JAMA Otolaryngology–Head & Neck Surgery | Original Investigation

Association of Plasma Metabolomic Biomarkers With Persistent Tinnitus
A Population-Based Case-Control Study

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IMPORTANCE Persistent tinnitus is common, disabling, and difficult to treat.

OBJECTIVE To evaluate the association between circulating metabolites and persistent tinnitus.

DESIGN, SETTING, AND PARTICIPANTS This was a population-based case-control study of 6477 women who were participants in the Nurses' Health Study (NHS) and NHS II with metabolomic profiles and tinnitus data. Information on tinnitus onset and frequency was collected on biennial questionnaires (2009-2017). For cases, metabolomic profiles were measured (2015-2021) in blood samples collected after the date of the participant's first report of persistent tinnitus (NHS, 1989-1999 and 2010-2012; NHS II, 1996-1999). Data analyses were performed from January 24, 2022, to January 14, 2023.

EXPOSURES In total, 466 plasma metabolites from 488 cases of persistent tinnitus and 5989 controls were profiled using 3 complementary liquid chromatography tandem mass spectrometry approaches.

MAIN OUTCOMES AND MEASURES Logistic regression was used to estimate odds ratios (ORs) of persistent tinnitus (per 1 SD increase in metabolite values) and 95% CIs for each individual metabolite. Metabolite set enrichment analysis was used to identify metabolite classes enriched for associations with tinnitus.

RESULTS Of the 6477 study participants (mean [SD] age, 52 [9] years; 6477 [100%] female; 6121 [95%] White individuals) who were registered nurses, 488 reported experiencing daily persistent (≥5 minutes) tinnitus. Compared with participants with no tinnitus (5989 controls), those with persistent tinnitus were slightly older (53.0 vs 51.8 years) and more likely to be postmenopausal, using oral postmenopausal hormone therapy, and have type 2 diabetes, hypertension, and/or hearing loss at baseline. Compared with controls, homocitrulline (OR, 1.32; (95% CI, 1.16-1.50); C38:6 phosphatidylethanolamine (PE; OR, 1.24; 95% CIs, 1.12-1.38), C52:6 triglyceride (TAG; OR, 1.22; 95% CIs, 1.10-1.36), C36:4 PE (OR, 1.22; 95% CIs, 1.10-1.35), C40:6 PE (OR, 1.22; 95% CIs, 1.09-1.35), and C56:7 TAG (OR, 1.21; 95% CIs, 1.09-1.34) were positively associated, whereas α-keto-β-methylvalerate (OR, 0.68; 95% CIs, 0.56-0.82) and levulinate (OR, 0.60; 95% CIs, 0.46-0.79) were inversely associated with persistent tinnitus. Among metabolite classes, TAGs (normalized enrichment score [NES], 2.68), PEs (NES, 2.48), and diglycerides (NES, 1.65) were positively associated, whereas phosphatidylcholine plasmalogens (NES, −1.91), lysophosphatidylcholines (NES, −2.23), and cholesteryl esters (NES,−2.31) were inversely associated with persistent tinnitus.

CONCLUSIONS AND RELEVANCE This population-based case-control study of metabolomic profiles and tinnitus identified novel plasma metabolites and metabolite classes that were significantly associated with persistent tinnitus, suggesting that metabolomic studies may help improve understanding of tinnitus pathophysiology and identify therapeutic targets for this challenging disorder.

Supplemental content

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JAMA Otolaryngol Head Neck Surg. 2023;149(5):404-415. doi:10.1001/jamaoto.2023.0052
Published online March 16, 2023.

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Persistent tinnitus can be disabling and adversely affect quality of life.[1,2] Approximately 50 million US individuals experience tinnitus,[3] yet its causes are often unknown, and for most individuals the effectiveness of proposed treatments is uncertain.[4] Identifying potentially modifiable risk factors could aid prevention efforts and inform targeted treatments for this challenging disorder.

There is a critical need to improve the understanding of the biologic underpinnings of tinnitus and the diverse underlying causes.[5] Accumulating evidence suggests complex interactions between individual-level and environmental factors that may influence the generation and persistence of tinnitus.[6] Metabolomic studies provide a comprehensive picture of an individual's metabolic status and have elucidated biological pathways underlying several neurodegenerative disorders. The metabolome encompasses the collection of small molecules in biologic samples. Metabolite levels are *downstream* of transcriptional and translational processes and reflect direct input from the diet, environment, and intestinal microbiome, thus the systematic analysis of metabolites can uncover valuable insights into a disorder as complex as tinnitus.

Previous studies identified metabolomic biomarkers for neurodegenerative disorders,[7,8] but human metabolomic studies of tinnitus are lacking. A study in rats identified perturbations in several metabolic pathways with tinnitus,[9] and multiple metabolite alterations demonstrated in brain tissue were also demonstrated in plasma.[9] However, to our knowledge, no human studies have evaluated plasma metabolomic profiles and persistent tinnitus. Therefore, we conducted a large cross-sectional study in 2 well-characterized cohorts of US women, the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS II), to identify metabolomic alterations associated with tinnitus.

Key Points

Question Are there differences in circulating plasma metabolites between individuals with persistent tinnitus and those who have never experienced this disorder?

Findings This population-based case-control study of 6477 participants from the Nurses' Health Study (NHS) and the NHS II, including 488 women with daily persistent tinnitus and 5989 controls, found several novel metabolites associated with persistent tinnitus. Homocitrulline, 3 phosphatidylethanolamines, and 2 triglycerides were significantly positively associated, whereas α-keto-β-methylvalerate and levulinate were significantly inversely associated with persistent tinnitus.

Meaning The findings of this population-based case-control study underscore the value of metabolomics studies for tinnitus biomarker discovery and demonstrate the potential for metabolomics to identify therapeutic targets for persistent tinnitus and other challenging disorders.

Methods

The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T. H. Chan School of Public Health. The return of the self-administered questionnaire and blood sample was considered to imply consent when the study protocols were approved in 1976 (NHS) and 1989 (NHS II).

Study Populations

The NHS began in 1976 when 121 700 female registered nurses (age range, 30-55 years) enrolled by returning a mailed questionnaire; then in 1986, the NHS II enrolled 116 429 female nurses (25-42 years) using the same method.[10] Since then, participants have been followed up biennially with questionnaires on reproductive history, lifestyle factors, diet, medication use, and new disease diagnoses. In 1989 to 1990 and 1996 to 1999, 32 826 NHS and 29 611 NHS II participants provided blood samples and completed a short questionnaire.[11] In brief, the participants arranged to have their blood drawn and the sample shipped with an ice pack via overnight courier to our laboratory, the Brigham and Women's Hospital/Harvard Cohort Biorepository. These blood samples were processed and separated into plasma and red and white blood cell components and frozen in gasketed cryovials in the vapor phase of liquid nitrogen freezers. Using the same protocol, 18 743 female participants in the NHS and 17 275 in the NHS II provided a second blood sample in 2000 through 2002 and 2010 through 2012.

Tinnitus Assessment

Information on tinnitus was collected on the biennial questionnaires in 2012 and 2016 for the NHS, and in 2009, 2013, and 2017 for the NHS II. These questionnaires included the question, "In the past 12 months, have you had ringing, roaring, or buzzing in your ears or head?" The 6 response options ranged from *never* to *daily*. Information on age of onset and how long the perception of sound lasts was also collected. The outcome in this study was self-reported persistent daily tinnitus; a case was defined as tinnitus experienced daily with a duration of 5 minutes or more during 1 year or more (hereafter referred to as tinnitus). We used a stringent definition of tinnitus to mitigate potential misclassification and focus on cases of tinnitus that were most likely to be clinically meaningful. A control was defined as *never* tinnitus. This study included 488 participants with prevalent tinnitus (cases) and 5989 participants who had never experienced tinnitus (controls). For all of the cases, metabolomic measurements were obtained from blood samples collected after the date of the participant's first report of persistent tinnitus.

Assessment of Covariates

Information on covariates (**Table**) was self-reported, including race and ethnicity, and obtained from substudy questionnaires completed at the time of blood collection and from biennial questionnaires completed nearest to and before the date of the blood collection. Covariate information on many of these factors has been formally validated.[12-16]

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Table. Participant Characteristics According to Tinnitus Case-Control Status at the Time of Sample Collection, NHS and NHS II

Characteristic	Participant status, No. (%)[a]		*P* value
	No tinnitus ever (controls)	Persistent tinnitus (cases)	
Participants, No.	5989	488	NA
Age, mean (SD), y	51.8 (8.8)	53.0 (7.9)	<.01
Race and ethnicity, White[b]	5666 (94.6)	455 (93.2)	.24
BMI, mean (SD)	25.5 (5.0)	25.8 (4.5)	.31
Missing data	58 (1.0)	5 (1.0)	
Menopausal status			
Premenopausal	2835 (47.3)	198 (40.6)	.02
Postmenopausal	2957 (49.4)	269 (55.1)	
Indeterminate	187 (3.1)	21 (4.3)	
Missing data	10 (0.2)	0	
Current hormone use			
No PMH	2892 (48.3)	196 (40.2)	<.01
Oral PMH	1907 (31.8)	199 (40.8)	
Other PMH type	33 (0.6)	6 (1.2)	
Premenopausal/missing data	1157 (19.3)	87 (17.8)	
Current oral contraceptive use			
No	5830 (97.3)	481 (98.6)	.20
Yes	140 (2.3)	7 (1.4)	
Missing data	19 (0.3)	0	
Diabetes, ever	889 (14.8)	86 (17.6)	.17
Missing data	10 (0.2)	0	
Hypertension, ever	2472 (41.3)	222 (45.5)	.13
Missing data	10 (0.2)	0	
Depression, ever[c]	1737 (29.1)	128 (26.2)	.28
Missing data	10 (0.2)	0	
Smoking			
Never	3350 (56.9)	262 (53.7)	.77
Past	2087 (34.8)	179 (36.7)	
Current	534 (8.9)	46 (9.4)	
Missing data	18 (0.3)	1 (0.2)	
Physical activity in MET-h/wk, mean (SD)	17.3 (22.2)	16.8 (19.9)	.61
Missing data	52 (0.9)	5 (1.0)	
DASH score, by quintile			
1	1169 (19.5)	82 (16.8)	.28
2	1196 (20.0)	99 (20.3)	
3	1042 (17.4)	99 (20.3)	
4	1181 (19.7)	106 (21.7)	
5	1106 (18.5)	83 (17.0)	
Missing data	295 (4.9)	19 (3.9)	
Alcohol intake, g/d			
None	2214 (37.0)	182 (37.3)	.46
1.0-4.9	1946 (32.5)	168 (34.4)	
5.0-14.9	1045 (17.4)	73 (15.0)	
15.0-29.9	335 (5.6)	29 (5.9)	
≥30	154 (2.6)	17 (3.5)	
Missing data	295 (4.9)	19 (3.9)	

(continued)

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Table. Participant Characteristics According to Tinnitus Case-Control Status at the Time of Sample Collection, NHS and NHS II (continued)

Characteristic	Participant status, No. (%)[a]		P value
	No tinnitus ever (controls)	Persistent tinnitus (cases)	
Cohort, NHS II	2178 (36.4)	157 (32.2)	.07
Hearing loss			
No	3928 (65.6)	129 (26.4)	<.01
Mild	1378 (23.0)	196 (40.2)	
Moderate	543 (9.1)	122 (25.0)	
Severe	132 (2.2)	38 (7.8)	
Missing data	8 (0.1)	3 (0.6)	

Abbreviations: BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; DASH, Dietary Approaches to Stop Hypertension, calculated on the distribution of the entire cohort; MET, metabolic equivalent of task; NA, not applicable; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; PMH, postmenopausal hormone therapy.

[a] These data represent mean values and SDs for continuous variables, and counts and percentages for categorical variables. *P* values were estimated from a *t* test for continuous variables or from a χ^2 test for categorical variables and are based on nonmissing values only.

[b] Race and ethnicity included Asian, Black, Hispanic, White, and other; however, the number of participants who did not self-report as White was small and could potentially identify study participants; therefore, they are not reported in these findings.

[c] Defined as the use of antidepressants in the past 2 years or self-reported diagnosis.

Metabolite Profiling

Metabolomics assays were performed within 15 prospective case-control studies (eTable 1 in Supplement 1) nested within the NHS and NHS II, including studies of different cancers (breast, ovarian, colon, pancreatic), diabetes, stroke, glaucoma, inflammatory bowel disease, amyotrophic lateral sclerosis, Parkinson disease, rheumatoid arthritis, and posttraumatic stress disorder. In eTable 1 in Supplement 1 are the number of tinnitus cases and controls by nested case-control study. We used the probit transformation within individual studies to correct for batch effects before merging individual metabolomic data across studies.

In total, 6477 women responded to the tinnitus questions and had metabolomic data previously measured. Plasma metabolites were profiled at the Broad Institute of the Massachusetts Institute of Technology and Harvard University using 3 complementary liquid chromatography tandem mass spectrometry methods designed to measure amino acids, amines, lipids, free fatty acids, bile acids, sugars, organic acids, purines, and pyrimidines, as described previously.[17-21] For each method, metabolite identities were confirmed using authentic reference standards or reference samples. Additional details describing the metabolomic assays are provided in the eMethods of Supplement 2.

During quality control analysis, 192 metabolites were excluded because they were not robust with respect to sample collection characteristics,[20] and an additional 132 metabolites were excluded because they had been measured in fewer than 100 participants.

In total, 466 metabolites were included in the study, of which 389 (83%) metabolites had a coefficient of variation equal to 25% or less. Metabolite sample sizes varied from 100 participants to 6477, which was mostly because of the selected extraction methods in the individual nested case-control studies (up to 4 methods selected from hydrophilic interaction liquid chromatography positive and negative, $C8^+$, $C18^-$) and to a lesser extent, missing metabolite values.

Statistical Analysis

Missing value imputation for metabolites was performed within individual nested case-control studies. Missing values for metabolites with less than 25% missingness within an individual nested case-control study were imputed with half of the minimum value measured for that metabolite in that individual study (n = 422 across all 15 studies). Metabolites missing in 25% or more of the samples within an individual nested case-control study were not imputed in that individual study. Metabolites were evaluated among samples with nonmissing or imputed values. Continuous metabolite values were transformed to probit scores within a nested case-control study to correct for batch effects, reduce the influence of skewed distributions, and heavy tails on the results, and to scale the measured metabolite values to the same range. Logistic regression was used to evaluate metabolite associations, modeled continuously, with tinnitus.

We present the odds ratios (ORs) and 95% CIs for an increase of 1 SD in probit-transformed metabolite levels estimated with 4 statistical models: model 1 adjusts for age, race and ethnicity, fasting status, season of blood draw, collection, cohort, and end point; model 2 adjusts for all covariates in model 1 plus body mass index (calculated as weight in kilograms divided by height in meters squared); model 3 adjusts for all covariates in model 2 plus menopausal status, current oral hormone use, and current oral contraceptive use; and model 4 adjusts for all covariates in model 3 plus smoking status, physical activity, Dietary Approaches to Stop Hypertension dietary score, and alcohol intake. We used residuals to adjust for categorical variables given the large number of resulting cross-classified subgroups compared with the sample size when including categorical variables in the models. We used the number of effective tests (NEF) to account for testing multiple correlated hypotheses.[22] We used metabolite set enrichment analysis[23] and the false discovery rate (FDR)[24] to identify metabolite classes enriched for associations with tinnitus. We present and discuss individual me-

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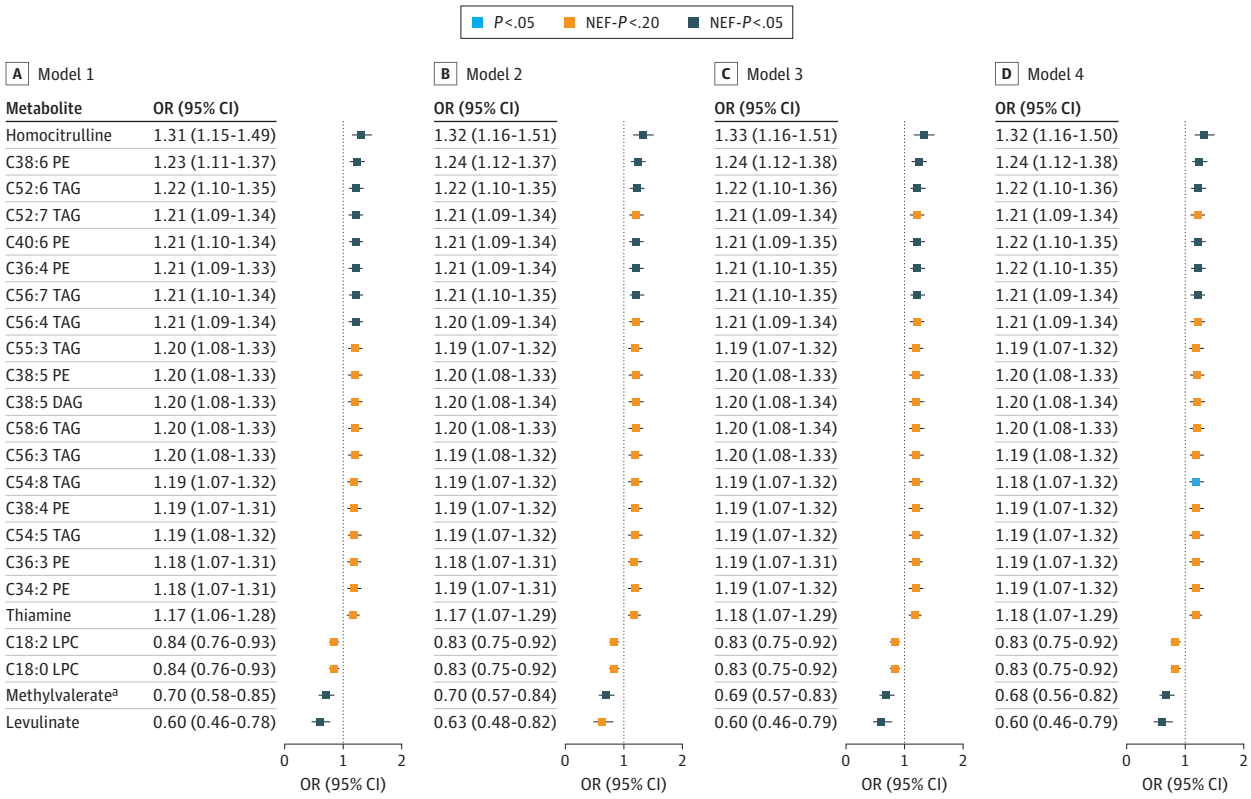
Figure 1. Individual Metabolites Associated With Persistent Daily Tinnitus Among 488 Participants With Tinnitus (Cases) and 5989 Participants Without Tinnitus (Controls), NEF-*P* < .20 in at Least 1 Model, NHS and NHS II



Metabolite	A Model 1 OR (95% CI)	B Model 2 OR (95% CI)	C Model 3 OR (95% CI)	D Model 4 OR (95% CI)
Homocitrulline	1.31 (1.15-1.49)	1.32 (1.16-1.51)	1.33 (1.16-1.51)	1.32 (1.16-1.50)
C38:6 PE	1.23 (1.11-1.37)	1.24 (1.12-1.37)	1.24 (1.12-1.38)	1.24 (1.12-1.38)
C52:6 TAG	1.22 (1.10-1.35)	1.22 (1.10-1.35)	1.22 (1.10-1.36)	1.22 (1.10-1.36)
C52:7 TAG	1.21 (1.09-1.34)	1.21 (1.09-1.34)	1.21 (1.09-1.34)	1.21 (1.09-1.34)
C40:6 PE	1.21 (1.10-1.34)	1.21 (1.09-1.34)	1.21 (1.09-1.35)	1.22 (1.10-1.35)
C36:4 PE	1.21 (1.09-1.33)	1.21 (1.09-1.34)	1.21 (1.10-1.35)	1.22 (1.10-1.35)
C56:7 TAG	1.21 (1.10-1.34)	1.21 (1.10-1.35)	1.21 (1.10-1.35)	1.21 (1.09-1.34)
C56:4 TAG	1.21 (1.09-1.34)	1.20 (1.09-1.34)	1.21 (1.09-1.34)	1.21 (1.09-1.34)
C55:3 TAG	1.20 (1.08-1.33)	1.19 (1.07-1.32)	1.19 (1.07-1.32)	1.19 (1.07-1.32)
C38:5 PE	1.20 (1.08-1.33)	1.20 (1.08-1.33)	1.20 (1.08-1.33)	1.20 (1.08-1.33)
C38:5 DAG	1.20 (1.08-1.33)	1.20 (1.08-1.34)	1.20 (1.08-1.34)	1.20 (1.08-1.34)
C58:6 TAG	1.20 (1.08-1.33)	1.20 (1.08-1.33)	1.20 (1.08-1.34)	1.20 (1.08-1.33)
C56:3 TAG	1.20 (1.08-1.33)	1.19 (1.08-1.32)	1.20 (1.08-1.33)	1.19 (1.08-1.32)
C54:8 TAG	1.19 (1.07-1.32)	1.19 (1.07-1.32)	1.19 (1.07-1.32)	1.18 (1.07-1.32)
C38:4 PE	1.19 (1.07-1.31)	1.19 (1.07-1.32)	1.19 (1.07-1.32)	1.19 (1.07-1.32)
C54:5 TAG	1.19 (1.08-1.32)	1.19 (1.07-1.32)	1.19 (1.07-1.32)	1.19 (1.07-1.32)
C36:3 PE	1.18 (1.07-1.31)	1.18 (1.07-1.31)	1.19 (1.07-1.31)	1.19 (1.07-1.32)
C34:2 PE	1.18 (1.07-1.31)	1.19 (1.07-1.31)	1.19 (1.07-1.32)	1.19 (1.07-1.32)
Thiamine	1.17 (1.06-1.28)	1.17 (1.07-1.29)	1.18 (1.07-1.29)	1.18 (1.07-1.29)
C18:2 LPC	0.84 (0.76-0.93)	0.83 (0.75-0.92)	0.83 (0.75-0.92)	0.83 (0.75-0.92)
C18:0 LPC	0.84 (0.76-0.93)	0.83 (0.75-0.92)	0.83 (0.75-0.92)	0.83 (0.75-0.92)
Methylvalerate[a]	0.70 (0.58-0.85)	0.70 (0.57-0.84)	0.69 (0.57-0.83)	0.68 (0.56-0.82)
Levulinate	0.60 (0.46-0.78)	0.63 (0.48-0.82)	0.60 (0.46-0.79)	0.60 (0.46-0.79)

[a] α-keto-β-methylvalerate.

Odds ratios (ORs) of tinnitus were calculated per 1 SD increase in probit-transformed metabolite values. Model 1 adjusts for age, race and ethnicity, fasting status, season of blood draw, collection, cohort, end point; model 2 adjusts for all covariates in model 1 plus BMI; model 3 adjusts for all covariates in model 2 plus menopausal status, current oral hormone use, and current oral contraceptive use; model 4 adjusts for all covariates in model 3 plus smoking status, physical activity, DASH score, and alcohol intake. BMI refers to body mass index calculated as weight in kilograms divided by height in meters squared; DAG, diglycerides; DASH, Dietary Approaches to Stop Hypertension; LPC, lysophosphatidylcholines; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; PE, phosphatidylethanolamines; and TAG, triglycerides.

tabolites with NEF-corrected *P* < .20, and metabolite classes with FDR < .20.

The main analysis included 488 cases (defined as participants with daily tinnitus of ≥5 minutes duration) and 5989 controls (participants with no tinnitus). We conducted stratified analyses restricted to those without self-reported hearing loss (129 cases vs 3928 controls) and to those with moderate or severe hearing loss (160 cases vs 675 controls). We conducted sensitivity analyses restricted to women who participated in the first NHS collection (268 cases vs 2683 controls), the second NHS collection (63 cases vs 1128 controls), the first NHS II collection (147 cases vs 2021 controls), and to controls as defined within an individual study (235 cases vs 3021 controls; ie, controls in the breast cancer or ovarian cancer prospective case-control study).

Statistical significance was defined as NEF-*P* < .05 or FDR < .05. Given the hypothesis-generating nature of this study, we also reported and discuss the findings with NEF-*P* < .20 or FDR < .20. Data analyses were performed from January 24, 2022, to January 14, 2023, using R, version 4.0.3 (R Foundation for Statistical Computing) and the R packages, Stats, Fast Gene Set Enrichment Analysis,[25] and ggplot2.[26]

Results

Study Population

The 6477 study participants were all female registered nurses, had a mean (SD) age of 52 (9) years, and most (95%; 6121 women) were White. Of these, 488 women reported experiencing daily persistent (≥5 minutes) tinnitus. Compared with the participants with no tinnitus (n = 5989), those with persistent tinnitus were slightly older (53.0 vs 51.8 years) and were more likely to be postmenopausal, using oral postmenopausal hormone therapy, and/or have type 2 diabetes, hypertension, and/or hearing loss (Table).

Individual Metabolites and Metabolite Classes

In the fully adjusted model 4, we found 8 metabolites associated with tinnitus at the NEF-corrected *P* value (NEF-*P*) < .05 (**Figure 1** and eTable 2 in Supplement 1). Homocitrulline (OR, 1.32; 95% CI, 1.16-1.50; NEF-*P* = .01), C38:6 phosphatidylethanolamine (PE; OR, 1.24; 95% CI, 1.12-1.38; NEF-*P* = .01), C52:6 triglyceride (TAG; OR, 1.22; 95% CI, 1.10-1.36; NEF-*P* = .03), C36:4 PE (OR, 1.22; 95% CI, 1.10-1.35; NEF-*P* = .03); C40:6 PE

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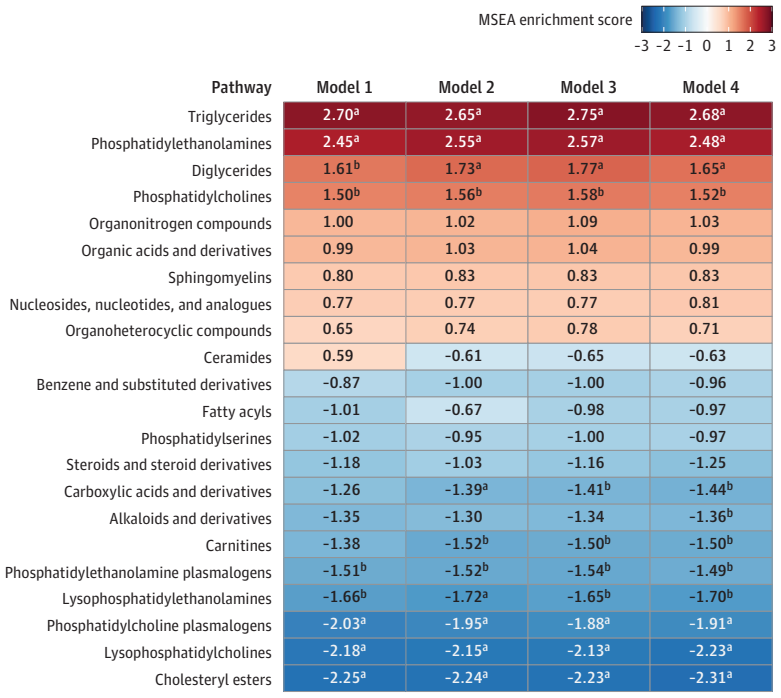
Figure 2. Metabolite Classes Associated With Persistent Daily Tinnitus Among 488 Participants With Tinnitus (Cases) and 5989 Participants Without Tinnitus (Controls), NHS and NHS II



Positive associations are shown in shades of red while inverse associations are shown in shades of blue. Model 1 adjusts for age, race and ethnicity, fasting status, season of blood draw, collection, cohort, and end point; model 2 adjusts for all covariates in model 1 plus BMI; model 3 adjusts for all covariates in model 2 plus menopausal status plus current oral hormone use and current contraceptive use; model 4 adjusts for all covariates in model 3 plus smoking status, physical activity, DASH score, and alcohol intake. BMI refers to body mass index, calculated as weight in kilograms divided by height in meters squared; DASH, Dietary Approaches to Stop Hypertension; FDR, false discovery rate; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; and MSEA, metabolite set enrichment analysis.

[a] FDR < .20.

[b] FDR < .05.

(OR, 1.22; 95% CI, 1.09-1.35; NEF-*P* = .04), and C56:7 TAG (OR, 1.21; 95% CI, 1.09-1.34; NEF-*P* = .05) were positively associated with tinnitus; whereas, α-keto-β-methylvalerate (OR, 0.68; 95% CI, 0.56-0.82; NEF-*P* = .01) and levulinate (OR, 0.60; 95% CI, 0.46-0.79; NEF-*P* = .04) were inversely associated with tinnitus. An additional 12 metabolites (6 TAGs, 4 PEs, 1 diglyceride [DAG], and thiamine) were positively associated and an additional 2 lysophosphatidylcholines (LPCs) were inversely associated with tinnitus at NEF-*P* < .20. Odds ratios and significance levels were similar across the 4 logistic regression models tested. eFigure 1 in Supplement 2 shows pairwise Spearman correlations across all metabolites associated with tinnitus at NEF-*P* < .20. Similar associations were observed in sensitivity analyses restricted to women who participated in the first NHS collection, the second NHS collection, the first NHS II collection, or to controls as defined within an individual study.

In total, 337 of the 466 metabolites were mapped to 34 metabolite classes, of which 22 classes included at least 3 metabolites and were tested with metabolite set enrichment analysis. The TAGs (normalized enrichment score [NES], 2.68; FDR < .001), PEs (NES, 2.48; FDR < .001), and DAGs (NES, 1.65; FDR = .05) were positively associated with tinnitus, whereas phosphatidylcholine (PC) plasmalogens (NES, −1.91; FDR = .02), LPCs (NES, −2.23; FDR < .001), and cholesteryl esters (CEs; NES, −2.31; FDR < .001) were inversely associated (FDR < .05) (**Figure 2**; eTable 3 in Supplement 1). The PCs were positively associated with tinnitus, whereas alkaloids and derivatives, carboxylic acids and derivatives, PE plasmalogens, carnitines, and lysophosphatidylethanolamines were inversely associated (FDR < .20).

Metabolites and Metabolite Classes by Hearing Status

In total, 129 participants with tinnitus (cases) and 3928 participants without tinnitus (controls) reported no hearing loss, while 160 cases and 675 controls reported moderate or severe hearing loss. Based on the findings of fully adjusted model 4, the associations with tinnitus observed among those with no hearing loss were statistically significant and stronger than among participants with moderate or severe hearing loss for these individual metabolite ORs: vitamin A (OR, 1.89 [95% CI, 1.29-2.77], NEF-*P* = .19 vs 0.84 [95% CI, 0.6-1.19], NEF-*P* > .99); C52:6 TAG (OR, 1.46 [95% CI, 1.20-1.77], NEF-*P* = .02 vs 1.17 [95% CI, 0.96-1.43], NEF-*P* > .99); C52:5 TAG (OR, 1.62 [95% CI, 1.30-2.02], NEF-*P* < .001 vs 1.06 [95% CI, 0.81-1.37], NEF-*P* > .99); C36:3 DAG (OR, 1.41 [95% CI, 1.16-1.72], NEF-*P* = .10 vs 1.17 [95% CI, 0.95-1.44], NEF-*P* > .99); C52:4 TAG (OR, 1.39 [95% CI, 1.14-1.69], NEF-*P* = .19 vs 1.18 [95% CI, 0.96-1.46], NEF-*P* > .99); C20:4 CE (OR, 0.70 [95% CI, 0.57-0.85], NEF-*P* = .07 vs 0.87 [95% CI, 0.72-1.05], NEF-*P* > .99); and C20:3 CE (OR, 0.69 [95% CI, 0.57-0.84], NEF-*P* = .04 vs 0.85 [95% CI, 0.71-1.03], NEF-*P* > .99). **Figure 3** and eTable 4 in Supplement 1 illustrate these data further. In contrast, the association between α-keto-β-methylvalerate and tinnitus was stronger and statistically significant among those with hearing loss (OR, 0.24 [95% CI, 0.10-0.54], NEF-*P* = .11) than among those with no hearing loss (OR, 0.70 [95% CI, 0.50-0.98], NEF-*P* > .99).

Based on the metabolite set enrichment analyses, TAGs and PEs were positively associated with tinnitus among those with and without hearing loss (**Figure 4**; eTable 5 in Supplement 1). Based on the fully multivariable-adjusted model 4, the observed associations with tinnitus were stronger and

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Figure 3. Individual Metabolites Associated With Persistent Daily Tinnitus, Moderate or Severe Hearing Loss (160 Cases, 675 Controls) and No Hearing Loss (129 Cases, 3928 Controls), NHS and NHS II

Metabolite	A Model 1 All OR (95% CI)	A Model 1 Hearing loss OR (95% CI)	A Model 1 No hearing loss OR (95% CI)	B Model 4 All OR (95% CI)	B Model 4 Hearing loss OR (95% CI)	B Model 4 No hearing loss OR (95% CI)
Homocitrulline	1.31 (1.15-1.49)	1.18 (0.91-1.54)	1.32 (1.05-1.66)	1.32 (1.16-1.50)	1.13 (0.86-1.49)	1.34 (1.06-1.69)
C38:6 PE	1.23 (1.11-1.37)	1.18 (0.98-1.43)	1.21 (1.00-1.47)	1.24 (1.12-1.38)	1.17 (0.96-1.43)	1.24 (1.02-1.50)
C52:6 TAG	1.22 (1.10-1.35)	1.18 (0.98-1.43)	1.47 (1.22-1.78)	1.22 (1.10-1.36)	1.17 (0.96-1.43)	1.46 (1.20-1.77)
C52:5 TAG	1.21 (1.07-1.36)	1.09 (0.86-1.40)	1.62 (1.31-2.00)	1.20 (1.06-1.36)	1.06 (0.81-1.37)	1.62 (1.30-2.02)
C36:4 PE	1.21 (1.09-1.33)	1.20 (0.99-1.46)	1.15 (0.96-1.39)	1.22 (1.10-1.35)	1.16 (0.95-1.42)	1.18 (0.98-1.44)
C56:4 TAG	1.21 (1.09-1.34)	1.18 (0.97-1.45)	1.29 (1.06-1.56)	1.21 (1.09-1.34)	1.14 (0.93-1.40)	1.26 (1.04-1.53)
C52:7 TAG	1.21 (1.09-1.34)	1.14 (0.94-1.37)	1.36 (1.13-1.65)	1.21 (1.09-1.34)	1.13 (0.93-1.37)	1.35 (1.11-1.63)
C40:6 PE	1.21 (1.10-1.34)	1.21 (1.00-1.46)	1.20 (1.00-1.45)	1.22 (1.10-1.35)	1.18 (0.97-1.44)	1.23 (1.01-1.49)
C56:7 TAG	1.21 (1.10-1.34)	1.10 (0.92-1.32)	1.31 (1.08-1.58)	1.21 (1.09-1.34)	1.09 (0.90-1.32)	1.30 (1.07-1.58)
C38:5 PE	1.20 (1.08-1.33)	1.24 (1.02-1.52)	1.19 (0.98-1.44)	1.20 (1.08-1.33)	1.22 (1.00-1.50)	1.19 (0.98-1.45)
C58:6 TAG	1.20 (1.08-1.33)	1.05 (0.87-1.28)	1.13 (0.93-1.37)	1.20 (1.08-1.33)	1.03 (0.84-1.26)	1.13 (0.93-1.38)
C55:3 TAG	1.20 (1.08-1.33)	1.27 (1.04-1.55)	1.30 (1.07-1.57)	1.19 (1.07-1.32)	1.23 (1.00-1.51)	1.28 (1.06-1.56)
C38:5 DAG	1.20 (1.08-1.33)	1.13 (0.93-1.37)	1.31 (1.08-1.58)	1.20 (1.08-1.34)	1.10 (0.91-1.35)	1.32 (1.09-1.61)
C56:3 TAG	1.20 (1.08-1.33)	1.22 (1.01-1.48)	1.28 (1.06-1.54)	1.19 (1.08-1.32)	1.18 (0.97-1.44)	1.27 (1.04-1.54)
C54:8 TAG	1.19 (1.07-1.32)	1.11 (0.92-1.35)	1.36 (1.12-1.65)	1.18 (1.07-1.32)	1.10 (0.90-1.35)	1.35 (1.11-1.64)
C38:4 PE	1.19 (1.07-1.31)	1.21 (1.00-1.47)	1.14 (0.94-1.38)	1.19 (1.07-1.32)	1.17 (0.96-1.42)	1.16 (0.96-1.41)
C54:5 TAG	1.19 (1.08-1.32)	1.31 (1.07-1.62)	1.30 (1.07-1.57)	1.19 (1.07-1.32)	1.30 (1.05-1.61)	1.29 (1.06-1.56)
C36:3 PE	1.18 (1.07-1.31)	1.30 (1.08-1.58)	1.25 (1.04-1.52)	1.19 (1.07-1.32)	1.27 (1.05-1.54)	1.27 (1.05-1.54)
C34:2 PE	1.18 (1.07-1.31)	1.23 (1.02-1.50)	1.23 (1.02-1.48)	1.19 (1.07-1.32)	1.20 (0.98-1.46)	1.26 (1.04-1.53)
C36:3 DAG	1.17 (1.06-1.30)	1.20 (0.99-1.45)	1.41 (1.17-1.71)	1.17 (1.05-1.30)	1.17 (0.95-1.44)	1.41 (1.16-1.72)
Thiamine	1.17 (1.06-1.28)	1.20 (1.00-1.44)	1.26 (1.06-1.51)	1.18 (1.07-1.29)	1.23 (1.02-1.48)	1.25 (1.05-1.50)
C52:4 TAG	1.14 (1.02-1.26)	1.19 (0.97-1.45)	1.39 (1.15-1.69)	1.13 (1.02-1.26)	1.18 (0.96-1.46)	1.39 (1.14-1.69)
Vitamin A	1.11 (0.92-1.32)	0.87 (0.63-1.20)	1.81 (1.26-2.62)	1.08 (0.90-1.29)	0.84 (0.60-1.19)	1.89 (1.29-2.77)
C36:4 DAG	1.11 (1.01-1.23)	1.15 (0.95-1.41)	1.37 (1.13-1.65)	1.11 (1.00-1.23)	1.14 (0.93-1.41)	1.35 (1.11-1.64)
C20:4 CE	0.87 (0.79-0.97)	0.85 (0.70-1.02)	0.71 (0.59-0.86)	0.87 (0.79-0.97)	0.87 (0.72-1.05)	0.70 (0.57-0.85)
C20:3 CE	0.86 (0.78-0.95)	0.86 (0.71-1.03)	0.69 (0.57-0.83)	0.86 (0.78-0.95)	0.85 (0.71-1.03)	0.69 (0.57-0.84)
C18:0 LPC	0.84 (0.76-0.93)	0.93 (0.77-1.12)	0.86 (0.71-1.04)	0.83 (0.75-0.92)	0.90 (0.75-1.09)	0.84 (0.70-1.03)
C18:2 LPC	0.84 (0.76-0.93)	0.94 (0.78-1.14)	0.85 (0.71-1.03)	0.83 (0.75-0.92)	0.96 (0.79-1.17)	0.85 (0.69-1.04)
Methylvalerate[a]	0.70 (0.58-0.85)	0.46 (0.25-0.79)	0.76 (0.55-1.05)	0.68 (0.56-0.82)	0.24 (0.10-0.54)	0.70 (0.50-0.98)
Levulinate	0.60 (0.46-0.78)	0.43 (0.24-0.72)	0.47 (0.29-0.76)	0.60 (0.46-0.79)	0.50 (0.28-0.90)	0.38 (0.22-0.66)

[a] α-keto-β-methylvalerate.

This figure includes all metabolites identified in the main analysis and all metabolites with NEF-*P* < .20 in the stratified analyses. *All* shows results of the main analysis, which included all tinnitus cases and controls; *hearing loss* shows results of the analysis restricted to those with moderate/severe hearing loss; *no hearing loss* shows results of the analysis restricted to those without hearing loss. Odds ratios of tinnitus were calculated per 1 SD increase in probit-transformed metabolite values. Model 1 adjusts for age, race and ethnicity, fasting status, season of blood draw, collection, cohort, and end point; model 4 adjusts for age, fasting status, season of blood draw, collection, cohort, end point, BMI, menopausal status, current oral hormone use, current oral contraceptive use, smoking status, physical activity, DASH score, and alcohol intake. BMI refers to body mass index, calculated as weight in kilograms divided by height in meters squared; CE, cholesteryl esters; DAG, diglycerides; DASH, Dietary Approaches to Stop Hypertension; LPC, lysophosphatidylcholines; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; PE, phosphatidylethanolamines; and TAG, triglycerides.

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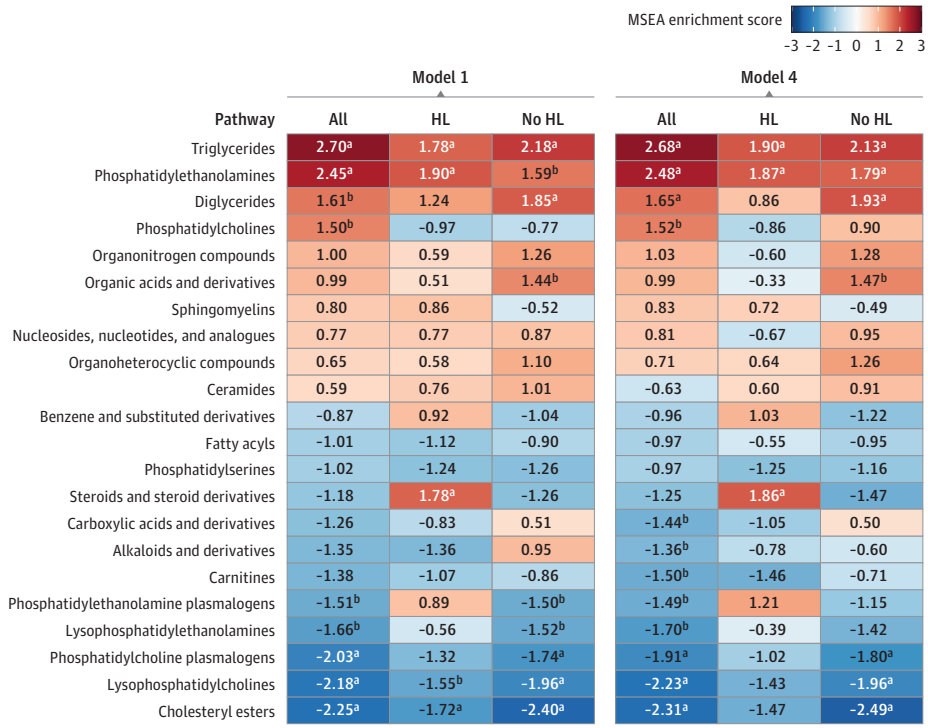
Figure 4. Metabolite Classes Associated With Persistent Daily Tinnitus, Moderate or Severe Hearing Loss (160 Cases, 675 Controls) and No Hearing Loss (129 Cases, 3928 Controls), NHS and NHS II



Pathway	Model 1 All	Model 1 HL	Model 1 No HL	Model 4 All	Model 4 HL	Model 4 No HL
Triglycerides	2.70[a]	1.78[a]	2.18[a]	2.68[a]	1.90[a]	2.13[a]
Phosphatidylethanolamines	2.45[a]	1.90[a]	1.59[b]	2.48[a]	1.87[a]	1.79[a]
Diglycerides	1.61[b]	1.24	1.85[a]	1.65[a]	0.86	1.93[a]
Phosphatidylcholines	1.50[b]	-0.97	-0.77	1.52[b]	-0.86	0.90
Organonitrogen compounds	1.00	0.59	1.26	1.03	-0.60	1.28
Organic acids and derivatives	0.99	0.51	1.44[b]	0.99	-0.33	1.47[b]
Sphingomyelins	0.80	0.86	-0.52	0.83	0.72	-0.49
Nucleosides, nucleotides, and analogues	0.77	0.77	0.87	0.81	-0.67	0.95
Organoheterocyclic compounds	0.65	0.58	1.10	0.71	0.64	1.26
Ceramides	0.59	0.76	1.01	-0.63	0.60	0.91
Benzene and substituted derivatives	-0.87	0.92	-1.04	-0.96	1.03	-1.22
Fatty acyls	-1.01	-1.12	-0.90	-0.97	-0.55	-0.95
Phosphatidylserines	-1.02	-1.24	-1.26	-0.97	-1.25	-1.16
Steroids and steroid derivatives	-1.18	1.78[a]	-1.26	-1.25	1.86[a]	-1.47
Carboxylic acids and derivatives	-1.26	-0.83	0.51	-1.44[b]	-1.05	0.50
Alkaloids and derivatives	-1.35	-1.36	0.95	-1.36[b]	-0.78	-0.60
Carnitines	-1.38	-1.07	-0.86	-1.50[b]	-1.46	-0.71
Phosphatidylethanolamine plasmalogens	-1.51[b]	0.89	-1.50[b]	-1.49[b]	1.21	-1.15
Lysophosphatidylethanolamines	-1.66[b]	-0.56	-1.52[b]	-1.70[b]	-0.39	-1.42
Phosphatidylcholine plasmalogens	-2.03[a]	-1.32	-1.74[a]	-1.91[a]	-1.02	-1.80[a]
Lysophosphatidylcholines	-2.18[a]	-1.55[b]	-1.96[a]	-2.23[a]	-1.43	-1.96[a]
Cholesteryl esters	-2.25[a]	-1.72[a]	-2.40[a]	-2.31[a]	-1.47	-2.49[a]

All shows results from the main analysis, which includes all tinnitus cases and controls; *HL* shows results restricted to those with moderate/severe hearing loss; and *No HL* shows results restricted to those without hearing loss. Positive associations are shown in shades of red while inverse associations are shown in shades of blue. Model 1 adjusts for age, race and ethnicity, fasting status, season of blood draw, collection, cohort, and end point; model 4 adjusts for age, race and ethnicity, fasting status, season of blood draw, collection, cohort, end point, BMI, menopausal status, current oral hormone use, current oral contraceptive use, smoking status, physical activity, DASH score, and alcohol intake. BMI refers to body mass index, calculated as weight in kilograms divided by height in meters squared; DASH, Dietary Approaches to Stop Hypertension; FDR, false discovery rate; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; and MSEA, metabolite set enrichment analysis.

[a] FDR < .001.

[b] FDR < .05.

statistically significant among those with no hearing loss than among those with moderate or severe hearing loss for these metabolite classes: DAGs (NES, 1.93; FDR = .02 vs NES, 0.86; FDR > .99), organic acids and derivates (NEF, 1.47; FDR = .16 vs NES, −0.33; FDR > .99), PC plasmalogens (NES, −1.80; FDR = .04 vs NES, −1.02; FDR = .93), LPCs (NES, −1.96; FDR = .01 vs NES, −1.43; FDR = .41), and CEs (NES, −2.49; FDR < .001 vs NES, −1.47; FDR = .40). Steroids and steroid derivatives were associated with tinnitus among those with moderate or severe hearing loss (NES, 1.86; FDR = .01), but not among those with no hearing loss (NES, −1.47; FDR = .28). Although LPCs and CEs were associated with tinnitus among those with moderate or severe hearing loss based on the least adjusted model (model 1), the associations were no longer statistically significant in the fully multivariable-adjusted model. Given the hypothesis-generating nature of this study, all metabolites associated with tinnitus at $P < .05$ based on the fully adjusted model 4 are shown in eFigure 2 in Supplement 2, and among those with and without hearing loss in eFigures 3 and 4 in Supplement 2.

Discussion

To our knowledge, this is the largest population-based investigation of plasma metabolite profiles and tinnitus, assessing 466 plasma biomarkers among 6477 participants. We conducted a broad search for plasma tinnitus biomarkers using a high throughput, agnostic metabolomic approach. We identified significant associations of a number of individual metabolites and metabolite classes with persistent tinnitus. Metabolites positively associated with tinnitus included the amino acid homocitrulline, 3 PEs (C38:6 PE, C36:4 PE, C40:6 PE), and 2 TAGs (C56:7 TAG and C52:6 TAG); α-keto-β-methylvalerate and levulinate were inversely associated. The metabolites classes TAGs, PEs, and DAGs were positively associated, and PC plasmalogens and CEs were inversely associated. These study findings suggest that metabolomic profiles offer a promising approach for identifying tinnitus biomarkers and gaining valuable insights into tinnitus pathophysiology.

We observed striking differences in metabolite profiles among participants experiencing persistent tinnitus with moderate or severe hearing loss and without hearing loss. For example, significant associations for certain metabolites and metabolite classes were observed only among women with tinnitus and no hearing loss. Several TAGs and vitamin A were positively associated with tinnitus without hearing loss, and CEs were inversely associated. For metabolite classes, positive associations were observed for DAGs and organic acids, whereas inverse associations were observed for PC plasmalogens, LPCs, and CEs, only among those without hearing loss. In contrast, strong positive associations were observed for steroids and steroid derivatives only among those with moderate or severe hearing loss. Strong positive associations of TAGs and PEs with tinnitus were observed in both groups.

These study findings indicate that metabolic dysregulation may contribute to tinnitus, although further research is needed to clarify which metabolites and metabolic pathways

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may be most influential. Although individual metabolites showed statistically significant associations, the observed effect-sizes were modest. This finding suggests that dysregulation of whole metabolic pathways involving the complex interplay of several individual metabolites may drive tinnitus development. This finding also underscores that tinnitus is a complex disorder with metabolic perturbations associated with hyperlipidemia, atherosclerosis, hypertension, diabetes, and genetic and environmental factors that may affect inflammation, oxidative stress, neural transmission, and neuroplasticity, which may all play a role. This study substantially augments the scarce literature on plasma metabolomic biomarkers for tinnitus. Specifically, a few small metabolomic investigations of sensorineural hearing loss in animals and humans have examined metabolomic profiles in perilymph, plasma, urine, temporal bone or brain tissue,[27] and evaluated metabolomic alterations after noise or cisplatin exposure. Plasma metabolomic alterations after noise exposure were observed for several individual metabolites and pathways, such as those involving glycerophospholipid, choline, and fatty acid metabolism.[28] We identified significant associations with metabolites and metabolite classes, which suggest that dysregulation of lipid metabolism and other metabolic pathways may be influential.

Homocitrulline, an amino acid and by-product of ornithine metabolism, was positively associated with tinnitus. Altered plasma homocitrulline levels have been identified among individuals with autism spectrum disorder.[29] In addition, higher plasma homocitrulline has been shown to be associated with chronic kidney disease,[30] liver disease,[31,32] coronary artery disease,[33] and a higher risk of mortality among individuals with diabetes.[34] Plasma homocitrulline reflects the carbamylation rate of serum proteins, promotes autoimmune and inflammatory responses,[35-38] and may be associated with neuroinflammation[39] and proteinopathy.[35,40-42] Homocitrulline also promotes endothelial dysfunction and vascular disease,[43] possibly indicating a neurovascular component of tinnitus pathophysiology.

Several TAGs and DAGs, key components of fatty acid metabolism, were positively associated with tinnitus, consistent with a small Turkish case-control study (n = 156) that found higher serum TAG, total cholesterol, and low-density lipoprotein levels among those with tinnitus.[44] In our study, higher plasma TAGs were strongly associated with tinnitus among individuals with and without hearing loss, whereas higher plasma DAGs were significantly associated with tinnitus only among those without hearing loss. Specific lipid species are structural components of cellular membranes and regulate a range of critical aspects of brain function.[45] Furthermore, lipids play a fundamental role in the pathophysiology of neurodegenerative diseases[46,47]; lipid metabolism perturbations have been shown to be associated with amyotrophic lateral sclerosis (ALS),[48-50] Parkinson disease,[46] and Alzheimer disease.[47,51] Whether lipidomic profiles aid in distinguishing tinnitus subgroups or whether targeted interventions that modify plasma lipids affect tinnitus merits further exploration.

Phosphatidylethanolamines were positively associated with tinnitus among those with and without hearing loss. Phosphatidylethanolamines, the second-most abundant cellular phospholipids, are involved in protein biogenesis, oxidative phosphorylation, membrane fusion, mitochondrial stability; serve as precursors to other lipids; and their dysregulation has been implicated in neurodegenerative disease.[47,52] A study among men with occupational noise exposure (n = 124) found lower plasma PE levels among those with hearing loss.[28,53]

Phosphatidylcholine plasmalogens—common glycerophospholipids that rigidify membranes, facilitate signaling processes, act as free radical scavengers, and protect membrane lipids from oxidation[54]—were inversely associated with tinnitus. Plasmalogens decrease with aging, and altered plasma PC plasmalogens have been demonstrated to be involved in Alzheimer disease, Parkinson disease, multiple sclerosis, and ALS.[55,56] Among individuals with Alzheimer disease, significantly lower plasma PC have been observed among those with hearing loss compared with those without hearing loss.[53] The role of PC plasmalogens in tinnitus is unclear, but studies in rats suggest that PCs may help protect cochlear mitochondrial function.[57] Further investigation into the protective role for PC plasmalogens in tinnitus could yield promising findings.

We observed inverse associations of plasma LPCs and CEs with persistent tinnitus, findings that are consistent with previous studies[58-63] suggesting that low LPCs and CEs may be important in the pathobiology of neurodegenerative disease. Given that LPC is the preferred carrier of long-chain polyunsaturated fatty acids across the blood-brain barrier, insufficient LPC could limit their supply to the brain.[63] Also, LPC-related neuroprotection may involve signaling pathways that protect against apoptosis and enhanced production of nerve growth factor.[64-68] Cholesterol is an essential membrane component, signaling molecular cofactor and steroid precursor, and CEs are a major component of high density lipids. Altered cholesterol metabolism and lower plasma CEs have been demonstrated in several neurodegenerative conditions, including Alzheimer disease, Parkinson disease, multiple sclerosis, and ALS.[58-62,69] Taken together, these findings indicating alterations in plasma TAGs, PCs, LPCs, PEs, and cholesterol-related metabolites suggest a role of lipid dysregulation in tinnitus pathogenesis and are consistent with findings for several other neurodegenerative disorders.

We observed inverse associations for 2 novel plasma metabolites not previously examined in relation to tinnitus, α-keto-β-methylvalerate and levulinate. A catabolic product of isoleucine, α-keto-β-methylvalerate is a branched-chain amino acid often used as a B-complex vitamin marker in laboratory tests.[70] Levulinate is a food additive and calcium supplement. Further studies to investigate the role of these 2 metabolites in the pathogenesis of tinnitus are needed.

Strengths and Limitations

The strengths of this study include use of 2 richly characterized cohorts, enabling adjustment for a broad range of covariates, and a well-characterized metabolomics platform that measured a large set of metabolites with robust coefficients

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of variation and low missingness. Limitations include the cross-sectional study design using nonrandom samples from previous studies, metabolomic profiling methods that covered only a subset of the full metabolome, and potential unmeasured confounding. Previous examinations of metabolite profile stability in NHS showed most measured metabolites demonstrate reasonable within-person stability for 1 to 2 years as well as for 10 years, showing 1 plasma sample reasonably represents long-term exposure.[20,71] Subjective tinnitus is perceived only by the individual, and therefore, tinnitus diagnosis relies on self-report.[72] Notably, among those who reported persistent tinnitus in 2009, 88% reported persistent tinnitus again 4 years later. Tinnitus definitions vary greatly.[73] We examined a stringent definition to reduce potential misclassification and evaluate cases of tinnitus most likely to be clinically meaningful. The study findings are not generalizable to the general population because the participants were predominantly White and female registered nurses; research among other populations is warranted.

Conclusions

This large population-based metabolomic case-control study of tinnitus identified plasma metabolites and metabolite classes that were significantly associated with persistent tinnitus, and which differed by hearing status. These findings provide new insights into metabolic pathways that may be involved in tinnitus and suggest that metabolomic profiling offers a promising approach to identifying tinnitus biomarkers and informing investigations of novel therapeutic targets for this challenging disorder.

ARTICLE INFORMATION

Accepted for Publication: January 17, 2023.

Published Online: March 16, 2023. doi:10.1001/jamaoto.2023.0052

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Obtained funding: Welling, Stankovic, G. Curhan, S. Curhan.
Administrative, technical, or material support: Zeleznik, Stankovic, G. Curhan, S. Curhan.
Supervision: G. Curhan, S. Curhan.

Conflict of Interest Disclosures: Dr Welling reported consulting and/or advisory fees from the scientific advisory boards of Recursion Therapeutics, Science 24/7, NF2 BioSolutions, NF Bio, Daybreak Vision Project, Mulberry Therapeutics, Skylark Biotherapy, and Salubritus Bio, all outside the submitted work. Dr G. Curhan reported being an employee of OM1, equity in Allena Pharmaceuticals, royalties from UpToDate, and grant funding from Decibel Therapeutics, all outside the submitted work. Dr S. Curhan reported consulting fees and grants from Decibel Therapeutics and grants from GlaxoSmithKline, outside the submitted work. No other disclosures were reported.

Funding/Support: This research was funded by the Royal National Institute for Deaf People (No. G103 to Dr S. Curhan), the Massachusetts Eye and Ear Foundation, the Allan and Muriel Greenblatt Philanthropic Fund, Bertarelli Foundation, and the US National Institutes of Health (No. R21 DC020777, U01 DC010811, UM1 CA186107, P01 CA87969, R01 CA49449, R01 HL034594, R01 HL088521, R01 CA67262, and U01 CA176726).

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 3.

REFERENCES

1. Baguley D, McFerran D, Hall D. Tinnitus. *Lancet*. 2013;382(9904):1600-1607. doi:10.1016/S0140-6736(13)60142-7

2. Nondahl DM, Cruickshanks KJ, Dalton DS, et al. The impact of tinnitus on quality of life in older adults. *J Am Acad Audiol*. 2007;18(3):257-266. doi:10.3766/jaaa.18.3.7

3. Shargorodsky J, Curhan GC, Farwell WR. Prevalence and characteristics of tinnitus among US adults. *Am J Med*. 2010;123(8):711-718. doi:10.1016/j.amjmed.2010.02.015

4. Hoare DJ, Kowalkowski VL, Kang S, Hall DA. Systematic review and meta-analyses of randomized controlled trials examining tinnitus management. *Laryngoscope*. 2011;121(7):1555-1564. doi:10.1002/lary.21825

5. Cederroth CR, Gallus S, Hall DA, et al. Editorial: towards an understanding of tinnitus heterogeneity. *Front Aging Neurosci*. 2019;11:53. doi:10.3389/fnagi.2019.00053

6. McFerran DJ, Stockdale D, Holme R, Large CH, Baguley DM. Why is there no cure for tinnitus? *Front Neurosci*. 2019;13:802. doi:10.3389/fnins.2019.00802

7. Escobar MQ, Tasic L, Costa TBBCD, et al. Serum metabolic profiles based on nuclear magnetic resonance spectroscopy among patients with deep vein thrombosis and healthy controls. *Metabolites*. 2021;11(12):874. doi:10.3390/metabo11120874

8. Pathan M, Wu J, Lakso HA, Forsgren L, Öhman A. Plasma Metabolite markers of Parkinson's disease and atypical Parkinsonism. *Metabolites*. 2021;11(12):860. doi:10.3390/metabo11120860

9. He J, Zhu Y, Aa J, et al. Brain metabolic changes in rats following acoustic trauma. *Front Neurosci*. 2017;11:148. doi:10.3389/fnins.2017.00148

10. Hankinson SE, Willett WC, Michaud DS, et al. Plasma prolactin levels and subsequent risk of breast cancer in postmenopausal women. *J Natl Cancer Inst*. 1999;91(7):629-634. doi:10.1093/jnci/91.7.629

11. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. *Cancer Res*. 2006;66(4):2476-2482. doi:10.1158/0008-5472.CAN-05-3369

12. Bao Y, Bertoia ML, Lenart EB, et al. Origin, methods, and evolution of the Three Nurses' Health Studies. *Am J Public Health*. 2016;106(9):1573-1581. doi:10.2105/AJPH.2016.303338

13. Salvini S, Hunter DJ, Sampson L, et al. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol*. 1989;18(4):858-867. doi:10.1093/ije/18.4.858

14. Wolf AM, Hunter DJ, Colditz GA, et al. Reproducibility and validity of a self-administered physical activity questionnaire. *Int J Epidemiol*. 1994;23(5):991-999. doi:10.1093/ije/23.5.991

15. Colditz GA, Martin P, Stampfer MJ, et al. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. *Am J Epidemiol*. 1986;123(5):894-900. doi:10.1093/oxfordjournals.aje.a114319

16. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology*. 1990;1(6):466-473. doi:10.1097/00001648-199011000-00009

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17. Mascanfroni ID, Takenaka MC, Yeste A, et al. Metabolic control of type 1 regulatory T cell differentiation by AHR and HIF1-α. *Nat Med*. 2015;21(6):638-646. doi:10.1038/nm.3868

18. O'Sullivan JF, Morningstar JE, Yang Q, et al. Dimethylguanidino valeric acid is a marker of liver fat and predicts diabetes. *J Clin Invest*. 2017;127(12):4394-4402. doi:10.1172/JCI95995

19. Paynter NP, Balasubramanian R, Giulianini F, et al. Metabolic predictors of incident coronary heart disease in women. *Circulation*. 2018;137(8):841-853. doi:10.1161/CIRCULATIONAHA.117.029468

20. Townsend MK, Clish CB, Kraft P, et al. Reproducibility of metabolomic profiles among men and women in 2 large cohort studies. *Clin Chem*. 2013;59(11):1657-1667. doi:10.1373/clinchem.2012.199133

21. Bajad SU, Lu W, Kimball EH, Yuan J, Peterson C, Rabinowitz JD. Separation and quantitation of water soluble cellular metabolites by hydrophilic interaction chromatography-tandem mass spectrometry. *J Chromatogr A*. 2006;1125(1):76-88. doi:10.1016/j.chroma.2006.05.019

22. Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol*. 2008;32(4):361-369. doi:10.1002/gepi.20310

23. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005;102(43):15545-15550. doi:10.1073/pnas.0506580102

24. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B*. 1995;57(1):289-300. doi:10.1111/j.2517-6161.1995.tb02031.x

25. Korotkevich G, Sukhov V, Budin N, Shpak B, Artyomov MN, Sergushichev A. Fast gene set enrichment analysis. BioRxiv: 2016:060012. doi:10.1101/060012

26. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag; 2016. Accessed February 6, 2023. Available from: https://ggplot2.tidyverse.org

27. Boullaud L, Blasco H, Trinh T-T, Bakhos D. Metabolomic studies in inner ear pathologies. *Metabolites*. 2022;12(3):214. doi:10.3390/metabo12030214

28. Miao L, Wang B, Zhang J, Yin L, Pu Y. Plasma metabolomic profiling in workers with noise-induced hearing loss: a pilot study. *Environ Sci Pollut Res Int*. 2021;28(48):68539-68550. doi:10.1007/s11356-021-15468-z

29. West PR, Amaral DG, Bais P, et al. Metabolomics as a tool for discovery of biomarkers of autism spectrum disorder in the blood plasma of children. *PLoS One*. 2014;9(11):e112445. doi:10.1371/journal.pone.0112445

30. Grams ME, Tin A, Rebholz CM, et al. Metabolomic alterations associated with cause of CKD. *Clin J Am Soc Nephrol*. 2017;12(11):1787-1794. doi:10.2215/CJN.02560317

31. Ascha M, Wang Z, Ascha MS, et al. Metabolomics studies identify novel diagnostic and prognostic indicators in patients with alcoholic hepatitis. *World J Hepatol*. 2016;8(10):499-508. doi:10.4254/wjh.v8.i10.499

32. Yang Y, Huang Z, Yang Z, et al. Serum metabolomic profiling reveals an increase in homocitrulline in Chinese patients with nonalcoholic fatty liver disease: a retrospective study. *PeerJ*. 2021;9:e11346. doi:10.7717/peerj.11346

33. Jaisson S, Kerkeni M, Santos-Weiss IC, Addad F, Hammami M, Gillery P. Increased serum homocitrulline concentrations are associated with the severity of coronary artery disease. *Clin Chem Lab Med*. 2015;53(1):103-110. CCLM. doi:10.1515/cclm-2014-0642

34. Ottosson F, Smith E, Fernandez C, Melander O. Plasma metabolites associate with all-cause mortality in individuals with type 2 diabetes. *Metabolites*. 2020;10(8):E315. doi:10.3390/metabo10080315

35. Gallart-Palau X, Serra A, Lee BST, Guo X, Sze SK. Brain ureido degenerative protein modifications are associated with neuroinflammation and proteinopathy in Alzheimer's disease with cerebrovascular disease. *J Neuroinflammation*. 2017;14(1):175. doi:10.1186/s12974-017-0946-y

36. Pruijn GJ. Citrullination and carbamylation in the pathophysiology of rheumatoid arthritis. *Front Immunol*. 2015;6:192. doi:10.3389/fimmu.2015.00192

37. Jang B, Kim E, Choi J-K, et al. Accumulation of citrullinated proteins by up-regulated peptidylarginine deiminase 2 in brains of scrapie-infected mice: a possible role in pathogenesis. *Am J Pathol*. 2008;173(4):1129-1142. doi:10.2353/ajpath.2008.080388

38. Gallart-Palau X, Serra A, Sze SK. Uncovering neurodegenerative protein modifications via proteomic profiling. *Int Rev Neurobiol*. 2015;121:87-116. doi:10.1016/bs.irn.2015.06.002

39. Currais A, Fischer W, Maher P, Schubert D. Intraneuronal protein aggregation as a trigger for inflammation and neurodegeneration in the aging brain. *FASEB J*. 2017;31(1):5-10. doi:10.1096/fj.201601184

40. Rimon S, Perlmann GE. Carbamylation of pepsinogen and pepsin. *J Biol Chem*. 1968;243(13):3566-3572. doi:10.1016/S0021-9258(19)34178-X

41. Kalim S, Karumanchi SA, Thadhani RI, Berg AH. Protein carbamylation in kidney disease: pathogenesis and clinical implications. *Am J Kidney Dis*. 2014;64(5):793-803. doi:10.1053/j.ajkd.2014.04.034

42. Jaisson S, Lorimier S, Ricard-Blum S, et al. Impact of carbamylation on type I collagen conformational structure and its ability to activate human polymorphonuclear neutrophils. *Chem Biol*. 2006;13(2):149-159. doi:10.1016/j.chembiol.2005.11.005

43. Speer T, Owala FO, Holy EW, et al. Carbamylated low-density lipoprotein induces endothelial dysfunction. *Eur Heart J*. 2014;35(43):3021-3032. doi:10.1093/eurheartj/ehu111

44. Avcı D. Increased Serum Lipid Levels in Patients with Subjective Tinnitus. *Iran J Otorhinolaryngol*. 2021;33(114):31-36.

45. Mielke MM, Haughey NJ, Bandaru VVR, et al. Plasma sphingomyelins are associated with cognitive progression in Alzheimer's disease. *J Alzheimers Dis*. 2011;27(2):259-269. doi:10.3233/JAD-2011-110405

46. Dong M-X, Wei Y-D, Hu L. Lipid metabolic dysregulation is involved in Parkinson's disease dementia. *Metab Brain Dis*. 2021;36(3):463-470. doi:10.1007/s11011-020-00665-5

47. Liu Y, Thalamuthu A, Mather KA, et al. Plasma lipidome is dysregulated in Alzheimer's disease and is associated with disease risk genes. *Transl Psychiatry*. 2021;11(1):344. doi:10.1038/s41398-021-01362-2

48. Area-Gomez E, Larrea D, Yun T, et al. Lipidomics study of plasma from patients suggest that ALS and PLS are part of a continuum of motor neuron disorders. *Sci Rep*. 2021;11(1):13562. doi:10.1038/s41598-021-92112-3

49. FernÁndez-Eulate G, Ruiz-Sanz JI, Riancho J, et al. A comprehensive serum lipidome profiling of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*. 2020;21(3-4):252-262. doi:10.1080/21678421.2020.1730904

50. Lim WLF, Huynh K, Chatterjee P, et al; AIBL research group. Relationships Between Plasma Lipids Species, Gender, Risk Factors, and Alzheimer's Disease. *J Alzheimers Dis*. 2020;76(1):303-315. doi:10.3233/JAD-191304

51. Zhang X, Liu W, Zan J, Wu C, Tan W. Untargeted lipidomics reveals progression of early Alzheimer's disease in APP/PS1 transgenic mice. *Sci Rep*. 2020;10(1):14509. doi:10.1038/s41598-020-71510-z

52. Llano DA, Devanarayan V; Alzheimer's Disease Neuroimaging Initiative. Serum phosphatidylethanolamine and lysophosphatidylethanolamine levels differentiate Alzheimer's disease from controls and predict progression from mild cognitive impairment. *J Alzheimers Dis*. 2021;80(1):311-319. doi:10.3233/JAD-201420

53. Llano DA, Issa LK, Devanarayan P, Devanarayan V; Alzheimer's Disease Neuroimaging Initiative Adni. Hearing loss in Alzheimer's disease is associated with altered serum lipidomic biomarker profiles. *Cells*. 2020;9(12):2556. doi:10.3390/cells9122556

54. Braverman NE, Moser AB. Functions of plasmalogen lipids in health and disease. *Biochim Biophys Acta*. 2012;1822(9):1442-1452. doi:10.1016/j.bbadis.2012.05.008

55. Bozelli JC Jr, Azher S, Epand RM. Plasmalogens and chronic inflammatory diseases. *Front Physiol*. 2021;12:730829. doi:10.3389/fphys.2021.730829

56. Goutman SA, Boss J, Guo K, et al. Untargeted metabolomics yields insight into ALS disease mechanisms. *J Neurol Neurosurg Psychiatry*. 2020;91(12):1329-1338. doi:10.1136/jnnp-2020-323611

57. Seidman MD, Khan MJ, Tang WX, Quirk WS. Influence of lecithin on mitochondrial DNA and age-related hearing loss. *Otolaryngol Head Neck Surg*. 2002;127(3):138-144. doi:10.1067/mhn.2002.127627

58. Di Natale C, Monaco A, Pedone C, et al. The level of 24-hydroxycholesteryl esters decreases in plasma of patients with Parkinson's disease. *Neurosci Lett*. 2018;672:108-112. doi:10.1016/j.neulet.2018.02.041

59. Proitsi P, Kim M, Whiley L, et al. Plasma lipidomics analysis finds long chain cholesteryl esters to be associated with Alzheimer's disease. *Transl Psychiatry*. 2015;5(1):e494-e494. doi:10.1038/tp.2014.127

60. Benussi L, Ghidoni R, Dal Piaz F, Binetti G, Di Iorio G, Abrescia P. The level of 24-hydroxycholesteryl esters is an early marker of

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Alzheimer's disease. *J Alzheimers Dis*. 2017;56(2):825-833. doi:10.3233/JAD-160930

61. Anchisi L, Dessì S, Pani A, Mandas A. Cholesterol homeostasis: a key to prevent or slow down neurodegeneration. *Front Physiol*. 2013;3:486. doi:10.3389/fphys.2012.00486

62. Albers JJ, Marcovina SM, Christenson RH. Lecithin cholesterol acyltransferase in human cerebrospinal fluid: reduced level in patients with multiple sclerosis and evidence of direct synthesis in the brain. *Int J Clin Lab Res*. 1992;22(3):169-172. doi:10.1007/BF02591418

63. Semba RD. Perspective: the potential role of circulating lysophosphatidylcholine in neuroprotection against Alzheimer disease. *Adv Nutr*. 2020;11(4):760-772. doi:10.1093/advances/nmaa024

64. Paoletti L, Domizi P, Marcucci H, et al. Lysophosphatidylcholine drives neuroblast cell fate. *Mol Neurobiol*. 2016;53(9):6316-6331. doi:10.1007/s12035-015-9528-0

65. Wuhanqimuge, Itakura A, Matsuki Y, Tanaka M, Arioka M. Lysophosphatidylcholine enhances NGF-induced MAPK and Akt signals through the extracellular domain of TrkA in PC12 cells. *FEBS Open Bio*. 2013;3:243-251. doi:10.1016/j.fob.2013.05.003

66. Ikeno Y, Cheon SH, Konno N, Nakamura A, Kitamoto K, Arioka M. Lysophosphatidylcholine protects cerebellar granule neurons from apoptotic cell death. *J Neurosci Res*. 2009;87(1):190-199. doi:10.1002/jnr.21821

67. Yalagala PCR, Sugasini D, Dasarathi S, Pahan K, Subbaiah PV. Dietary lysophosphatidylcholine-EPA enriches both EPA and DHA in the brain: potential treatment for depression. *J Lipid Res*. 2019;60(3):566-578. doi:10.1194/jlr.M090464

68. Sugasini D, Thomas R, Yalagala PCR, Tai LM, Subbaiah PV. Dietary docosahexaenoic acid (DHA) as lysophosphatidylcholine, but not as free acid, enriches brain DHA and improves memory in adult mice. *Sci Rep*. 2017;7(1):11263. doi:10.1038/s41598-017-11766-0

69. La Marca V, Maresca B, Spagnuolo MS, et al. Lecithin-cholesterol acyltransferase in brain: Does oxidative stress influence the 24-hydroxycholesterol esterification? *Neurosci Res*. 2016;105:19-27. doi:10.1016/j.neures.2015.09.008

70. Campbell P. α-keto-β-methylvalerate. In: Cammack R, Atwood T, Parish H, Smith A, Vella F, Stirling J, eds. *Oxford Dictionary of Biochemistry and Molecular Biology*. Oxford University Press; 2006.

71. Zeleznik OA, Wittenbecher C, Deik A, et al. Intrapersonal stability of plasma metabolomic profiles over 10 years among women. *Metabolites*. 2022;12(5):372. doi:10.3390/metabo12050372

72. Heller AJ. Classification and epidemiology of tinnitus. *Otolaryngol Clin North Am*. 2003;36(2):239-248. doi:10.1016/S0030-6665(02)00160-3

73. McCormack A, Edmondson-Jones M, Somerset S, Hall D. A systematic review of the reporting of tinnitus prevalence and severity. *Hear Res*. 2016;337:70-79. doi:10.1016/j.heares.2016.05.009

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