Effect of Lorenzo's Oil on Hepatic Gene Expression and the Serum Fatty Acid Level in *abcd1*-Deficient Mice

Masashi Morita • Ayako Honda • Akira Kobayashi •
Yuichi Watanabe • Shiro Watanabe •
Kosuke Kawaguchi • Shigeo Takashima •
Nobuyuki Shimozaawa • Tsuneo Imanaka

Received: 12 January 2016 / Revised: 19 April 2017 / Accepted: 2 May 2017 / Published online: 31 May 2017
© SSIEM and Springer-Verlag Berlin Heidelberg 2017

Abstract Lorenzo's oil is known to decrease the saturated very long chain fatty acid (VLCFA) level in the plasma and skin fibroblasts of X-linked adrenoleukodystrophy (ALD) patients. However, the involvement of Lorenzo's oil in in vivo fatty acid metabolism has not been well elucidated. To investigate the effect of Lorenzo's oil on fatty acid metabolism, we analyzed the hepatic gene expression together with the serum fatty acid level in Lorenzo's oil-treated wild-type and *abcd1*-deficient mice. The change in the serum fatty acid level in Lorenzo's oil-treated *abcd1*-deficient mice was quite similar to that in the plasma fatty acid level in ALD patients supplemented with Lorenzo's oil. In addition, we found that the hepatic gene expression of two peroxisomal enzymes, *Dbp* and *Scp2*, and three microsomal enzymes, *Elovl1*, 2, and 3, were significantly

stimulated by Lorenzo's oil. Our findings indicate that Lorenzo's oil activates hepatic peroxisomal fatty acid β -oxidation at the transcriptional level. In contrast, the transcriptional stimulation of *Elovl1*, 2, and 3 by Lorenzo's oil does not cause changes in the serum fatty acid level. It seems likely that the inhibition of these elongation activities by Lorenzo's oil results in a decrease in saturated VLCFA. Thus, these results not only contribute to a clarification of the mechanism by which the saturated VLCFA level is reduced in the serum of ALD patients by Lorenzo's oil-treatment, but also suggest the development of a new therapeutic approach to peroxisomal β -oxidation enzyme deficiency, especially mild phenotype of DBP deficiency.

Communicated by: Robert Steiner

Masashi Morita and Ayako Honda contributed equally to this work.

Electronic supplementary material: The online version of this chapter (doi:10.1007/8904_2017_32) contains supplementary material, which is available to authorized users.

M. Morita (✉) • A. Kobayashi • Y. Watanabe • K. Kawaguchi • T. Imanaka

Department of Biological Chemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan
e-mail: masa@pha.u-toyama.ac.jp

A. Honda • S. Takashima • N. Shimozaawa
Division of Genomic Research, Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

S. Watanabe
Division of Nutritional Biochemistry, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

T. Imanaka
Faculty of Pharmaceutical Sciences, Hiroshima International University, 5-1-1 Hirokoshinkai, Kure, Hiroshima 737-0112, Japan

Introduction

X-linked adrenoleukodystrophy (ALD) (OMIM 300100) is a rare, inherited metabolic disease characterized by an abnormal accumulation of saturated very long chain fatty acids (VLCFAs) such as C24:0 and C26:0 in all tissues (Moser 1997). The abnormal accumulation of lipid molecules containing saturated VLCFAs is thought to be involved in the pathological mechanism underlying ALD. This disease is caused by mutation of the *ABCD1* gene that encodes the peroxisomal ABC protein ABCD1. ABCD1 is a transporter of VLCFA-CoA into peroxisomes (van Roermund et al. 2011). The dysfunction of ABCD1 causes a reduction in peroxisomal VLCFA β -oxidation and an increase in the VLCFA-CoA level in the cytosol (Ofman et al. 2010). The increased cytosolic VLCFA-CoA is used as a substrate for microsomal fatty acid elongation, which results in the accumulation of VLCFAs (Schackmann et al.

2015). Therefore, lowering the saturated VLCFAs by stimulating peroxisomal fatty acid β -oxidation or by inhibiting microsomal fatty acid elongation is a rational therapeutic approach to ALD.

Until now, two therapeutic approaches have been reportedly investigated: hematopoietic cell transplantation and dietary treatment based on Lorenzo's oil (LO). LO, a 4:1 mixture of glycerol trioleate and glycerol trierucate, is used as a diet supplement to ALD patients (Rizzo et al. 1989). It has been known that LO administration significantly reduces the plasma C26:0 levels in ALD patients (Rizzo et al. 1989; Aubourg et al. 1993; Deon et al. 2008). However, LO does not halt the clinical progression of patients with preexisting neurological dysfunction (Restuccia et al. 1999; Berger and Gartner 2006). On the other hand, it is suggested LO may have a preventative effect in asymptomatic patients (Moser et al. 2005).

Trioleate and trierucate are absorbed from the intestine after being hydrolyzed by lipases and then transported to the liver. Incorporation of erucic acid into the liver was demonstrated in ALD patients who had been administered LO (Murphy et al. 2008). As the bulk of plasma VLCFAs exists as neutral lipids in low density lipoprotein, it is likely that the fatty acid level in plasma is largely associated with lipid metabolism in the liver. We administered LO to both wild-type and *abcd1*-deficient mice, and then analyzed the hepatic gene expression together with the serum level of variety of fatty acids in order to investigate the effect of LO on the hepatic fatty acid metabolism.

Materials and Methods

Mice and Dietary Treatment

The C57BL/6 mice obtained from Clea Japan Inc. and the *abcd1*-deficient mice generated by Kobayashi et al. (1997) were kept at $24\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ under a dark-light cycle as described previously (Morita et al. 2015). Males aged 4 weeks were used in this experiment. Male C57BL/6 mice and *abcd1*-deficient mice were fed with a powdered chow (CE2, Clea Japan, Inc), supplemented with or without 20% (w/w) LO (SHS International Ltd., Liverpool, UK). All of the mice had free access to food and water. The applied dose of LO was deduced from the daily dose administered to ALD patients as described in De Craemer et al. (1998). The daily intake of LO by the mice on a diet with 20% (w/w) LO was an average of 26 g/kg body weight. After the

treatment, the serum and each tissue were prepared for fatty acid analysis and gene expression analysis and kept at $-80\text{ }^{\circ}\text{C}$ before analysis. All of the procedures used in the animal laboratory research were approved by the University Committee for Animal Use and Care at the University of Toyama.

VLCFA Level Analysis

VLCFAs, plasmalogen (C16:0 hexadecanal dimethyl acetal), and phytanic acid in the mouse serum were analyzed as described previously (Takemoto et al. 2003). In screw-capped glass tube, 100 μl of serum were mixed with 2 ml of methanolic hydrochloric acid and the mixture was heated for 2 h at $100\text{ }^{\circ}\text{C}$. The methyl derivatives were extracted twice with *n*-hexane and subjected to GC/MS system (QP5050A, Shimadzu, Kyoto, Japan), equipped with a capillary INNOWAX column (Hewlett-Packard, Palo Alto, CA, USA) and an autosampler/autoinjector (AOC-20, Shimadzu). The fatty acid analysis in liver was also performed as described previously (Morita et al. 2015).

Quantitative Real-Time PCR

After the mice were sacrificed, the livers and brains were removed and quickly frozen in lipid N_2 . Total RNA was purified with Isogen (Nippon Gene, Japan) and cDNA was synthesized with M-MLV reverse transcriptase (Invitrogen, CA). Gene expression analysis was performed with fluorescent Taqman methodology, using the StepOnePlus™ Real-Time PCR System (Applied Biosystems, CA). Real-time quantitative PCR was performed for each of the following genes, using ready-to use primer and probe sets pre-developed by Applied Biosystems (TaqMan gene expression assays): *Acox1* (Mm00443579_m1), acetyl-Coenzyme A acyltransferase 1A (*Acaa1a*, Mm00728460_s1), *Dbp* (Mm0050043_m1), *Scp2* (Mm01257982_m1), *Abcd2* (Mm00496455_m1), *Ppara-1 and 2* (Mm00627559_m1 for boundary between exon 3 and 4, and Mm00440939_m1 for 7 and 8, respectively), *Elovl1* (Mm00517077_m1), *Elovl2* (Mm00517086_m1), *Elovl3* (Mm00468164_m1), *Elovl4* (Mm00521704_m1), *Elovl5* (Mm00506717_m1), *Elovl6* (Mm00851223_s1), and *Gapdh* (Hs99999905_m1) as an endogenous control. The mRNA Ct values for these genes were normalized to *Gapdh* and expressed as a relative increase or decrease in the tissues from the non-treated mice to those in the LO-treated mice.

Statistical Analysis

Statistical analysis was performed with unpaired *t*-test, using the Bonferroni correction for multiple comparisons.

Results and Discussion

Administration of LO to Wild-Type and *abcd1*-Deficient Mice

Wild-type or *abcd1*-deficient 4-week-old mice were fed a diet containing LO for 5 weeks. The average consumptions of laboratory chow in wild-type mice with or without LO were 2.45 g/day and 2.80 g/day, respectively. The average consumptions were 2.50 g/day and 2.87 g/day in *abcd1*-deficient mice with or without LO, respectively. From the intake of diet, the mice were fed approximately 26 g of LO per kg of body weight daily. This almost corresponds to the treatment of LO for ALD therapy in patients. The intake of LO had no effect on body weight in either the wild-type or *abcd1*-deficient mice (Supplementary Fig. 1). After 5

weeks, the serum fatty acid level and hepatic gene expression were analyzed.

Changes in Fatty Acid Level in Serum from LO-Treated Mice

We previously reported that a high-fat diet consisting mainly of C16:0, C18:1, and C18:2 activates hepatic peroxisomal VLCFA metabolism (Kozawa et al. 2011). To date, however, the effect of LO on hepatic fatty acid metabolism has not been reported. In the present study, we first analyzed the fatty acid level in the serum from wild-type and *abcd1*-deficient mice fed a diet with or without LO. In *abcd1*-deficient mice, the level of saturated VLCFAs such as C20:0, C22:0, C24:0, and C26:0 was higher than those in the wild-type mice. In particular, the level of C26:0 in *abcd1*-deficient mice was nearly four times higher than that in the wild-type mice. As a result of treatment with LO, the concentration ($\mu\text{g}/100 \mu\text{l}$ serum) of C26:0 and the ratio of C26:0/C22:0 were decreased to a level similar to that in wild-type mice (Table 1 and Fig. 1). These results clearly indicate that LO has the capacity to reduce the saturated

Table 1 Effect of Lorenzo's oil on the level of serum fatty acids

Fatty acid	WT	WT + LO	KO	KO + LO
C14:0	0.643 $\pm$ 0.082	0.378 $\pm$ 0.015	0.941 $\pm$ 0.061	0.375 $\pm$ 0.007***
C14:1	0.018 $\pm$ 0.008	0.004 $\pm$ 0.001	0.033 $\pm$ 0.006	0.005 $\pm$ 0.001*
C16:0DMA	0.897 $\pm$ 0.059	0.769 $\pm$ 0.026	0.920 $\pm$ 0.027	0.883 $\pm$ 0.040
C16:0	29.96 $\pm$ 1.674	24.62 $\pm$ 0.982	41.01 $\pm$ 0.834 ^{##}	26.70 $\pm$ 1.047**
C16:1	3.986 $\pm$ 0.763	0.904 $\pm$ 0.102	7.580 $\pm$ 0.502	0.995 $\pm$ 0.083***
Phytanic acid	0.443 $\pm$ 0.037	0.255 $\pm$ 0.009**	0.515 $\pm$ 0.018	0.258 $\pm$ 0.013***
C18:0	12.02 $\pm$ 0.207	16.01 $\pm$ 0.399***	13.76 $\pm$ 0.239 [#]	16.07 $\pm$ 0.763
C18:1	26.42 $\pm$ 2.652	48.83 $\pm$ 3.745*	34.95 $\pm$ 0.438	48.87 $\pm$ 3.003*
C18:2	39.77 $\pm$ 1.267	33.59 $\pm$ 0.893	47.68 $\pm$ 1.034 [#]	35.46 $\pm$ 0.867***
C18:3	1.142 $\pm$ 0.136	0.378 $\pm$ 0.048*	1.952 $\pm$ 0.113 [#]	0.366 $\pm$ 0.043***
C20:0	0.325 $\pm$ 0.023	0.334 $\pm$ 0.037	0.615 $\pm$ 0.009 ^{###}	0.394 $\pm$ 0.038*
C20:1	0.844 $\pm$ 0.141	2.300 $\pm$ 0.111***	1.356 $\pm$ 0.052	1.889 $\pm$ 0.139
C20:4 (ARA)	5.039 $\pm$ 0.187	8.483 $\pm$ 0.465***	5.891 $\pm$ 0.162	8.991 $\pm$ 0.206***
C20:5 (EPA)	5.069 $\pm$ 0.178	3.417 $\pm$ 0.146***	7.095 $\pm$ 0.112 ^{###}	3.772 $\pm$ 0.170***
C22:0	0.530 $\pm$ 0.014	0.314 $\pm$ 0.044*	0.646 $\pm$ 0.013 ^{##}	0.461 $\pm$ 0.031*
C22:1	0.126 $\pm$ 0.024	2.852 $\pm$ 0.497*	0.255 $\pm$ 0.005 [#]	3.297 $\pm$ 0.715
C24:0	0.386 $\pm$ 0.023	0.126 $\pm$ 0.014***	0.544 $\pm$ 0.004 ^{###}	0.202 $\pm$ 0.011***
C24:1	1.455 $\pm$ 0.083	3.877 $\pm$ 0.061***	1.211 $\pm$ 0.027	4.232 $\pm$ 0.128***
C22:6 (DHA)	19.05 $\pm$ 0.484	15.84 $\pm$ 0.321*	19.24 $\pm$ 0.983	16.90 $\pm$ 0.679
C25:0	0.010 $\pm$ 0.001	0.005 $\pm$ 0.000	0.021 $\pm$ 0.003	0.009 $\pm$ 0.000
C26:0	0.008 $\pm$ 0.001	0.005 $\pm$ 0.001	0.033 $\pm$ 0.002 ^{###}	0.010 $\pm$ 0.001***

Wild-type (WT) (*n* = 4) and *abcd1*-deficient (KO) mice (*n* = 4) were fed with (+LO) or without LO for 5 weeks, as in Supplementary Fig. 1. The serum was prepared from each mouse and subjected to gas chromatography/mass spectrometry (GC/MS) analysis. Values are expressed as μg fatty acid/100 μl serum and are the mean $\pm$ S.E. for 4 determinations. Where “*” is indicated, the values for LO-treated mice are significantly different from non-treated mice, * <0.02, ** <0.005, *** <0.002. Where “#” is indicated, the values for *abcd1*-deficient mice were significantly different from wild-type mice, # <0.02, ## <0.005, ### <0.002. Statistical analysis was analyzed by unpaired *t*-test, using the Bonferroni correction for multiple comparisons

Fig. 1 Serum fatty acids level in LO-treated mice. The ratios of C20:0/C18:0 (a), C24:0/C22:0 (b), C26:0/C22:0 (c), and C22:6/C20:5 (d) calculated from Table 1 are shown. The values are the mean $\pm$ S.

E. ($n = 4$ animals. *, $p < 0.005$, **, $p < 0.002$). Statistical analysis was performed with unpaired t -test, using the Bonferroni correction for multiple comparisons

VLCFA level in serum of *abcd1*-deficient mice. This is consistent with reports that the C26:0 level in plasma from ALD patients was decreased to a normal level by 4-week-treatment with LO (Rizzo et al. 1989). In addition, as reported in LO-treated ALD patients (Moser et al. 1999), an increase of the mono-unsaturated fatty acids such as C18:1, C20:1, C22:1, and C24:1 along with a decrease of n-3 polyunsaturated fatty acids such as linolenic acid (C18:3n-3) and eicosapentanoic acid (EPA) (C20:5n-3) were also observed in the LO-treated *abcd1*-deficient mice. These results indicate that the effect of LO on the fatty acid metabolism was quite similar in *abcd1*-deficient mice and ALD patients.

EPA (C20:5n-3) is known to be converted sequentially to C22:5, C24:5, C24:6 and then DHA (C22:6n-3) via fatty acid elongation, desaturation, and β -oxidation, respectively (Ferdinandusse et al. 2001). As the final β -oxidation is a limiting step in the peroxisome, the C24:6/C22:6 ratio is a

reliable measure of the impairment of peroxisomal β -oxidation. In addition, the C26:1 level is reported to be elevated in the insufficiency of peroxisomal β -oxidation (Fourcade et al. 2010). However, C26:1 as well as the intermediate fatty acids such as C22:5, C24:5, and C24:6 during the synthesis of C22:6 from C20:5 could not be detected in our assay system using GC/MS (Takemoto et al. 2003). Therefore, we showed C22:6/C20:5 ratio as a marker of peroxisomal fatty acid β -oxidation in this study (Fig. 1). As shown, C22:6/C20:5 ratio in *abcd1*-deficient mice was lower than in wild-type mice, but was significantly increased by LO, suggesting that peroxisomal β -oxidation in *abcd1*-deficient mice may be partly recovered. Peroxisomal VLCFA β -oxidation consists of the ABCD1-dependent and -independent pathways (Morita et al. 2012). In *abcd1*-deficient mice, LO seems to stimulate the latter pathway in which VLCFA-CoA is imported into peroxisomes by functionally related homologs, ABCD2 or

ABCD3, or VLCFA is diffused through the lipid barrier and activated to VLCFA-CoA by very long chain acyl-CoA synthetases, such as ACSVL1 and ACSVL5. In contrast to the ratio of C22:6/C20:5, the ratios of C20:0/C18:0 and C24:0/C22:0 were largely decreased in LO-treated wild-type and *abcd1*-deficient mice (Fig. 1), suggesting that the elongation of C18:0 and C22:0 to C20:0 and C24:0 may be depressed by LO treatment. It is therefore possible that the level of saturated VLCFAs was decreased by either activating peroxisomal fatty acid β -oxidation or by inhibiting microsomal fatty acid elongation. Further detailed analyses for the other markers representing the above fatty acid metabolic systems are believed to provide a complete view of the efficacies of LO.

In mice, the level of phytanic acid and palmitoleic acid (C16:1) is decreased and the level of arachidonic acid (C20:4) is increased by LO treatment, which is different from result in ALD patients supplemented with LO (Moser et al. 1999).

Changes in Hepatic Gene Expression in LO-Treated Mice

As the fatty acid level in serum is thought to be affected by the fatty acid metabolism in the liver, we next analyzed the effect of LO on hepatic gene expression in both wild-type and *abcd1*-deficient mice. The level of C26:0 in the liver from *abcd1*-deficient mice (6.0 ± 0.5 $\mu\text{g/g}$ tissue weight) was higher than that from wild-type mice (1.3 ± 0.2 $\mu\text{g/g}$

tissue weight). However, the level was significantly decreased (4.0 ± 0.1 $\mu\text{g/g}$ tissue weight) in LO-treated *abcd1*-deficient mice (Supplementary Fig. 2). Figure 2 shows the gene expression of the representative enzymes that are involved in peroxisomal fatty acid β -oxidation and microsomal fatty acid elongation. *Abcd2* gene encodes ABCD2 protein. *Acox1* and *Acaa1a* genes encode an acyl-CoA oxidase protein and a straight-chain 3-oxo-acyl thiolase, respectively. *Dbp* gene encodes D-bifunctional protein that has enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities, and generates 3-ketoacyl-CoAs (Ferdinandusse et al. 2006a). *Scp2* gene encodes SCPx, a peroxisome-associated thiolase, which involved in the oxidation of branched-chain fatty acids (Seedorf et al. 1994; Ferdinandusse et al. 2006b). *Elovl* genes encode enzymes responsible for the first and rate-limiting step of fatty acid elongation reaction in ER. In contrast, *Ppar α* gene encodes PPAR α , a transcription factor and a major regulator of lipid metabolism in the liver. The mRNA expression of these genes was almost the same in the wild-type and *abcd1*-deficient mice fed by normal diet (Fig. 2) although the expression of *Ppar α* in the *abcd1*-deficient mice was higher, but not significantly, compared with wild-type mice. When wild or *abcd1*-deficient mice were administered with LO, the gene expression of *Dbp*, *Scp2*, *Elovl1*, 2, 3, and 5 was significantly increased in both the wild-type and *abcd1*-deficient mice. The expression of *Abcd2* and *Ppar α* was also increased by the treatment with

Fig. 2 Gene expression profiles in LO-treated mice liver. The relative gene expressions of liver from wild-type (WT) or *abcd1*-deficient (KO) mice with or without LO were analyzed by real-time PCR. *Pparα-1* and *Pparα-2* are genes detected by probes for recognizing

distinct position of *Pparα* sequence. The gene expression of *Elovl4* gene was not detected (ND). Values are mean $\pm$ S.E. ($n = 4$ animals). *, $p < 0.05$

LO in both the wild-type and *abcd1*-deficient mice, although there was no significant difference in *abcd1*-deficient mice. The up-regulation of *Abcd2*, *Dbp*, *Scp2*, and *Ppar α* gene indicates that LO has the capacity to activate hepatic peroxisomal fatty acid β -oxidation, which may thereby participate in the decrease of saturated VLCFA. This is consistent with the reports that show high-fat diets rich in erucic acid induced hepatic peroxisomal fatty acid β -oxidation in rodents (Neat et al. 1981; Bremer and Norum 1982; Thomassen et al. 1985; Veerkamp and Zevenbergen 1986). As DBP and SCPx are involved in the peroxisomal β -oxidation of branched-chain fatty acids and bile acid intermediates (Seedorf et al. 1994; Ferdinandusse et al. 2006a, b), LO might exert an effect on the β -oxidation of not only saturated VLCFAs, but also branched-chain fatty acids or bile acid intermediates, in peroxisomes.

In microsomal fatty acid elongation reactions, the ELOVLs are responsible for the first and rate-limiting steps in the reaction cycle (Jump 2009). Among them, ELOVL1 and 3, encoded by *ELOVL1* and 3, respectively, are involved in the synthesis of saturated and mono-unsaturated fatty acids (Sassa and Kihara 2014). In contrast, ELOVL2 and 5, encoded by *ELOVL2* and 5, respectively, are involved in poly-unsaturated fatty acid metabolism. In ALD, ELOVL1 is thought to mainly be involved in the accumulation of saturated VLCFAs, because the accumulation of C26:0 was decreased by a silencing of the *ELOVL1* gene in ALD fibroblasts (Ofman et al. 2010). However, the hepatic gene expression of *Elovl*s was the same in the wild-type and *abcd1*-deficient mice. In addition, we found that the *Elovl1* and 3 genes were quite stimulated by the treatment with LO, while the level of C26:0 was decreased (Table 1 and Supplementary Fig. 2). These results indicate that the decrease of saturated VLCFAs by LO was not regulated at the transcriptional level. Recently, Sassa et al. reported that in an in vitro experiment LO reduced the synthesis of saturated VLCFAs by inhibiting ELOVL1 at the protein but not transcriptional level (Sassa et al. 2014). The elongations of both C18:0 to C20:0 and C22:0 to C24:0, which are catalyzed by ELOVL1 and 3, appear to be largely inhibited by the treatment with LO. Therefore, we speculated that LO suppressed these fatty acid elongation activities, which resulted in a decrease in saturated VLCFAs. The decrease in the saturated VLCFA level can stimulate *Elovl1* and 3 genes expression. It has been reported that *Elovl3* expression is elevated in mice lacking the ABCD2 protein that is involved in the peroxisomal β -oxidation of monounsaturated VLCFA (Brolinson et al. 2008). In LO-treated mice, the gene expression of *Elovl1* and *Elovl3* in the *abcd1*-deficient mice was lower than that in wild-type mice. It is therefore possible that the *Elovl1*

and 3 gene expression is tightly regulated by the level of mono-unsaturated and saturated VLCFAs.

In LO-treated mice, the level of n-3 polyunsaturated VLCFA (C20:5 and C22:6) was decreased (Table 1). As the synthesis of n-3 polyunsaturated VLCFAs is known to be mediated by ELOVL2 and 5, it seems likely that the decrease of n-3 polyunsaturated VLCFAs was, at least in part, due to the inhibition of ELOVL2 and 5 activities by LO.

In the present study, we found that effect of LO on the serum fatty acid level in *abcd1*-deficient mice was quite similar to that on the plasma fatty acid level in ALD patients as reported by Moser et al. (1999). Therefore, *abcd1*-deficient mice are useful for investigating the effect of LO on in vivo fatty acid metabolism. In the present study, we first reported that LO has remarkable effects on the hepatic gene expression involved in peroxisomal fatty acid β -oxidation and microsomal fatty acid elongation. More recently, van Engen et al. showed that mutation of *Cyp4f2* decreases the conversion of VLCFA into very long chain dicarboxylic acids by ω -oxidation (van Engen et al. 2016). As the ω -oxidation is a potential escape route for the deficient peroxisomal VLCFA β -oxidation in ALD, it is interesting whether LO induces expression of *Cyp4f2* gene.

Much less is known about the relevance of these findings in mice for human physiology. Nonetheless, our results can help provide novel insights into the metabolic impact of LO on in vivo fatty acid metabolism. Furthermore, we found that the hepatic gene expression of *Dbp* was increased by LO-treatment in both the wild-type and *abcd1*-deficient mice, which suggests that LO-treatment may be a candidate therapy for DBP deficiency. DBP deficiency has been classified into 3 subgroups (Wanders et al. 2001) and recently, several patients with a less severe form of DBP have been reported by whole-exome sequencing (PMID: 27790638). Mutant DBP in patients with less severe form seems to possess some degree of enzyme activity. Our results suggest that LO may have a capacity to increase hepatic *DBP* gene expression as well as decrease serum saturated VLCFAs in DBP patients. Further investigation is required, not only studies using *dbp*-deficient mice but also clinical research for patients with mild phenotype of DBP deficiency.

Acknowledgements We thank Akiko Ohba, Kayoko Toyoshi, and Noritake Taniguchi for technical assistance. This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (16K09961, 15H04875, 15K15389). Pacific Edit reviewed the manuscript prior to submission.

Take-Home Message

Lorenzo's oil activates hepatic peroxisomal fatty acid β -oxidation at the transcriptional level.

Conflict of Interest

Masashi Morita, Ayako Honda, Akira Kobayashi, Yuichi Watanabe, Shiro Watanabe, Kosuke Kawaguchi, Shigeo Takashima, Nobuyuki Shimozaawa, and Tsuneo Imanaka declare that they have no conflict of interest.

Informed Consent

This article does not contain any studies with human participants performed by any of the authors.

Animal Rights

All institutional and national guidelines for the care and use of laboratory animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the University Committee for Animal Use and Care at the University of Toyama.

Author Contributions

TI conceived and supervised the study; TI, MM, AH, and NS designed experiments; MM, AH, AK, YW, KK, ST, and SW performed the experiments; MM, TI, and NS wrote the manuscript, which was discussed by all authors.

References

- Aubourg P, Adamsbaum C, Lavallard-Rousseau MC et al (1993) A two-year trial of oleic and erucic acids ("Lorenzo's oil") as treatment for adrenomyeloneuropathy. *N Engl J Med* 329:745–752
- Berger J, Gartner J (2006) X-linked adrenoleukodystrophy: clinical, biochemical and pathogenetic aspects. *Biochim Biophys Acta* 1763:1721–1732
- Bremer J, Norum KR (1982) Metabolism of very long-chain monounsaturated fatty acids (22:1) and the adaptation to their presence in the diet. *J Lipid Res* 23:243–256
- Brolinson A, Fourcade S, Jakobsson A, Pujol A, Jacobsson A (2008) Steroid hormones control circadian Elov13 expression in mouse liver. *Endocrinology* 149:3158–3166
- De Craemer D, Van den Branden C, Fontaine M, Vamecq J (1998) Effects of Lorenzo's oil on peroxisomes in healthy mice. *Prostaglandins Other Lipid Mediat* 55:237–244
- Deon M, Garcia MP, Sitta A et al (2008) Hexacosanoic and docosanoic acids plasma levels in patients with cerebral childhood and asymptomatic X-linked adrenoleukodystrophy: Lorenzo's oil effect. *Metab Brain Dis* 23:43–49
- Ferdinandusse S, Denis S, Mooijer PA et al (2001) Identification of the peroxisomal β -oxidation enzymes involved in the biosynthesis of docosahexaenoic acid. *J Lipid Res* 42:1987–1995
- Ferdinandusse S, Denis S, Mooyer PA et al (2006a) Clinical and biochemical spectrum of D-bifunctional protein deficiency. *Ann Neurol* 59:92–104
- Ferdinandusse S, Kostopoulos P, Denis S et al (2006b) Mutations in the gene encoding peroxisomal sterol carrier protein X (SCPx) cause leukoencephalopathy with dystonia and motor neuropathy. *Am J Hum Genet* 78:1046–1052
- Fourcade S, Ruiz M, Guilera C et al (2010) Valproic acid induces antioxidant effects in X-linked adrenoleukodystrophy. *Hum Mol Genet* 19:2005–2014
- Jump DB (2009) Mammalian fatty acid elongases. *Methods Mol Biol* 579:375–389
- Kobayashi T, Shinnoh N, Kondo A, Yamada T (1997) Adrenoleukodystrophy protein-deficient mice represent abnormality of very long chain fatty acid metabolism. *Biochem Biophys Res Commun* 232:631–636
- Kozawa S, Honda A, Kajiwarra N et al (2011) Induction of peroxisomal lipid metabolism in mice fed a high-fat diet. *Mol Med Rep* 4:1157–1162
- Morita M, Kawamichi M, Shimura Y, Kawaguchi K, Watanabe S, Imanaka T (2015) Brain microsomal fatty acid elongation is increased in *abcd1*-deficient mouse during active myelination phase. *Metab Brain Dis* 30:1359–1367
- Morita M, Shinbo S, Asahi A, Imanaka T (2012) Very long chain fatty acid β -oxidation in astrocytes: contribution of the ABCD1-dependent and -independent pathways. *Biol Pharm Bull* 35:1972–1979
- Moser AB, Jones DS, Raymond GV, Moser HW (1999) Plasma and red blood cell fatty acids in peroxisomal disorders. *Neurochem Res* 24:187–197
- Moser HW (1997) Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy. *Brain* 120:1485–1508
- Moser HW, Raymond GV, Lu SE et al (2005) Follow-up of 89 asymptomatic patients with adrenoleukodystrophy treated with Lorenzo's oil. *Arch Neurol* 62:1073–1080
- Murphy CC, Murphy EJ, Golovko MY (2008) Erucic acid is differentially taken up and metabolized in rat liver and heart. *Lipids* 43:391–400
- Neat CE, Thomassen MS, Osmundsen H (1981) Effects of high-fat diets on hepatic fatty acid oxidation in the rat. Isolation of rat liver peroxisomes by vertical-rotor centrifugation by using a self-generated, iso-osmotic, Percoll gradient. *Biochem J* 196:149–159
- Ofman R, Dijkstra IM, van Roermund CW (2010) The role of ELOVL1 in very long-chain fatty acid homeostasis and X-linked adrenoleukodystrophy. *EMBO Mol Med* 2:90–97
- Restuccia D, Di Lazzaro V, Valeriani M et al (1999) Neurophysiologic follow-up of long-term dietary treatment in adult-onset adrenoleukodystrophy. *Neurology* 52:810–816
- Rizzo WB, Leshner RT, Odone A et al (1989) Dietary erucic acid therapy for X-linked adrenoleukodystrophy. *Neurology* 39:1415–1422
- Sassa T, Kihara A (2014) Metabolism of very long-chain fatty acids: genes and pathophysiology. *Biomol Ther* 22:83–92
- Sassa T, Wakashima T, Ohno Y, Kihara A (2014) Lorenzo's oil inhibits ELOVL1 and lowers the level of sphingomyelin with a saturated very long-chain fatty acid. *J Lipid Res* 55:524–530
- Schackmann MJ, Ofman R, Dijkstra IM, Wanders RJ, Kemp S (2015) Enzymatic characterization of ELOVL1, a key enzyme in very long-chain fatty acid synthesis. *Biochim Biophys Acta* 1851:231–237
- Seedorf U, Brysch P, Engel T, Schrage K, Assmann G (1994) Sterol carrier protein X is peroxisomal 3-oxoacyl coenzyme A thiolase with intrinsic sterol carrier and lipid transfer activity. *J Biol Chem* 269:21277–21283

- Takemoto Y, Suzuki Y, Horibe R, Shimozawa N, Wanders RJ, Kondo N (2003) Gas chromatography/mass spectrometry analysis of very long chain fatty acids, docosahexaenoic acid, phytanic acid and plasmalogen for the screening of peroxisomal disorders. *Brain Dev* 25:481–487
- Thomassen MS, Norseth J, Christiansen EN (1985) Long-term effects of high-fat diets on peroxisomal β -oxidation in male and female rats. *Lipids* 20:668–674
- van Engen CE, Ofman R, Dijkstra IM et al (2016) CYP4F2 affects phenotypic outcome in adrenoleukodystrophy by modulating the clearance of very long-chain fatty acids. *Biochim Biophys Acta* 1862:1861–1870
- van Roermund CW, Visser WF, Ijlst L, Waterham HR, Wanders RJ (2011) Differential substrate specificities of human ABCD1 and ABCD2 in peroxisomal fatty acid β -oxidation. *Biochim Biophys Acta* 1811:148–152
- Veerkamp JH, Zevenbergen JL (1986) Effect of dietary fat on total and peroxisomal fatty acid oxidation in rat tissues. *Biochim Biophys Acta* 878:102–109
- Wanders RJA, Barth PG, Heymans HAS (2001) Single peroxisomal enzyme deficiencies. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) *The molecular and metabolic basis of inherited disease*. McGraw-Hill, New York, pp 3219–3256