Peroxisomal disorders

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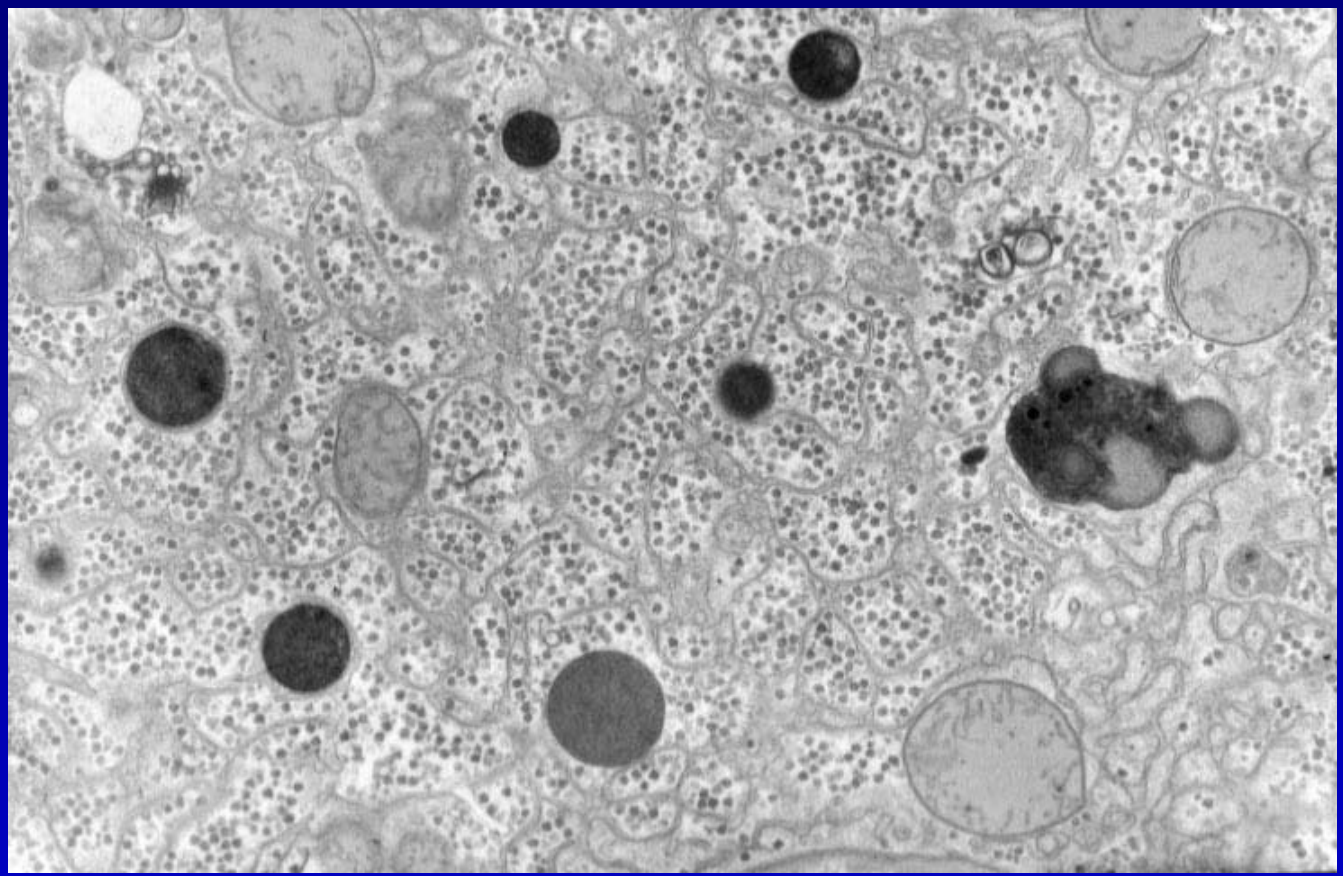
Peroxisomes

- Found in all nucleated mammalian cells
- Initially identified histologically as microbodies in 1950s
- First evidence of biological function in 1960s
- Catalase 'marker' enzyme to remove H_2O_2 generated by several oxidases
- Many other important functions now known

Peroxisome morphology

- Generally appear as spherical single membrane-bound, bodies of 0.1 – 1.0μm in diameter
- The number per cell varies depending on demand. Most numerous in liver and kidney, few in fibroblasts
- Localised by histochemical staining for catalase (DAB) or using immunocytochemical methods
- Peroxisomes are formed from pre-existing organelles
- In rodents but not man induction can be stimulated by clofibrate, plasticisors or hypolipidaemic drugs

Liver EM – DAB stain



Peroxisomal functions

- The importance of these functions is illustrated by the severe consequences of peroxisomal dysfunction, especially in peroxisome biogenesis defects
- Although these defects were first reported in 1973 by Goldfischer, it was not until a decade later that the biochemical abnormalities were really recognised

Peroxisomal functions in man

- Distinct fatty acid β-oxidation system, especially of very long straight-chain fatty acids (C12-27)
- Biosynthesis of ether phospholipids (plasmalogens)
- The α-oxidation of 3-methyl branched chain fatty acids (phytanate)
- Biosynthesis of isoprenoids

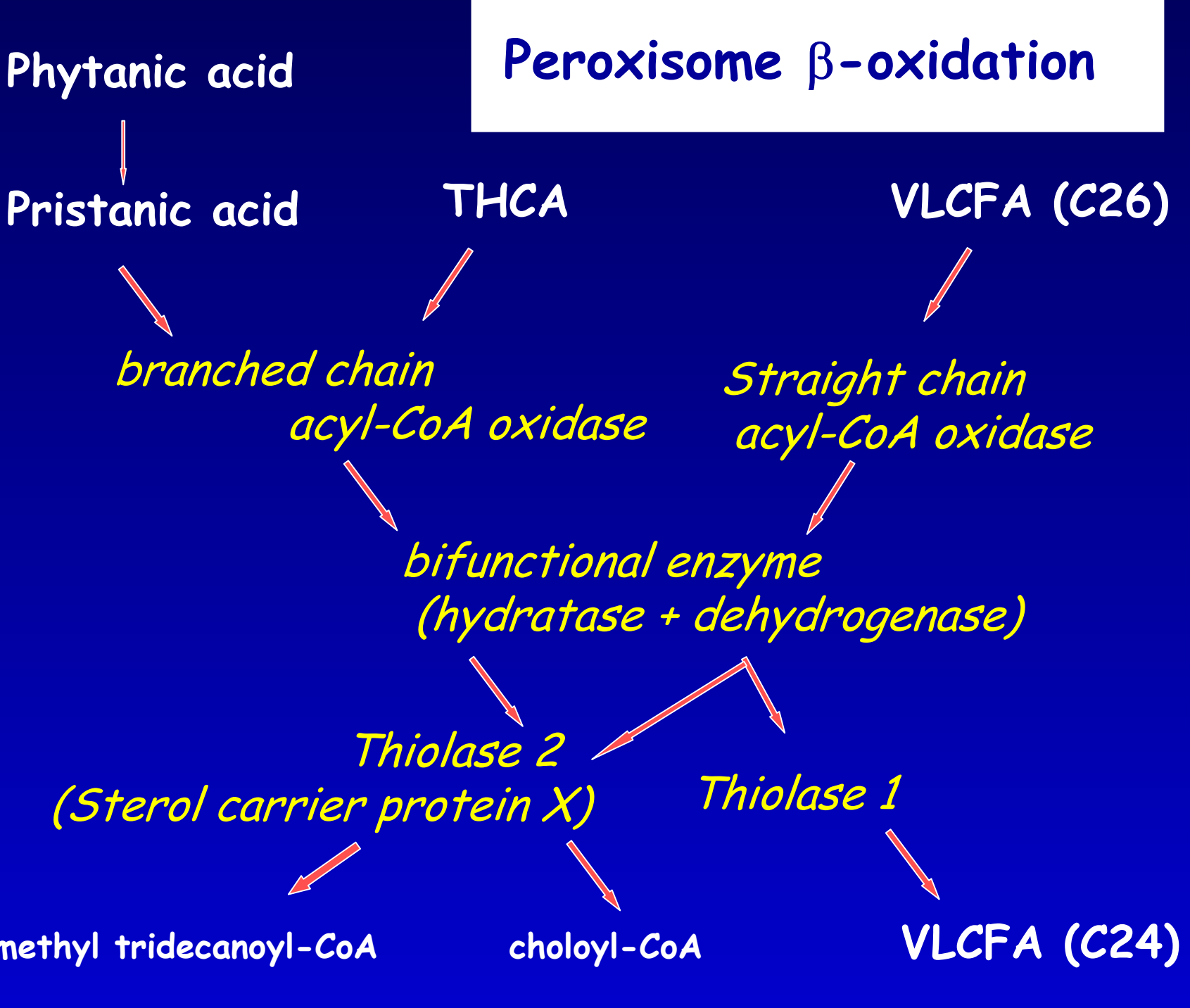
Other peroxisomal functions in man

- Removal of glyoxylate via alanine:glyoxylate amino transferase
- Oxidation of pipecolic acid derived from L-lysine
- Oxidation of glutaryl-CoA
- Elongation of fatty acids
- Unlike mitochondria, the energy generated by various oxidation steps generates heat and H_2O_2, not ATP.

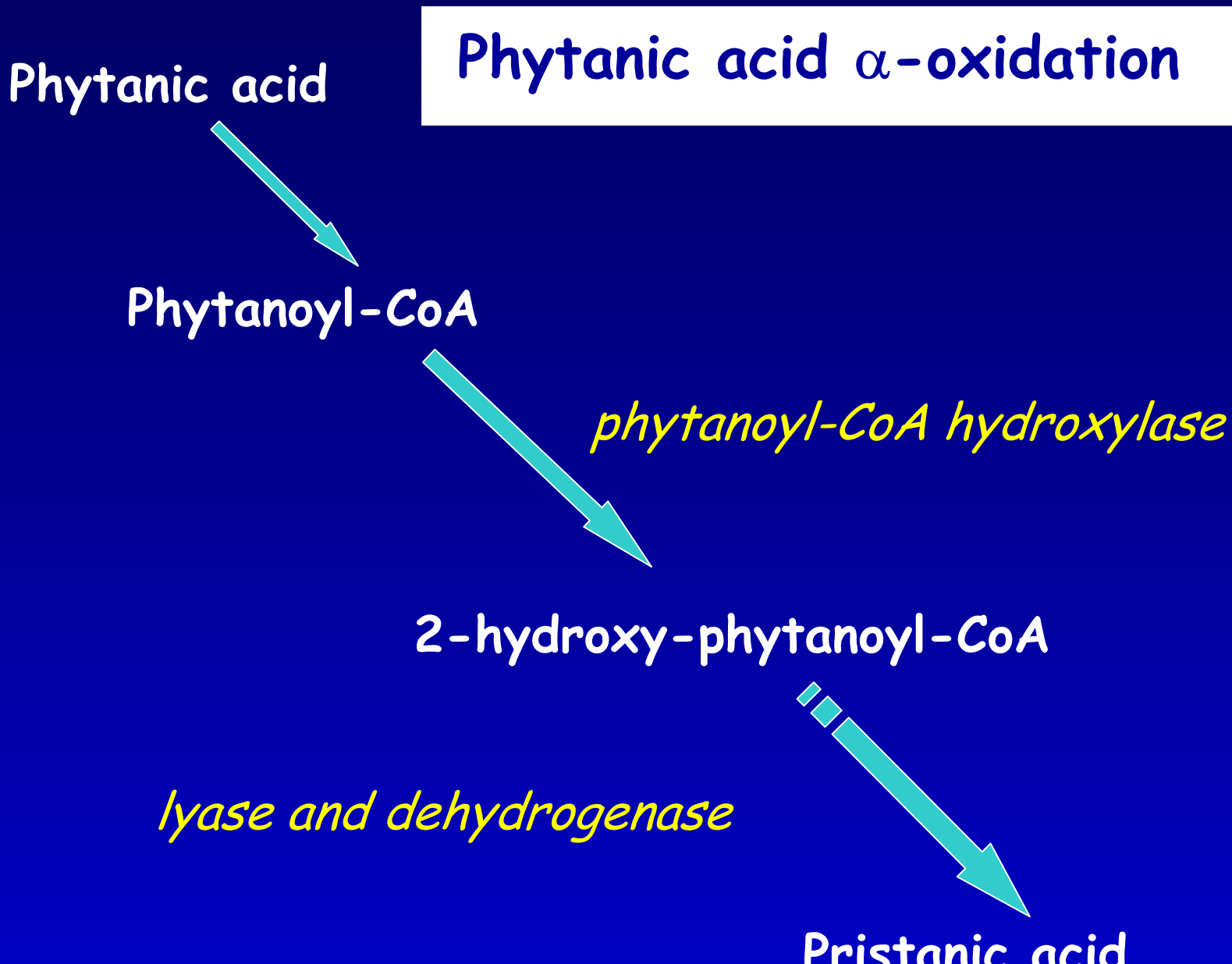
Very long chain fatty acid metabolism

- Very long chain fatty acids (C22-26) are preferentially oxidised by the peroxisome.
- They are taken up into peroxisomes following activation to their CoA ester
- Uptake is via a specific ABC-transporter in the peroxisomal membrane, which is ATP-dependent
- Referred to as ALDP which exists as a homo- or heterodimer

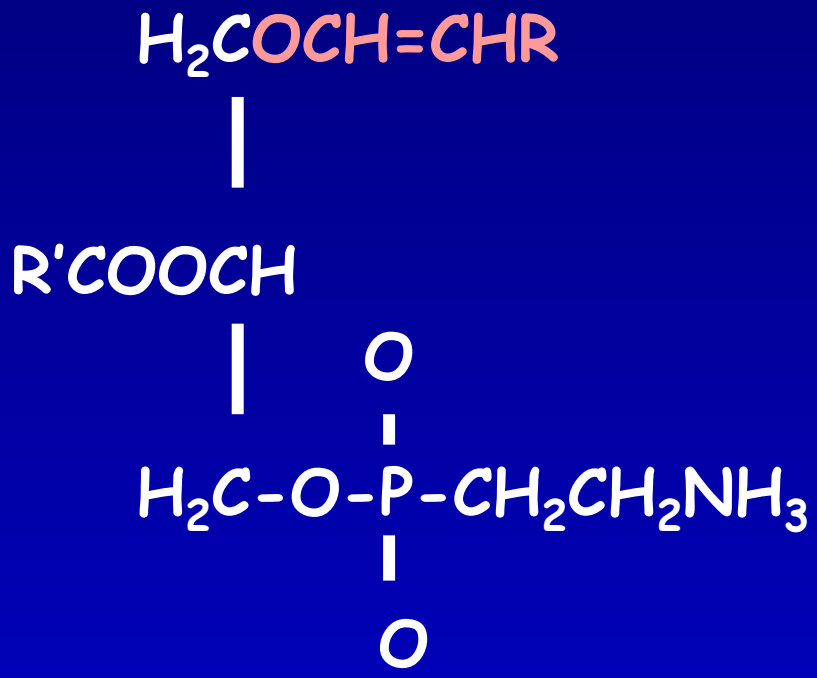
Peroxisome β-oxidation



Phytanic acid α-oxidation

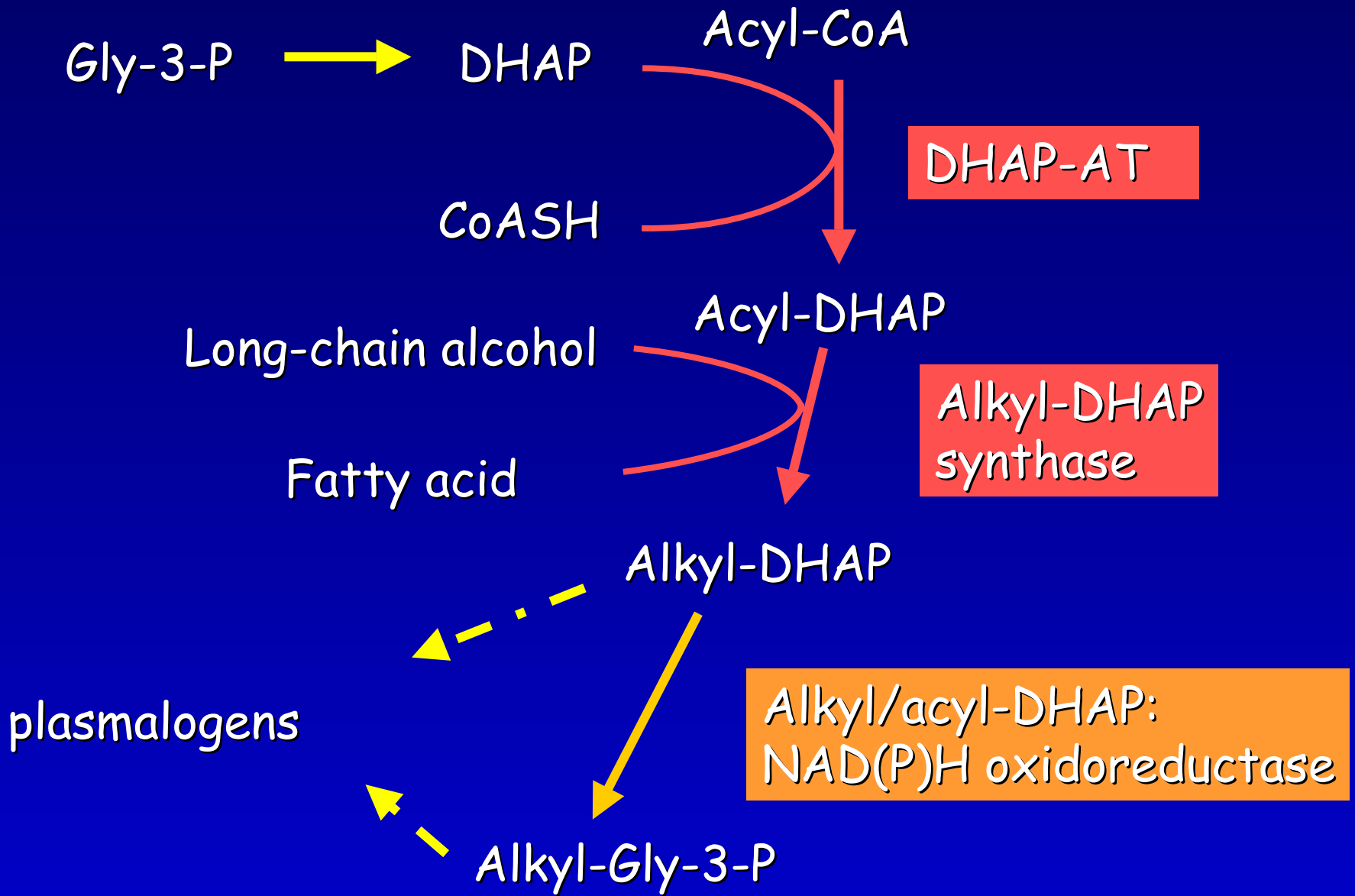


Plasmalogens – ether-linked phospholipids



Plasmalogens represent 5 – 20% membrane phospholipids (30% in myelin) and protect against photosensitivity

Plasmalogen biosynthesis



Peroxisome biogenesis

- Peroxisomal proteins are synthesised on free polyribosomes
- They must carry a specific peptide signal to direct them to pre-existing peroxisomes
- Most matrix proteins carry the Peroxisome Targetting Signal (PTS1) serine-lysine-leucine (SKL)
- A second signal (PTS2) is required for some enzyme proteins, including thiolase, phytanoyl-CoA hydroxylase and DHAP-AT, alkyl-DHAP synthase
- Peroxisome membrane proteins do not use PTS1 or PTS2 for targetting

PEX genes

- Through studies on peroxisome function and defects in biogenesis a number of genes have been identified
- These genes encode mostly peroxisome integral membrane proteins essential for the assembly and biosynthesis of peroxisomes
- The proteins are referred to as peroxins and the genes PEX genes
- Mutations in PEX genes are responsible for peroxisome biogenesis disorders (Zellweger) and RCDP

Peroxisomal disorders and PEX gene disorders: two major categories (www.peroxisome.org)

- PEX gene defects
  - Peroxisome biogenesis disorders including: Zellweger syndrome, neonatal adrenoleukodytrophy and infantile Refsum disease and Rhizomelic chondrodysplasia punctata (PTS2 defect - PEX7 mutations)
- Single enzyme defects (X-ALD, β-oxidation defects, Refsum etc)

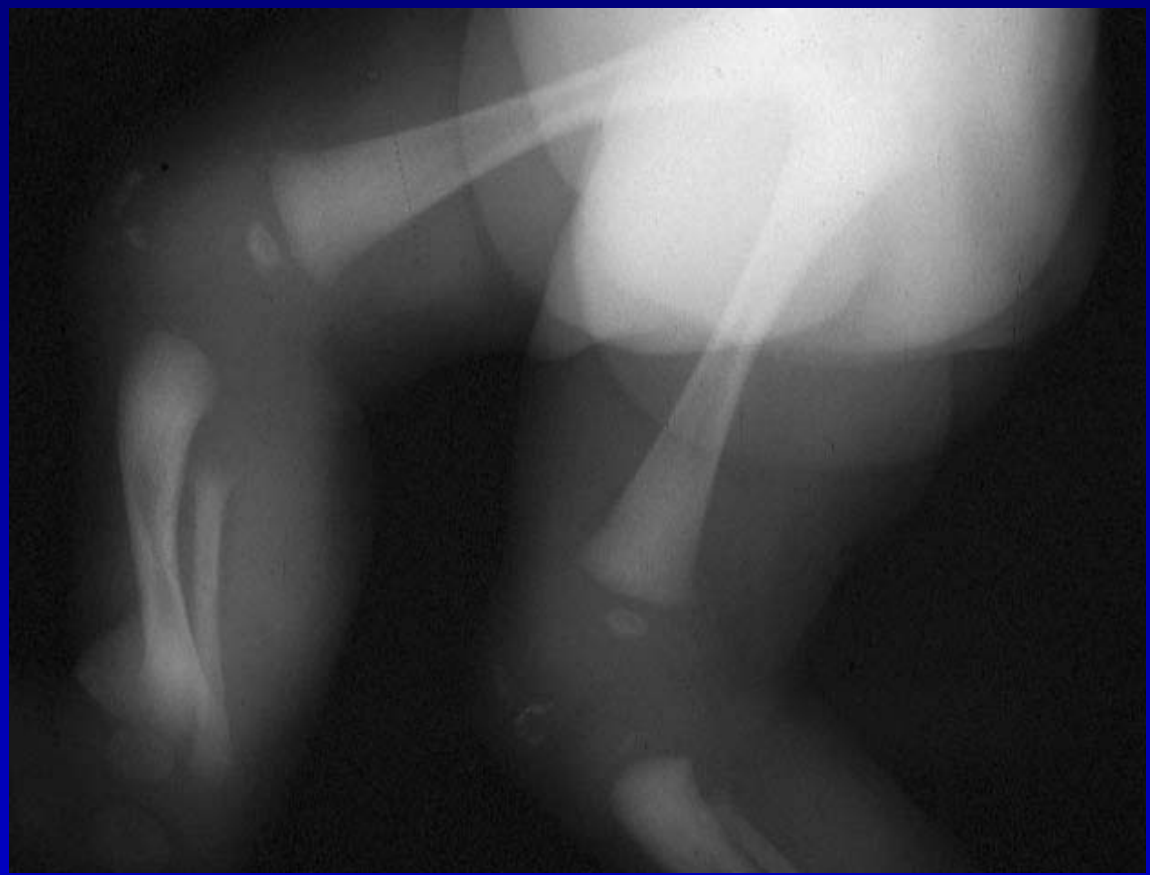
Peroxisome biogenesis disorders

- Zellweger syndrome – most severe phenotype
  - Typical dysmorphology, profound hypotonia, hepatic dysfunction, neonatal presentation
- Neonatal adrenoleukodystrophy
  - Intermediate phenotype
- Infantile Refsum disease – milder phenotype
  - Retinopathy, deafness, mild dysmorphism
- 11 complementation groups, group 1 (PEX1 mutations account for 65% patients) includes all phenotypes

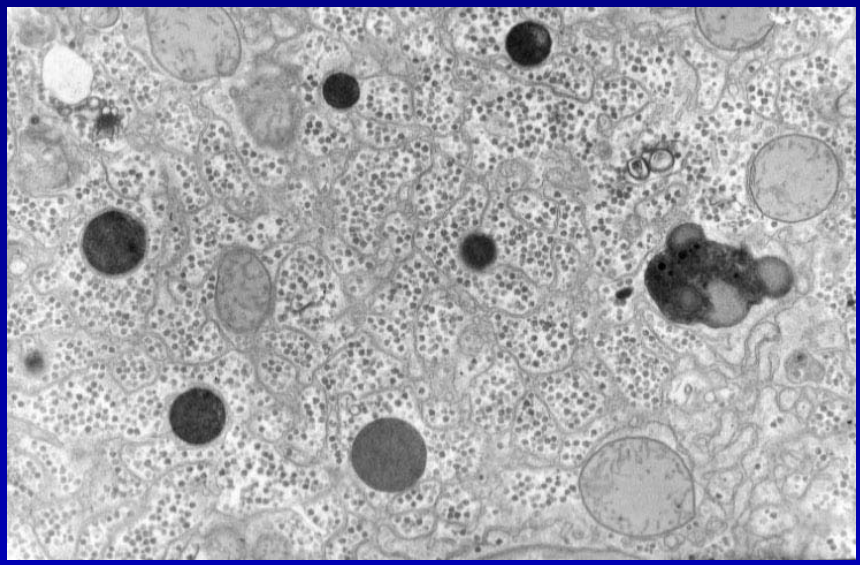
Zellweger syndrome

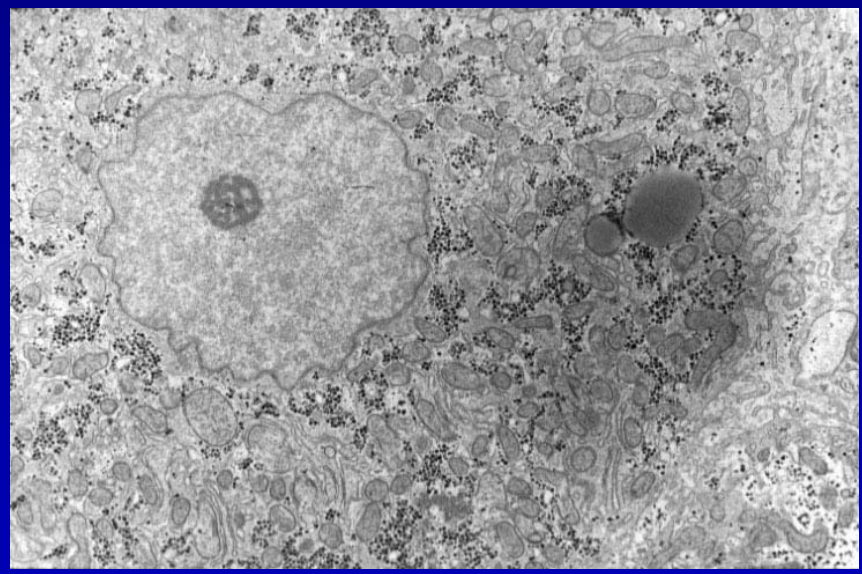
- Typical facial dysmorphism: high forehead, large anterior fontanelle, hypoplastic supraorbital ridges, epicanthic folds
- Profound hypotonia and seizures
- Retinopathy, cataracts, hearing loss
- Enlarged liver
- Renal cysts
- Punctate stippling esp. patella and epiphyses

Zellweger - punctate stippling



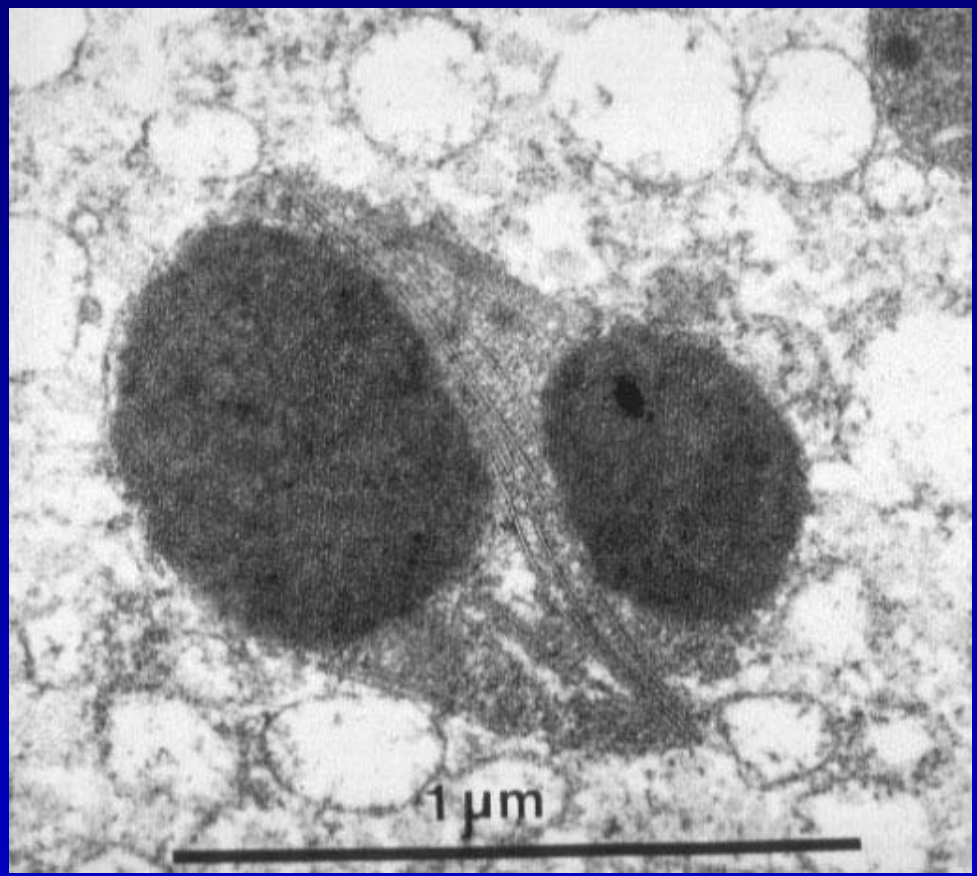
Normal and Zellweger liver





Feature	ZS	NALD	IRD
Age of death	<1y	2y	6.5y
Dysmorphism	++	+	+
Cataract	80%	45%	7%
Retinopathy	70%	80%	95%
Deafness	100%	100%	93%
Psychomotor delay	+++	++	+
Hypotonia	99%	82%	52%
Seizures	80%	82%	20%
Liver	100%	80%	83%

Trilamellar body in IRD



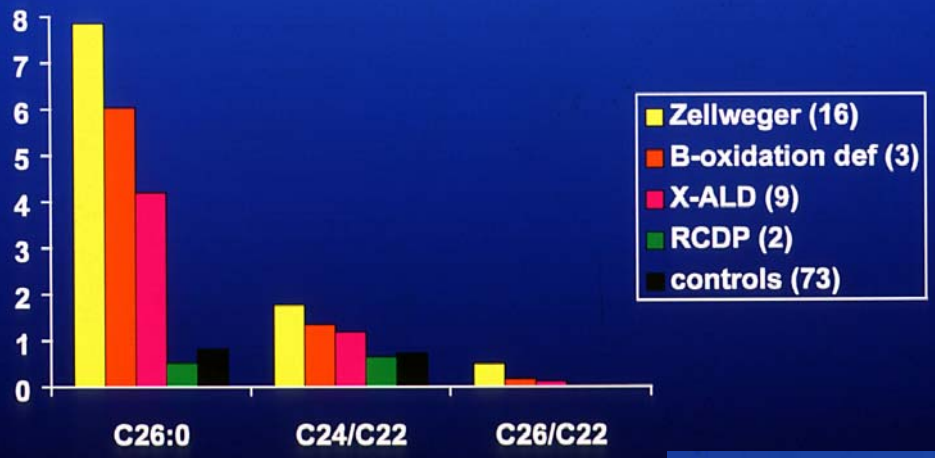
Biochemical abnormalities

- All PBD patients have the same basic biochemical abnormalities, however some abnormalities maybe less marked in milder patients
- They all lack functional peroxisomes
- Many of the 50 matrix proteins are lost
- However peroxisome membrane proteins (PMPs) may remain as ghosts

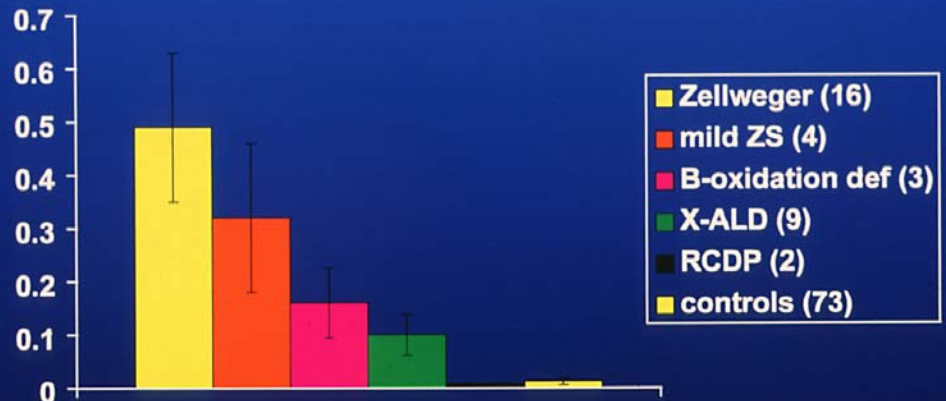
Diagnosis of PBD patients

- Plasma very long chain fatty acid analysis
- Plasma phytanic acid
- Bile acid analysis
- Erythrocyte plasmalogens
- DHAP-AT activity

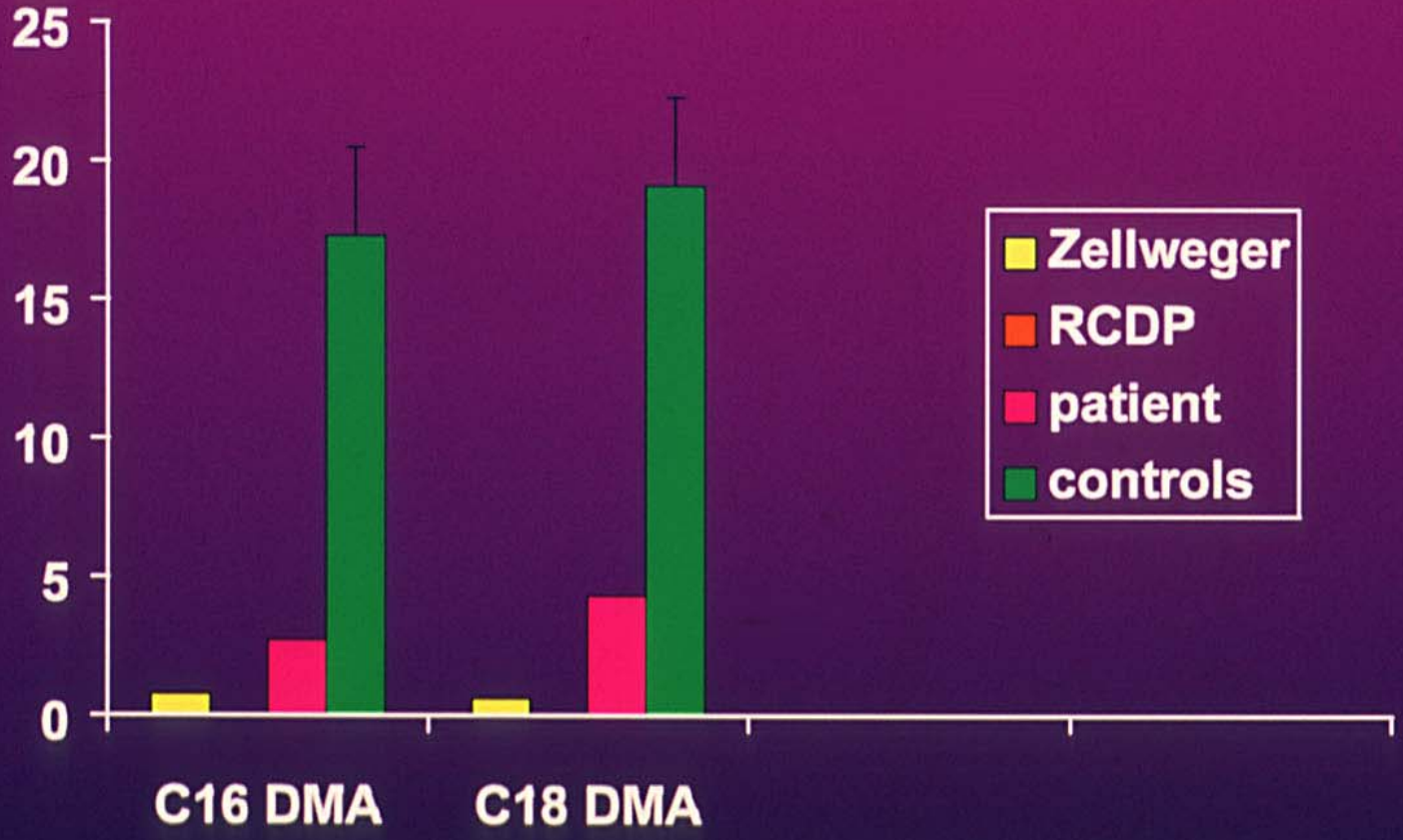
plasma VLCFA
(μmol/L or ratio)



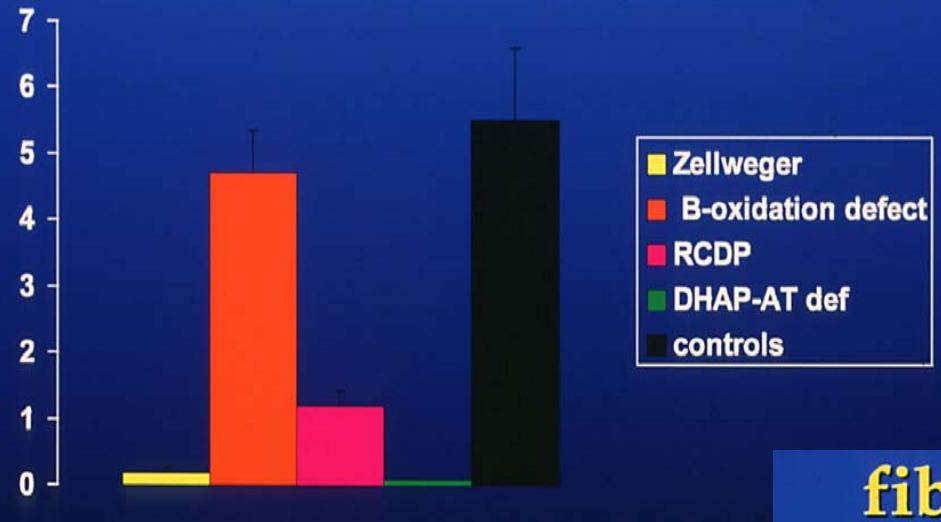
plasma C26/C22 ratios



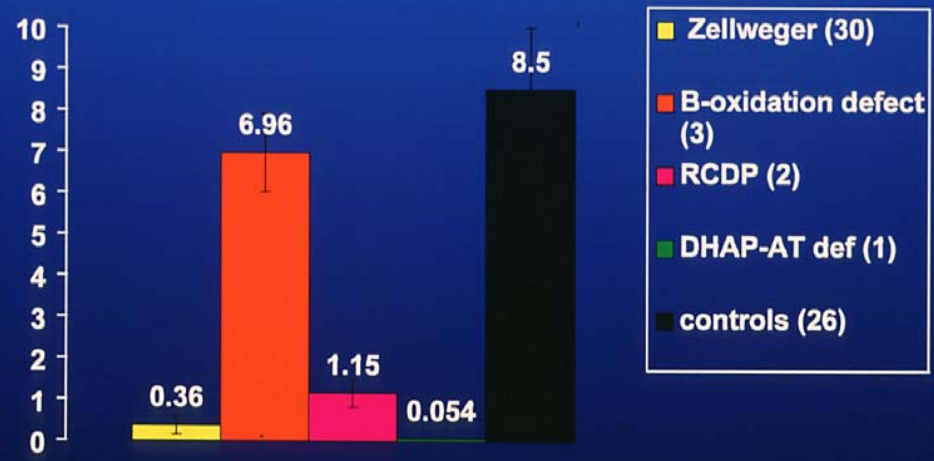
RBC plasmalogens
dimethylacetal:palmitate ratio



platelet DHAP-AT activity
(μmol/h per g protein)



fibroblast DHAP-AT activity
(μmol/h per g protein)



Rhizomelic chondrodysplasia punctata

- Type 1
  - PEX7 gene defect with PTS2 receptor defect
    - Plasmalogen biosynthesis impaired, low DHAP-AT activity
    - Phytanate raised but pristanate low and VLCFAs normal
    - Immature thiolase
  - Note some patients present with Refsum phenotype
- Type 2
  - DHAP-AT isolated deficiency, low plasmalogens
- Type 3
  - Alkyl DHAP synthase deficiency, low plasmalogens

Rhizomelic chondrodysplasia punctata

- Proximal limb shortening, joint contractures
- Punctate calcific stippling esp. of epiphyses, skeletal dysplasia
- Microcephaly, psychomotor retardation and FTT
- Cataracts, deafness
- Ichthyosis

Single peroxisomal defects

- X-linked adrenoleukodystropy
- Peroxisome β-oxidation defects
  - Acyl-CoA oxidase deficiency
  - Bifunctional protein deficiency
  - Thiolase deficiency
- Refsum disease
- Racemase deficiency

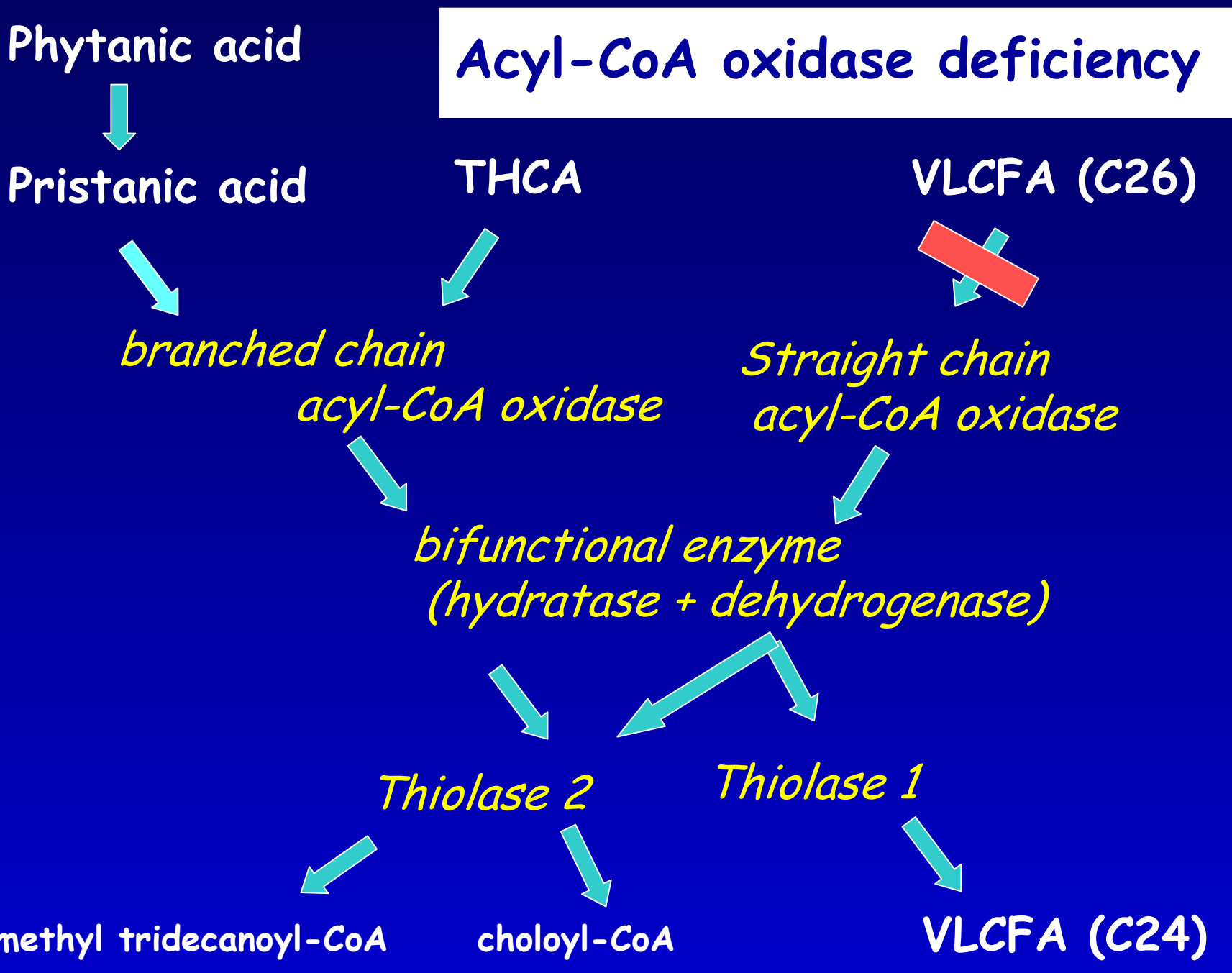
X-linked adrenoleukodystrophy (www.x-ald.nl)

- Mutations in the ALDP gene
- Phenotype: classical juvenile to milder adrenomyeloleukodystrophy. Female carriers (15% may manifest symptoms)
- Diagnosis by plasma VLCFA analysis
- ALDP expression in fibroblasts (70% cases -ve)
- Mutation analysis, but mostly private (over 500)
- Prenatal diagnosis on cultured cell VLCFA or by mutation analysis

Acyl-CoA oxidase deficiency (pseudo-neonatal adrenoleukodystrophy)

- Peroxisomes present
- Diagnosis by increased VLCFAs, but normal pristanate, phytanate, THCA and normal DHAP-AT activity/plasmalogen levels

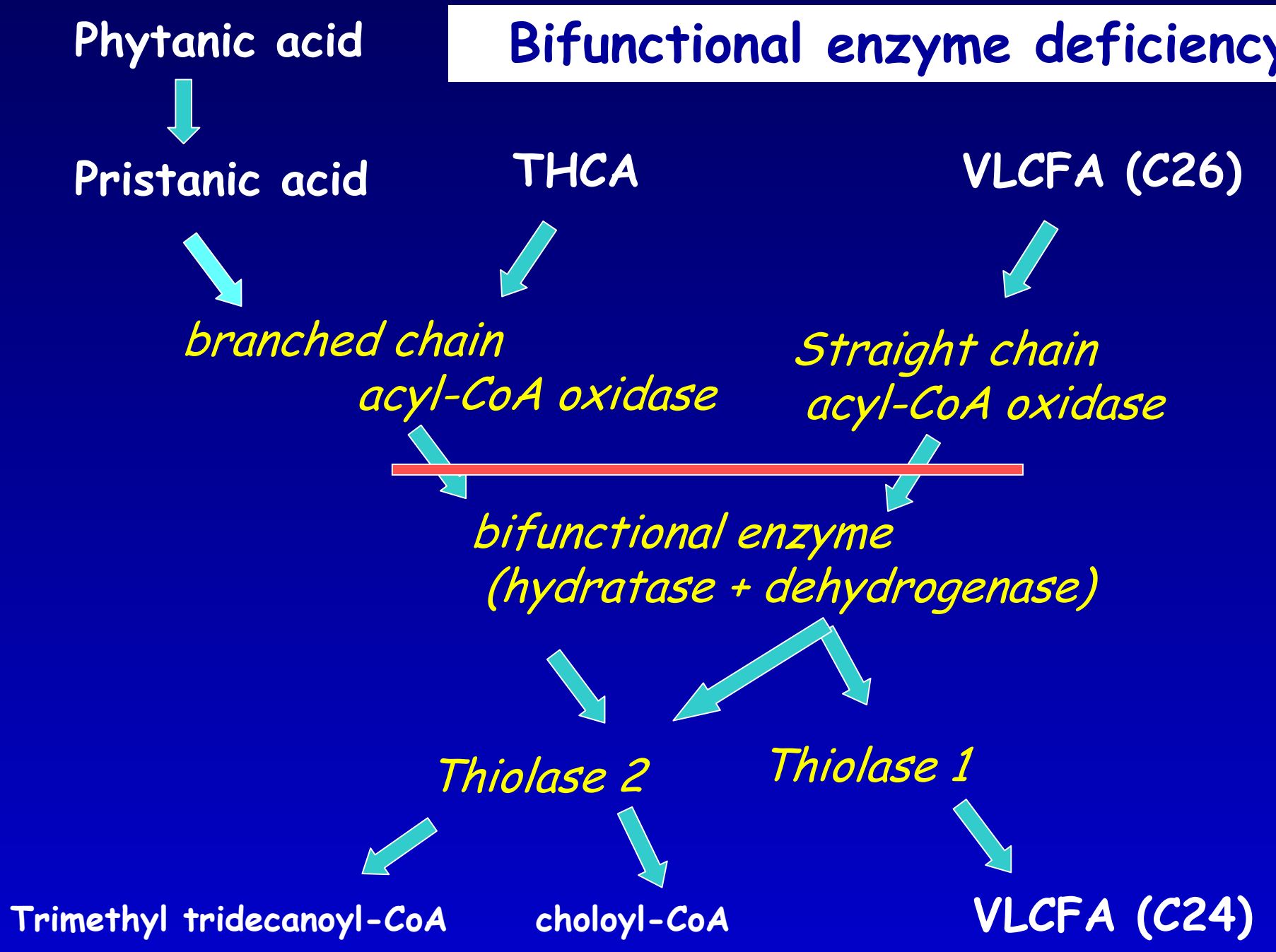
Acyl-CoA oxidase deficiency



Bifunctional protein deficiency

- Severe disorder, presenting like Zellweger.
- Dysmorphia, seizures, hypotonia, death in first year, disordered neuronal migration.
- Most common of β-oxidation defects
- Peroxisomes present
- Diagnosis by increased VLCFAs, increased pristanate and THCA, but normal DHAP-AT or plasmalogens

Bifunctional enzyme deficiency



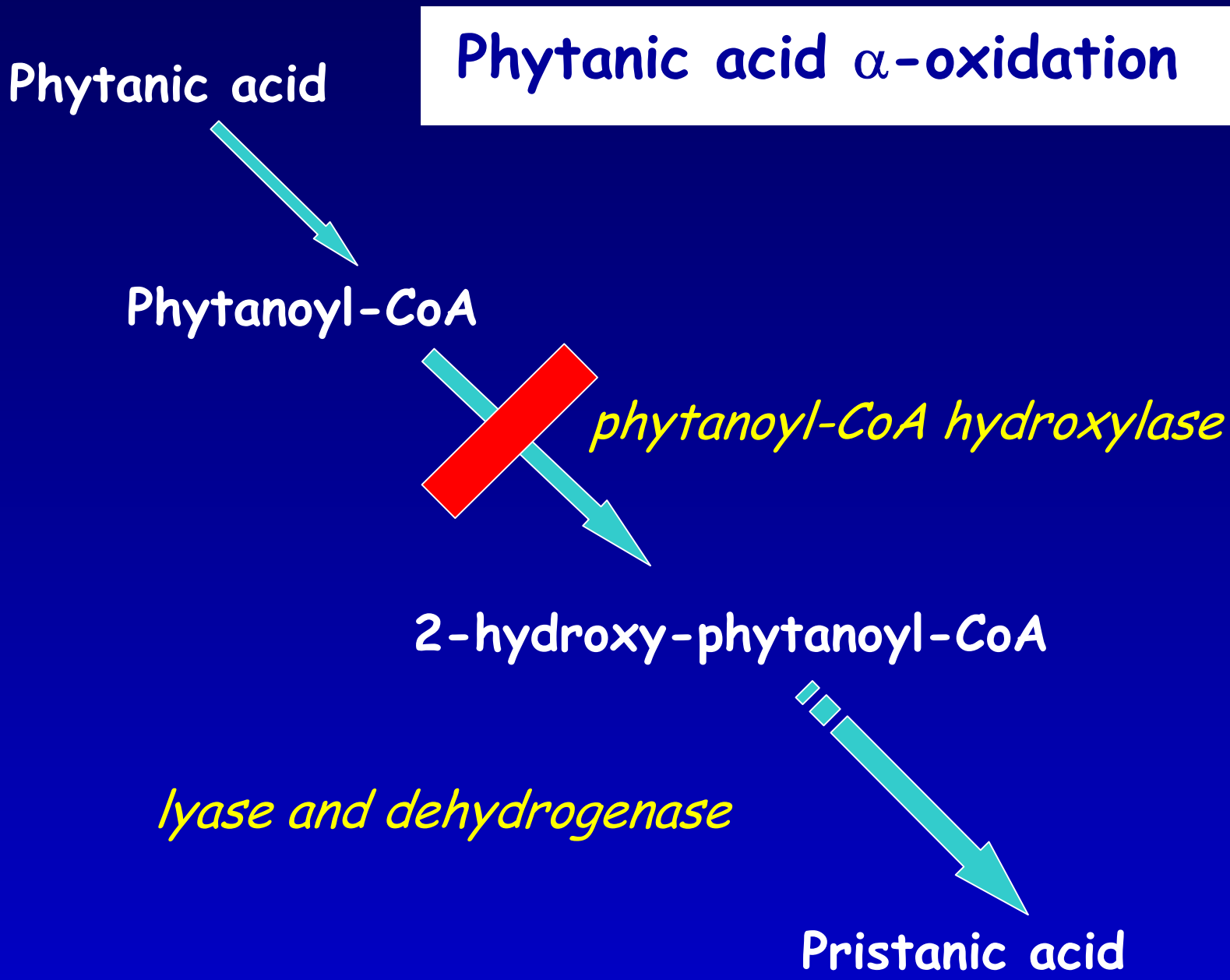
Thiolase deficiency

- Rare, 1 patient originally reported
- Peroxisomes present
- Diagnosis similar to bifunctional protein defect (increased VLCFAs, THCA and ?pristanate)
- Identification of defect by fibroblast complementation studies.

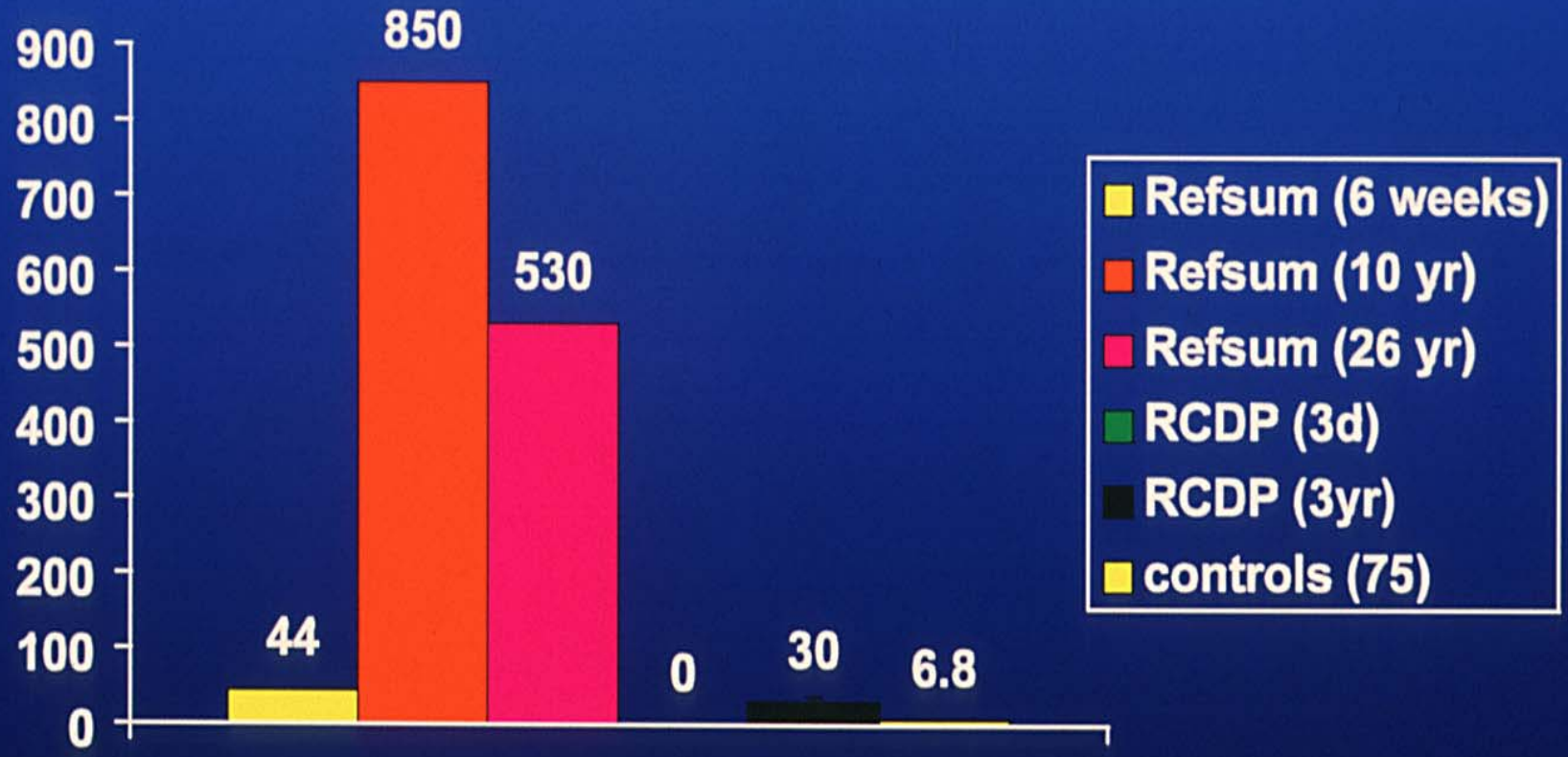
Refsum disease

- Retinitis pigmentosum, ataxia, chronic polyneuropathy, deafness, anosmia, ichthyosis, cardiac abnormalities, skeletal abnormalities (metatarsal shortening)
- Onset usually in late childhood to adults
- Diagnosis: raised phytanate, but normal VLCFAs but low pristanate
- Note some patients have PEX7 mutation (RCDP variant)

Phytanic acid α-oxidation



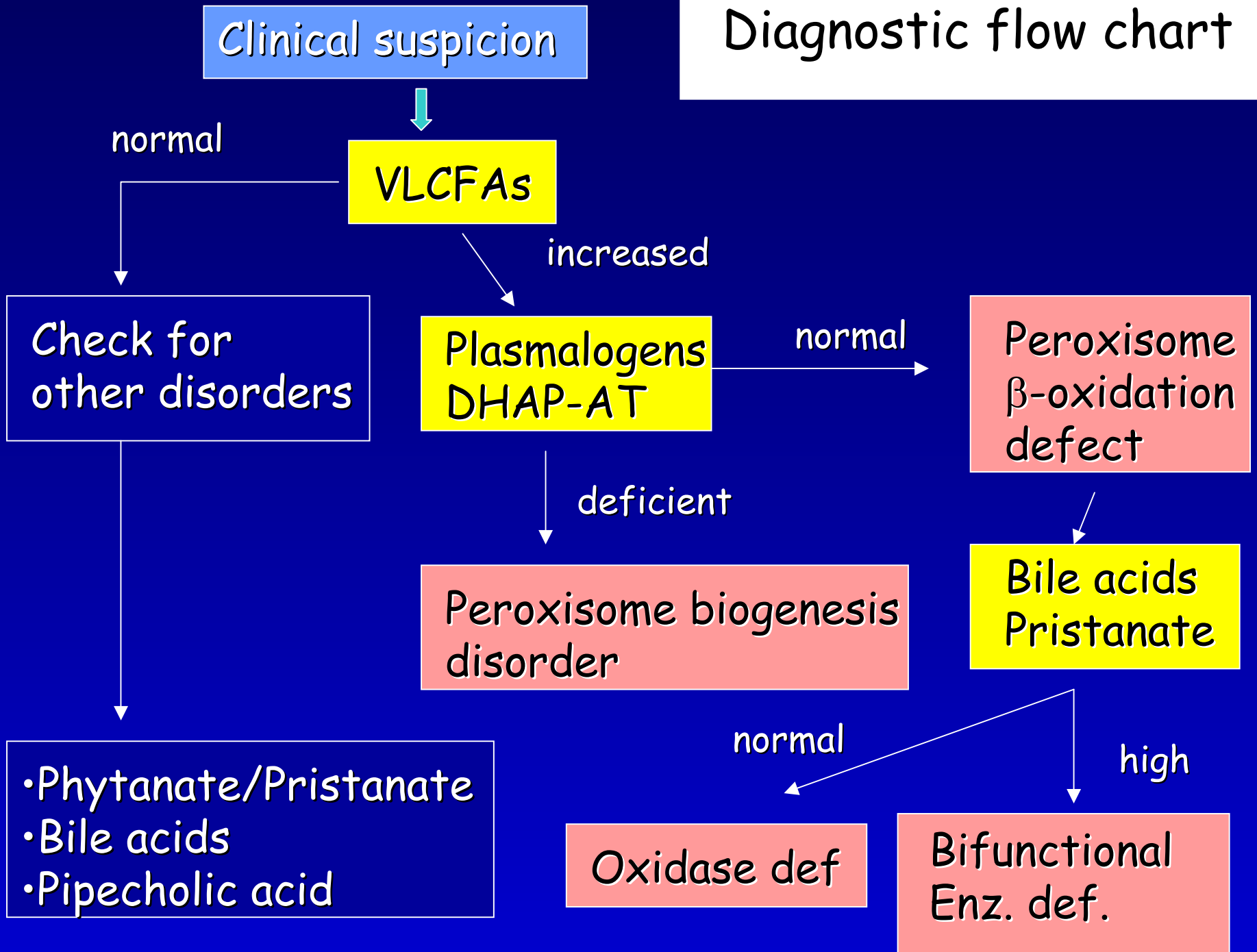
plasma phytanic acid (μmol/L)



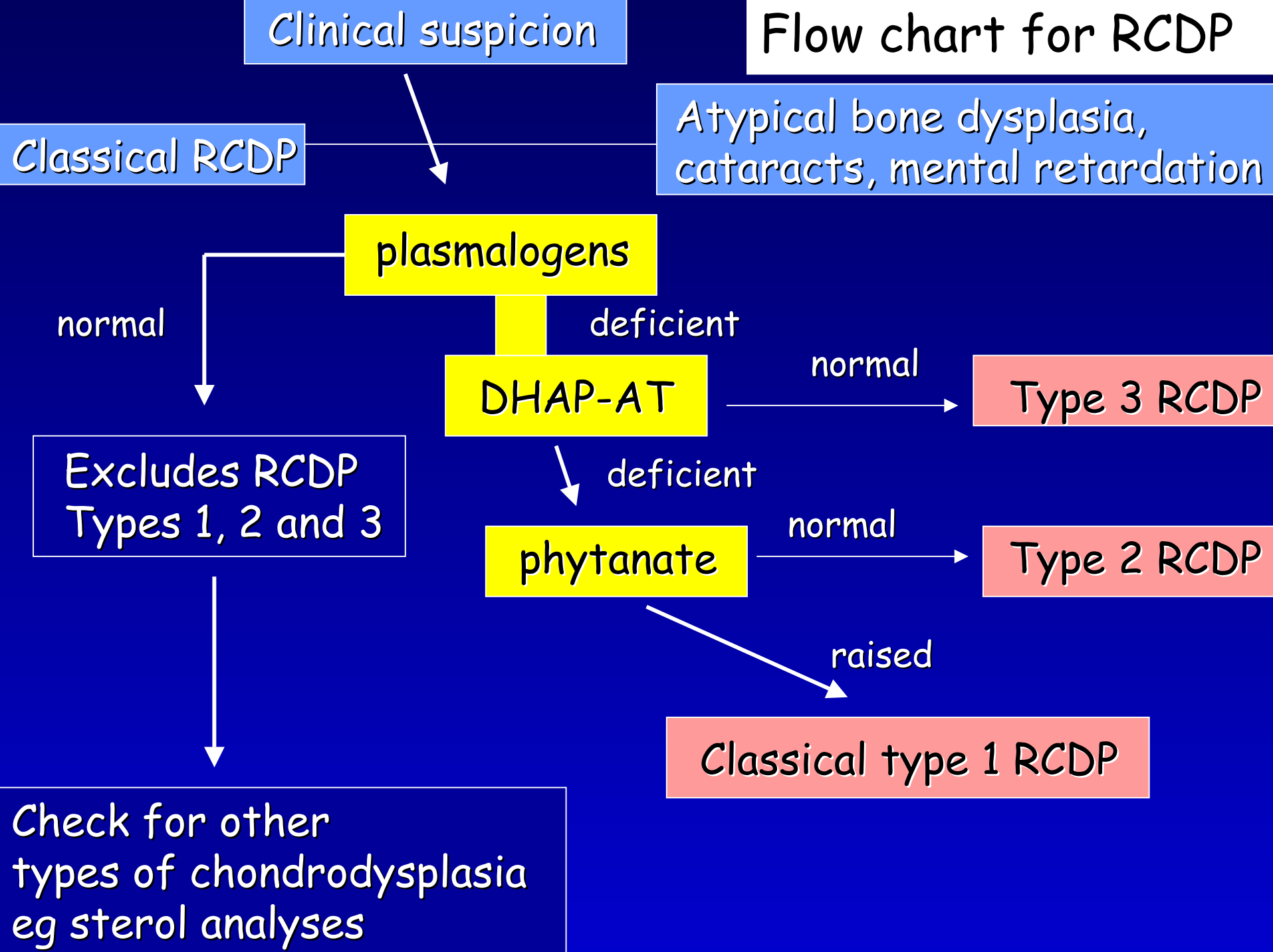
2-methylacyl-CoA racemase deficiency

- Presentation similar to adrenomyeloneuropathy and Refsum disease
- Increased pristanate and bile acids (THCA) but normal VLCFAs
- Suggests a defect in branched-chain oxidase, but this was normal
- However the 2-methyl esters exist as 2R and 2S stereoisomers, but β-oxidation requires conversion to 2S form by racemase enzyme
- Defect in the methylacyl-CoA racemase was recently identified

Diagnostic flow chart



Flow chart for RCDP



That's all, folks