −0.362 | −0.119 | 0.311 | −0.012 | 0.053 |
| Lipoprotein (a) | −0.168 | −0.274 | −0.138 | 0.019 | −0.159 | −0.129 |
| Insulin | −0.163 | 0.204 | **0.498*** | 0.281 | **0.596*** | **0.460*** |
| Leptin | −0.160 | 0.153 | **0.560*** | 0.030 | 0.361 | 0.330 |
| Glucose | −0.388 | −0.227 | 0.049 | −0.097 | 0.141 | 0.068 |
| HOMA-IR | −0.108 | 0.167 | **0.519*** | 0.282 | **0.553*** | **0.523*** |
| HbA1c | 0.133 | 0.095 | 0.373 | 0.096 | −0.048 | 0.233 |
| hs-CRP | 0.307 | −0.001 | **0.427** | 0.143 | **−0.408** | 0.055 |

Cell contents: Spearman's rank correlations. Boldfaced correlations with absolute value above 0.4. *$P < 0.05$. Analysis involving subcutaneous adipose tissue cholesterol concentration are performed in 18 subjects.
Abbreviations: BMI, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.
[a]Analysis involving subcutaneous adipose tissue cholesterol concentration are performed in 18 subjects.

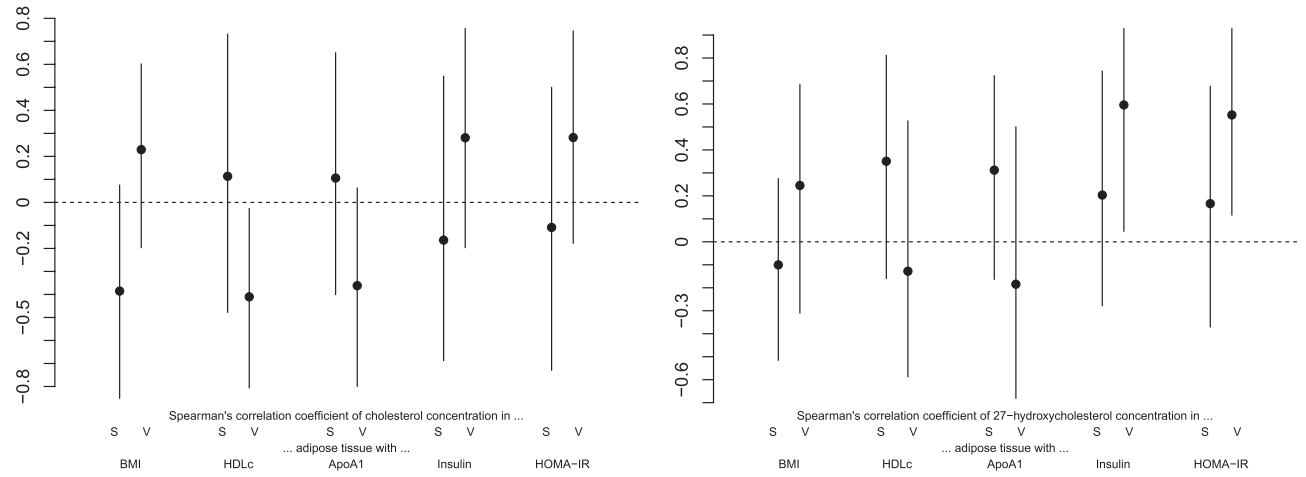


**Figure 1.** Spearman's correlation coefficient of cholesterol (upper panel) and 27- hydroxycholesterol (lower panel) concentration in subcutaneous and visceral adipose tissue with those clinical parameters in which the correlations differ most between territories. Abbreviations: ApoA1, apolipoprotein A1; BMI, body mass index; HDLc, serum high-density lipoprotein cholesterol, S, subcutaneous; V, visceral.

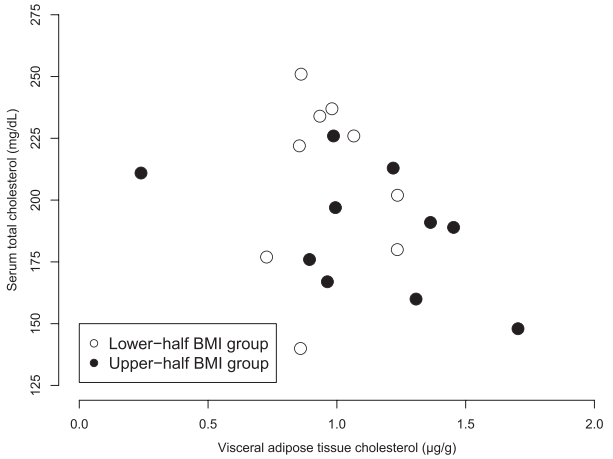


**Figure 2.** Scatter plot of visceral adipose tissue cholesterol concentration and serum total cholesterol stratified by body mass index group.

diastolic blood pressure (visceral adipose concentration) and inflammation, as measured by hs-CRP (subcutaneous adipose concentration). In contrast, 27-hydroxycholesterol seems to behave oppositely as higher levels associate with lower serum triglycerides and lower inflammation (both in visceral adipose tissue). Several studies highlight that oxysterols can modulate aspects of the metabolic syndrome such as insulin sensitivity and glucose homeostasis (35), acting through different mechanisms, including acting as signal mediators or producing direct cellular toxicity (36, 37). The local 27-hydroxycholesterol biosynthesis pathway may be regarded as a protective mechanism (24). The synthesis of 27-hydroxycholesterol from cholesterol by CYP27A1 in adipocytes could signify an adaptive mechanism to eliminate the excess of cholesterol from adipose tissue. It may prevent the formation of new fat cells in the context of excess cholesterol induced by dietary habits (24). In this line, patients with CYP27A1 deficiency (cerebrotendinous xanthomatosis) display a lack of 27-hydroxycholesterol, and this disease is characterized by an accumulation of lipids and an enhanced tendency to develop atherosclerosis (38). Furthermore, hyperlipidemic mice, following a high-fat diet, that were treated with 27-hydroxycholesterol showed reduced hepatic inflammation in nonalcoholic fatty liver disease (39).

In nationwide epidemiological studies, we evaluated the cross-sectional relationship between BMI and serum cholesterol concentration in 2 population cohorts (11). We observed that as BMI increased, serum total and LDL cholesterol also increased, but above BMI 27.1 kg/m², total and LDL cholesterol decreased significantly with higher BMI. In this study, we describe that a possible explanation for the trend to an inverse association between BMI and serum cholesterol in overweight and obese women may be due, at least in part, to a higher cholesterol concentration in adipose tissue associated with lower serum concentration, which could multiply a deposit effect of an increased fat mass. Blood cholesterol concentration depends mostly on its absorption, hepatic synthesis, storage, and digestive excretion through the bile. In obesity, cholesterol metabolism is characterized by increased cholesterol synthesis and absorption (14, 15). However, the storage of cholesterol in obesity—in particular, the differences in cholesterol metabolism in adipose tissue territories—has not been previously thoroughly studied. Adipocytes accrue cholesterol as they expand (40), and this may impact cholesterol distribution between compartments as the amount of circulating serum cholesterol is very small compared to the total cholesterol in the human body. The sequential adipose expansion mentioned earlier, with "saturated" subcutaneous tissue and actively expanding visceral tissue, would be compatible with the different association of tissue cholesterol concentration with BMI: cholesterol accumulation is inversely associated with BMI in subcutaneous tissue while directly associated in visceral tissue. Interestingly, we found a tendency for an inverse correlation between serum HDL cholesterol and visceral adipose cholesterol concentration, showing a convergence of visceral adipose tissue expansion, an increase of visceral adipose cholesterol with BMI, increased insulin stimulus associated with both cholesterol oxidative visceral adipose metabolites, and a decrease of serum HDL cholesterol associated with that cholesterol deposition (40).

Likewise, the hypothesis suggested in this study on the buffering function of adipose tissue could be part of the theory of adipose tissue dysfunction, since we observed that in

obesity, cholesterol accumulated in adipose tissue with different metabolic consequences. In visceral adipose tissue, this cholesterol could suffer an oxidative degradation to 24S-hydroxycholesterol, a different pathway from that happening in subcutaneous adipose tissue. The association of BMI and adipose cholesterol that we described, opposite between territories, seems to indicate that subcutaneous adipose tissue cholesterol concentration behaves similarly to that in serum, accounting for less cholesterol stored per gram of tissue as BMI increases. Along that process, it is the recruiting of visceral tissue for lipid storage, which shows a different behavior, that could increase cholesterol storage, justify decreased concentrations in serum and subcutaneous adipose tissue, and increase the 24S-hydroxycholesterol oxidative pathway that we found associated with metabolic syndrome and inflammation. Previous studies in animal models support that obesity induces measurable changes in oxysterol synthesis in liver and adipose tissue (30). Whether the increase in cholesterol and its metabolites in visceral adipose tissue associated with BMI that we observed is primary or secondary, whether they play any role in the dysfunction of adipose tissue in obesity, and to what extent they affect overall metabolic abnormalities will need to be elucidated in future research.

Our study has several limitations, mainly derived from the complexity of intracellular cholesterol metabolism. Studying cholesterol and oxysterols tissue concentration may at most reflect only partial aspects of this complexity. Although it has been described that enrichment of free cholesterol on the lipid droplets' surface is associated with impaired adipose tissue function (41), our methods cannot discern cholesterol location and cannot help in extend the knowledge in that regard. The relatively small sample size may have limited the power to find statistically significant associations. Only women were studied to avoid sex heterogeneity, given that the methods conditioned that the sample size should be kept below reasonable boundaries. This study used an exploratory statistical approach to pioneer in the field. In that regard, we do not consider a strict statistical significance framework. Multiple tests have been performed, and thus some of the statistically significant reported associations may be false positives. However, we find value in the overall description of this understudied matter, and accordingly, we have described tendencies disregarding statistical significance, acknowledging that there is unavoidable random error. Altogether, findings were consistent, and this study will serve as exploratory analysis for other potential links.

In summary, adipose tissue oxysterols are associated with blood insulin and insulin resistance. Tissue cholesterol correlated more with 27-hydroxycholesterol in subcutaneous adipose tissue and with 24S-hydroxycholesterol in visceral adipose tissue. Levels of adipose 24S-hydroxycholesterol seem to be correlated with some metabolic syndrome symptoms and inflammation while adipose 27-hydroxycholesterol could represent some protection against them. Cholesterol could be deposited in adipose tissue during weight gain. Subcutaneous and visceral adipose tissues seem to behave differently with respect to the association of their cholesterol and oxysterols concentrations and some clinical traits.

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## Authors' Contributions

M.L., A.C., F.C., and J.M.A-M. conceived and designed the study. L.B-R., A.C., and M.L. wrote the manuscript. L.B-R. and A.C. performed laboratory measurements. M.C.C-F. and J.M.A-M. collected and contributed the biological specimens and part of the clinical data. J.M.A-M. leads the FATe study. V.M-B. and F.C. contributed in clinical and laboratory data collection. M.L., F.C., and A.C. interpreted data. I.L-M., I.G-R., V.M-B., and F.C. reviewed the manuscript for important intellectual content. All authors corrected and approved the final manuscript. M.L. is the guarantor and takes responsibility for the contents of the article.

## Disclosures

The authors declare no conflict of interest.

## Data Availability

Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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