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Extrinsic effectors regulating genes for plasmalogen biosynthetic enzymes in HepG2 cells

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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 Pl 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 Pl biosynthetic enzymes remain unknown. To gain insight into the manipulation of Pl biosynthesis and the relationship between Pl 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 Pl precursor alkylglycerol, all of which supply materials or coenzymes for Pl biosynthesis, unexpectedly reduced the expression of the genes for Pl biosynthetic enzymes. These results suggest the presence of strict regulation of Pl homeostasis. In contrast, pitavastatin induced peroxisome biogenesis and promoted the expression of peroxisomal Pl 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 Pl 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 Plasmalogen; PlsEtn: Ethanolamine Plasmalogen; 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 Pls 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 Pls are synthesized mainly in the liver and secreted into the blood as lipoprotein components. To investigate the clinical significance of plasma Pls, 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 Pl are a risk factor for atherosclerosis and dementia [14-18]. Serum levels of Pl showed a strong positive correlation with high-density lipoprotein (HDL) cholesterol concentration [14,15], suggesting that Pls may be involved in metabolism or HDL functions.

Accordingly, we attempted to increase the levels of plasma Pls 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 Pl 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 Pl biosynthetic enzymes remain unknown. To gain insight into the mechanisms mediating the enhancement of Pl 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. Fenofibrate 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),

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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 TSUNO 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% CO_2. 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 $MgCl_2$, 0.5 µM of the upstream and downstream PCR primers, and 2 mL 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).

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

Gene	Protein		5'Primer 3'	GeneBank accession no.
PPARA	peroxisome proliferator-activated receptor alpha	Forward primer	ATGGTGGACACGGAAAGCC	NM-005036
		Reverse primer	CGATGGATTGCGAAATCTCTTGG	
PPARG	peroxisome proliferator-activated receptor gamma	Forward primer	GGGATCAGCTCCGTGGATCT	NM-138711
		Reverse primer	TGCACTTTGGTACTCTTGAAGTT	
TYSND1	trypsin domain containing 1	Forward primer	TGCAGCGGGGTAATCCTGA	NM-173555
		Reverse primer	CCTCCGACACTTCGTCATCC	
CROT	carnitine *O*-octanoyltransferase	Forward primer	GTGGTGGCTGAATGTTGCCTA	NM-021151
		Reverse primer	TTGGAGGCCAGTAGTGTTCAA	
EHHADH	enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	Forward primer	AAACTCAGACCCGGTTGAAGA	NM-001166415
		Reverse primer	TTGCAGAGTCTACGGGATTCT	
ACSL1	acyl-CoA synthetase long-chain family member 1	Forward primer	GCCGAGTGGATGATAGCTGC	NM-004457
		Reverse primer	ATGGCTGGACCTCCTAGAGTG	
CTP1A	carnitine palmitoyltransferase 1A (liver)	Forward primer	TCCAGTTGGCTTATCGTGGTG	NM-001876
		Reverse primer	TCCAGAGTCCGATTGATTTTTGC	
ACAA1	acetyl-Coenzyme A acyltransferase 1	Forward primer	GCGGTTCTCAAGGACGTGAAT	NM-001607
		Reverse primer	GTCTCCGGGATGTCACTCAGA	
ACOX1	acyl-Coenzyme A oxidase 1, palmitoyl	Forward primer	ACTCGCAGCCAGCGTTATG	NM-007292
		Reverse primer	AGGGTCAGCGATGCCAAAC	
GNPAT	glyceronephosphate O-acyltransferase	Forward primer	GAGGAGGCATGTCAGTGACTT	NM-014236
		Reverse primer	ACAAAACCGAATGGCTCCAAG	
AGPS	alkylglycerone phosphate synthase	Forward primer	TGAGTACCAATGAGTGCAAAGC	NM-003659
		Reverse primer	GGTAAACCCATGCCACTAAGAG	
FAR1	fatty acyl-CoA reductase 1:	Forward primer	AGACACCACAAGAGCGAGTG	NM-032228
		Reverse primer	CCAGTTTAGGTTGGGTGAGTTC	
PEMT	phosphatidylethanolamine N-methyltransferase	Forward primer	CTGGAATGTGGTTGCACGATG	NM-148172
		Reverse primer	GCTTAGAGAGTAGCAGGCCA	
FASN	fatty acid synthase	Forward primer	AAGGACCTGTCTAGGTTTGATGC	NM-004104
		Reverse primer	TGGCTTCATAGGTGACTTCCA	
FABP1	fatty acid binding protein 1 (liver)	Forward primer	ATGAGTTTCTCCGGCAAGTACC	NM-001443
		Reverse primer	CTCTTCCGGCAGACCGATTG	
APOA1	apolipoprotein A1	Forward primer	CCCTGGGATCGAGTGAAGGA	NM-000039
		Reverse primer	CTGGGACACATAGTCTCTGCC	
LIPG	lipase, endothelial	Forward primer	GGGAGCCCCGTACCTTTTG	NM-006033
		Reverse primer	CCTCACAGATGGTTTGACCTCA	
ABCA1	ATP-binding cassette, sub-family A1	Forward primer	GGAAGAACAGTCATTGGGACAC	NM-080282
		Reverse primer	GCTACAAACCCTTTTAGCCAGT	
SCARF1	scavenger receptor class B, member 1 (SR-B1)	Forward primer	CCGATCAGACCTCAAGGACAG	NM-145352
		Reverse primer	CCCAGGGTAGCTTGTGGGA	
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Forward primer	GGAGCGAGATCCCTCCAAAAT	NM-001256799
		Reverse primer	GGCTGTTGTCATACTTCTCATGG	
β-Actin	beta actin	Forward primer	CATGTACGTTGCTATCCAGGC	NM-001101
		Reverse primer	CTCCTTAATGTCACGCACGAT	

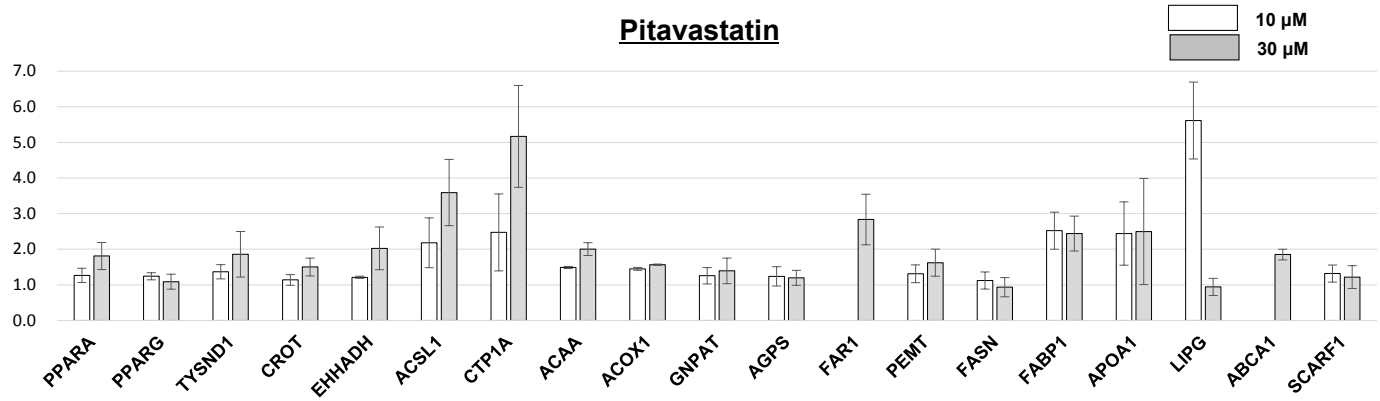

Figure 1. Effects of pitavastatin on target gene expression in HepG2 cells. Values were calculated as fold changes relative to each target mRNA expression of control cells without pitavastatin treatment. The effects of 10 uM of pitavastatin was not tested for *FAR1*an *ABCA1*. Columns and bars represent means ± standard error (SE).

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: *APOA1*), 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) (*CTP1A*). Peroxisome proliferator-activated receptor (PPAR) agonists, fenofibrate and Wy14643, also enhanced *ACSL1* and *CTP1A* expression. These agonists reduced the expression of genes for other peroxisomal β-oxidation enzymes, such as enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase (*EHHADH*) 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 *APOA1*, 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 *APOA1*, *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 *APOA1* expression and DHA (C22:6) increased *FAR1* and *ABCA1* expression. MI treatment resulted in decreased expression of multiple genes such as *APOA1*, *PEMT*, and *FASN*, as well as Pl biosynthetic enzymes such as glyceronephosphate *O*-acyltransferase (*GNPAT*) and alkylglycerone phosphate synthase (*AGPS*). MI also reduced the expression of peroxisomal β-oxidation enzymes such as carnitine *O*-octanoyltransferase (*CROT*), *ACSL1*, and *ACAA1*. 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 (*TYSND1*) [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 *APOA1*, *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 plasmalogen (PlsEtn; 1-*O*-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine) to choline plasmalogen (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

Table 2. Effects of test substances on target gene expression in HepG2 cells.

Wy14643: Agonist of PPARα and PPARγ; POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; SM18: Sphingomyelin containing stearic acid; SM24: Sphingomyelin containing lignoceric acid; E2: β-estradiol; MI: Myo-inositol; HG: 1-o-hexadecyl-sn-glycerol.

Test substances	**Pitavastatin**	**Simvastatin**	**Fenofibrate**	**Wy14643**	**POPC**	**Oleic acid**	**Erucic acid**	**Nervonic acid**	**Linoleic acid**	**Arachidonic acid**	**EPA**	**DHA**	**SM18**	**SM24**	**E2**	**MI**	**HG**		
						C18:1	C22:1	C24:1	C18:2	C20:4	C20:5	C22:6							
Concentrations	10-100 μM	10-30 μM	10-100 μM	10-100 μM	50-250 μM	10-50 μM	10-50 μM	10-100 μM	10-100 μM	10-100 μM	10-100 μM	10-100 μM	10-50 μM	10-50 μM	1-100 nM	0.2-5 mM	60 μM		
Number of tests	5 times	Twice	Twice	Once	Once	5 times	5 times	Twice	Twice	Twice	Twice	3 times	5 times	5 times	Twice	Once	Once		
Gene name																		++	Obvious rise (more than 2-fold)
PPARA	+		±	±	±	±	±	±	±	±	±	±			±	±	±	+	Moderate rise (more than 1.5-fold)
PPARG	±		±	±		±	±	±	±	±	±	±			±	±	±	±	No change
TYSND1	+		±	±		±	±	±	±	±	±	±	±	±	±	±	±	–	Moderate descent (less than 0.75)
CROT	+		±	–		+	±	±	±	±	±	+	±	±	±	–	±	– –	Obvious descent (less than 0.5)
EHHADH	+		– –	–		±	±	±	±	–	±	±			±	±	±		
ACSL1	++	+	++	++	±	±	±	±	++	+	±	±			±	–			
CTP1A	++	+	++	++	– –	±	±	±	±	±	±	±	±	±	±	++	++		
ACAA1	+		–	–		±	±	±	±	±	±	±	±	±	±	–			
ACOX1	+		±	±		±	±	±	±	±	±	±			±	±			
GNPAT	+	±	±	–	±	±	±	±	±	±	±	±	±	±	±	–	–		
AGPS	+	±	±	±	±	±	±	±	±	±	±	±	±	±	±	–	±		
FAR1	++	±	±			–	–	±	±	±	±	+					–		
PEMT	+	±	±	±	+	+	+	±	±	±	±	±	±	±	±	– –	+		
FASN	±	±	±	±	++	+	+						±	±	±	– –	– –		
FABP1	++	++	– –	–		–	–	±	–	–	±	±			±	+	±		
APOA1	++	+	±	±	+	++	++	±	±	±	+	±	±	±	±	– –	±		
LIPG	++				++	±	±						±	±	±	±	–		
ABCA1	+	+	±			±	±	±	±	±	±	+				±			
SCARF1	±				±	±	±						±	±	±	±	±		

FAR1 expression, the enzyme fatty acyl CoA reductase 1 (Far 1) supplies the fatty alcohols used in the formation of ether-linked alkyl bonds. Pitavastatin, but not simvastatin, facilitated the gene expression of peroxisomal Pl biosynthetic enzymes such as *GNPAT*, *AGPS*, and *FAR1*. The first two steps in Pl biosynthesis, which are catalyzed by the enzymes encoded by *GNPAT* and *AGPS*, exclusively occur in peroxisomes [4,34]. In addition, the rate-limiting enzyme of Pl biosynthesis, is also peroxisomal [5]. Pitavastatin further enhanced the expression of peroxisomal *PPARA* and *TYSND1*, as well as β-oxidation enzymes (Table 2, Figure 1). This suggests that pitavastatin may increase Pl biosynthesis by facilitating peroxisome biogenesis. In addition, pitavastatin increased the expression of *PEMT*, which may be involved in the conversion of PlsEtn to PlsCho. Our clinical observational studies indicated that serum levels of Pls, particularly PlsCho 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 Pl 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 alkylglycerol, and NADPH from MI catabolism, suppressed the expression of Pl biosynthetic enzymes (Table 2). These results suggest that Pl homeostasis is strictly regulated, and the supplementation of these materials may be effective in restoring normal levels of Pls in Pl-deficient individuals. Since peroxisome biogenesis induced by treatment with pitavastatin promoted both the gene expression of 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 the serum levels of Pls, especially PlsCho and HDL-cholesterol

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.

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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. *BiochimBiophys Acta* 1822: 1358-1362. [Crossref]

3. Lavrovsky Y, Chatterjee B, Clark RA, Roy AK (2000) Role of redox-regulated transcription factors in inflammation, aging and age-related diseases. *ExpGerontol*35: 521-532. [Crossref]

4. Waterham HR, Ferdinandusse S2, Wanders RJ2 (2016) Human disorders of peroxisome metabolism and biogenesis. *BiochimBiophys Acta* 1863: 922-933. [Crossref]

5. Honsho M, Asaoku S, Fujiki Y (2010) Posttranslational regulation of fatty acyl-CoA reductase 1, Far1, controls ether glycerophospholipid synthesis. *J BiolChem* 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, Nagan N, Gaposchkin 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. Nagan N, Zoeller RA (2001) Plasmalogens: biosynthesis and functions. *Prog Lipid Res* 40: 199-229. [Crossref]

9. Braverman NE, Moser AB (2012) Functions of plasmalogen lipids in health and disease. *BiochimBiophys Acta* 1822: 1442-1452. [Crossref]

10. Maeba R, Ueta N (2004) Determination of choline and ethanolamine plasmalogens in human plasma by HPLC using radioactive triiodide (1-) ion (125I3-). *Anal Biochem* 331: 169-176. [Crossref]

11. Maeba R, Yamazaki Y, Nezu T, Okazaki T (2012) Improvement and validation of ^{125}I-high-performance liquid chromatography method for determination of total human serum choline and ethanolamine plasmalogens. *Ann ClinBiochem*49: 86-93. [Crossref]

12. Nishimukai M, Yamashita M, Watanabe Y, Yamazaki Y, Nezu T, et al. (2011) Lymphatic 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, Sugimori D (2016) Hydrolysis of plasmalogen by phospholipase A1 from Streptomyces albidoflavus 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 N, Kamiya K, et al. (2014) Serum choline plasmalogens-those with oleic acid in sn-2-are biomarkers for coronary artery disease. *ClinChim Acta* 437C: 147-154. [Crossref]

16. Sutter I, 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. Goodenowe 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 excretion levels of urinary myo-inositol: A cross-sectional study. *ClinChim Acta* 453: 134-140. [Crossref]

19. Wood PL, Smith T, Lane N, Khan MA, Ehrmantraut G, et al. (2011) Oral bioavailability of the ether lipid plasmalogen precursor, 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 plasmalogen 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 RI Jr, Smith CC, Farber HW, Zoeller RA (2006) Synthesis and biological properties of the fluorescent ether lipid precursor 1-O-[9'-(1''-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. *BiochimBiophys 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, Oohashi M (1995) Incorporation of acetyl-CoA generated from peroxisomal beta-oxidation into ethanolamine plasmalogen of rat liver. *BiochimBiophys Acta* 1254: 319-325. [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 Biochem* 121: 978-983. [Crossref]

31. Pettegrew JW, Panchalingam K, Levine J, McClure RJ, Gershon S, et al. (2001) Chronic myo-inositol increases rat brain phosphatidylethanolamine plasmalogen. *Biol Psychiatry* 49: 444-453. [Crossref]

32. Honsho M, Asaoku S, Fukumoto K, Fujiki Y (2013) Topogenesis and homeostasis of fatty acyl-CoA reductase 1. *J BiolChem*288: 34588-34598. [Crossref]

33. Farooqui AA, Horrocks LA (2001) Plasmalogens, phospholipase A2, and docosahexaenoic acid turnover in brain tissue. *J MolNeurosci*16: 263-272. [Crossref]

34. Ofman R, Lajmir S, Wanders RJ (2001) Etherphospholipid biosynthesis and dihydroxyactetone-phosphate acyltransferase: resolution of the genomic organization of the human gnpat gene and its use in the identification of novel mutations. *BiochemBiophys Res Commun* 281: 754-760. [Crossref]

35. Chapman MJ (2011) Pitavastatin: novel effects on lipid parameters. *AtherosclerSuppl*12: 277-284. [Crossref]

36. Orsoni A, Thérond P, Tan R, Giral P, Robillard P, et al. (2016) Statin action enriches HDL3 in polyunsaturated 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, Riwanto 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]