Involvement of microsomal fatty aldehyde dehydrogenase in the α-oxidation of phytanic acid

N.M. Verhoeven[a,*], C. Jakobs[a], G. Carney[b], M.P. Somers[b], R.J.A. Wanders[c], W.B. Rizzo[b]

[a] *Department of Clinical Chemistry, Metabolic Unit, Free University Hospital Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands*
[b] *Departments of Human Genetics and Pediatrics, Medical College of Virginia, Virginia Commonwealth University, 1101 E Marshall St Room 12-026, Richmond, VA, USA*
[c] *Departments of Pediatrics and Clinical Chemistry, Academic Medical Center, Meibergdreef 9, Amsterdam, The Netherlands*

Received 27 April 1998

Abstract We investigated the role of microsomal fatty aldehyde dehydrogenase (FALDH) in the conversion of pristanal into pristanic acid. Cultured skin fibroblasts from controls and patients with Sjögren-Larsson syndrome (SLS) who are genetically deficient in FALDH activity were incubated with [2,3-^{3}H]phytanic acid. The release of aqueous-soluble radioactivity by the SLS cells was decreased to 25% of normal, consistent with an intact formation of pristanal but a deficiency of further oxidation. SLS cells also accumulated four-fold more radioactivity in *N*-alkyl-phosphatidyl ethanolamine, which arises from incorporation of free aldehyde into phosphatidyl ethanolamine. Recombinant human FALDH expressed in Chinese hamster ovary cells readily oxidized pristanal and cultured fibroblasts from SLS patients showed a severe deficiency in FALDH activity (13% of normal) when pristanal was used as substrate. Nevertheless, SLS patients did not accumulate phytanic acid in their plasma. We conclude that FALDH is involved in the oxidation of pristanal to pristanic acid and that this reaction is deficient in patients with SLS.

© 1998 Federation of European Biochemical Societies.

Key words: Sjögren-Larsson syndrome; Peroxisomal disorder; Aldehyde dehydrogenase; Phytanic acid

1. Introduction

The human diet contains a branched chain fatty acid, phytanic acid (3,7,11,15-tetramethylhexadecanoic acid), that can be rapidly metabolized. The mechanism of this degradation is different from the β-oxidation by which straight chain fatty acids are degraded, as the β-methyl group in the phytanic acid molecule blocks formation of a 3-ketoacyl-CoA intermediate. Instead, phytanic acid is initially oxidized by α-oxidation, shortening the chain by one carbon atom. After activation to phytanoyl-CoA, 2-hydroxyphytanoyl-CoA is formed by phytanoyl-CoA hydroxylase, a dioxygenase type of enzyme using 2-ketoglutarate as cofactor [1–3]. Hereafter, 2-hydroxyphytanoyl-CoA is converted to pristanal and formyl-CoA [4–6]. The enzyme that catalyzes this reaction is not known, but probably functions as a lyase. After this decarboxylation reaction, pristanal is oxidized to pristanic acid in a $NAD(P)^+$ dependent reaction [6,7]. Pristanic acid can, after activation, be degraded by peroxisomal β-oxidation.

The conversion of 2-hydroxyphytanoyl-CoA to pristanic acid was found to be localized in microsomes in human liver [7]. This was a surprising finding, as it is well known that the first step in α-oxidation, which is formation of 2-hydroxyphytanoyl-CoA from phytanoyl-CoA, occurs in peroxisomes. However, the microsomal localization of the decarboxylation step is consistent with the normal activity of this reaction in peroxisome deficient liver obtained from a patient with Zellweger syndrome [7].

Phytanic acid α-oxidation is impaired in three peroxisomal disorders: classical Refsum disease, generalized peroxisomal disorders like the Zellweger syndrome and in rhizomelic chondrodysplasia punctata (RCDP). In all three disorders, the first step of α-oxidation, the conversion of phytanoyl-CoA to 2-hydroxyphytanoyl-CoA, is deficient [1,8–10]. In classical Refsum disease, phytanoyl-CoA hydroxylase is deficient due to mutations in the gene encoding this enzyme [11,12]. In generalized peroxisomal disorders, there is an absence of functional peroxisomes, resulting in a deficiency of various peroxisomal processes, among which is the phytanoyl-CoA hydroxylase reaction [1]. In RCDP, an import defect of proteins containing a peroxisomal targeting sequence 2 results in mistargeting and thus severe deficiency of phytanoyl-CoA hydroxylase [13–15]. Patients affected with classical Refsum disease, generalized peroxisomal disorders and RCDP accumulate phytanic acid in blood and tissues [16].

Aldehyde dehydrogenases comprise a family of enzymes that oxidize a variety of aliphatic and aromatic aldehydes to their corresponding acids. Recently, a microsomal fatty aldehyde dehydrogenase (FALDH) was purified from human liver [17]. This enzyme was found to be deficient in patients affected with Sjögren-Larsson syndrome (SLS), an autosomal recessive disorder presenting with mental retardation, congenital ichthyosis and spastic di- and tetraplegia [17–19]. The biochemical defect, an inability to oxidize specific fatty aldehydes, results in accumulation of fatty alcohol in cultured fibroblasts and plasma of most SLS patients [20,21].

In search for the enzyme that oxidizes pristanal to pristanic acid, we investigated the possible role of the microsomal FALDH in this process. Here we report that FALDH is indispensable for the complete degradation of phytanic acid.

2. Materials and methods

Phytanic acid, [3-methyl-$^2H_3$]phytanic acid, pristanic acid and [2-methyl-$^2H_3$]pristanic acid were synthesized as described previously [22]. Pristanal was synthesized in the Free University Hospital Amsterdam and purified by thin layer chromatography (TLC) on silica gel G plates using a solvent system consisting of petroleum ether/diethyl ether (90/10). The plate was stained with rhodamine, after which the region corresponding to the free aldehyde was scraped off and eluted with hexane/benzene (3/2). [2,3-^{3}H]Phytanic acid was synthesized as described [23]. Octadecanal-modified phosphatidyl ethanolamine (PE) was synthesized by reacting octadecanal with PE in the presence of $NaBH_3CN$ in methanol as described [24]. Radioactive

*Corresponding author. Fax: (31) (20) 4440305.

0014-5793/98/$19.00 © 1998 Federation of European Biochemical Societies. All rights reserved.
PII S0014-5793(98)00574-2

octadecanal-modified PE was synthesized in a similar fashion using [^{14}C]octadecanal.

2.1. Cells

Cultured skin fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C in an atmosphere of 95% air/5% CO_2. Human skin fibroblasts from SLS patients were confirmed to be deficient in FALDH activity. Cultured fibroblasts from patients with RCDP and classical Refsum disease were kindly provided by A. Moser, Kennedy-Krieger Institute, Baltimore, MD. Fibroblasts from patients with Zellweger syndrome (GM4340) were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ. Dr. A. Zoeller, Boston University School of Medicine, kindly provided a Chinese hamster ovary cell line (FAA-K1A) with deficient FALDH activity [24]. FAA-K1A cells were routinely grown in Ham's F12 medium containing 5% fetal bovine serum.

2.2. Expression of recombinant human FALDH by Chinese hamster ovary cells

A cDNA encoding the entire human FALDH protein [25] was cloned into the mammalian expression vector pCI-neo (Promega Corp) and was designated pCI-neo-FALDH. Vector DNA was amplified and purified using standard techniques.

FAA-K1A cells were seeded in 6 well dishes containing 2 ml of F12 medium per well and allowed to attach for 2 h. 3 μg of pCI-neo-FALDH DNA was incubated with Fugene-6 (Boehringer-Mannheim) according to the manufacturer's instructions and added to each well. After 3 days the cells were harvested, washed and homogenized as described [18].

2.3. Incubation of intact fibroblasts with [2,3-^{3}H]phytanic acid

Cultured skin fibroblasts were plated in 6 well dishes in DMEM containing 10% fetal bovine serum and penicillin/streptomycin 3 days prior to testing. On the day prior to testing, the media were renewed. On the day of the experiment when the cells were confluent, the medium was replaced by 1 ml of DMEM containing 1% fetal bovine serum and 1.5 μCi of [2,3-^{3}H]phytanic acid (final concentration of phytanic acid was 50 nM). After 24 h, 0.2 ml of the medium was removed and water-soluble radioactivity was determined as described [23]. All cell incubations were done in duplicate wells. Water-soluble radioactivity in the media of duplicate wells varied by less than 5%. Cell protein was determined according to Lowry et al. using replicate dishes.

To measure the formation of radioactive aldehyde-modified PE, the cell monolayers were washed twice with PBS and incubated with 25 mM $NaBH_3CN$ at 37°C for 2 h as described [24] to stabilize the aldehyde adducts. Cells were collected by scraping, pelleted by centrifugation and extracted twice with 2 ml of chloroform/methanol (1/1). The chloroform/methanol extracts were pooled, dried under nitrogen atmosphere, dissolved in 5 ml hexane and applied to disposable 3 ml silica gel columns (J.T. Baker, Philipsburg, NJ) that were pre-equilibrated with hexane. The columns were washed with 10 ml hexane and the radioactive *N*-alkyl-PE was eluted with 10 ml dichloromethane, dried under nitrogen and purified by TLC on a channeled silica gel G plate using a solvent system consisting of chloroform/methanol (98/2). Synthetic *N*-alkyl-PE was co-chromatographed to localize the radioactive lipid. Lipids were visualized under UV light after spraying the plate with rhodamine G. The regions corresponding to *N*-alkyl-PE (Rf = 0.8) were collected by scraping and the radioactive lipid eluted twice with 5 ml of hexane/benzene (3/2). After drying under nitrogen, the *N*-alkyl-PE was resuspended in 2 ml of 0.3 M ethanolic-NaOH and hydrolyzed overnight at 90°C. After cooling to room temperature, 2 ml of water was added to each tube and the [^{3}H]*N*-alkyl-PE hydrolysis product was extracted twice with 2 ml hexane. The pooled hexane extracts were dried under nitrogen and the [^{3}H]*N*-alkyl-PE hydrolysis product (now free of acyl-linked fatty acids) was purified by TLC on a channeled silica gel G plate using a solvent system consisting of hexane/ether/acetic acid (60/40/1). The hydrolysis product of non-radioactive octadecanyl-modified PE (25 μg) was co-chromatographed with each sample to permit visualization by staining the plate with rhodamine and examining under UV light. The regions corresponding to the hydrolysis product of *N*-alkyl-PE (Rf = 0.45) were collected by scraping and the radioactivity was determined. Using synthetic [^{14}C]octadecanal-modified PE, the overall recovery of the radioactive hydrolysis product of *N*-alkyl-PE was 71 ± 4%. The data shown in Section 3 were not corrected for recovery.

To measure incorporation of [2,3-^{3}H]phytanic acid into PE, radioactive phospholipids were eluted off the silica gel columns (after elution of *N*-alkyl-PE as described above) with 10 ml of methanol and dried under nitrogen. PE was purified by TLC [25].

2.4. Fatty aldehyde dehydrogenase activity in cultured fibroblasts using pristanal and octadecanal as substrates

FALDH was assayed according to Rizzo and Craft [18] as modified by Kelson et al. [17]. Both substrates were used at 175 μM final concentration in the assays.

2.5. Quantification of phytanic acid and pristanic acid in plasma

The concentrations of phytanic acid and pristanic acid in plasma samples from controls and patients affected with SLS were determined by stable isotope dilution gas chromatography mass spectrometry as previously described [16].

3. Results

3.1. Incubation of intact fibroblasts with [2,3-^{3}H]phytanic acid

To determine whether microsomal FALDH was involved in the oxidation of phytanic acid, we incubated [2,3-^{3}H]phytanic acid with intact fibroblasts from SLS patients, normal controls and patients with defective α-oxidation. The ability of cells to release aqueous-soluble radioactivity into the medium was used as a measure of phytanic acid α-oxidation [23]. As shown in Table 1, the cells from patients with SLS released only 25% of the aqueous-soluble radioactivity compared to normal controls, suggesting that FALDH activity is involved in phytanic acid α-oxidation. In classical Refsum disease, the release of radioactivity was more severely depressed to about 10% of control values. In fibroblasts from the Zellweger patient, no activity could be detected, whereas the cells from the

Table 1
Metabolism of [2,3-^{3}H]phytanic acid by cultured fibroblasts

Fibroblasts	Aqueous-soluble radioactivity released into the medium (cpm/h/mg cell protein)	Radioactivity incorporated into *N*-alkyl-PE (cpm/mg cell protein)	Radioactivity incorporated into PE (cpm/mg cell protein)
Normal controls ($n = 4$)[a]	70 440 ± 16 690[b]	708 ± 614	9 640 ± 4 780
SLS ($n = 4$)	17 760 ± 10 190	3 084 ± 586	7 770 ± 1 260
Classical Refsum disease ($n = 1$)	8 160	558	26 770
Zellweger syndrome ($n = 1$)	0	667	5 040
RCDP ($n = 1$)	2 880	853	9 450

Cells were incubated for 24 h. The release of aqueous-soluble radioactivity into the medium, incorporation of radioactivity into the alkyl linkage of *N*-alkyl-PE and into PE were determined as described in Section 2.
[a]Number of different cell lines tested.
[b]Mean ± standard deviation.

Table 2
Fatty aldehyde dehydrogenase activity in cultured fibroblast homogenates using pristanal and octadecanal as substrates

Cells	FALDH activity (pmol/min/mg protein)	
	Pristanal	Octadecanal
Normal controls ($n = 6$)[a]	916 ± 203	8351 ± 2105
SLS ($n = 9$)	116 ± 92	569 ± 275
% of mean normal FALDH activity in SLS cells	13	7

Enzyme assays were performed with 1.5 mM NAD^+.
[a]Number of different cell lines tested.

RCDP patient released a small amount of radioactivity (4% of normal).

If the impaired tritium release by SLS cells were due to a block in pristanal oxidation, accumulation of *N*-alkyl-modified PE, which arises from Schiff base formation of free pristanal with the ethanolamine moiety of PE, might occur [24]. We therefore measured radioactive *N*-alkyl-PE in the same fibroblast cultures used to measure tritium release. As shown in Table 1, the SLS cells accumulated four-fold more radioactivity in *N*-alkyl-PE than did normal control cells. In contrast, incorporation of radioactivity into non-modified PE was normal in SLS cells.

3.2. Fatty aldehyde dehydrogenase activity using pristanal as substrate

To determine whether pristanal was a substrate for FALDH, we used recombinant human FALDH overexpressed in FALDH-deficient Chinese hamster ovary cells (FAA.K1A). The FALDH activity in homogenates of FAA-K1A cells that were transfected with an expression vector containing the human FALDH cDNA was 200–350-fold higher than in untransfected cells (data not shown). The recombinant enzyme readily oxidized pristanal and displayed an apparent K_m of 40 μM. The enzyme activity with saturating concentrations of pristanal (175 μM) and 1.5 mM $NADP^+$ was 30% of that measured with an equivalent NAD^+ concentration. FALDH activity was considerably higher (7–8-fold) with octadecanal as substrate than with pristanal.

FALDH activity was measured in cultured skin fibroblasts homogenates from normal controls using pristanal and octadecanal as substrates. As seen with the recombinant FALDH, the fibroblast enzyme showed a similar apparent K_m (40 μM) with pristanal (data not shown). In addition, enzyme activity with octadecanal was about nine-fold higher than with pristanal (Table 2). FALDH activity was also assayed in SLS fibroblasts using both substrates (Table 2). In SLS cells, FALDH activity was severely decreased. When pristanal was used as substrate, SLS fibroblasts had a residual activity of about 13% of normal. Using octadecanal as substrate, the FALDH activity in SLS cells was about 7% of that found in normal controls.

3.3. Quantification of phytanic acid and pristanic acid in plasma

We wondered whether a defect in pristanal oxidation that occurs after the decarboxylation step in α-oxidation would lead to phytanic acid accumulation in SLS. As shown in Table 3 there were no significant differences in the concentrations of phytanic acid and pristanic acid and in the pristanic acid/phytanic acid ratios in plasma from controls and patients with SLS.

4. Discussion

To further elucidate the phytanic acid α-oxidation pathway, we investigated the oxidation of pristanal to pristanic acid. The nature of the substrate and the product suggested the enzyme catalyzing this reaction would be an aldehyde dehydrogenase (ALDH). In man, several ALDHs are known which differ in subcellular localization, amino acid sequence, substrate specificity and biophysical properties. In human microsomes, FALDH oxidizes straight chain aliphatic aldehydes of 6–24 carbons to their corresponding fatty acids [17]. This FALDH also acts on dihydrophytal, a 20 carbon saturated branched chain aldehyde, and other 3-methyl and 2-methyl branched-chain aliphatic substrates (Rizzo, unpublished). Activity of this enzyme is deficient in SLS, resulting in impaired fatty alcohol oxidation [18,21].

Our results imply FALDH is involved in the conversion of pristanal into pristanic acid. In intact SLS fibroblasts, the release of radioactivity from [2,3-^{3}H]phytanic acid was about 25% of the values found in controls. With normal phytanic acid oxidation, the release of tritium label is expected to be 25% during the formation of 2-hydroxyphytanoyl-CoA from phytanoyl-CoA. The conversion of 2-hydroxyphytanoyl-CoA to pristanal is not expected to release additional label, whereas subsequent formation of pristanic acid from pristanal would release another 25% of the label. Further β-oxidation of pristanic acid liberates the remainder of the label in propionyl-CoA. The observation that in SLS cells the tritium release is only 25% of the values in controls points to an intact formation of 2-hydroxyphytanoyl-CoA but deficient oxidation of pristanal. In the fibroblasts from patients affected with classical Refsum disease, Zellweger syndrome and RCDP, the tritium release was 10% or lower. This is consistent with the findings of Jansen et al. and Verhoeven et al. that the dioxygenase which converts phytanoyl-CoA into 2-hydroxyphytanoyl-CoA is deficient in these disorders [1,8–10].

The results reported here are in accordance with our previous findings that formation of pristanic acid from 2-hy-

Table 3
Concentrations of phytanic acid and pristanic acid in plasma from normal controls and patients affected with SLS

	Phytanic acid (μM)	Pristanic acid (μM)	Pristanic/Phytanic
Controls ($n = 90$)	0.04–9.88	0.01–2.98	0.05–0.40
SLS patients ($n = 10$)	0.45–8.86	0.06–2.88	0.09–0.18

droxyphytanoyl-CoA is localized in microsomes in human liver, and that the enzyme catalyzing the conversion of pristanal to pristanic acid uses NAD^+ and/or $NADP^+$ as cofactors [7]. The impaired release of aqueous soluble radioactivity from [2,3-^{3}H]phytanic acid and the accumulation of radioactive *N*-alkyl-PE by SLS fibroblasts strongly support the involvement of FALDH in phytanic acid oxidation. This conclusion is further supported by the demonstration that recombinant FALDH utilizes pristanal as substrate and FALDH activity using pristanal is deficient in SLS cells.

Our results argue against a significant contribution by an alternative ALDH enzyme catalyzing the oxidation of pristanal. However, Croes et al. [6] have recently reported that rat liver peroxisomes are capable of oxidizing 2-methyl-pentadecanal to 2-methyl-pentadecanoic acid in the presence of NAD^+. It is unclear whether this activity is specific to rodents or is physiologically relevant to phytanic acid oxidation. If this peroxisomal activity is present in humans, our results suggest that it makes a minor contribution to overall pristanal oxidation.

Two transcripts from the FALDH gene have been found to arise from alternate splicing [26]. The major transcript (which was expressed in the FAA-K1A cells) encodes a protein with a microsomal targeting sequence at the carboxy terminal region. A minor transcript, accounting for less than 10% of the major transcript, encodes a protein containing most of the microsomal targeting sequence but possessing an additional carboxy-terminal sequence of unknown function. This FALDH protein lacks any known peroxisomal targeting sequence. Moreover, it is unlikely that this minor FALDH isoform is directed to peroxisomes, since no FALDH enzyme activity was found in human liver peroxisomes.

Patients with SLS do not accumulate phytanic acid in their plasma. This may be due to several reasons. First, it is possible that the residual activity of FALDH is sufficient to avoid accumulation of phytanic acid. The residual enzyme activity measured in whole cell homogenates (approximately 6% of normal) is due to other ALDH enzymes [18,27]. Another fatty aldehyde dehydrogenase, such as a cytosolic or mitochondrial isozyme may partially take over the role of the deficient FALDH in certain tissues. The normal concentrations of pristanic acid and the normal ratio between pristanic acid and phytanic acid are consistent with this explanation. Second, the impaired pristanal oxidation in SLS may result in diversion of this aldehyde intermediate into other metabolites, thereby mitigating pristanal and phytanic acid accumulation. Aldehydes are very reactive molecules that form Schiff bases with amino groups of proteins and lipids. Recently, SLS fibroblasts were shown to accumulate fatty-aldehyde-modified PE [24], and our results demonstrate accumulation of aldehyde modified PE derived from phytanic acid metabolism. It is therefore possible that SLS patients divert pristanal into other amino-containing molecules such as proteins.

Future experiments will focus on the elucidation of the mechanism that is responsible for the absence of phytanic acid accumulation in SLS, and the mechanism by which the α-oxidation intermediates are transported from one organelle to another.

Acknowledgements: This work was supported by National Institutes of Health Grant AR44552 to W.B.R. and by the European Concerted Action CT 96-1621 'Peroxisomal leukodystrophy' (DGXII, Biomedical Research). The authors thank Drs. P. Watkins and S. Mihalik for stimulating discussions.

References

[1] Jansen, G.A., Mihalik, S.J., Watkins, P., Moser, H.W., Jakobs, C., Denis, S. and Wanders, R.J.A. (1996) Biochem. Biophys. Res. Commun. 229, 205–210.
[2] Mihalik, S.J., Rainville, A.M. and Watkins, P.A. (1995) Eur. J. Biochem. 232, 545–551.
[3] Croes, K., Casteels, M., De Hoffmann, E., Mannaerts, G.P. and Van Veldhoven, P.P. (1996) Eur. J. Biochem. 240, 674–683.
[4] Verhoeven, N.M., Schor, D.S.M., ten Brink, H.J., Wanders, R.J.A. and Jakobs, C. (1997) Biochem. Biophys. Res. Commun. 237, 33–36.
[5] Croes, K., Casteels, M., Asselberghs, S., Herdewijn, P., Mannaerts, G.P. and van Veldhoven, P.P. (1997) FEBS Lett. 412, 643–645.
[6] Croes, K., van Veldhoven, P.P., Mannaerts, G.P. and Casteels, M. (1997) FEBS Lett. 407, 197–200.
[7] Verhoeven, N.M., Wanders, R.J.A., Schor, D.S.M., Jansen, G.A. and Jakobs, C. (1997) J. Lipid Res. 38, 2062–2070.
[8] Jansen, G.A., Wanders, R.J.A., Watkins, P.A. and Mihalik, S.J. (1996) New Engl. J. Med. 337, 133–134.
[9] Jansen, G.A., Mihalik, S.J., Watkins, P.A., Moser, H.W., Jakobs, C., Heijmans, H.S.A. and Wanders, R.J.A. (1997) J. Inher. Metab. Dis. 20, 444–446.
[10] Verhoeven, N.M., Schor, D.S.M., Roe, C.R., Wanders, R.J.A. and Jakobs, C. (1997) Biochim. Biophys. Acta 1361, 281–286.
[11] Jansen, G.A., Ofman, R., Ferdinandusse, S., Ijlst, L., Muijsers, A.O., Skjeldal, O.H., Stokke, O., Jakobs, C., Besley, G.T.N., Wraith, J.E. and Wanders, R.J.A. (1997) Nature Genet. 17, 190–193.
[12] Mihalik, S.J., Morrell, J.C., Kim, D., Sacksteder, K.A., Watkins, P.A. and Gould, S.J. (1997) Nature Genet. 17, 185–189.
[13] Motley, A.M., Hettema, E.H., Hogenhout, E.M., Brites, P., Tenasbroek, A.L.M.A., Wijburg, F.A., Baas, F., Heijmans, H.S., Tabak, H.F., Wanders, R.J.A. and Distel, B. (1997) Nature Genet. 15, 377–380.
[14] Braverman, N., Steel, G., Obie, C., Moser, A., Moser, H., Gould, S.J. and Valle, D. (1997) Nature Genet. 15, 369–376.
[15] Purdue, P.E., Zhang, J.W., Skoneczny, M. and Lazarow, P.B. (1997) Nature Genet. 15, 381–384.
[16] ten Brink, H.J., Stellaard, F., van den Heuvel, C.M.M., Kok, R.M., Schor, D.S.M., Wanders, R.J.A. and Jakobs, C. (1992) J. Lipid Res. 33, 31–37.
[17] Kelson, T.L., Secor McVoy, J.R. and Rizzo, W.B. (1997) Biochim. Biophys. Acta 1335, 99–110.
[18] Rizzo, W.B. and Craft, D.A. (1991) J. Clin. Invest. 88, 1643–1648.
[19] Rizzo, W.B. (1993) Semin. Dermatol. 12, 210–218.
[20] Rizzo, W.B., Dammann, A.L. and Craft, D.A. (1988) J. Clin. Invest. 81, 738–744.
[21] Rizzo, W.B., Dammann, A.L., Craft, D.A., Black, S.H., Henderson Tilton, A., Africk, D., Chaves-Caballo, E., Holmgren, G. and Jagell, S. (1989) J. Pediatr. 115, 228–234.
[22] ten Brink, H.J., Jakobs, C., van der Baan, J.L. and Bickelhaupt, F. (1989) in: Synthesis and Applications of Isotopically Labeled Compounds (Baillie, T.A. and Jones, J.R., Eds.), pp. 712–722, Elsevier, Amsterdam.
[23] Zenger-Hain, J., Craft, D.A. and Rizzo, W.B. (1992) in: New Developments in Fatty Acid Oxidation (Coates, P.M. and Tanaka, K., Eds.), pp. 399–407, Wiley-Liss, New York.
[24] James, P.F. and Zoeller, R.A. (1997) J. Biol. Chem. 272, 23532–23539.
[25] Blackard, W.G., Li, J., Clore, J.N. and Rizzo, W.B. (1997) Lipids 32, 193–198.
[26] Rogers, G.R., Markova, N.G., De Laurenzi, V., Rizzo, W.B. and Compton, J.G. (1997) Genomics 39, 127–135.
[27] De Laurenzi, V., Rogers, G.R., Tarcsa, E., Carney, G., Marekov, L., Bale, S.J., Compton, J.G., Markova, N., Steinert, P.M. and Rizzo, W.B. (1997) J. Invest. Dermatol. 109, 79–83.