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Interdependence of Oxysterols with Cholesterol Profiles in Multiple Sclerosis

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Abstract

Purpose—To investigate levels of oxysterols in healthy control (HC) and multiple sclerosis (MS) patients and their inter-dependence with demographic, clinical characteristics and cholesterol biomarkers.

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DISCLOSURE

Kelly Fellows, Shreya Mukhopadhyay, Prachi Khare and Sandhya Krishnan Radhakrishnan have nothing to disclose.

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AUTHOR CONTRIBUTIONS

Kelly Fellows – Data acquisition and analysis, manuscript drafting.

Shreya Mukhopadhyay – Data acquisition and analysis, manuscript drafting.

Prachi Khare – Data acquisition

Sandhya Krishnan Radhakrishnan – Data acquisition

Richard W. Browne – Data acquisition, manuscript preparation.

Bianca Weinstock-Guttman, – Data interpretation, manuscript preparation

Robert Zivadinov – MRI data analysis, manuscript preparation

Murali Ramanathan – Study concept and design, data analysis, manuscript preparation.

Methods—This study included 550 subjects (203 HC, 221 relapsing-remitting MS (RR-MS), 126 progressive MS (P-MS)). A complete lipid profile including total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), apolipoproteins (Apo) A1, A2, B, E, C-reactive protein (CRP), 24-hydroxycholesterol (24HC), 25HC, 27HC, 7 α HC and 7-ketocholesterol (7KC) was obtained. Lipoprotein particle sizing by H¹ NMR was available for 432 subjects.

Results—The levels of 24HC, 27HC, and 7 α HC (all $p < 0.015$) were lower in MS compared to HC, and 7KC was higher in P-MS compared to RR-MS ($p < 0.001$). TC, LDL-C and ApoB were associated with higher levels of all oxysterols (all $p < 0.05$) in HC. In MS, LDL-C was associated with higher levels of 24HC, 25HC, 7KC, and 7 α HC (all $p < 0.05$), while TC and ApoB were associated with increased levels of all oxysterols (all $p < 0.005$).

Conclusions—The findings of lower 24HC, 27HC and 7 α HC in MS compared to HC and higher 7KC in P-MS compared to RR-MS indicate that the oxysterol network is disrupted in MS.

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease characterized by demyelination in the central nervous system (CNS). Cholesterol is required for myelin structure and proper functioning of neuronal, vascular and immune cells in the CNS. About 25% of cholesterol in the human body is found in the brain.

The brain is dependent on *de novo* cholesterol synthesis from acetyl-coA because lipoprotein cholesterol from the peripheral circulation does not cross the blood-brain barrier (BBB). In our previous work, we have shown that higher levels of high-density lipoprotein cholesterol (HDL-C) are associated with lower measures of BBB breakdown, assessed using CSF measures of macromolecules, and CNS inflammation¹. Additionally, we found a significant association between low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) levels and inflammatory MRI activity measures², as well as worsening disability measured using the Expanded Disability Status Scale (EDSS) and the MS Severity Scale (MSSS) (10–13). However, the mechanisms underlying the associations of cholesterol biomarkers with disease progression in MS are unknown.

Oxysterols are oxygenated cholesterol metabolites that can cross the BBB, and are therefore thought to play an important role in brain cholesterol regulation^{3–5}. Oxysterols such as 24HC, 27HC and 25HC are endogenous agonists for the liver X receptor (LXR), a nuclear receptor involved in regulating metabolic and immune processes⁶. 24HC is exclusively produced in the brain via cytochrome P450 (CYP) 46A1 in neurons, and is able to traverse the BBB; its plasma levels reflect neuronal loss and brain atrophy⁴⁵. 27HC, produced by CYP27A1, also has the ability to cross the BBB, and is the most abundant oxysterol in the circulation⁵⁷. Both 7 α HC, formed via CYP7A, and 7KC, formed by auto-oxidation of cholesterol, stimulate an inflammatory phenotype in human endothelial cells. 25HC, which is produced by macrophages, has inflammatory and antiviral effects⁸.

Oxysterols thus regulate cholesterol homeostasis, enhance inflammatory reactions, contribute to endothelial cell dysfunction, and exhibit pro-oxidant effects⁷⁹. There is now

increasing evidence that oxysterols play a role in the pathophysiological processes mediating MS disease progression¹⁰. Based on these findings, our working hypothesis was that levels of oxysterols, particularly 24HC, 25HC, 27HC, 7KC and 7aHC, would be altered by MS disease course. The goals of this translational study are to identify the demographic, clinical and cholesterol biomarker covariates associated with oxysterols profiles in MS patients.

METHODS

Study Population

Study Setting—Single-center, prospective observational study.

Clinical Study Design—Samples and clinical data from an ongoing prospective study of clinical, genetic and environmental risk factors in MS at the MS Center of the State University of New York at Buffalo were used. The study included patients with MS, clinically isolated syndrome (CIS), healthy controls (HC) and controls with other neurological diseases (OND).

Patients and controls underwent neurological and MRI examinations, provided blood samples and responded to a structured questionnaire administered in-person¹¹. Body mass index (BMI) was derived from the patient's height and weight. Smoking status was obtained from patient responses to the structured questionnaire.

Informed Consent—The University at Buffalo Human Subjects Institutional Review Board approved the study protocol, and all participants provided written informed consent.

Inclusion and Exclusion Criteria—Patients with the following characteristics: 18–65 years of age. Children younger than 18 years, adults over 65 years of age, CIS, neuromyelitis optica, or OND were excluded from this sub-study.

Oxysterol Assays

Oxysterols (7aHC, 24HC, 25HC, 27HC, 7KC) were determined in EDTA plasma samples by liquid chromatography-selected ion monitoring-stable isotope dilution-atmospheric pressure chemical ionization-mass spectroscopy (LC-SIM-SID-APCI-MS) as previously described¹².

Total sterols and oxysterols were released from sterol esters by cold saponification (0.35M KOH in ethanol containing 0.05% BHT and under argon atmosphere), neutralized and extracted on HyperSep C-18 solid phase extraction tubes before being concentrated and injected into a reversed phase, C-18 LC system using a gradient mobile phase of methanol and water. The Shimadzu 10ADVP LC system was interfaced with the 2010A MS by APCI interface in positive ion mode. Oxysterols were monitored by selected ion monitoring of the $[M+H-H_2O]^+$ molecular ion of the hydroxyl groups present on each (oxy)sterol. 7KC was monitored as the $[M+H]^+$ ion of the 7-keto group. Deuterated (d7) 24-hydroxycholesterol, (d7) 7a-hydroxycholesterol and (d7) 7-ketocholesterol were used as stable isotope-labeled internal standards.

We, and others¹³, have found that oxysterol analytes are stable at -80°C storage. The mild room temperature saponification in the presence of butylated hydroxytoluene (BHT) under argon ameliorated autooxidation of cholesterol or degradation of pre-formed oxysterols, particularly 7KC. This method successfully resolves the most critical isomeric pairs including separation of 24HC from 25HC, 7 α HC from 7 β HC and 7KC from 7 α -hydroxycholesten-3-one¹². The assay was systematically validated in accordance with the FDA guidance for bioanalytical methods. The coefficient of variation (CV) of each oxysterol analyte was 8.7%.

Lipids and Apolipoproteins

EDTA plasma samples for lipid and apolipoprotein analyses were obtained in the non-fasted state. Our previously published¹⁴ methods for lipid and apolipoprotein analyses were used. Analysts were blinded to the clinical status of samples.

TC, HDL-C, phospholipids and triglycerides were measured with diagnostic reagent kits from Sekisui Diagnostics (Lexington, MA) an ABX Pentra 400 (Horiba Instruments, Irvine, CA) automated chemistry analyzer. LDL-C was obtained from the Friedewalde equation¹⁵. Immunoturbidimetric diagnostic kits (Kamiya Biomedical, Thousand Oaks, CA) were used for the apolipoprotein (AI, AII, B and E) and high sensitivity C-reactive protein (CRP) assays. The coefficient of variation of these assays is $< 5\%$.

Lipoprotein Particle Size Analysis

Lipoprotein particle number and size were measured using H^1 NMR spectroscopy (LipoScience, Raleigh, NC). The measured amplitudes of the lipid methyl group NMR signals is used to calculate particle concentration, and the particles sizes are the sum of the diameter of each subclass multiplied by the relative mass percentages from the NMR signals.

Data Analysis

SPSS (IBM Inc., Armonk, NY, version 22.0) statistical program was used. In view of the multiple testing, the Benjamini-Hochberg method was used to assess significance with a target false discovery rate of $q = 0.05$ ¹⁶. The Tables and Results summarize the raw, unadjusted p -values. Adjusted p -values (q -values) are shown only for variables with unadjusted p -values ≤ 0.05 .

Secondary progressive (SP) and primary-progressive (PP) MS were categorized as progressive MS (P-MS)¹⁷.

The Mann-Whitney test was used to assess differences in oxysterol levels (24HC, 25HC, 27HC, 7 α HC, 7KC) for dichotomous variables, e.g., between HC vs. MS patients RR-MS vs. PMS, and MS patients with borderline or high cholesterol (defined as $\text{TC} > 200 \text{ mg/dl}$) vs. desirable cholesterol levels ($\text{TC} \leq 200 \text{ mg/dl}$),.

The associations of oxysterol variables (24HC, 25HC, 27HC, 7 α HC, and 7KC) with lipid profile variables (HDL-C, LDL-C, TC, ApoA-I, ApoA-II, ApoB, ApoE and CRP) were investigated using linear regression analyses where the lipid variable of interest was the

dependent variable and age, gender, BMI and quartiles of either 24HC, 25HC, 27HC, 7 α HC, or 7KC were treated as covariates.

The Kruskal-Wallis test was used to assess whether oxysterol levels were significantly different between patients receiving interferon, glatiramer acetate or no current treatment. A Mann-Whitney test was used to assess whether oxysterols differed between patients who were presently receiving interferon and those who were not currently on an interferon regimen.

RESULTS

Demographic and Clinical Characteristics

The clinical, demographic and MRI characteristics of the study sample at baseline in healthy controls, RR-MS and P-MS are summarized in Table 1. The age, disability and MRI differences between RR-MS and P-MS groups are representative of the respective disease courses. The distribution of oxysterol levels and baseline lipid profiles (TC, HDL-C, and LDL-C) are shown in Table 2.

Associations of Oxysterols with Clinical Factors in MS Patients

MS Disease Course—Figure 1 compares the baseline oxysterol levels between HC vs. MS and RR-MS vs. P-MS groups. The levels of 24HC ($p = 0.015$), 27HC ($p = 0.004$), and 7 α HC ($p < 0.001$) were significantly lower in MS patients compared to HC. There were no differences in 25HC and 7KC levels between HC and RR-MS patients.

7KC levels were significantly higher in P-MS patients compared to RR-MS patients ($p < 0.001$). No differences were found between the two MS groups for 24HC, 25HC, 27HC, and 7 α HC.

Disability Measures—The associations of oxysterols with disability measures (MSSS and EDSS) in both RR-MS and P-MS patients were investigated (data not shown). Increased levels of 24HC were significantly associated with MSSS ($p = 0.045$) in P-MS patients. There were no significant associations between any other oxysterol levels and MSSS and EDSS in the P-MS patient population. No associations were found between oxysterol levels and disability measures in RR-MS patients.

Disease-Modifying Therapies—We compared the levels of oxysterols between patients currently being treated with interferon and patients who are not currently being treated with interferon. The differences in 25HC and 7 α HC levels between untreated, glatiramer acetate treated and interferon treated patients are summarized in Figure 2. We found that 25HC ($p = 0.038$) and 7 α HC ($p = 0.003$) were significantly higher in interferon-treated patients. No significant differences were observed for 24HC, 27HC and 7KC.

Associations of Oxysterols with Lipid Profile Variables

The associations between oxysterols and lipid variables (TC, HDLC, LDLC), apolipoproteins (ApoAI, ApoAII, ApoB, ApoE) and C-reactive protein (CRP) in both HC and MS patients are summarized in Table 3.

TC—Higher levels of all five of the oxysterols were associated with TC for both HC and MS populations (all $p < 0.001$ except 27HC in MS patients, $p = 0.005$).

In the HC group, 25HC ($p = 0.007$) and 7 α HC ($p = 0.028$) were greater in subjects with borderline and high TC (TC > 200 mg/dl). MS patients with borderline and high TC had greater 24HC ($p = 0.011$), 25HC ($p = 0.019$) and 27HC ($p = 0.002$).

LDL-C—LDL-C was associated with higher levels of 24HC ($p < 0.001$), 25HC ($p = 0.024$), 27HC ($p = 0.001$), 7KC ($p = 0.005$) and 7 α HC ($p < 0.001$) in HC. In MS patients, LDL-C was significantly associated with higher levels of 24HC ($p < 0.001$), 25HC ($p = 0.028$), 7KC ($p = 0.030$) and 7 α HC ($p < 0.001$).

HDL-C—None of the oxysterols were significantly associated with HDL-C in either HC or MS patients. Thus, the associations of oxysterols with TC are principally driven by the associations of oxysterols with LDL-C. To further support this hypothesis, we conducted linear regression analyses that corrected for HDL-C values. The significant findings persisted.

Apolipoproteins—Apolipoproteins are functionally critical components of lipoproteins that enable physiological interactions. ApoB is the major apolipoprotein of LDL-C, whereas ApoAI and ApoAII are characteristic of HDL-C.

ApoB was significantly associated with increased levels of all oxysterols in both HC and MS patients (all $p < 0.001$), which is concordant with the LDL-C associations. ApoAII was associated with 27HC and 7 α HC in both HC and MS. ApoAI was associated with 7 α HC in HC. We did not obtain evidence for associations with ApoE and CRP.

Associations of Oxysterols with Lipoprotein Particle Sub-Classes

To further delineate the associations between oxysterols and lipoprotein particle sub-classes, we examined lipoprotein particle concentrations obtained by NMR particle sizing. The results are summarized in Table 4.

Overall, the oxysterols were associated with the VLDL and also, as expected from earlier analyses, with LDL lipoprotein particle concentrations in both MS and HC.

VLDL Particle Sub-Classes—Within the VLDL particle sub-classes, 25HC, 27HC, 7KC and 7 α HC were associated with large and medium VLDL particle concentrations whereas levels of 24HC were only associated with large VLDL particle concentrations for both MS and HC.

LDL-C Particle Sub-classes—Within the LDL particle sub-classes, 25HC, 27HC, 7KC and 7 α HC were associated with small LDL particle concentrations for both MS and HC. 24HC was associated with small LDL particle concentrations in MS but not for HC.

HDL-C Particle Sub-classes—By comparison, associations of oxysterols with HDL particle sub-class concentrations were weaker and sporadic. However, 7 α HC was associated with small HDL particle concentrations for both MS and HC.

DISCUSSION

We investigated whether the levels of 24HC, 25HC, 27HC, 7 α HC and 7KC differed between MS and HC and between RR-MS and P-MS, and whether the levels of these oxysterols were associated with lipid variables (HDL-C, LDL-C, TC, ApoAI, ApoAII, ApoB, ApoE and CRP) and demographic variables. We found that the levels of 24HC, 27HC and 7 α HC were significantly lower in MS patients compared to HC, and that 7KC levels were significantly higher in P-MS patients compared to RR-MS patients, which supports findings from previous studies¹⁸¹⁹. Additionally, oxysterol levels in both MS and HC were pre dominantly associated with levels of LDL-C and its cognate apolipoprotein ApoB. The large and medium VLDL and small LDL particle sub-classes exhibited the strongest associations with the majority of oxysterols. 24HC is produced exclusively in the CNS and we hypothesize that the reduction in 24HC levels in MS is a marker of CNS cholesterol metabolism; the decrease therefore reflects changes to brain cholesterol homeostasis as a result of neurodegeneration. In contrast, the reductions in 27HC and 7 α HC observed in MS are a result of changes to cholesterol metabolism pathways in the periphery and could facilitate in pro-inflammatory changes that enable MS disease progression. The observed increases in 7KC in PMS compared to RRMS reflect oxidative stress that promotes neurodegeneration in PMS.

Results from animal models offer insights into the effector mechanisms of oxysterols. Oxysterols exert immunomodulatory effects on dendritic cell, T cell, macrophage and B cell function via LXR and LXR-independent, EBI2-mediated signaling pathways. Oxysterols amplify and sustain inflammatory processes²⁰ and regulate the expression of inflammatory mediators such as IL-6 and matrix metalloproteinase-9 (MMP-9)²¹²². LXR signaling is critical for polarization of CD4 helper T cells into Th17 cells in humans²³ and it also potently suppresses T cell proliferation²²²⁴. LXR activation ameliorates experimental allergic encephalomyelitis (EAE) whereas LXR deficiency exacerbates it²³. Oxysterols inhibit EAE by down-regulating IL-17 production and suppressing Th17 cells²³. Dendritic cells produce cholesterol 27 hydroxylase during EAE²⁵. Thus the reductions in 24HC, 27HC and 7 α HC levels and the increases in 7KC levels that we observed could potentially contribute to pathophysiological immune effects in MS.

There is increasing evidence for associations of cholesterol with disease progression in MS. We²⁶, and others^{27–29}, have shown that increased levels of LDL-C and TC are significantly associated with worsening disability on the EDSS scale. LDL-C and TC levels were associated with new T2 lesions and grey matter atrophy². Increased levels of HDL-C and ApoAI were associated with less BBB breakdown as assessed with surrogate CSF measures¹.

The pleiotropic effects of oxysterols on metabolic and neuroimmune processes, coupled with their ability to permeate the BBB, has generated interest in investigating the role of

oxysterols in MS. 24HC, 25HC and 27HC are LXR ligands that coordinately down-regulate cholesterol synthesis as well as up-regulate the transporters such as the ABCA1 transporter involved in the removal of cellular cholesterol⁹. There is evidence that interferon induces 25-hydroxylase, the enzyme responsible for the conversion of cholesterol to 25HC³⁰. This may explain our finding that 25HC was higher in interferon-treated MS patients.

7KC is an oxidative stress marker that stimulates phenotypic changes in macrophages that promote, invasion of the vascular endothelium, and leads to secretion of inflammatory factors that can lead to the formation of MS lesions³¹. Demyelinating MS lesions have been found to contain foam cells containing ingested myelin-derived lipids³². Boven *et al.*³² used an *in vitro* model of myelin ingestion to assess anti-inflammatory and pro-inflammatory markers based on location within the lesion. They found that the lipid-filled macrophages located at the center of the lesions expressed anti-inflammatory cytokines, such as IL-1ra, CCL18, IL-10, TGF- β and IL-4, but the cells did not express the pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-12p40/70, regardless of location within the lesion³². These authors proposed that lipid-ingested macrophages may have anti-inflammatory properties in the CNS and may also contribute to repair. They may contribute to the characteristic partial recovery that follows exacerbations in patients with RR-MS³².

In a cross-sectional analysis, van de Kraats *et al.* compared the serum and CSF levels of 24HC, 27HC and lanosterol between MS patients and HC, and investigated the associations with MRI measures. They found that levels of serum 24HC were negatively associated with normalized gray matter volume ($p = 0.004$) in RR-MS patients³³. Additional studies have shown that serum levels of 24HC and 27HC were significantly lower in MS patients compared to HC, while lanosterol levels were increased in HC relative to MS patients¹⁸¹⁹. In our analysis we found supporting evidence that MS patients have lower levels of 24HC and 27HC compared to HC. Our findings indicating that age is negatively correlated with 24HC levels in HC and that 7 α HC is lower in females compared to males is concordant with the results from a study by Stiles *et al.*, who measured an extensive panel of sterols in a multi-ethnic, population-based sample³⁴.

To further delineate the significant associations between oxysterol levels and TC, LDL-C and ApoB, we investigated whether oxysterols were associated with particular sub-classes of lipoprotein particles. To the best of our knowledge, this was the first assessment of oxysterol associations with lipoprotein particle concentrations in MS patients. We found that the large and medium VLDL and small LDL particle sub class concentrations were most significantly associated with oxysterols. These results are consistent with the cardiovascular disease literature, where previous examinations of the oxysterols in lipoproteins revealed that small dense LDL are associated with the highest levels of oxidized lipids and have the strongest pro-atherogenic effect³⁵.

Although we were able to investigate numerous lipid profile and clinical covariates, our study is limited by its cross sectional design and the lack of data on statins. However, we expect the intriguing results of this study in MS will provide the rationale for well-controlled longitudinal studies.

In conclusion, the findings that lower levels of 24HC, 27HC and 7 α HC are seen in MS patients compared to HC, and that higher 7KC levels are observed in P-MS compared to RR-MS indicate that the oxysterol network is disrupted in MS. Our findings also highlight the interdependence between oxysterols and LDL-C, which may explain the evidence that supports a role for LDL-C in the pathophysiology of MS.

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References

1. Fellows K, Uher T, Browne RW, et al. Protective Associations of HDL with Blood Brain Barrier Injury in Multiple Sclerosis Patients. *Journal of lipid research*. 2015
2. Weinstock-Guttman B, Zivadinov R, Horakova D, et al. Lipid profiles are associated with lesion formation over 24 months in interferon-beta treated patients following the first demyelinating event. *Journal of neurology, neurosurgery, and psychiatry*. 2013; 84(11):1186–91.
3. Chen J, Costa LG, Guizzetti M. Assessment of cholesterol homeostasis in astrocytes and neurons. *Methods in molecular biology*. 2011; 758:403–14. [PubMed: 21815081]
4. Leoni V, Caccia C. 24S-hydroxycholesterol in plasma: a marker of cholesterol turnover in neurodegenerative diseases. *Biochimie*. 2013; 95(3):595–612. [PubMed: 23041502]
5. Vitali C, Wellington CL, Calabresi L. HDL and cholesterol handling in the brain. *Cardiovascular research*. 2014; 103(3):405–13. [PubMed: 24907980]
6. Olkkonen VM, Beaslas O, Nissila E. Oxysterols and their cellular effectors. *Biomolecules*. 2012; 2(1):76–103. [PubMed: 24970128]
7. Poli G, Biasi F, Leonarduzzi G. Oxysterols in the pathogenesis of major chronic diseases. *Redox biology*. 2013; 1:125–30. [PubMed: 24024145]
8. Gold ES, Diercks AH, Podolsky I, et al. 25-Hydroxycholesterol acts as an amplifier of inflammatory signaling. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111(29):10666–71. [PubMed: 24994901]
9. Spann NJ, Glass CK. Sterols and oxysterols in immune cell function. *Nat Immunol*. 2013; 14(9):893–900. [PubMed: 23959186]
10. Zhornitsky S, McKay KA, Metz LM, et al. Cholesterol and markers of cholesterol turnover in multiple sclerosis: relationship with disease outcomes. *Multiple sclerosis and related disorders*. 2016; 5:53–65. [PubMed: 26856944]
11. Dolic K, Weinstock-Guttman B, Marr K, et al. Risk factors for chronic cerebrospinal venous insufficiency (CCSVI) in a large cohort of volunteers. *PloS one*. 2011; 6(11):e28062. [PubMed: 22140507]
12. Narayanaswamy R, Iyer V, Khare P, et al. Simultaneous determination of oxysterols, cholesterol and 25-hydroxy-vitamin D3 in human plasma by LC-UV-MS. *PloS one*. 2015; 10(4):e0123771. [PubMed: 25875771]
13. Helmschrodt C, Becker S, Thiery J, et al. Preanalytical standardization for reactive oxygen species derived oxysterol analysis in human plasma by liquid chromatography-tandem mass spectrometry. *Biochemical and biophysical research communications*. 2014; 446(3):726–30. [PubMed: 24370823]
14. Browne RW, Weinstock-Guttman B, Horakova D, et al. Apolipoproteins are associated with new MRI lesions and deep grey matter atrophy in clinically isolated syndromes. *Journal of neurology, neurosurgery, and psychiatry*. 2014; 85(8):859–64.

15. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*. 1972; 18(6):499–502. [PubMed: 4337382]
16. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc B*. 1995; 57:289–300.
17. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology*. 1996; 46(4):907–11. [PubMed: 8780061]
18. Leoni V, Masterman T, Diczfalusy U, et al. Changes in human plasma levels of the brain specific oxysterol 24S-hydroxycholesterol during progression of multiple sclerosis. *Neuroscience letters*. 2002; 331(3):163–6. [PubMed: 12383922]
19. Teunissen CE, Dijkstra CD, Polman CH, et al. Decreased levels of the brain specific 24S-hydroxycholesterol and cholesterol precursors in serum of multiple sclerosis patients. *Neuroscience letters*. 2003; 347(3):159–62. [PubMed: 12875910]
20. Poli G, Biasi F, Leonarduzzi G. Oxysterols in the pathogenesis of major chronic diseases. *Redox Biology*. 2013; 1(1):125–30. [PubMed: 24024145]
21. Gargiulo S, Sottero B, Gamba P, et al. Plaque oxysterols induce unbalanced up-regulation of matrix metalloproteinase-9 in macrophagic cells through redox-sensitive signaling pathways: Implications regarding the vulnerability of atherosclerotic lesions. *Free radical biology & medicine*. 2011; 51(4):844–55. [PubMed: 21664966]
22. Glass CK, Saijo K. Immunology: Oxysterols hold T cells in check. *Nature*. 2008; 455(7209):40–1. [PubMed: 18769427]
23. Cui G, Qin X, Wu L, et al. Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. *J Clin Invest*. 2011; 121(2):658–70. [PubMed: 21266776]
24. Bensinger SJ, Bradley MN, Joseph SB, et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell*. 2008; 134(1):97–111. [PubMed: 18614014]
25. Chalmin F, Rochemont V, Lippens C, et al. Oxysterols regulate encephalitogenic CD4(+) T cell trafficking during central nervous system autoimmunity. *Journal of autoimmunity*. 2015; 56:45–55. [PubMed: 25456971]
26. Weinstock-Guttman B, Zivadinov R, Mahfooz N, et al. Serum lipid profiles are associated with disability and MRI outcomes in multiple sclerosis. *Journal of neuroinflammation*. 2011; 8:127. [PubMed: 21970791]
27. Mandoj C, Renna R, Plantone D, et al. Anti-annexin antibodies, cholesterol levels and disability in multiple sclerosis. *Neuroscience letters*. 2015; 606:156–60. [PubMed: 26342534]
28. Palavra F, Marado D, Mascarenhas-Melo F, et al. New markers of early cardiovascular risk in multiple sclerosis patients: oxidized-LDL correlates with clinical staging. *Disease markers*. 2013; 34(5):341–8. [PubMed: 23478275]
29. Tetley P, Simpson S Jr, Taylor BV, et al. Vascular comorbidities in the onset and progression of multiple sclerosis. *Journal of the neurological sciences*. 2014; 347(1–2):23–33. [PubMed: 25454639]
30. Chen Y, Wang S, Yi Z, et al. Interferon-inducible cholesterol-25-hydroxylase inhibits hepatitis C virus replication via distinct mechanisms. *Scientific reports*. 2014; 4:7242. [PubMed: 25467815]
31. Cui X, Kushiya A, Yoneda M, et al. Macrophage foam cell formation is augmented in serum from patients with diabetic angiopathy. *Diabetes research and clinical practice*. 2010; 87(1):57–63. [PubMed: 19939487]
32. Boven LA, Van Meurs M, Van Zwam M, et al. Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. *Brain*. 2006; 129(Pt 2):517–26. [PubMed: 16364958]
33. van de Kraats C, Killestein J, Popescu V, et al. Oxysterols and cholesterol precursors correlate to magnetic resonance imaging measures of neurodegeneration in multiple sclerosis. *Multiple sclerosis*. 2014; 20(4):412–7. [PubMed: 23959711]
34. Stiles AR, Kozlitina J, Thompson BM, et al. Genetic, anatomic, and clinical determinants of human serum sterol and vitamin D levels. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111(38):E4006–14. [PubMed: 25201972]

35. Chancharme L, Therond P, Nigon F, et al. LDL particle subclasses in hypercholesterolemia. Molecular determinants of reduced lipid hydroperoxide stability. *Journal of lipid research*. 2002; 43(3):453–62. [PubMed: 11893782]

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Figure 1.

Oxysterol levels in healthy controls (HC), relapsing remitting MS (RR-MS) and progressive MS (P-MS). Figure 1 shows the values of 24HC (Figure 1A), 25HC (Figure 1B), 27HC (Figure 1C), 7KC (Figure 1D), and 7αHC (Figure 1E) in ng/ml, by disease course (HC, RR-MS and P-MS). The bars compare mean oxysterol values shown on the y-axis by disease course shown on the x-axis. The error bars indicate the standard error of the mean. The *p*-values from a Mann-Whitney test are provided for the HC versus MS and RR-MS versus P-MS comparisons.

Figure 2. 25HC and 7αHC levels (ng/ml) in MS patients on no treatment, glatiramer acetate or IFN treatment. The error bars indicate the standard error of the mean.

Table 1

Demographic and clinical characteristics at baseline by disease course.

Characteristic	All	HC	RR-MS	PMS
Sample size <i>n</i>	550	203	221	126
% Female	377 (69%)	120 (59%)	167 (76%)	90 (71%)
Age, years	47.1 ± 12.0	45.6 ± 14	44.4 ± 10	54.3 ± 8.5
Disease duration, years	15.7 ± 10.6	–	12.2 ± 8.7	21.7 ± 11.1
BMI	25.8 ± 2.0	27.5 ± 5.8	27.2 ± 6.1	25.4 ± 5.2
EDSS ^a	3.0 (4.0)	–	2.0 (2.0)	6.0 (2.0)

^a All continuous variables (age, disease duration) are mean ± standard deviation. For the ordinal EDSS, the median (inter-quartile range) is given.

Table 2

Baseline oxysterol and lipid values by disease course.

Characteristic	All Subjects	HC	RR-MS	PMS
<i>n</i>	550	203	221	126
24HC, ng/ml	40.7 ± 36	44.4 ± 44.	40.4 ± 34	35.3 ± 20
25HC, ng/ml	17.3 ± 22	17.8 ± 17	15.9 ± 11	18.6 ± 38
27HC, ng/ml	235.5 ± 121	251.1 ± 117	233.2 ± 128	214.5 ± 114
7αHC, ng/ml	88.7 ± 130	116.6 ± 188	73.4 ± 72	69.9 ± 74
7KC, ng/ml	14.7 ± 11	14.0 ± 7	13.5 ± 8	18.1 ± 17
LDL-C, mg/dL	92.9 ± 27	95.7 ± 28	90.8 ± 28	91.9 ± 25
HDL-C, mg/dl	68.0 ± 15	68.7 ± 16	66.8 ± 14	68.8 ± 15
TC, mg/ml	188.4 ± 35	193.0 ± 36	185.3 ± 35	186.2 ± 33

^a All variables are mean ± standard deviation. 24HC: 24 hydroxy cholesterol; 25HC: 25 hydroxy cholesterol; 27HC: 27 hydroxy cholesterol; 7αHC: 7α hydroxyl cholesterol; 7KC: 7 ketocholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

Associations between oxysterols and lipid profile variables in healthy control and MS patients. The partial correlation coefficient (r -value) are shown

Table 3

Lipid Variable	HC vs. MS	24HC	25HC	27HC	7KC	7aHC
TC	HC	0.37 (< 0.001)	0.27 (< 0.001)	0.35 (< 0.001)	0.34 (< 0.001)	0.52 (< 0.001)
	MS	0.26 (< 0.001)	0.24 (< 0.001)	0.16 (0.005)	0.24 (> 0.001)	0.35 (< 0.001)
HDLc	HC	-0.10 (0.17)	0.113 (0.13)	0.05 (0.49)	0.02 (0.79)	0.10 (0.18)
	MS	-0.056 (0.32)	0.089 (0.11)	-0.06 (0.33)	-0.015 (0.79)	0.06 (0.28)
LDLc	HC	0.39 (< 0.001)	0.17 (0.024)	0.25 (0.001)	0.21 (0.005)	0.36 (< 0.001)
	MS	0.22 (< 0.001)	0.12 (0.028)	0.10 (0.09)	0.12 (0.03)	0.21 (< 0.001)
ApoAI	HC	0.025 (0.73)	0.13 (0.081)	0.10 (0.17)	0.013 (0.86)	0.24 (0.001)
	MS	-0.047 (0.40)	0.06 (0.28)	-0.075 (0.18)	-0.07 (0.21)	0.10 (0.077)
ApoAII	HC	0.19 (0.012)	0.051 (0.49)	0.26 (< 0.001)	0.16 (0.03)	0.35 (< 0.001)
	MS	0.091 (0.10)	0.087 (0.12)	0.12 (0.030)	-0.40 (0.48)	0.19 (0.001)
ApoB	HC	0.32 (< 0.001)	0.29 (< 0.001)	0.39 (< 0.001)	0.32 (< 0.001)	0.36 (< 0.001)
	MS	0.29 (< 0.001)	0.27 (< 0.001)	0.31 (< 0.001)	0.22 (< 0.001)	0.21 (< 0.001)
ApoE	HC	-0.14 (0.069)	-0.089 (0.23)	-0.093 (0.21)	-0.09 (0.26)	-0.10 (0.18)
	MS	-0.07 (0.22)	-0.032 (0.57)	-0.024 (0.67)	0.08 (0.16)	0.05 (0.35)
CRP	HC	0.011 (0.88)	0.042 (0.57)	0.083 (0.26)	0.08 (0.30)	0.06 (0.42)
	MS	0.04 (0.47)	0.075 (0.18)	-0.003 (0.96)	0.03 (0.64)	-0.007 (0.90)

Associations between oxysterols and lipoprotein particle sub-class concentrations in HC and MS patients.

Table 4

Lipoprotein	HC vs. MS	24HC	25HC	27HC	7KC	7aHC
Total VLDL	HC	0.033 (0.69)	0.22 (0.007)	0.098 (0.23)	0.13 (0.11)	0.24 (0.003)
	MS	0.16 (0.009)	0.22 (< 0.001)	0.19 (0.002)	0.13 (0.030)	0.17 (0.005)
Large VLDL	HC	0.19 (0.019)	0.29 (< 0.001)	0.27 (0.001)	0.29 (< 0.001)	0.51 (< 0.001)
	MS	0.19 (0.002)	0.20 (0.001)	0.18 (0.003)	0.18 (0.003)	0.32 (< 0.001)
Medium VLDL	HC	0.064 (0.44)	0.26 (0.001)	0.17 (0.042)	0.32 (< 0.001)	0.42 (< 0.001)
	MS	0.088 (0.15)	0.18 (0.002)	0.14 (0.025)	0.19 (0.002)	0.29 (< 0.001)
Small VLDL	HC	-0.045 (0.59)	0.085 (0.30)	-0.017 (0.84)	-0.085 (0.30)	-0.051 (0.53)
	MS	0.11 (0.078)	0.13 (0.039)	0.11 (0.067)	0.053 (0.39)	0.011 (0.86)
Total LDL	HC	0.21 (0.011)	0.30 (< 0.001)	0.45 (< 0.001)	0.36 (< 0.001)	0.46 (< 0.001)
	MS	0.34 (< 0.001)	0.32 (< 0.001)	0.34 (< 0.001)	0.23 (< 0.001)	0.30 (< 0.001)
Total IDL	HC	0.056 (0.49)	0.18 (0.029)	0.10 (0.21)	0.092 (0.26)	0.20 (0.012)
	MS	0.041 (0.50)	0.15 (0.012)	0.088 (0.15)	0.064 (0.29)	0.008 (0.89)
Large LDL	HC	0.25 (0.002)	0.11 (0.17)	0.21 (0.010)	0.068 (0.41)	0.092 (0.26)
	MS	0.11 (0.073)	0.068 (0.27)	0.031 (0.61)	0.063 (0.30)	-0.038 (0.53)
Small LDL	HC	0.032 (0.70)	0.20 (0.015)	0.30 (< 0.001)	0.34 (< 0.001)	0.41 (< 0.001)
	MS	0.22 (< 0.001)	0.23 (< 0.001)	0.27 (< 0.001)	0.15 (0.011)	0.33 (< 0.001)
Total HDL	HC	-0.081 (0.32)	0.009 (0.91)	-0.057 (0.49)	0.050 (0.54)	0.16 (0.047)
	MS	0.051 (0.40)	0.056 (0.36)	0.070 (0.25)	0.015 (0.80)	0.074 (0.23)
Large HDL	HC	0.071 (0.39)	-0.13 (0.13)	-0.12 (0.13)	-0.13 (0.12)	-0.090 (0.27)
	MS					

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Lipoprotein	HC vs. MS	24HC	25HC	27HC	7KC	7aHC
	MS	0.003 (0.97)	-0.029 (0.64)	-0.14 (0.020)	0.021 (0.74)	-0.087 (0.16)
	HC	-0.024 (0.77)	0.033 (0.68)	-0.065 (0.42)	0.001 (0.99)	0.11 (0.18)
Medium HDL	MS	0.058 (0.34)	0.005 (0.94)	0.081 (0.19)	-0.008 (0.90)	0.060 (0.33)
	HC	-0.13 (0.11)	0.14 (0.098)	0.12 (0.15)	0.208 (0.010)	0.26 (0.001)
Small HDL	MS	0.036 (0.56)	0.10 (0.099)	0.15 (0.013)	0.039 (0.53)	0.12 (0.045)

^aValues provided represent the Spearman correlation coefficient with *p*-values in parenthesis.