Metabolism of phytanic acid and 3-methyl-adipic acid excretion in patients with adult Refsum disease

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Abstract Adult Refsum disease (ARD) is associated with defective α-oxidation of phytanic acid (PA). ω-Oxidation of PA to 3-methyl-adipic acid (3-MAA) occurs although its clinical significance is unclear. In a 40 day study of a new ARD patient, where the plasma half-life of PA was 22.4 days, ω-oxidation accounted for 30% initially and later all PA excretion. Plasma and adipose tissue PA and 3-MAA excretion were measured in a cross-sectional study of 11 patients. The capacity of the ω-oxidation pathway was 6.9 (2.8–19.4) mg [20.4 (8.3–57.4) μmol] PA/day. 3-MAA excretion correlated with plasma PA levels ($r = 0.61$; $P = 0.03$) but not adipose tissue PA content. ω-Oxidation during a 56 h fast was studied in five patients. 3-MAA excretion increased by 208 ± 58% in parallel with the 158 (125–603)% rise in plasma PA. Plasma PA doubled every 29 h, while 3-MAA excretion followed second-order kinetics. Acute sequelae of ARD were noted in three patients (60%) after fasting. The ω-oxidation pathway can metabolise PA ingested by patients with ARD, but this activity is dependent on plasma PA concentration. ω-Oxidation forms a functional reserve capacity that enables patients with ARD undergoing acute stress to cope with limited increases in plasma PA levels.—Wierzbicki, A. S., P. D. Mayne, M. D. Lloyd, D. Burston, G. Mei, M. C. Sidey, M. D. Feher, and F. B. Gibberd. **Metabolism of phytanic acid and 3-methyl-adipic acid excretion in patients with adult Refsum disease.** ***J. Lipid Res.*** **2003.** 44: **1481–1488.**

Supplementary key words organic acid • α-oxidation • ω-oxidation

Adult Refsum disease (ARD) (*Heredopathia atactica polyneutiformis*) (On-line Mendelian Inheritance in Man; OMIM 265000) is an autosomal recessive disease that presents with retinitis pigmentosa, anosmia, peripheral neuropathy, deafness, and ataxia with additional ichthyosis and cardiomyopathy in severe cases (1–3). Many cases are caused by mutations in phytanoyl-CoA hydroxylase, resulting in increased plasma phytanic (3,7,11,15-tetramethyl-hexadecanoic) acid levels and accumulation of phytanic acid (PA) in all fat-containing tissues (1, 2). Normal metabolism of PA involves peroxisomal α-oxidation, but an additional low-capacity pathway through ω-oxidation and subsequent cycles of β-oxidation result in production of a variety of 3-methyl-organic acids, including 3-methyl-adipic acid (3-MAA; 3-methyl-hexanedioic acid) (4–6). Refsum disease is treated by the restriction of PA intake or physical removal of PA by plasmapheresis or apheresis (7–9). In patients on an appropriate diet, PA levels fall gradually due to ω-oxidation. In fasting and acutely ill patients, PA 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 ω-oxidation pathway to the metabolism of PA by measuring 3-MAA excretion in patients with ARD.

METHODS

This study received ethical approval, and all patients and controls gave informed consent for the investigations performed.

Patient selection

Healthy controls and heterozygotes for ARD. Four healthy volunteers [three male, one female; median age 32 (range 30–41) years] and two obligate heterozygotes (one male, one female) from a consanguineous family with Refsum disease were recruited. Each subject was stabilized on the low-PA diet (Westminster) for 24 h, and the next morning blood and urine samples were taken (7, 10, 11). Oral PA loading was performed using 25 ml of Maxepa oil (7.5 mg; 22.2 μmol PA/ml) in 75 ml orange juice. Blood and double-void technique urine samples were collected at 6 h intervals.

Manuscript received 19 March 2003 and in revised form 9 April 2003.

Published, JLR Papers in Press, April 16, 2003.
DOI 10.1194/jlr.M300121-JLR200

Abbreviations: 2,6-DMOA, 2,6-dimethyloctanedioic acid; 3-MAA, 3-methyl-adipic acid.

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Dietary stabilization of patients with ARD. Eleven patients with ARD diagnosed clinically and with plasma PA levels >200 μmol/l (normal <30 μmol/l) were recruited and had initial biochemical investigations performed. Patients were stabilized on a low-PA diet for over 12 weeks with a monthly dietetic review. Clinical biochemical analyses comprising blood PA, triglycerides, glucose, urinary 3-MAA, creatinine, and ketones were taken at the start and end of the dietary stabilization 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 stabilization. Tissue PA content was measured and compared with blood and urine PA, creatinine and 3-MAA measurements.

Fasting studies in patients with ARD

A subset of five patients stabilized 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 h. Clinical biochemical analyses comprising blood PA, triglycerides, and glucose and urinary 3-MAA, creatinine, and ketones were performed every 12 h. At the end of the study, patients were restarted on an oral low-PA diet, were discharged home after 3 days, and were reviewed monthly until well.

Biochemical assays

Measurement of PA. PA was measured by established methods using methanol-chloroform extraction and gas chromatography (12). Plasma (0.5 ml) was extracted with 6 ml chloroform-methanol (2:1, v/v) and centrifuged at 1,900 *g* for 10 min at 4°C. One hundred microliters of 3-methyl-pentadecanoate (Sigma, Poole, UK) dissolved in hexane (0.728 mg/ml) was added as an internal standard to a 3 ml aliquot of the organic phase followed by 5 ml of 0.5 M methanoic sodium hydroxide (20 g/l methanol). The solution was heated at 100°C for 5 min, cooled, and 5 ml of boron trifluoride (14% w/v) was added and the sample was heat treated as before for 4 min. After cooling 30 ml of saturated sodium chloride, 15 ml of petroleum ether was added and the aqueous layer removed and filtered through phase separation paper (Whatman, Maidstone, UK). The aqueous layer was reextracted with 20 ml of petroleum ether and then the extract was evaporated to dryness, resuspended in 1 ml hexane, and reevaporated. The purified extract was dissolved in 0.3 ml hexane for injection.

PA was measured by gas chromatography (Varian 3700; Varian Ltd., Walton-on-Thames, UK) fitted with a 25 m 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 flame ionization detector temperature 300°C. Peaks were integrated on a Varian CDS100 integrator. The PA peak was identified from log retention time after injection of 1 μl and concentration calculated relative to methylphytanate (Phase Separations, Chester, UK) and methyl-pentadecanoate internal standards. The between-batch coefficient of variation was 5.6% (n = 5) and biological variation 7.7% (n = 9).

Measurement of 3-MAA. 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 (50 μl; 1 mg/ml) were added to 1 ml of fresh urine. The urine was acidified with three 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 were separated by centrifugation. The aqueous phase was reextracted twice more. Supernatant fractions were combined and dried down under nitrogen. The residue was redissolved in ethyl acetate and redried. The organic acids were derivatized in 30 μl of acetonitrile and 30 μl bis-(tri-methylsilyl)trifluoroacetamide.

Organic acids were analyzed by gas chromatography using a 25 m BP1 column (SGE). One microliter sample was injected with a split ratio of 1:100 and a temperature gradient from 90–250°C increasing at a rate of 8°C/min after an initial 4 min isothermal stage. Peaks were detected by flame ionization 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 were analyzed using GBStat 7.0 (Dynamic Microsystems, Silver Spring, MD). As only small numbers of patients were investigated and distributions of results were non-Gaussian, data were analyzed by nonparametric statistics or after log transformation. Multiple regression analysis was performed to investigate the dependence of plasma PA and 3-MAA levels on demographic, anthropometric, and biochemical variables. $P <$ 0.05 was considered significant. Kinetic plot analysis was conducted using sequential data in one newly presented patient and in five patients in response to fasting. Linearity of plots against time of natural logarithm plot of current to basal concentrations ([C]) (i.e., Ln $[C]/[C_o]$) and respective inverse concentrations (i.e., $1/[C]$ $1/[C_o]$) 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 PA and 3-MAA elimination on a low-PA diet (**Fig. 1**). PA elimination followed an approximately exponential course with first-order kinetics ($\ln \{[S]/[So]\} = -kt$) over 40 days, with a plasma half-life for PA 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 (Fig. 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 PA ($\beta = -1{,}962$; $P = 0.05$), 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 $1/[C] - 1/[C_o]$, and therefore demonstrated second-order kinetics.

PA loading studies in healthy controls and heterozygotes for ARD

Baseline levels of PA (2,000%) and 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 two heterozygotes for ARD (5.2 mg 3-MAA/g creatinine) compared with four healthy controls (3.4 mg 3-MAA/g creatinine). Ingestion of 187.5 mg (555 μmol) of PA by the control group led to an ~500% increase in urinary

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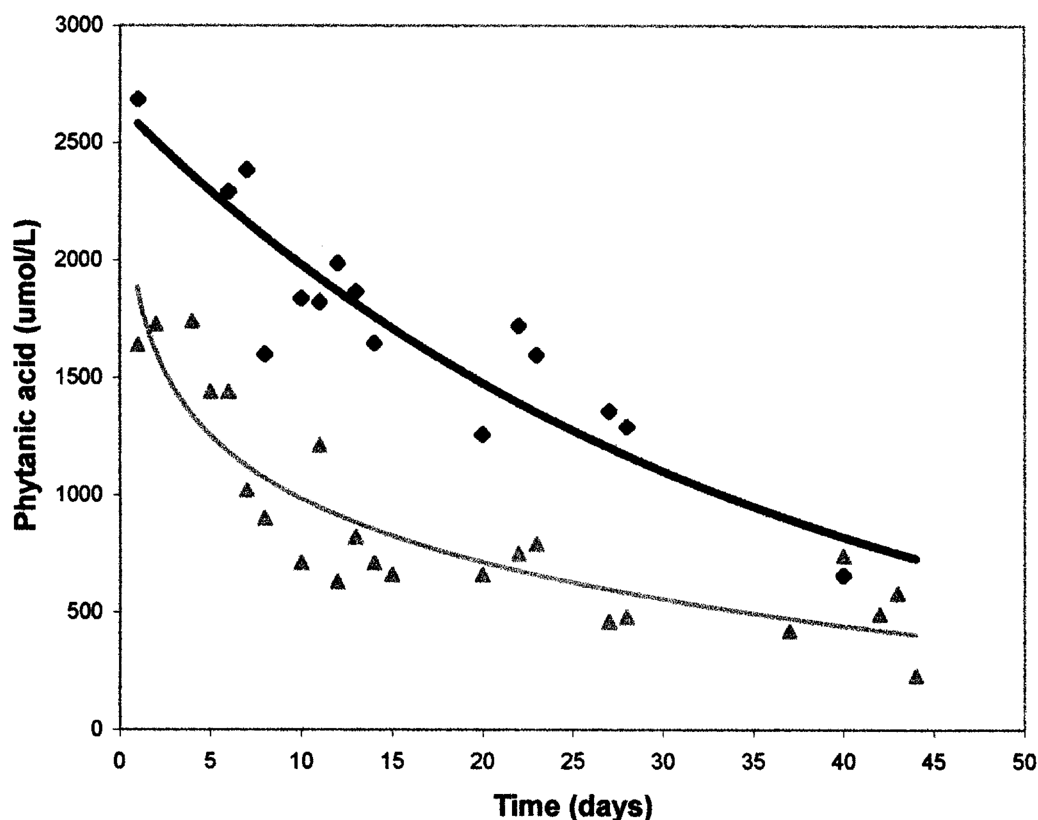

Fig. 1. Plasma PA concentration in response to fasting in five patients with ARD assuming first-order kinetics.

3-MAA levels within 14 h, with no increase in plasma PA levels in both groups. No difference in 3-MAA production in response to the PA load was seen between the healthy controls and the ARD heterozygotes.

Studies with supervised low-PA diets in patients with ARD

Responses to a supervised low-PA 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 PA levels despite a 3.2% increase in fat-derived calories from an initial median intake of 2.7 mg (8.0 μmol) PA/day, and plasma levels of 628 (46–2,336) μmol/l of PA. However, two patients did not complete the diet assessment and were excluded from the analysis. Two patients, despite supervision, showed an increase in PA intake as measured by the food intake questionnaire, yet their PA levels fell over the period of the study, demonstrating that either acute variations in dietary intake were identified in the questionnaire or the slow kinetics of plasma PA response to dietary-induced changes. The median subcutaneous fat biopsy PA concentration was 782 (217–1,458) mg [2.31 (0.64–4.31) mmol] 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 PA content correlated nonsignificantly with age ($\beta = 30.4$; $P = 0.1$) and log PA level ($\beta = 318$; $P = 0.19$), indicating the need for high-concentration gradients and exposure that lead to accumulation of PA.

TABLE 1. Plasma phytanic acid concentrations and urinary 3-methyladipic acid excretion in controls, obligate heterozygotes, and patients with adult Refsum disease in response to phytanic acid loading

		Plasma PA		Urine 3-MAA	
Group	Number	Pre	Post	Pre	14 h
		μmol/l		*mg/g creatinine*	
Controls	4	2.7 (2–4)	2.7 (2–4)	3.35 (2.3–4.3)[b]	15.3 (10.3–26.0)[b]
Heterozygotes	2	2.5 (2–3)	3.5 (3–4)	5.2 (3.6–6.9)	12.4 (8.8–15.9)[b]
ARD	11	410 (46–1127)[a]	ND	5.4 (1.9–16.0)[c]	ND

ND, not done; PA, phytanic acid; 3-MAA, 3-methyl-adipic acid.
[a] $P < 0.001$.
[b] Mean value.
[c] $P = 0.05$.

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TABLE 2. Effects of low-PA diet on nutritional parameters in patients with adult Refsum disease prior to initiation (pre) and after (post) 6 weeks of dietary therapy

Case	PA Intake		Protein		Fat		P:S Ratio		Plasma PA		3-MAA Excretion
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
	mg/d		*g/d*		*% total calories*				*μmol/l*		*mg/day*
1	14.0	0.0	96	78	27.5	33.6	1.7	2.2	833	589	11.6
2	10.0	0.0	127	59	32.5	36.1	2.0	1.7	709	422	8.1
3	0.46	0.0	72	114	26.0	37.6	1.1	1.0	652	446	7.8
4	1.3	0.9	67	49	28.9	31.6	1.2	1.8	565	455	6.8
5	2.7	0.5	89	119	23.4	38.1	1.4	1.2	941	546	14.4
6	1.5	5.3	86	84	21.6	27.0	0.8	0.6	53	16	1.9
7	0.8	2.1	68	99	21.7	18.0	1.2	0.5	413	676	6.0
8	52.4	16.0	82	85	29.2	31.4	0.5	0.8	173	7	2.7
9	5.8	0.0	66	62	25.2	27.4	1.8	2.3	113	262	4.8
Average(median)	2.7	0.5	82	86	27.6	30.8	1.2	1.3	565	446	6.8
SD (range)	0.4–52.4	0–16	19	21	4.7	5.8	0.5	0.7	53–948	7–676	2–14

P:S, polyunsaturated:saturated fatty acid intake. Two patients did not complete the assessment protocol and are excluded.

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

Response of patients with ARD to prolonged fasting

Five patients agreed to fast, and all developed ketonuria within 48 h. Four were able to tolerate fasting for >48 h and one for 36 h. Peak 3-MAA excretion increased by 208 ± 58% in all patients up to 52 h, after which rates seemed to plateau (two 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 three patients, this was correlated with a rise

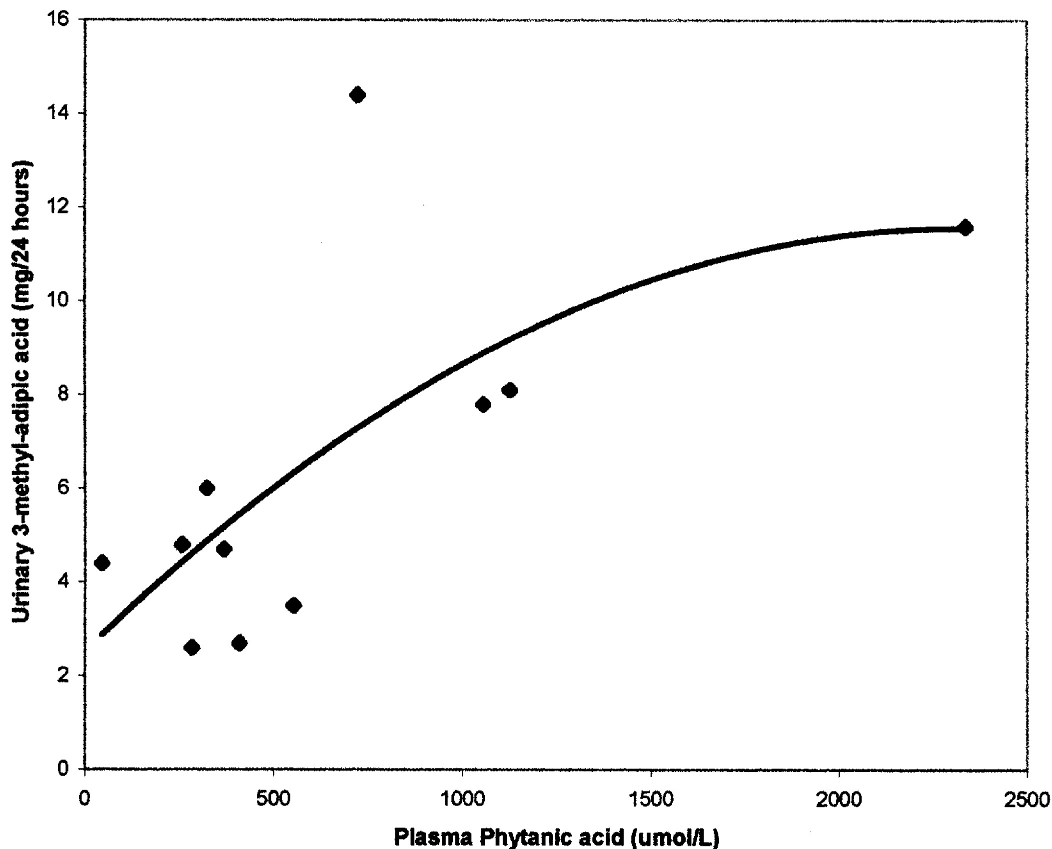

Fig. 2. Excretion of 3-MAA acid and plasma PA in 11 patients with adult Refsum disease (ARD).

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TABLE 3. Plasma PA and urinary 3-MAA excretion in five patients with adult Refsum disease in response to a prolonged (56 h) fast

Number	Plasma PA Initial	Plasma PA Final	Urine 3-MAA Initial	Urine 3-MAA Final	Duration
	μmol/l		*μg/mg creatinine*		*h*
1	151	632	5.8	10.5	56
2	258	409	4.8	11.3	32
3	388	1,028	3.6	7.8	56
4	410	474	2.7	5.8	40
5	1,210	1,417	13.0	10.7	56
Median	388	632	4.8	10.5	56
% Change	—	163(115–419)	—	219(−16–235)	

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. PA release into plasma followed first-order kinetics in all five patients (**Fig. 3**) and showed a doubling time of 29.2 h. The production of 3-MAA showed no obvious latency and was consistent with second-order kinetics in the three patients who could be evaluated (**Fig. 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 and 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 three cases. One lost 8 kg in weight, a second felt nonspecifically unwell for 12 weeks and needed hospital admission for exhaustion, while a third patient developed leg cramps for 3 days afterwards.

DISCUSSION

PA 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-MAA (13).

This study included seven patients whose genetic defect has been mapped to chromosome 10, indicating they were likely to have mutations in phytanoyl-CoA hydroxylase (14). Family sizes were too small to allow mapping of the disease in four other patients. In one newly presenting ARD patient, long-term treatment with a low-PA diet that reduced intake from 60 mg (178 μmol)/day to <20 mg (60 μmol)/day resulted in a gradual fall in plasma and tissue PA levels, with a plasma half-life for PA of 22.4 days following classical first-order kinetics (1, 10). This study, like others, shows that significant ω-oxidation occurs in patients with acute and chronic ARD, with increased levels of 3-MAA compared with normal

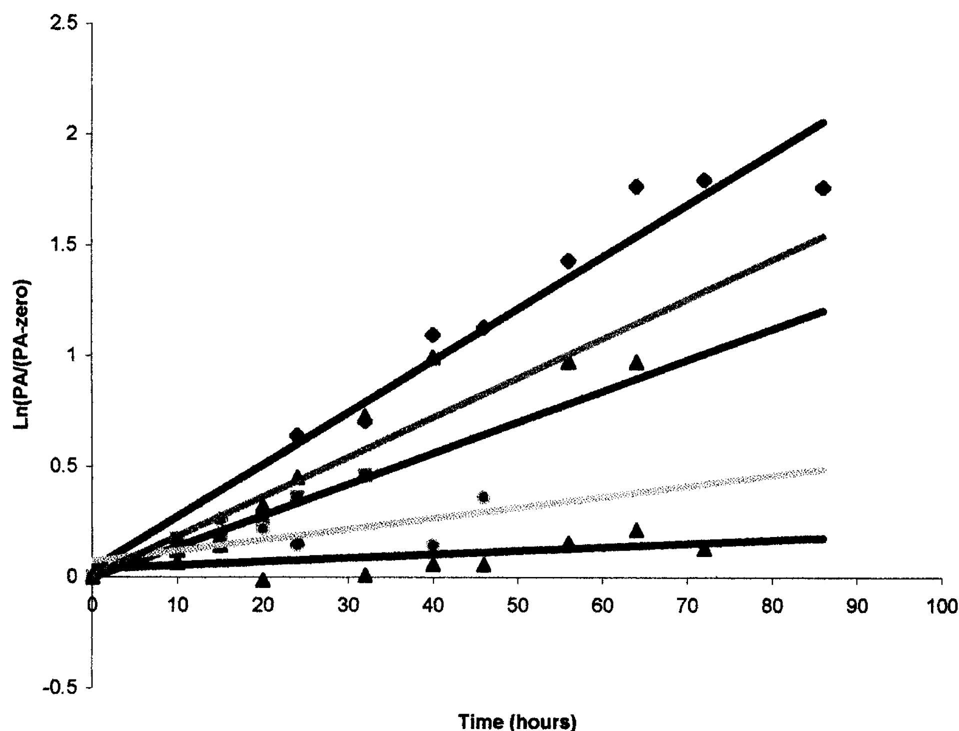

Fig. 3. Fall in plasma phytanic acid (PA) (diamonds) and excretion of 3-methyl-adipic acid (3-MAA) (triangles) expressed as molar equivalent PA metabolized in a newly presented patient with Refsum disease presented as best-fit exponential and hyperbolic trend lines.

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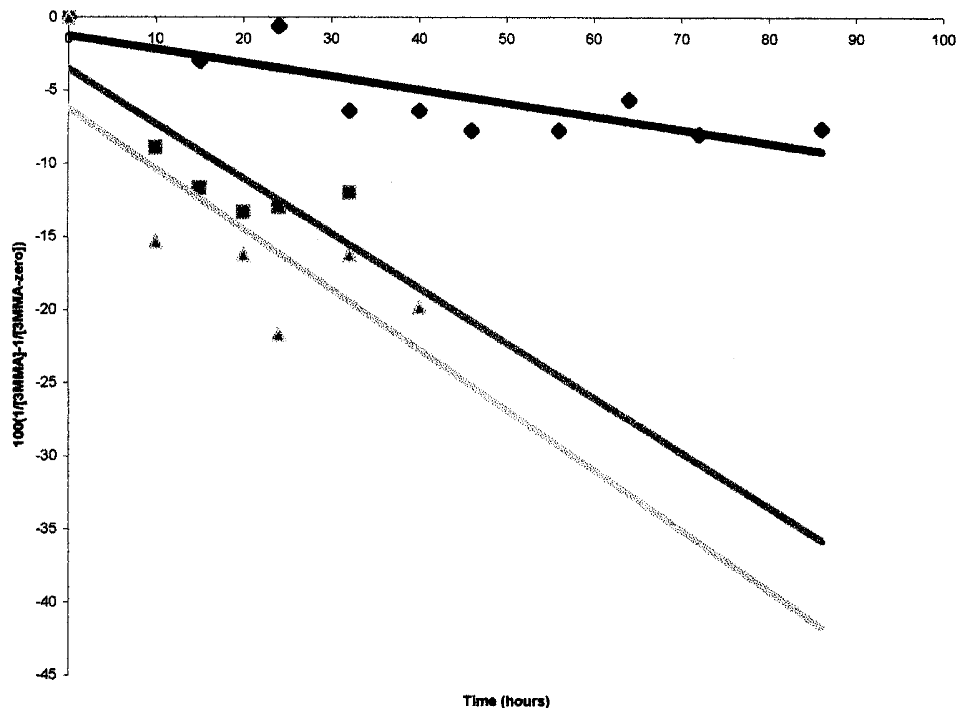

Fig. 4. Changes in plasma 3-MAA in response to fasting in three patients with ARD assuming second-order kinetics.

controls (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 postprandial 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 (**Fig. 5**). Based on 2,6-DMOA excretion in two patients, the capacity of the ω-oxidation pathway has been suggested to be 30 mg PA (89 μmol)/day (15). 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) μmol]/day in patients consuming 2.7 (0–53) mg [8(0–16) μmol]/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 with a normal daily intake of 165 mg (0.5 mmol)/day of PA, but no data were provided on the success of dietary therapy (15). 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 that occur in ARD (16) 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 stabilization 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. ω-Oxidation did not show any lag phase, suggesting that metabolism is dependent on existing enzyme molecules.

PA loading was considered unethical in patients with established ARD, so the acute response of ω-oxidation in patients with ARD was assessed in a fasting state. Fasting induces ketosis, lipolysis, and acute mobilization of PA in hepatocyte and adipocyte fatty acid pools, resulting in secretion of VLDL enriched in PA. This process can induce release of 5,000 mg (14.8 mmol)/day of PA (50-fold normal) (2). In four individuals who developed ketosis following acute starvation, plasma PA increased as expected with a doubling time of 29 h, and in four 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 noncompliance. The release kinetics of PA showed a doubling time of 29.2 h, which is slower than that associated with secretion of VLDL (2–4 h) and implies that PA must be mobilized slowly from an internal hepatocyte pool or from adipose tissue. The acute stress used in this study was insufficient to saturate the ω-oxidation pathway as rises in PA levels paralleled those in 3-MAA. The findings suggest that ω-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 three

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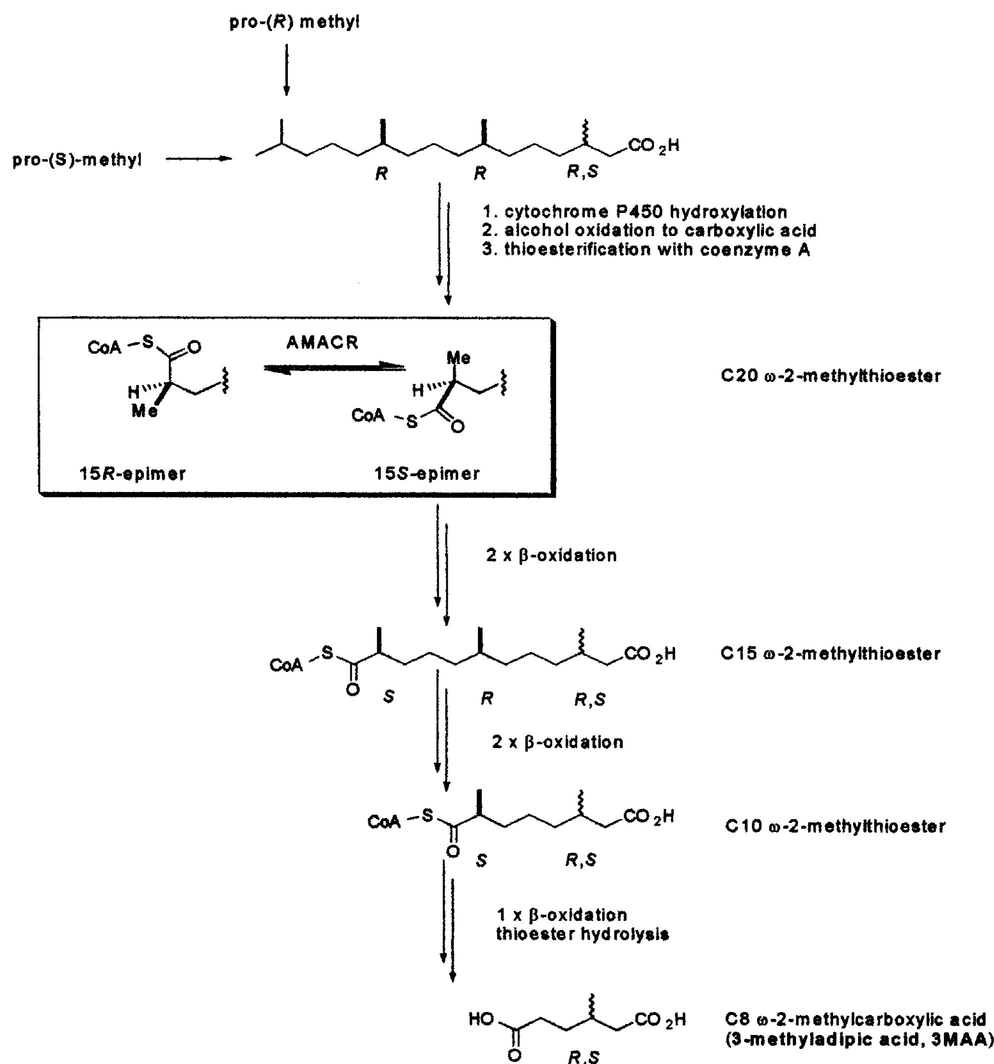

Fig. 5. The metabolism of PA by ω-oxidation and subsequent β-oxidation to 3-MAA.

patients, with increases in two 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 PA stores.

In one patient studied, sequentially, PA clearance through ω-oxidation was initially 32% of total PA 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 P_{450}CYP4A system (17, 18), while structurally related tocopherols (vitamin E) are metabolized by a cytochrome CYP3A enzyme (19). The stereochemistry of the ω-oxidation reaction may be relevant to its activity but has not been investigated. PA exists in a 2:1 racemic mixture of *R*- and *S*-epimers, and α-oxidation occurs through initial nonstereospecific steps before α-methylacyl-CoA racemase is required to allow entry of *R*-epimers into the β-oxidation pathway (1). ω-Oxidation of the terminal methyl groups of PA introduces a new chiral center (Fig. 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 25*S*-alcohol and 25*S*-carboxylic acid derivatives (20). The cytochrome P_{450} enzyme CYP27A that performs this reaction has a wide substrate range, but whether it has a role in the metabolism of PA is unknown. If the hydroxylation is not stereoselective, then the same racemase that functions in the α-oxidation pathway may be able to convert the *R*-epimer to the *S*-epimer (as its CoA derivative). Degradation of the 15*S*-methyl fatty acyl-CoA derivative by β-oxidation would give the C15 and C10 2-methyl fatty acyl-CoA esters with the *S*-stereochemistry. PA itself exists as a 2:1 mixture of 3*R*- and 3*S*-epimers, and it is anticipated that this stereochemistry will be preserved in 3-MAA (21). 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 allow some suggestions to be made about the metabolism of PA in

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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. PA is hydrophobic and is usually transported in lipoproteins, so it would need to be made into a hydrophilic metabolite for renal excretion (22). Hepatic conjugation would fulfill this function. One candidate for this role would be glucuronidation of PA, which would be consistent with the finding of PA in urine (23). Another possibility is nonspecific renal loss due to nephropathy. Though nephropathy has been described in some patients with ARD (24), only one of the patients studied here showed any evidence of hypokalaemia, but no evidence of aminoaciduria or glycosuria. Later, ω-oxidation is the principal route for PA excretion. This pathway is predominantly located in the liver, because fibroblasts from patients with ARD have only a limited capacity to metabolise PA (13, 25–27); therefore, the process of PA elimination is dependent on reverse PA transport from tissues, probably in HDL cholesterol (22). The relationship of ω-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 α-oxidation capacity, because most mutations in phytanoyl-CoA hydroxylase do not completely inhibit activity (28) and PA concentration-dependent ω-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 the ω-oxidation pathway can theoretically metabolize all the PA ingested in patients with ARD on a low-PA diet. However, its main role seems to be to provide a functional reserve capacity that enables patients with ARD undergoing acute stress to cope with limited increases in plasma PA levels. As ω-oxidation is capable of large increases in activity, and is most likely mediated through P_{450} cytochrome enzymes, it forms a good candidate for therapeutic interventions to induce enzyme activity and reduce PA levels in ARD.

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.

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