Metabolism of phytanic acid and 3-methyl-adipic acid excretion in patients with Adult Refsum’s disease

Anthony S. Wierzbicki¹²
Phillip D. Mayne¹
Matthew D. Lloyd³
David Burston¹
Guam Mei¹
Margaret C. Sidey²
Michael D. Feher²
F. Brian Gibberd²

Department of Chemical Pathology¹ and Refsum Disease Clinic², Chelsea & Westminster Hospital, 369 Fulham Road, London SW10 9NH, United Kingdom

Department of Pharmacy & Pharmacology³, University of Bath, Claverton Down, Bath BA2 7AY

Key words:

Refsum disease; phytanic acid; 3-methyl-adipic acid; organic acid; alpha-oxidation; omega-oxidation

Running Title : Refsums and 3-MAA excretion

Address for Correspondence
Dr. Anthony S. Wierzbicki
Senior Lecturer in Chemical Pathology
St. Thomas’ Hospital
Lambeth Palace Road
London SE1 7EH

Tel: (44)20-7928-9292x2027
Fax: (44)20-7928-4226
E-mail: Anthony.Wierzbicki@kcl.ac.uk
Abstract

Adult Refsum’s disease (ARD) is associated with a biochemical defect in the metabolism of phytanic acid (PA) by alpha-oxidation. Limited metabolism of phytanic acid to 3-methyl-adipic acid (3-MAA) occurs through omega (ω)-oxidation. The contribution of ω-oxidation to metabolism of phytanic acid has not been systematically assessed in patients with ARD.

In a 40 day study of a newly presented ARD patient, where the plasma half-life of PA was 22.4 days, ω-oxidation accounted for 30% initially and later all PA excretion. A PA loading test in 2 obligate ARD heterozygotes and 4 controls showed no difference in baseline or stimulated activity. Plasma and adipose tissue PA and 3-MAA excretion were measured in a cross-sectional study of 11 patients (5 men and 6 women) with ARD. The capacity of the ω-oxidation pathway was 6.9 (2.8-19.4) mg (20.4[8.3- 57.4] µmols) PA/day in ARD patients. In ARD, 3-MAA excretion correlated with plasma PA levels (r = 0.61; p=0.03) but not adipose tissue PA content.

Omega-oxidation during a 56 hour fast was studied in 5 ARD patients. 3-MAA excretion increased by 208±58% in parallel with the 158 [125-603]% rise in plasma PA. The plasma PA release curve showed a 29-hour doubling time while 3-MAA excretion followed second order kinetics with no obvious latency. Acute sequelae of ARD were noted in 3 patients (60%) after fasting.

The ω-oxidation pathway can metabolise all the phytanic acid ingested in patients with ARD on a low phytanic acid diet but this activity is dependent on plasma phytanic acid concentration. Omega oxidation forms a functional reserve capacity that enables patients with ARD undergoing acute stress to cope with limited increases in plasma PA levels.
Introduction

Adult Refsum’s disease (ARD) (Heredopathia atactica polyneutiformis) (On-line Mendelian Inheritance in Man; OMIM 265000) is an autosomal recessive disease, which presents with retinitis pigmentosa, anosmia, peripheral neuropathy, deafness and ataxia with additional ichthyosis and cardiomyopathy in severe cases. Many cases are caused by mutations in phytanoyl-CoA hydroxylase resulting in increased plasma phytanic (3,7,11,15-tetramethylhexadecanoic) acid levels and accumulation of phytanic acid (PA) in all fat-containing tissues. Normal metabolism of phytanic acid involves peroxisomal alpha-oxidation, but an additional low capacity pathway through omega-oxidation and subsequent cycles of beta-oxidation result in production of a variety of 3-methyl-organic acids including 3-methyl-adipic acid (3-MAA; 3-methyl-hexanedioic acid). Refsum’s disease is treated by restriction of phytanic acid intake or physical removal of phytanic acid by plasmapheresis or apheresis. In patients on an appropriate diet phytanic acid levels fall gradually due to omega-oxidation. In fasting and acutely ill patients phytanic acid is mobilized from liver and adipose tissue pools as a result of the activation of lipolytic pathways resulting in an acute increase in plasma levels and acute symptoms.

This study assessed the contribution made by the omega-oxidation pathway to the metabolism of phytanic acid (PA) by measuring 3-methyl-adipic acid (3-MAA) excretion in patients with ARD.

Methods

This study received ethical approval and all patients and controls gave informed consent to the investigations performed.
Patient Selection

Healthy controls and heterozygotes for ARD

Four healthy volunteers (3 male; one female; median age 32 (range 30-41) years) and 2 obligate heterozygotes (1 male, 1 female) from a consanguineous family with Refsum’s disease were recruited. Each subject was stabilized on the low phytanic acid diet (Westminster) diet for 24 hours and next morning blood and urine samples were taken\textsuperscript{7,10,11}. Oral phytanic acid loading was performed using 25 mLs of Maxepa oil (7.5 mg; 22.2 µmols phytanic acid/mL) in 75 mLs orange juice. Blood and double-void technique urine samples were collected at 6-hourly intervals.

Dietary stabilisation of patients with ARD

Eleven patients with ARD diagnosed clinically and with plasma phytanic acid levels > 200 µmol/L (normal <30 µmol/L) were recruited and had initial biochemical investigations performed. Patients were stabilized on a low phytanic acid diet over 12 weeks with a monthly dietetic review. Clinical biochemical analyses comprising blood phytanic acid, triglycerides, and glucose and urinary 3-MAA, creatinine and ketones were taken at the start and end of the dietary stabilisation period. Serial blood and urine sampling on a more frequent basis was possible in one newly presented 24-year old woman with ARD admitted over a 90-day period.

Ten patients underwent abdominal wall fat biopsy after dietary stabilisation. Tissue phytanic acid content was measured and compared with blood and urine phytanic acid, creatinine and 3-methyl-adipic acid measurements.

Fasting studies in patients with ARD

A subset of 5 patients stabilised on the low phytanic diet agreed to be admitted for investigation of their biochemical response to acute starvation. Patients agreed to be deprived of food and to be allowed only water to drink for a maximum period of 72 hours. Clinical
biochemical analyses comprising blood phytanic acid, triglycerides, and glucose and urinary 3-
MAA, creatinine and ketones were performed every 12 hours. At the end of the study patients
were re-started on an oral low phytanic acid diet and discharged home after 3 days and were
reviewed monthly until well.

**Biochemical assays**

**Measurement of Phytanic acid**

Phytanic acid was measured by established methods using methanol-chloroform
extraction and gas chromatography. Plasma (0.5mls) was extracted with 6 mls chloroform:
methanol (2:1), centrifuged at 1900 g for 10 minutes at 4°C. 100µL of 3-methyl-pentadecanoate
(Sigma, Poole, UK) dissolved in hexane (0.728mg/mL) was added as an internal standard to a 3
ml aliquot of the organic phase followed by 5 mLs of 0.5M methanoic sodium hydroxide (20g/L
methanol). The solution was heated at 100°C for 5 minutes, cooled, and 5mLs of boron trifluoride
(14% BF w/v) were added and the sample heat-treated as before for 4 minutes. After cooling 30
mL of saturated sodium chloride and 15 mL of petroleum ether were added and the aqueous layer
removed and filtered through phase separation paper (Whatman, Maidstone, UK). The aqueous
layer was re-extracted with 20 mLs of petroleum ether and then the extract was evaporated to
dryness, resuspended in 1 ml hexane and re-evaporated. The purified extract was dissolved in 0.3
mL hexane for injection.

Phytanic acid was measured by gas chromatography (Varian 3700; Varian Ltd, Walton-on-
Thames, UK) fitted with a 25m BPX70 (70% cyanopropyl siloxane) capillary column (SGE;
Milton Keynes UK) with a temperature gradient from 150°C to 250°C. The injector temperature
was 240°C and FID detector temperature 300°C. Peaks were integrated on a Varian CDS100
integrator. The phytanic acid peak was identified from log retention time after injection of 1 μL and concentration calculated relative to methyl-phytanate (Phase Separations, Chester, UK) and methyl-pentadecanoate internal standards. The between batch co-efficient of variation was 5.6% (n=5) and biological variation 7.7% (n=9).

**Measurement of 3-methyl-adipic acid**

3-methyl-adipic acid (3-MAA) was measured by standard gas chromatographic methods following ethyl-acetate extraction from acidified plasma. Lauric acid (50 μL; 1 mg/mL) and dodecanedioic acid (50μL; 1mg/mL) were added to 1 ml of fresh urine. The urine was acidified with 3 drops of concentrated hydrochloric acid and sodium chloride added in excess. The acidified urine was extracted with an equal volume of ethyl acetate and phases separated by centrifugation. The aqueous phase was re-extracted twice more. Supernatant fractions were combined and dried down under nitrogen. The residue as re-dissolved in ethyl acetate and re-dried. The organic acids were derivatised in 30 μl of acetonitrile and 30 μL bis-(tri-methylsilyl)trifluoroacetamide (BSTFA).

Organic acids were analysed by gas chromatography using a 25m BP1 column (SGE, Milton Keynes, UK). 1μL sample was injected with a split ratio of 1:100 and a temperature gradient from 90-250°C increasing at 8°C/min after an initial 4 minute isothermal stage. Peaks were detected by flame ionisation and integrated. 3-MAA concentrations were calculated from internal reference standards.

Triglycerides, glucose and creatinine were measured by standard automated techniques on a Hitachi 717 analyzer (Boehringer-Mannheim; Lewes, UK).
**Statistical analysis**

Data from the studies was analysed using GBStat 7.0 (Dynamic Microsystems, Silver Spring, Maryland, USA). As only small numbers of patients were investigated and distributions of results were non-Gaussian, data was analysed by non-parametric statistics or after log transformation. Multiple regression analysis was performed to investigate the dependence of plasma phytanic acid and 3-MAA levels on demographic and anthropometric and biochemical variables. A p-value of <0.05 was considered significant. Kinetic plot analysis was conducted using sequential data in one newly presented patient and in 5 patients in response to fasting. Linearity of plots against time of natural logarithm plot of current to basal concentrations([C]) (i.e. Ln [C]/[C₀]) and respective inverse concentrations (i.e. 1/[C] - 1/[C₀]) were used to confirm first or second order kinetics respectively.

**Results**

**Response to dietary therapy in a new patient**

In one newly presented patient with ARD it was possible to follow phytanic acid and 3-MAA elimination on a low phytanic acid diet (figure 1). Phytanic acid elimination followed an approximately exponential course with first order kinetics \( \ln \{[S]/[S₀]\} = -kt \) over 40 days with a plasma half-life for phytanic acid of 22.4 days. Plasma PA correlated weakly with 3-MAA excretion \( r=0.54; p=0.02 \) over the period of admission in this patient (figure 1). Multiple regression analysis of the production of 3-MAA \( r=0.825 \) showed it was dependent on time \( (\beta=-42; P<0.001) \) and the log of concentration of phytanic acid \( (\beta=-1962; p=0.05) \) but independent of other anthropometric and biochemical factors. Analysis of the kinetics of 3-MAA levels in this
patient showed they were linear when plotted against $1/[C]-1/[C_0]$ and therefore demonstrated second order kinetics.

**Phytanic acid loading studies in healthy controls and heterozygotes for ARD**

Baseline levels of phytanic acid (PA) (2000%) and 3-methyl-adipic acid (3-MAA) (50%) were raised in patients with previously diagnosed ARD as opposed to both healthy controls and obligate heterozygotes whose levels were similar (table 1). Urinary 3-MAA concentrations were raised, but not significantly, in 2 heterozygotes for ARD (5.2 mg 3-MAA/ g creatinine) compared with 4 healthy controls (3.4 mg 3-MAA/ g creatinine). Ingestion of 187.5 mg (555 µmols) of phytanic acid by the control group led to an approximate 500% increase in urinary 3-MAA levels within 14 hours with no increase in plasma phytanic acid levels in both groups. No difference in 3-MAA production in response to the phytanic acid load was seen between the healthy controls and the ARD heterozygotes.

**Studies with supervised low phytanic acid diets in patients with ARD**

Responses to a supervised low phytanic acid diet were assessed over 12 weeks prior to admission for fat biopsy in 11 patients with ARD (table 2). Institution of supervised dietary therapy led to a median 21% fall in plasma phytanic acid levels despite a 3.2% increase in fat-derived calories from an initial median intake of 2.7 mg (8.0 µmols) PA/day and plasma levels of 628 (46-2336) µmol/L of PA. However, 2 patients did not complete the diet assessment and were excluded from the analysis. Two patients, despite supervision, showed an increase in phytanic acid intake as measured by food intake questionnaire yet their phytanic acid levels fell over the period of the study demonstrating either acute variations in dietary intake were identified in the questionnaire or the slow kinetics of plasma phytanic acid response to dietary-induced changes.
The median subcutaneous fat biopsy PA concentration was 782 (217-1458) mg (2.31 [0.64-4.31] mmols) PA /100 mg tissue (n=8) and did not correlate with age, weight, or plasma PA or urinary 3-MAA levels pre- or post diet. Fat biopsy phytanic acid content correlated non-significantly with age (β=30.4; p=0.1) and log phytanic acid level (β= 318; p=0.19) indicating the need for high concentration gradients and exposure to lead to accumulation of PA.

Cross-sectional analysis of the group showed a non-linear relationship between plasma PA and 3-MAA production after dietary stabilisation (figure 2). Urinary 3-MMA production was 6.8 (1.9-14.4mg/day) with a calculated phytanic acid clearance from urine 3-MAA levels of 6.9 (2.8-19.4) mg (20.4[8.3-57.4] µmols)/day in this study. Production of 3-MAA correlated weakly with initial plasma PA level (r=0.61; p = 0.03) using a linear model. No correlation was observed with 3-MAA production with age, sex or levels of phytanic acid in the fat biopsies.

**Response of patients with ARD to prolonged fasting**

Five patients agreed to fast and all developed ketonuria within 48 hours. Four were able to tolerate fasting for >48 hours and one for 36 hours. Peak 3-MAA excretion increased by 208±58% in all patients up to 52 hours after which rates seemed to plateau (2 individuals) in parallel with the rise in plasma PA (158 [125-603]% ) (table 3). Four of five patients showed significant increases in plasma PA during the fasting period and in 3 this was correlated with a rise in 3-MAA. In one patient with highly elevated PA levels, 3-MAA levels fell during the fast and in another levels did not change. These patients may not have complied with the protocol. Phytanic acid release into plasma followed first order kinetics in all 5 patients (figure 3) and showed a doubling time of 29.2 hours. The production of 3-MAA showed no obvious latency and was consistent with second order kinetics in the three evaluable patients (figure 4). Assessment of the
rate of change of 3-MAA and PA showed that the 3-MAA production rate correlated weakly with fat biopsy PA content (r=0.65; p=0.06) or plasma PA (r=0.86; p=0.01) but that PA production was independent of plasma or biopsy PA. No significant long-term changes in plasma PA or 3-MAA were seen after the study had terminated.

Though no patients developed acute symptoms as a result of fasting, clinical sequelae were noted in 3 cases. One lost 8 kg in weight, a second felt non-specifically unwell for 12 weeks and needed hospital admission for exhaustion while a third patient developed leg cramps for 3 days afterwards.

Discussion

Phytanic acid is derived by bacterial metabolism of chlorophyll in the digestive systems of ruminants and is not normally present in significant levels in human plasma or tissues. In contrast, in patients with ARD, PA is present in large quantities in plasma and accumulates in nervous and adipose tissues. Patients with ARD cannot metabolize PA by α-oxidation and so rely on ω-oxidation with the consequent production of 3-methyl-adipic acid (3-MAA)\textsuperscript{13}.

This study included 7 patients whose genetic defect has been mapped to chromosome 10 indicating they were likely to have mutations in phytanoyl-CoA hydroxylase\textsuperscript{14}. Family sizes were too small to allow mapping of the disease in 4 other patients. In one newly presenting ARD long term treatment with a low PA diet which reduces intake from 60 mg (178 $\mu$mols)/day to <20 mg (60 $\mu$mols)/day resulted in a gradual fall in plasma and tissue PA levels with a plasma half-life for phytanic acid of 22.4 days following classical first order kinetics \textsuperscript{1;10}. This study, like others, shows significant ω-oxidation occurs in patients with acute and chronic ARD with increased levels of 3-MAA compared to normal controls\textsuperscript{5;6;13}. After ingestion of a test load of PA, 3-MAA was
detected in the urine of healthy controls and ARD heterozygotes showing that ω-oxidation plays a significant role in post-prandial metabolism of PA in humans.

The capacity of the ω-oxidation pathway has been measured by excretion of 2,6 dimethyloctanedioic acid (2,6-DMOA), derived from the C10 ω-2-methyl thioester derivative of PA (figure 5). Based on 2,6-DMOA excretion in 2 patients, the capacity of the ω-oxidation pathway has been suggested to be 30 mg PA (89 µmols)/day. However, this present study measuring 3-MAA excretion showed a far lower capacity of 6.9 (2.8-19.4) mg (20.4[8.3- 57.4] µmols)/day in patients consuming 2.7 (0-53) mg (8[0-16] µmols/day of PA. The production of 3-MAA followed second order kinetics indicating that the activity of the pathway is dependent on initial plasma PA concentration. Patients in the original study underwent dietary PA restriction compared to a normal daily intake of 165 mg (0.5 mmol)/day of PA but no data was provided on the success of dietary therapy. However, these capacities may differ for an alternative reason. 2,6-DMOA and 3-hexanedioic acid are products of the initial steps of ω-oxidation and may be dependent on carnitine ester formation for activation for further metabolism. Phytanoyl-carnitines which occur in ARD may impair the activation reaction through competition and lead to urinary excretion of excess 2,6-DMOA and 3-methylhexanedioic acid so that the initial steps of ω-oxidation may seem to have a greater capacity than that of the whole pathway.

The results of this study suggest that patients on a low PA diet can maintain stable undetectable levels of PA in plasma if solely dependent on ω-oxidation. This aim has been achieved in 8/22 (36%) patients with ARD in the clinic. The cross-sectional study of dietary stabilisation showed that the rate of ω-oxidation was dependent on plasma PA levels and not overall adipose tissue stores. In contrast to the elimination of PA, which showed first order kinetics with a half-life of 22.4 days, in a patient followed for 40 days after presentation, 3-MAA
excretion followed second order kinetics. Omega-oxidation did not show any lag phase suggesting that metabolism is dependent on existing enzyme molecules.

Phytanic acid loading was considered unethical in patients with established ARD so the acute response of $\omega$-oxidation in patients with ARD was assessed in a fasting state. Fasting induces ketosis and lipolysis and acute mobilization of PA in hepatocyte and adipocyte fatty acid pools resulting in secretion of very-low density lipoprotein (VLDL) enriched in PA. This process can induce release of 5000 mg (14.8 mmols)/day of PA (50-fold normal)\(^2\). In 4 individuals, who developed ketosis following acute starvation plasma PA increased as expected with a doubling time of 29 hours and in 4 patients (80%) a rise in urinary 3-MAA levels was seen with the plasma PA following first order kinetics and 3-MAA second order kinetics respectively. One other patient showed no change in PA or 3-MAA in response to fasting possibly due to non-compliance. The release kinetics of phytanic acid showed a doubling time of 29.2 hours, which is slower than that associated with secretion of VLDL (2-4 hours) and implies that phytanic acid must be mobilised slowly from an internal hepatocyte pool or from adipose tissue. The acute stress used in this study was insufficient to saturate the $\omega$-oxidation pathway as rises in PA levels paralleled those in 3-MAA. The findings suggest that $\omega$-oxidation processes are similar in patients with ARD and healthy controls in response to acute PA loads. However, delayed symptoms associated with a relapse of disease were seen in 3 patients with increases in 2 patients with plasma PA\(^10\). Another patient showed an asymptomatic rise in plasma PA after the study. These findings confirm that the acute signs of ARD are secondary to increases in plasma levels rather than on total body phytanic acid stores.

In one patient studied sequentially PA clearance through $\omega$-oxidation was initially 32% of total phytanic acid metabolism but rose to 100% by 40 days as PA levels fell. The exact
enzymology of the ω-oxidation pathway in ARD is obscure but in plants and humans ω-oxidation of other fatty acids occurs through the microsomal cytochrome P₄₅₀CYP4A system while structurally related tocopherols (vitamin E) are metabolised by a cytochrome CYP3A enzyme. The stereochemistry of the ω-oxidation reaction may be relevant to its activity but has not been investigated. Phytanic acid exists in a 2:1 racemic mixture of R- and S-epimers and α-oxidation occurs through initial non-stereospecific steps before α-methylacyl-CoA racemase is required to allow entry of R-epimers into the β-oxidation pathway. ω-Oxidation of the terminal methyl groups of phytanic acid introduces a new chiral centre (figure 5). The stereochemistry of the ω-dicarboxylic acid would depend on whether the pro-R or pro-S methyl group was hydroxylated and oxidized to the corresponding carboxylic acid. It is unclear whether the initial hydroxylation step is stereospecific. In bile acid metabolism an analogous reaction occurs in which 27-hydroxylation of cholesterol gives rise to 25S-alcohol and 25S-carboxylic acid derivatives. The cytochrome P₄₅₀ enzyme CYP27A that performs this reaction has a wide substrate range but whether it has a role in the metabolism of phytanic acid is unknown. If the hydroxylation is not stereoselective then the same racemase which functions in the α-oxidation pathway may be able to convert the R-epimer to the S-epimer (as its coenzyme A derivative). Degradation of the 15S-methyl fatty acyl-CoA derivative by β-oxidation would give the C15 and C10 2-methyl fatty acyl-CoA esters with the S-stereochemistry. Phytanic acid itself exists as a 2:1 mixture of 3R- and 3S-epimers, and it is anticipated that this stereochemistry will be preserved in 3-MAA. Since 3-MAA is significantly soluble in aqueous environments it is likely that it will be excreted in preference to undergoing further β-oxidation.

The data from the newly presented patient allows some suggestions to be made about the metabolism of PA in ARD. Initially PA levels exceed the capacity of the residual α- and ω-
oxidation pathways. Excess PA is still excreted so another alternative low affinity pathway for PA excretion has to exist. Phytanic acid is hydrophobic and is usually transported in lipoproteins so it would need to be made into a hydrophilic metabolite for renal excretion\textsuperscript{23}. Hepatic conjugation would fulfil this function. One candidate for this role would be glucuronidation of phytanic acid, which would be consistent with the finding of phytanic acid in urine\textsuperscript{24}. Another possibility is non-specific renal loss due to nephropathy. Though nephropathy has been described in some patients with ARD\textsuperscript{25} only one of the patients studied here showed any evidence of hypokalaemia, though no evidence of aminoaciduria or glycosuria. Later, $\omega$-oxidation is he principal route for PA excretion. This pathway is predominantly located in the liver as fibroblasts from patients with ARD have only a limited capacity to metabolise phytanic acid\textsuperscript{13,26-28} so the process of PA elimination is dependent on reverse PA transport from tissues probably in high-density lipoprotein (HDL) cholesterol\textsuperscript{23}. The relationship of $\omega$-oxidation capacity to HDL was not assessed in this study. It is likely that long-term PA values in patients on stable diets are dependent on residual $\alpha$-oxidation capacity, as most mutations in phytanoyl-CoA hydroxylase do not completely inhibit activity\textsuperscript{29}, as well as PA concentration–dependent $\omega$-oxidation. This may explain the large heterogeneity and variability in response to diet despite high compliance seen in patients with ARD.

This study shows that $\omega$-oxidation pathway can theoretically metabolise all the phytanic acid ingested in patients with ARD on a low phytanic acid diet. However, its main role seems to be to provide functional reserve capacity that enables patients with ARD undergoing acute stress to cope with limited increases in plasma PA levels. As $\omega$-oxidation is capable of large increases in activity and is most likely mediated through P\textsubscript{450} cytochrome enzymes it forms a good candidate for therapeutic interventions to induce enzyme activity and reduce PA levels in ARD.
Acknowledgments

Professor J Billimoria and Dr. M Clemens developed the methods used to assay PA and 3-MAA. This study was supported by a grant from the North West Thames Regional Health Authority and European Community Biomed 5 grant RDDPT.
Table 1

Plasma phytanic acid (PA) concentrations and urinary 3-methyl-adipic acid (3-MAA) excretion in controls, obligate heterozygotes and patients with adult Refsum’s disease (ARD) in response to phytanic acid loading.

p=0.05; ***p <0.001  Note + = mean value ND= not done

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<th>Number</th>
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<td>3.35 (2.3-4.3)†</td>
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<td></td>
<td></td>
<td>2.7 (2-4)</td>
<td>15.3 (10.3-26.0)†</td>
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<td></td>
<td>2</td>
<td>2.5 (2-3)</td>
<td>5.2 (3.6-6.9)†</td>
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<td></td>
<td></td>
<td>3.5 (3-4)</td>
<td>12.4 (8.8-15.9)†</td>
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<tr>
<td>ARD</td>
<td>11</td>
<td>410 (46-1127)***</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ND</td>
<td>5.4 (1.9-16.0)*</td>
</tr>
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</table>

**Note:**
- †: Significant difference from baseline.
- *: Significant difference between groups.
Table 2

Effects of low phytanic acid diet on nutritional parameters in patients with adult Refsum’s disease (ARD) prior to initiation (pre) and after (post) 6 weeks of dietary therapy. P:S = Polyunsaturated: saturated fatty acid intake.

2 patients did not complete the assessment protocol and are excluded.

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<td>2.7 0.5</td>
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<td>27.6 30.8</td>
<td>1.2 1.3</td>
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<td>6.8</td>
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<td>53-948 7-676</td>
<td>2-14</td>
</tr>
</tbody>
</table>
Table 3

Plasma phytanic acid and urinary 3-methyl-adipic acid excretion in 5 patients with adult Refsum’s disease (ARD) in response to a prolonged (56-hour) fast.

<table>
<thead>
<tr>
<th>Number</th>
<th>Plasma phytanic acid</th>
<th>Urine 3-methyl-adipic acid</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/L</td>
<td>µg/mg creatinine</td>
<td>hours</td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>1</td>
<td>151</td>
<td>632</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>258</td>
<td>409</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>388</td>
<td>1028</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>410</td>
<td>474</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>1210</td>
<td>1417</td>
<td>13.0</td>
</tr>
<tr>
<td>Median</td>
<td>388</td>
<td>632</td>
<td>4.8</td>
</tr>
<tr>
<td>% change</td>
<td>-</td>
<td>163(115 - 419)</td>
<td>219 (-16 – +235)</td>
</tr>
</tbody>
</table>
Figure 1

Fall in plasma phytanic acid (♦) and excretion of 3-methyladipic acid (▲) expressed as molar equivalent phytanic acid metabolised in a newly-presented patient with Refsum’s disease presented as best-fit exponential and hyperbolic trend lines.
Figure 2

Excretion of 3-methyl-adipic acid and plasma phytanic acid in 11 patients with adult Refsum’s disease
Figure 3

Plasma phytanic acid (PA) concentration in response to fasting in 5 patients with adult Refsum’s disease assuming first order kinetics.
Figure 4. Changes in plasma 3-methylmalonic acid (3-MAA) in response to fasting in 3 patients with adult Refsum’s disease assuming second order kinetics.
Figure 5: The metabolism of phytanic acid by $\omega$-oxidation and subsequent $\beta$-oxidation to 3-methyladipic acid.

Abbreviations: CoA = coenzyme A, AMACR, $\alpha$-methylacyl-CoA racemase. Phytanic acid exists as a 2:1 mixture of the (3$R$)- and (3$S$)-epimers [1], and this stereochemistry is preserved upon degradation via the C-10 $\omega$-2 thioester (2,6 dimethyloctanoic acid [2,6-DMOA]-ester) to 3-methyladipic acid (3-MAA).
Reference List


