Extrinsic effectors regulating genes for plasmalogens biosynthetic enzymes in HepG2 cells

Ryouta Maeba* and Shin-ichi Nakahara

Department of Biochemistry, Teikyo University School of Medicine, Tokyo, Japan

Abstract

Plasma plasmalogens (Pls) may serve as potential biomarkers not only for rare peroxisomal diseases but also for general disorders related to oxidative stress and aging. Recent clinical observational studies demonstrated that low levels of plasma Pls are risk factors for atherosclerosis and dementia. Serum levels of Pls showed a strong positive correlation with high-density lipoprotein (HDL) cholesterol concentration, suggesting that Pls may be involved in metabolism or the function of HDL. Increasing the levels of plasma Pls may serve as a novel therapeutic strategy for preventing diseases associated with oxidative stress and aging. Therefore, we and other groups elevated plasma PI levels in laboratory animals or humans through administration of myo-inositol, monounsaturated long-chain fatty acids, and the hypolipidemic agent, statin. However, their effects on the gene expression of PI biosynthetic enzymes remain unknown. To gain insight into the manipulation of PI biosynthesis and the relationship between PI biosynthesis and HDL metabolism, we examined target gene expression by real-time reverse transcription polymerase chain reaction (RT-PCR) in hepatoma HepG2 cells treated with various test substances. Monounsaturated long-chain fatty acids such as oleic acid and erucic acid, myo-inositol, and the PI precursor alkylglycerol, all of which supply materials or coenzymes for PI biosynthesis, unexpectedly reduced the expression of the genes for PI biosynthetic enzymes. These results suggest the presence of strict regulation of PI homeostasis. In contrast, pitavastatin induced peroxisome biogenesis and promoted the expression of peroxisomal PI biosynthetic enzymes and HDL metabolism-associated proteins such as apoprotein A1 and ATP-binding cassette transporter A1. This was likely through enhancement of peroxisome proliferator-activated receptor (PPAR) expression. These findings suggest that there may be a physiological relationship between PI biosynthesis and HDL metabolism via peroxisomal status.

Abbreviations

DHA: Docosahexaenoic Acid; Far 1: Fatty Acyl CoA Reductase 1; HG: 1-O-hexadecyl-sn-glycerol; MI: myo-inositol; PlsCho: Choline Plasmalogens; PlsEtn: Ethanolamine Plasmalogens; PPARs: Peroxisome Proliferator-Activated Receptors.

Introduction

Age-related diseases, such as atherosclerosis and dementia, are associated with oxidative stress and chronic inflammation [1]. Peroxisomal as well as mitochondrial dysfunction may be related to aging and age-related pathologies, possibly through the derangement of redox homeostasis [2,3]. Plasmalogens (Pis), a subclass of glycerophospholipids possessing a vinyl-ether bond at the sn-1 position, are biosynthesized and regulated in peroxisomes [4-6]. Therefore, plasma PIs may reflect the systemic functional state of peroxisomes, and serve as potential biomarkers for diseases related to oxidative stress and aging [7-9]. Human plasma PIs are synthesized mainly in the liver and secreted into the blood as lipoprotein components. To investigate the clinical significance of plasma PIs, we developed three promising analytical methods [10-13]. Our research lab and other investigators have demonstrated in clinical observational studies that low levels of plasma PI are a risk factor for atherosclerosis and dementia [14-18]. Serum levels of PI showed a strong positive correlation with high-density lipoprotein (HDL) cholesterol concentration [14,15], suggesting that PIs may be involved in metabolism or HDL functions.

Accordingly, we attempted to increase the levels of plasma PIs as a preventative strategy for diseases associated with oxidative stress and aging. This was achieved in laboratory animals as well as humans through administration of the PI precursor alkylglycerol [19], myo-inositol (MI) [20,21], monounsaturated long-chain fatty acids [22], and the hypolipidemic agent, statin [23]. However, their effects on the gene expression of PI biosynthetic enzymes remain unknown. To gain insight into the mechanisms mediating the enhancement of PI biosynthesis, and its relationship with HDL metabolism, we examined target gene expression in HepG2 cells treated with various test substances.

Materials and methods

Materials

Pitavastatin and simvastatin were kindly provided by Nissan Chemical Industries, Ltd. (Tokyo, Japan) and Merck Research Laboratories (Rahway, NJ), respectively. Fenoibrate was a kind gift from ASKA Pharmaceutical Co., Ltd. (Tokyo, Japan). Wy14643 was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fatty acids such as oleic acid, erucic acid, nervonic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and β-estradiol (E2) were purchased from Sigma-Aldrich (St. Louis, MO). N-palmitoyl-D-erythro-sphingosylphosphorylcholine (SM18), N-lignoceroyl-D-erythro-sphingosylphosphorylcholine (SM24),
1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), and 1-O-hexadecyl-sn-glycerol (HG) were obtained from Avanti Polar Lipids (Alabaster, AL). Myo-inositol (MI) was a kind gift from TSUNOH Co., Ltd. (Wakayama, Japan).

Cell culture and treatment with test substances

HepG2 cells (RIKEN BioResource Center, Tukuba, Ibaragi, Japan) were cultured in Dulbecco’s Modified Eagle Medium (Gibco) containing 10% fetal bovine serum (Gibco), 100 μg/mL streptomycin sodium, and 100 U/mL penicillin G sodium (Meiji Seika Pharma Co., Ltd. Tokyo, Japan) at 37°C and 5% CO₂. Test substances were dissolved in distilled water, ethanol, or dimethyl sulfoxide and then passed through a membrane filter (0.45 μm) for sterilization. The test substance solution was added to the cell culture medium at a desired concentration, adjusted to a vehicle concentration of less than 0.1%. Cells were incubated with test substances at 37°C for 24 h.

Real-time RT-PCR

Total RNA was prepared using TriZol (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized from 1.0 μg RNA with GeneAmp® RNA PCR (Applied Biosystems, Branchburg, NJ, USA) using random hexamers. Real-time RT-PCR was performed using LightCycler-FastStart DNA Master SYBR-Green 1 (Roche, Tokyo, Japan), according to the manufacturer’s instructions. The reaction mixture (20 μL) contained LightCycler-FastStart DNA Master SYBR-Green 1, 4 mM MgCl₂, 0.5 μM of the upstream and downstream PCR primers, and 2 μL of the first-strand cDNA as a template. The target genes and their primers are shown in Table 1. To control variations in the reactions, all PCR reactions were normalized against GAPDH or β-actin expression. The results of pitavastatin are shown as the mean ± SEM (Figure 1). Statistical analyses were performed using Stat Flex ver.6 (Artech Co. Ltd., Osaka, Japan).

| Gene          | Protein                      | 5’Primer 3’       | GeneBank accession no. |
|---------------|------------------------------|-------------------|------------------------|
| PPARA         | peroxisome proliferator-activated receptor alpha Forward primer | ATGGTGGCACACGGAAAGCC | NM-005036               |
| PPARG         | peroxisome proliferator-activated receptor gamma Forward primer | GGGATCACCTCTGCTGGATCT | NM-138711               |
| TYSND1        | trypsin domain containing 1 Forward primer | TOACCTTGGTACCTGAATGGT | NM-173555               |
| CROT          | carnitine O-octanoyltransferase Forward primer | GTGGTGGCTAAGTGGTCTCA | NM-021151               |
| EHHADH        | enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase Forward primer | AAACCTGACACGGGCGTGGAGA | NM-001166445            |
| ACSL1         | acyl-CoA synthetase long-chain family member 1 Forward primer | GCCGAGTGGATGATAGCTGC | NM-0018876              |
| CTP1A         | carnitine palmitoyltransferase 1A (liver) Forward primer | GCTAGCTGCGATCTAGAGGCT | NM-001256799            |
| ACAA1         | acetyl-Coenzyme A acyltransferase 1 Forward primer | GCCTGGTCTCAAGGACGTTGAA | NM-0016077              |
| ACOX1         | acetyl-Coenzyme A oxidase 1, palmitoyl Forward primer | ACTGCCAGCAGCGTGTTGAGT | NM-007292               |
| GNPAT         | glyceronephosphate O-acyltransferase Forward primer | AGGGTGACGACGATCAACAC | NM-0014236              |
| AGPS          | alkylglycerone phosphate synthase Forward primer | TGATGATCACCTAGTGCAACAG | NM-003659               |
| FAR1          | fatty acyl-CoA reductase 1: Forward primer | AGACACACAAAGGGAGGATG | NM-032228               |
| PEMT          | phosphatidylethanolamine N-methyltransferase Forward primer | CTGAGATGTTGCGAGCATG | NM-148172               |
| FASN          | fatty acid synthase Forward primer | AAGGACCTGTCTAGGTTGAGT | NM-004104               |
| FABP1         | fatty acid binding protein 1 (liver) Forward primer | ATGGTCTTCCGCGATGCTTCCA | NM-001443               |
| APOA1         | apolipoprotein A1 Forward primer | CCTGGGACACATGCACGTGAC | NM-000039               |
| LIPG          | lipase, endothelial Forward primer | GGGACGCCCCGACTCTTTTGG | NM-006033               |
| ABCA1         | ATP-binding cassette, sub-family A1 Forward primer | GGAAGAAGCTGCTGCTAGGGAC | NM-080282               |
| SCARF1        | scavenger receptor class B, member 1 (SR-B1) Forward primer | CGGATGACCTGCAAGGACAG | NM-143532               |
| GAPDH         | glyceraldehyde-3-phosphate dehydrogenase Forward primer | GGAGCAGATCCCCTCAAAAGT | NM-001256799            |
| β-Actin       | beta actin Forward primer | CATGAGCTGCTATGCAGG | NM-001101               |

Table 1. Primers used for analysis for expression of target genes.
**Results**

**Effect of test substances on target gene expression in HepG2 cells**

The effects of several test substances on target mRNA expression in HepG2 cells were examined by real time RT-PCR (Table 2). The target genes were chosen such as to cover more or less the enzymes involved in Pl synthesis, functions of peroxisome and lipid metabolism.

Pitavastatin and simvastatin enhanced the expression of genes for apolipoprotein A1 (gene name: APOAI), ATP-binding cassette A1 (ABCA1), and fatty acid binding protein 1 (liver) (FABP1), as well as peroxisomal β-oxidation enzymes, such as acyl-CoA synthetase long-chain family member 1 (ACSL1) and carnitine palmitoyltransferase 1A (liver) (CPT1A). Peroxisome proliferator-activated receptor (PPAR) agonists, fenofibrate and Wy14643, also enhanced ACSL1 and CPT1A expression. These agonists reduced the expression of genes for other peroxisomal β-oxidation enzymes, such as enoyl-CoA hydratase/3-hydroxyacyl CoA dehydratase (EHHDH) and acetyl-Coenzyme A acyltransferase 1 (ACAA1), as well as FABP1 expression. 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), a proposed endogenous ligand for PPAR alpha [24], increased APOAI, phosphatidylethanolamine N-methyltransferase (PEMT), fatty acid synthase (FASN), and lipase, endothelial (LIPG) expression. Monounsaturated long-chain fatty acids such as oleic acid (C18:1) and erucic acid (C22:1) increased APOAI, PEMT, and FASN expression and decreased fatty acyl-CoA reductase 1 (FAR1) and FABP1 expression, while nervonic acid (C24:1) exhibited no significant effects on target gene expression. However, polyunsaturated fatty acids had different effects on gene expression. For instance, linoleic acid (C18:2) and arachidonic acid (C20:4) increased ACSL1 expression and lowered FABP1 expression. EPA (C22:5) also increased APOAI expression and DHA (C22:6) increased FAR1 and ABCA1 expression. MI treatment resulted in decreased expression of multiple genes such as APOAI, PEMT, and FASN, as well as Pl biosynthetic enzymes such as glyceronephosphate O-acetyltransferase (GNPAT) and alkylglycerol phosphate synthase (AGPS). MI also reduced the expression of peroxisomal β-oxidation enzymes such as carnitine O-octanoyltransferase (CROT), ACSL1, and ACACA1. 1-O-hexadecyl-sn-glycerol (HG), a precursor for Pl biosynthesis [25], lowered the expression of GNPAT and FAR1, as well as FASN and LIPG. Sphingomyelins (SM18 and SM24) and β-estradiol (E2) had no significant effects on target gene expression.

**Effects of pitavastatin on target gene expression in HepG2 cells**

Pitavastatin enhanced the expression of multiple genes (Figure 1). Increased expression of PPAR alpha (PPARA) and trypsin domain containing 1 (TYSNDI) [26], as well as peroxisomal β-oxidation enzymes were observed with pitavastatin treatment. Pitavastatin also enhanced the expression of HDL metabolism-associated proteins such as APOAI, ABCA1, and LIPG by greater than two-fold. Furthermore, pitavastatin enhanced the expression of Pl biosynthetic enzymes such as GNPAT, AGPS, and FAR1. Particularly, the expression of the rate-limiting enzyme of Pl biosynthesis, FAR1, was increased by nearly 3-fold. In addition, pitavastatin augmented by approximately 1.5-fold the expression of PEMT, which specifically localizes in the liver [27] and possibly participates in the conversion of ethanolamine plasmalogens (PlsEtn: 1-O-alk-1'-enyl-2-acyl-sn-glycero-3-phosphoethanolamine) to choline plasmalogens (PlsCho; 1-O-alk-1'-enyl-2-acyl-sn-glycero-3-phosphocholine) [28].

**Discussion**

Monounsaturated long-chain fatty acids such as oleic acid (C18:1) and erucic acid (C22:1), myo-inositol, and Pl precursors, alkylglycerol and HG, have been reported to increase Pl levels in laboratory animals and humans [19-23]. However, they unexpectedly reduced the gene expression of Pl biosynthetic enzymes in HepG2 cells (Table 2). Monounsaturated long-chain fatty acids are preferred substrates for peroxisomal β-oxidation, and the resulting acetyl CoA is preferentially utilized for the synthesis of ether phospholipids including Pls [29,30]. The decreased expression of FAR1 in HepG2 cells treated with C18:1 and C22:1 may have resulted from the negative feedback from the overproduction of Pls. MI is presumed to enhance Pl biosynthesis through NADPH generation during MI catabolism [31], since Far 1 is activated via NADPH binding [32]. Therefore, the suppressed expression of Pl biosynthetic enzymes in cells treated with MI could also be caused by the negative feedback from overproduction of Pl. Similarly, the reduced expression of Pl biosynthetic enzymes in cells treated with HG was thought to be attributed to overload of Pls in the cells.

However, DHA and pitavastatin increased the gene expression of Pl biosynthetic enzymes in HepG2 cells (Table 2, Figure 1). Because DHA is preferentially incorporated into Pls at the sn-2 position and Pls may function as reservoirs for these biologically active lipid mediators [33], DHA supplementation was considered to potentiate...
Serum total cholesterol, low-density lipoprotein cholesterol, and reductase inhibitor and is more potent than other statins in lowering concentration than PlsEtn [14,15]. Pitavastatin is a strong HMG-CoA reductase inhibitor and may increase Pl biosynthesis by facilitating peroxisome biogenesis. Our clinical observations indicated that serum levels of Pls, especially PlsCho and HDL-cholesterol were significantly but negatively associated with diverse risk factors for metabolic syndrome and/or atherosclerosis. Furthermore, PlsCho showed the stronger positive correlation with HDL cholesterol concentration than PlsEtn [14,15]. Pitavastatin is a strong HMG-CoA reductase inhibitor and is more potent than other statins in lowering serum total cholesterol, low-density lipoprotein cholesterol, and triglycerides with modest elevation of HDL cholesterol [35]. Recently, pitavastatin was reported to increase PI content in HDL particles in relation to improving HDL functionality [36]. Moreover, pitavastatin promoted the expression of HDL metabolism-associated proteins such as APOA1. It is proposed that this is probably via enhancement of PPAR expression, since APOA1 and ABCA1 expression are up-regulated by PPAR agonists [37].

In conclusion, the supply of materials or coenzymes for Pl biosynthesis such as acetyl CoA derived from peroxisomal β-oxidation of monounsaturated long-chain fatty acids, the Pl precursor materials may be effective in restoring normal levels of Pls in Pl-deficient individuals. Since peroxisome biogenesis induced by these materials may be effective in restoring normal levels of Pls in Pl-deficient individuals. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status. Our findings of the strong association between Pl biosynthetic enzymes and HDL metabolism-associated proteins, there may be a close relationship between them as a result of their peroxisomal status.
concentration, in clinical observational studies [14,15] might reflect their regulatory gene expression levels. Furthermore, their physiological association may extend to HDL functionality, specifically, PLs may induce atheroprotective effects of HDL, such as cholesterol efflux capacity, anti-inflammatory and antioxidant activities, and endothelial protection [36,38,39].

Authorship and contributorship
R.M. contributed conception of the work and drafting the article.
S.N. contributed data collection.
R.M. and S.N. contributed data analysis and interpretation and final approval of the version to be published.

Conflict of interest
The authors have no conflicts of interest to report.

Acknowledgments
We are very grateful for the assistance of our colleagues from the Department of Biochemistry, Teikyo University School of Medicine.

References
1. Chung HY, Sung B, Jung KJ, Zou Y, Yu BP (2006) The molecular inflammatory process in aging. Antioxid Redox Signal 8: 572-581. [Crossref]
2. Giordano CR, Terlecky SR (2012) Peroxisomes, cell senescence, and rates of aging. Biochim Biophys Acta 1822: 1358-1362. [Crossref]
3. Lavrovsky Y, Chatterjee B, Clark RA, Roy AK (2000) Role of redox-regulated transcription factors in aging, and age-related diseases. Expert Rev Mol Med 3: 521-532. [Crossref]
4. Waterham HR, Ferdinandsusse S2, Wanders RJ2 (2016) Human disorders of peroxisome metabolism and biogenesis. Biochim Biophys Acta 1863: 922-933. [Crossref]
5. Honsho M, Asaoku S, Fujiky Y (2010) Posttranslational regulation of fatty acyl-CoA reductase 1, Fab1, controls ether glycerophospholipid synthesis. J Biol Chem 285: 8537-8542. [Crossref]
6. Lessig J, Fuchs B (2009) Plasmalogens in biological systems: their role in oxidative processes in biological membranes, their contribution to pathological processes and aging and plasmalogen analysis.Curr Med Chem 16: 2021-2041. [Crossref]
7. Zoeller RA, Lake AC, Nagar N, Gaposhchin DP, Legner MA, et al. (1999) Plasmalogens as endogenous antioxidants: somatic cell mutants reveal the importance of the vinyl ether. Biochem J 338: 769-776. [Crossref]
8. Nagar N, Zoeller RA (2001) Plasmalogens: biosynthesis and functions. Prog Lipid Res 40: 199-229. [Crossref]
9. Braverman NE, Moser AB (2012) Functions of plasmalogens lipids in health and disease. Biochim Biophys Acta 1822: 1442-1452. [Crossref]
10. Maeba R, Ueta N (2004) Determination of choline and ethanolamine plasmalogens in serum by HPLC using radioactive triiodide (1-1) ion (125I-). Anal Biochem 313: 169-176. [Crossref]
11. Maeba R, Yamazaki Y, Nishimukai M, Nezu T, Okazaki T (2012) Improvement and validation of 125I-high-performance liquid chromatography method for determination of total human serum choline and ethanolamine plasmalogens. Ann Clin Biochem 49: 86-93. [Crossref]
12. Nishimukai M, Yamashita M, Watanabe Y, Yamazaki Y, Nezu T, et al. (2011) Lympathic absorption of choline plasmalogen is much higher than that of ethanolamine plasmalogen in rats. Eur J Nutr 50: 427-436. [Crossref]
13. Sakasegawa SI, Maeba R, Murayama K, Matsumoto H, Sugimoto D (2016) Hydrolysis of plasmalogens by phospholipase A1 from Streptomyces albolutea for early detection of dementia and arteriosclerosis. Biotechnol Lett 38: 109-116. [Crossref]
14. Nishimukai M, Maeba R, Yamazaki Y, Nezu T, Sakurai T, et al. (2014) Serum choline plasmalogens, particularly those with oleic acid in sn-2, are associated with proatherogenic state. J Lipid Res 55: 956-965. [Crossref]
15. Nishimukai M, Maeba R, Ikuta A, Asakawa K, Kamiya K, et al. (2014) Serum choline plasmalogens-those with oleic acid in sn-2-are biomarkers for coronary artery disease. Clin Chim Acta 437C: 147-154. [Crossref]
16. Sutter J, Klingenberg R, Othman A, Rohrer L, Landmesser U, et al. (2016) Decreased phosphatidylcholine plasmalogens - A putative novel lipid signature in patients with stable coronary artery disease and acute myocardial infarction. Atherosclerosis 246: 130-140. [Crossref]
17. Goodenow DB, Cook LL, Liu J, Lu Y, Jayasinghe DA, et al. (2007) Peripheral ethanolamine plasmalogen deficiency: a logical causative factor in Alzheimer’s disease and dementia. J Lipid Res 48: 2485-2498. [Crossref]
18. Maeba R, Araki A, Ishii K, Ogawa K, Tamura Y, et al. (2016) Serum ethanolamine plasmalogens improve detection of cognitive impairment among elderly with high exercise levels of urinary myo-inositol: A cross-sectional study. Clin Chim Acta 453: 134-140. [Crossref]
19. Wood PL, Smith T, Lane N, Khan MA, Ehrmantrout G, et al. (2011) Oral bioavailability of the ether lipid plasmalogen precursors, PPI-1011, in the rabbit: a new therapeutic strategy for Alzheimer’s disease. Lipids Health Dis 10: 227. [Crossref]
20. Maeba R, Hara H, Ishikawa H, Hayashi S, Yoshimura N, et al. (2008) Myo-inositol treatment increases serum plasmalogens and decreases small dense LDL, particularly in hyperlipidemic subjects with metabolic syndrome. J Nutr Sci Vitaminol (Tokyo) 54: 196-202. [Crossref]
21. Hoffman-Kuczynski B, Reo NV (2005) Administration of myo-inositol plus ethanolamine elevates phosphatidylethanolamine plasmalogens in the rat cerebellum. Neurochem Res 30: 47-60. [Crossref]
22. Yamazaki Y, Kondo K, Maeba R, Nishimukai M, Nezu T, et al. (2014) Proportion of nervonic acid in serum lipids is associated with serum plasmalogen levels and metabolic syndrome. J Oleo Sci 63: 527-537. [Crossref]
23. Khan M, Singh J, Singh I (2008) Plasmalogen deficiency in cerebral adrenoleukodystrophy and its modulation by lovastatin. J Neurochem 106: 1766-1779. [Crossref]
24. Wolf G (2010) Endogenous ligand for an orphan receptor. Nutr Rev 68: 316-318. [Crossref]
25. Zheng H, Duclos RJ Jr, Smith CC, Farber HW, Zoeller RA (2006) Synthesis and biological properties of the fluorescent ether lipid precursor 1-O-9-(4′-pyrenyl)nonyl-sn-glycerol. J Lipid Res 47: 633-642. [Crossref]
26. Mizuno Y, Ninomiya Y, Nakachi Y, Iseki M, Iwasa H, et al. (2013) Tysnd1 deficiency in mice interferes with the peroxisomal localization of PTS2 enzymes, causing lipid metabolic abnormalities and male infertility. PLoS Genet 9: e1003286. [Crossref]
27. Vance DE, Walkey CJ, Cui Z (1997) Phosphatidylethanolamine N-methyltransferase from liver. Biochim Biophys Acta 1348: 142-150. [Crossref]
28. Morita SY, Takeuchi A, Kitagawa S (2010) Functional analysis of two isoforms of phosphatidylethanolamine N-methyltransferase. Biochem J 432: 387-398. [Crossref]
29. Hayashi H, Ohashi M (1995) Incorporation of acetyl-CoA generated from peroxisomal dihydroxyacetone-phosphate acyltransferase: resolution of the genomic organization of the human gnpat gene and its use in the identification of novel mutations. J BiolChem 270: 277-284. [Crossref]
30. Hayashi H, Hara M (1997) 1-Alkenyl group of ethanolamine plasmalogen derives mainly from de novo-synthesized fatty alcohol within peroxisomes, but not extraperoxisomal fatty alcohol or fatty acid. J Lipid Res 121: 978-983. [Crossref]
31. Pettengrew JW, Panchalingam K, Levine J, McClure RJ, Gershon S, et al. (2001) Chronic myo-inositol increases rat brain phosphatidylethanolamine plasmalogens. Biol Psychiatry 49: 444-453. [Crossref]
32. Honsho M, Asaoku S, Fukumoto K, Fujiki Y (2012) Topogenesis and homeostasis of fatty acyl-CoA reductase 1. J Biochem 148: 3458-3459. [Crossref]
33. Farooqui AA, Horrocks LA (2001) Plasmalogens, phospholipase A2, and docosahexaenoic acid turnover in brain tissue. J MolNeurosci16: 263-272. [Crossref]
34. Ofman R, Lajmiri S, Wanders RJ (2001) Etherphospholipid biosynthesis and dihydroxyacetone-phosphate acetyltransferase: resolution of the genomic organization of the human gnpat gene and its use in the identification of novel mutations. BiochimBiophys Res Commun 281: 754-760. [Crossref]
35. Chapman MJ (2011) Pitavastatin: novel effects on lipid parameters. AtherosclerSuppl12: 277-284. [Crossref]
36. Orsoni A, Thörnd P, Tan R, Girai P, Robillard P, et al. (2016) Statin action enriches HDL3 in polysaturated phospholipids and plasmalogens and reduces LDL-derived phospholipid hydroperoxides in atherogenic mixed dyslipidemia. J Lipid Res 57: 2073-2087. [Crossref]
37. Miller M, Rhyne J, Hamlette S, Birnbaum J, Rodriguez A (2003) Genetics of HDL regulation in humans. *CurrOpinLipidol* 14: 273-279. [Crossref]

38. Mandel H, Sharf R, Berant M, Wanders RJ, Vreken P, et al. (1998) Plasmalogen phospholipids are involved in HDL-mediated cholesterol efflux: insights from investigations with plasmalogen-deficient cells. *BiochemBiophys Res Commun* 250: 369-373. [Crossref]

39. Sutter I, Velagapudi S, Othman A, Riawanto M, Manz J, et al. (2015) Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL. *Atherosclerosis* 241: 539-546. [Crossref]

**Copyright:** ©2017 Maeba R. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.