Treatment of inherited metabolic disorders
There is no effective therapy for many inherited metabolic disorders

- Symptoms developing in utero

- Primary affection of central nervous system

For many disorders only symptomatic therapy

---------------------

Effective therapy for a number of disorders

Novel developments: Enzyme replacement therapy
Substrate inhibition therapy
Pharmacological chaperones
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
   a) Substitution of deficient products
   b) Increasing the load of the substrate
   c) Supplementation of alternative substrates

3. Lowering toxic effects of metabolites
   a) Removal of toxic metabolites
   b) Blocking toxic effects

4. Stimulation of residual activity
   a) Treatment with coenzymes
   b) Pharmacological chaperones

4. Supplying deficient enzyme
   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) *Diet*
      b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
   a) Substitution of deficient products
   b) Increasing the load of the substrate
   c) Supplementation of alternative substrates

3. Lowering toxic effects of metabolites
   a) Removal of toxic metabolites
   b) Blocking toxic effects

4. Stimulation of residual activity
   a) Treatment with coenzymes
   b) Pharmacological chaperones

4. Supplying deficient enzyme
   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Reduction of substrate intake by diet

- successful in enzyme deficiencies where substantial part of the substrate originates from the diet (i.e. not synthesized in the body)
- semisynthetic diets
- special foods with reduced/absent substrate
- supplementation of minerals and trace elements
- monitoring of substrate levels
- aminoacidopathies
- disorders of sugar metabolism
Special foods

XLeu Maxamaid (Nutricia)

A leucine-free powdered medical food for the dietary management of isovaleric acidemia and other proven disorders of leucine metabolism in children aged 1 to 8 years. 25 g of protein equivalent per 100 g of powder.

**Nutrition Information:**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Per 100 g</th>
<th>Per 10 g</th>
<th>Protein Equivalent</th>
<th>Leucine: None</th>
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**Amino Acids, g**

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<td>L-Glutamine</td>
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**Vitamins**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Per 100 g</th>
<th>Per 10 g</th>
<th>Protein Equivalent</th>
<th>Leucine: None</th>
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<tbody>
<tr>
<td>Vitamin A, IU</td>
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<td>Vitamin E, IU</td>
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<td>Niacin, mg</td>
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<td>Folic Acid, mcg</td>
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<tr>
<td>Biotin, mcg</td>
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<td>Vitamin C, mg</td>
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<td>Choline, mg</td>
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<tr>
<td>Inositol, mg</td>
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</table>

**Minerals**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Per 100 g</th>
<th>Per 10 g</th>
<th>Protein Equivalent</th>
<th>Leucine: None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>810</td>
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</tr>
<tr>
<td>Phosphorus, mg</td>
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<td>Iron, mg</td>
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<tr>
<td>Zinc, mg</td>
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<tr>
<td>Manganese, mg</td>
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<td>Copper, mcg</td>
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<td>Iodine, mcg</td>
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<tr>
<td>Molybdenum, mcg</td>
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<tr>
<td>Chromium, mcg</td>
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<tr>
<td>Selenium, mcg</td>
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<td>None</td>
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<tr>
<td>Sodium, mg (mEq)</td>
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<td>None</td>
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<tr>
<td>Potassium, mg (mEq)</td>
<td>840 (21.5)</td>
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<td>Chloride, mg (mEq)</td>
<td>450  (12.9)</td>
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<td>4.5</td>
<td>None</td>
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</tbody>
</table>

**Ingredients:**


Phenylketonuria

http://www.chemie.fu-berlin.de/chemistry/bio/aminoacid/gif/phe.gif
Untreated HPA/PKU

- CZ 1:6,500, Turkey 1:3,000, very rare Finland, N Europe 1:15,000
- 1-2% HPA secondary due to primary pterine defects
- 30% patients BH4 sensitive
- newborn screening
- untreated HPA- mental retardation, typical mouse odour, light complexions, eczema, epilepsy
- maternal HPA-VCC, microcephaly a PMR

http://www.dshs.state.tx.us/newborn/images/PKU_untreated.jpg
Phenylketonuria and hyperphenylalaninemas

Deficiency of phenylalanine hydroxylase, hereditary disorders of biopterin metabolism

Pathogenesis of neuropathological changes is not clear, but they are clearly related to elevated phenylalanine levels in blood

Hyperphenylalaninemia: plasma phenylalanine > 120 μmol/l

Untreated classical phenylketouria: > 1000 μmol/l phenylalanine

Non-PKU hyperphenylalaninemas: <1000 μmol/l phenylalanine (persistent levels <600 μmol/l may lead to cognitive deficits)

Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency

Phenylalanine-restricted diet
(1) reversal of key biochemical abnormalities;
(2) improved neuropsychological performance;
(3) prevention of neurologic deterioration. (OMMBID, Ch157)

Maternal PKU:
Maternal hyperphenylalaninemia is harmful to the embryo and fetus, Phenylalanine-restricted diet during the pregnancy (ideally before conception) prevents the metabolic embryopathy
XPhe Maxamum Powder (Nutricia)

A phenylalanine-free powdered medical food for the dietary management of phenylketonuria (PKU) in children 9 years and older and adults, including pregnant women and women of child-bearing age.

**Nutrition Information:**

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Per 100 g</th>
<th>Per 10 g</th>
<th>Protein Equivalent</th>
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</thead>
<tbody>
<tr>
<td>Calories</td>
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<td>76</td>
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<td>Protein Equivalent</td>
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<td>Fat, g</td>
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<td>&lt;0.25</td>
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<tr>
<td>Carbohydrate, g</td>
<td>34</td>
<td>8.5</td>
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**Amino Acids, g**

| L-Alanine | 1.6 | 0.40 |
| L-Arginine| 3   | 0.75 |
| L-Aspartic Acid | 2.8 | 0.70 |
| L-Cystine | 1.1 | 0.28 |
| L-Glutamic Acid | None | None |
| Glycine | 2.8 | 0.70 |
| L-Histidine | 1.7 | 0.43 |
| L-Isoleucine | 2.7 | 0.68 |
| L-Leucine | 4.6 | 1.15 |
| L-Lysine | 3.5 | 0.88 |
| L-Methionine | 0.73 | 0.18 |
| L-Phenylalanine | None | None |
| L-Proline | 3.2 | 0.80 |
| L-Serine | 2   | 0.50 |
| L-Threonine | 2.2 | 0.55 |
| L-Tryptophan | 0.89 | 0.22 |
| L-Tyrosine | 4   | 1.00 |
| L-Valine | 2.9 | 0.73 |
| L-Carnitine | 0.039 | 0.01 |
| Taurine | 0.14 | 0.04 |
| L-Glutamine | 4.9 | 1.23 |

**Vitamins**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Per 100 g</th>
<th>Per 10 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A IU (mcg RE)</td>
<td>2228 (669)</td>
<td>557 (167)</td>
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<tr>
<td>Vitamin D IU (mcg)</td>
<td>332 (8.3)</td>
<td>83 (2.1)</td>
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<tr>
<td>Vitamin E IU(mg α TE)</td>
<td>18.3 (12.3)</td>
<td>4.6 (3.1)</td>
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<tr>
<td>Vitamin K, mcg</td>
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<tr>
<td>Thiamine, mg</td>
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<td>Riboflavin, mg</td>
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<td>Vitamin B₆, mcg</td>
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<td>Vitamin B₁₂, mcg</td>
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<td>Niacin, mg</td>
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<td>Pantothenic Acid, mg</td>
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<td>Biotin, mcg</td>
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<td>5.1</td>
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<td>Vitamin C, mg</td>
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<td>Choline, mg</td>
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<td>Inositol, mg</td>
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**Minerals**

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<td>Iron, mg</td>
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<td>Zinc, mg</td>
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<td>Manganese, mg</td>
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<td>Molybdenum, mcg</td>
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<td>9</td>
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<tr>
<td>Chromium, mcg</td>
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<td>7</td>
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<tr>
<td>Selenium, mcg</td>
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<td>15</td>
</tr>
<tr>
<td>Sodium, mg (mEq)</td>
<td>560 (24.3)</td>
<td>140 (6.1)</td>
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<td>Potassium, mg (mEq)</td>
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<tr>
<td>Chloride, mg (mEq)</td>
<td>560 (16)</td>
<td>140 (4)</td>
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**Ingredients:**

- Sugar, L-Arginine, L-Aspartate, L-lysine
- Acetate, L-Glutamine, L-Leucine, L-Tyrosine, Calcium Glycerophosphate, L-Proline, L-Valine, Glycine, L-Isoleucine, L-Threonine, Trispotassium Citrate, Magnesium Hydrogen Phosphate, and 2% or less of each of the following: L-Serine, L-Histidine, L-Alanine, Calcium Phosphate Dibasic, Choline Bitartrate, L-Cysteine, Tricalcium Phosphate, Sodium Chloride, N-Acetyl L-Methionine, Trisodium Citrate, L-Tryptophan, Soy Lecithin, Magnesium L-Aspartate, Taurine, L-Lactic Acid, M-Inositol, Ferrous Sulfate, L-Carnitine, Zinc Sulfate, DL-Alpha Tocopheryl Acetate, Niacinamide, Manganese Sulfate, Calcium D-Pantothenate, Cupric Sulfate, Pyridoxine Hydrochloride, Sodium Selenite, Thiamine Chloride Hydrochloride, Riboflavin, Vitamin A Acetate, Folic Acid, Potassium Iodide, Chromium Chloride, Sodium Molybdate, Pyridoxaline, D-Biotin, Vitamin D₃, Cyanocobalamin.

**Orange** flavored version also contains:

- Citric Acid, and 2% or less of each of the following: Artificial Flavor, Artificial Sweetener: Acesulfame K, Artificial Colors (Beta Carotene, Beet Red).
Dietary treatment of PKU

**Goal:** to lower phenylalanine levels and prevent its neuropathological effects
Treatment must start as soon as possible after birth – neonatal screening
Treatment is directed by phenylalanine levels in blood – monitoring of Phe (and Tyr)

Low protein diet + synthetic amino-acid mixture without phenylalanine

Infant formulas, mixtures for older children
Egg, substitute, low-protein flour, low-phe sweets, cookies, ...
Supplements : minerals, vitamins, trace elements, (tyrosine)

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<th>Phe tolerance (mg/day)</th>
<th>Target blood Phe levels (μmol/l)</th>
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<td>~ 130 - 400</td>
<td>120 - 360</td>
</tr>
<tr>
<td>3-6 y</td>
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<td>7 -9 y</td>
<td>~ 200 - 400</td>
<td>120 - 480</td>
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<tr>
<td>10 -12</td>
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<td>~ 350 - 800</td>
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<td>Adolescents/adults</td>
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<td>Maternal PKU</td>
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<td>120 - 360</td>
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Protein requirements

Protein intake
Phenylalanine-restricted diet

Supervised by a dietician

Sufficient protein intake

Calculation of phenylalanine intake in natural foods, protein intake is supplemented by phenylalanine-free amino-acid mixture

Low-protein flour, egg substitute, low-Phe drink, cookies, ...

Patients avoid meat, eggs, fish, cheese, nuts and seeds, flour-based foods (e.g. bread), aspartame

Some foods can be eaten in larger amounts: some fruits (apples, apricots, blackberries, blueberries, cherries, clementines, cranberries, currants (black, red), grapes, grapefruit, melon, nectarines, olives, oranges,...), some vegetables (beetroot, cucumber, marrow, onion, leek lettuce, ... ), butter, margarine, vegetable oils, sugar

Some foods can be eaten in limited amounts: some vegetables (potatoes, ...), corn, cereals, double cream, bananas, ...

Parents use tables listing Phe content of foods

In UK and US system of „exchanges“
### Jídla s vysokým obsahem Phe

<table>
<thead>
<tr>
<th>množství</th>
<th>obsah Phe/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>pstruh na grilu</td>
<td>200 g 1110</td>
</tr>
<tr>
<td>hranolky</td>
<td>100 g</td>
</tr>
<tr>
<td>pečené kuřecí stehno</td>
<td>150 g 1300</td>
</tr>
<tr>
<td>vařené brambory</td>
<td>250 g</td>
</tr>
<tr>
<td>smažený sýr Eidam</td>
<td>140 g 1900</td>
</tr>
<tr>
<td>hranolky</td>
<td>100 g</td>
</tr>
<tr>
<td>tatarská omáčka</td>
<td>25 g</td>
</tr>
<tr>
<td>smažený vepřový řízek</td>
<td>110 g 1170</td>
</tr>
<tr>
<td>vařené brambory</td>
<td>250 g</td>
</tr>
<tr>
<td>špagety milánské/boloňské (se sýrem)</td>
<td>1 porce 330 g 1320</td>
</tr>
</tbody>
</table>

Hodnoty jsou orientační (průměrné), nikdy nelze určit přesnou hodnotu jídel z důvodu rozdílných receptur v jednotlivých restauračních zařízeních.

<table>
<thead>
<tr>
<th>Snídaň</th>
<th>Dietní pečivo (100 g), máslo (10 g), džem/med (30 g), zelenina - rajské/paprika (40 g), čaj/dietní kakao (200 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Přesnídávka</td>
<td>NB pudínek s ovocem</td>
</tr>
<tr>
<td>Oběd</td>
<td>Zeleninová polévka s nudlemi</td>
</tr>
<tr>
<td></td>
<td>Bramborový knedlík (230 g), kysané zelí (120 g), smažená cibulka</td>
</tr>
<tr>
<td>Svačina</td>
<td>Linecké cukroví (popř. koláč – 100 g)</td>
</tr>
<tr>
<td>Večeře</td>
<td>NB těstoviny (200 g), rajská omáčka (250 ml), kompot</td>
</tr>
<tr>
<td>II. Večeře</td>
<td>Jablko/banán</td>
</tr>
</tbody>
</table>
Určeno pro věkovou kategorii nad 18 let (s přihlédnutím k dané denní toleranci Phe v přirozené stravě)

<table>
<thead>
<tr>
<th>Polévky</th>
<th>množství/ml</th>
<th>obsah Phe/mg</th>
<th>hodnocení</th>
<th>poznámka</th>
</tr>
</thead>
<tbody>
<tr>
<td>gulášová</td>
<td>250</td>
<td>355</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>Bramborová</td>
<td>250</td>
<td>185</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>hovězí vývar</td>
<td>250</td>
<td>170</td>
<td>☺️</td>
<td>bez masa</td>
</tr>
<tr>
<td>rajská s těstovinou, rýži</td>
<td>250</td>
<td>125</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>česnečka s brambory</td>
<td>250</td>
<td>110</td>
<td>☺️</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bezmasá jídla</th>
<th>množství/g</th>
<th>obsah Phe/mg</th>
<th>hodnocení</th>
<th>poznámka</th>
</tr>
</thead>
<tbody>
<tr>
<td>smažený květák</td>
<td>150</td>
<td>345</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>smažené žampiony</td>
<td>150</td>
<td>385</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>pizza Vegetaria</td>
<td>340</td>
<td>1100</td>
<td>☒️</td>
<td>bez sýru</td>
</tr>
<tr>
<td>zeleninové rizoto</td>
<td>300</td>
<td>360</td>
<td>☒️</td>
<td>bez sýru</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Základní přílohy</th>
<th>množství/ml</th>
<th>obsah Phe/mg</th>
<th>hodnocení</th>
<th>poznámka</th>
</tr>
</thead>
<tbody>
<tr>
<td>americké brambory</td>
<td>220</td>
<td>270</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>hranolky</td>
<td>150</td>
<td>185</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>bramborový knedlík</td>
<td>200</td>
<td>550</td>
<td>☒️</td>
<td></td>
</tr>
<tr>
<td>bramborová kaše</td>
<td>300</td>
<td>335</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>chléb konzumní</td>
<td>50</td>
<td>155</td>
<td>☺️</td>
<td>1 krajíc</td>
</tr>
<tr>
<td>houska</td>
<td>45</td>
<td>200</td>
<td>☺️</td>
<td>1 kus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Saláty</th>
<th>množství/g</th>
<th>obsah Phe/mg</th>
<th>hodnocení</th>
<th>poznámka</th>
</tr>
</thead>
<tbody>
<tr>
<td>míchaný</td>
<td>150</td>
<td>50</td>
<td>☺️</td>
<td>(rajče, okurka, paprika, cibule)</td>
</tr>
<tr>
<td>rajčatový</td>
<td>150</td>
<td>45</td>
<td>☼️</td>
<td></td>
</tr>
<tr>
<td>zelný</td>
<td>150</td>
<td>50</td>
<td>☾️</td>
<td>(rajče, okurka, paprika, olivy, salát, Feta/Balkán)</td>
</tr>
<tr>
<td>řecký</td>
<td>150</td>
<td>195</td>
<td>☽️</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Zeleninové přílohy</th>
<th>množství/ml</th>
<th>obsah Phe/mg</th>
<th>hodnocení</th>
<th>poznámka</th>
</tr>
</thead>
<tbody>
<tr>
<td>dušená mrkev s hráškem</td>
<td>120</td>
<td>100</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>dušené kysané zelí</td>
<td>150</td>
<td>80</td>
<td>☺️</td>
<td></td>
</tr>
<tr>
<td>dušený špenát</td>
<td>150</td>
<td>240</td>
<td>☺️</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moučníky</th>
<th>množství/g</th>
<th>obsah Phe/mg</th>
<th>hodnocení</th>
<th>poznámka</th>
</tr>
</thead>
<tbody>
<tr>
<td>palačinka s džemem</td>
<td>150</td>
<td>430</td>
<td>☾️</td>
<td>bez šlehačky</td>
</tr>
<tr>
<td>palačinka s ovoce</td>
<td>180</td>
<td>460</td>
<td>☾️</td>
<td>bez šlehačky</td>
</tr>
<tr>
<td>medovník</td>
<td>100</td>
<td>300</td>
<td>☽️</td>
<td>1 porce</td>
</tr>
<tr>
<td>jablčný štrúdl</td>
<td>150</td>
<td>330</td>
<td>☽️</td>
<td>1 porce</td>
</tr>
</tbody>
</table>
### Příklady jídelníčků pro dospělé

**s PKU dietou**  
*(v rozmezí 800 – 1000 mg Phe/den)*

<table>
<thead>
<tr>
<th>Snídaně</th>
<th>čaj, NB pečivo, máslo, zelenina</th>
<th>Snídaně</th>
<th>čaj/káva, NB perník</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Přesnídávka</strong></td>
<td>ovoce</td>
<td><strong>Přesnídávka</strong></td>
<td>smetanový jogurt</td>
</tr>
<tr>
<td><strong>Oběd</strong></td>
<td>pol. hovězí vývar se zeleninou</td>
<td><strong>Oběd</strong></td>
<td>pol. pórková</td>
</tr>
<tr>
<td></td>
<td>dušená mrkev, brambor,</td>
<td></td>
<td>dušený špenát, br. knedlík,</td>
</tr>
<tr>
<td></td>
<td>(bez masa)</td>
<td></td>
<td>(bez masa)</td>
</tr>
<tr>
<td><strong>Svačina</strong></td>
<td>pečivo, máslo, zelenina</td>
<td><strong>Svačina</strong></td>
<td>ovoce</td>
</tr>
<tr>
<td><strong>Večeře</strong></td>
<td>NB těstovinový salát se zeleninou</td>
<td><strong>Večeře</strong></td>
<td>NB těstoviny po Srbsku</td>
</tr>
<tr>
<td><strong>II. večeře</strong></td>
<td>ovoce</td>
<td><strong>II. večeře</strong></td>
<td>NB křehlý chléb, máslo, zelenina</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snídaně</th>
<th>čaj s mlékem/bílá káva</th>
<th>Snídaně</th>
<th>čaj, NB chléb, máslo, med</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Přesnídávka</strong></td>
<td>NB ovocné řezy</td>
<td><strong>Přesnídávka</strong></td>
<td>Krajanka</td>
</tr>
<tr>
<td><strong>Oběd</strong></td>
<td>smetanový jogurt</td>
<td><strong>Oběd</strong></td>
<td>pol. rajská s rýží</td>
</tr>
<tr>
<td></td>
<td>pol. zeleninová</td>
<td></td>
<td>vařená brokolice se sýr. přelivem,</td>
</tr>
<tr>
<td></td>
<td>bramboráky</td>
<td></td>
<td>brambor</td>
</tr>
<tr>
<td><strong>Svačina</strong></td>
<td>NB křehlý chléb, máslo, zelenina</td>
<td><strong>Svačina</strong></td>
<td>NB křehlý chléb, máslo, zelenina</td>
</tr>
<tr>
<td><strong>Večeře</strong></td>
<td>zapeč. NB těstoviny se zeleninou</td>
<td><strong>Večeře</strong></td>
<td>NB těstoviny, &quot;gulášová&quot; omáčka</td>
</tr>
<tr>
<td><strong>II. večeře</strong></td>
<td>hlávkový salát</td>
<td><strong>II. večeře</strong></td>
<td>ovocný salát</td>
</tr>
<tr>
<td></td>
<td>ovoce, NB sušenka</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Effect of diet in selected inherited metabolic disorders

+ minimal effect, ++++ complete or almost complete resolution of symptoms

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Diet</th>
<th>Effect of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorders of long chain fatty acid oxidation</td>
<td>- long chain fatty acids</td>
<td>+++</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>- galactose</td>
<td>++++ (liver, kidney, eyes) + (brain, ovary)</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>- methionine</td>
<td>++++</td>
</tr>
<tr>
<td>Hereditary fructose intolerance</td>
<td>- fructose</td>
<td>+++++</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>- phenylalanine</td>
<td>+++++</td>
</tr>
<tr>
<td>Glycogen storage disorders</td>
<td>+ saccharides</td>
<td>+++</td>
</tr>
<tr>
<td>Organic acidemias</td>
<td>- protein</td>
<td>+</td>
</tr>
<tr>
<td>Maple sirup urine disease</td>
<td>- valin, leucin, isoleucine</td>
<td>+++</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase def.</td>
<td>- saccharides</td>
<td>+</td>
</tr>
<tr>
<td>Urea cycle disorders</td>
<td>- protein</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosinemia I</td>
<td>- phenylalanine, tyrosine</td>
<td>++</td>
</tr>
</tbody>
</table>

According to Walter, Wraith in Fernandes et al (eds), Inborn metabolic diseases, diagnosis and treatment, 4th ed., Chapter 5
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
   a) Substitution of deficient products
   b) Increasing the load of the substrate
   c) Supplementation of alternative substrates

3. Lowering toxic effects of metabolites
   a) Removal of toxic metabolites
   b) Blocking toxic effects

4. Stimulation of residual activity
   a) Treatment with coenzymes
   b) Pharmacological chaperones

4. Supplying deficient enzyme
   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

„Substrate inhibition (reduction) therapy“
Tyrosinemia type I

- Fumarylacetoacetase deficiency
- Acute manifestation in infancy
- Hepatorenal involvement with acute hepatic dysfunction and Fanconi syndrome
- Porphyric crises - abdominal cramps
- Chronic - ca hepatis and ca in cirrhosis
- Diet, nitisone, liver transplant
Tyrosinemia 1

PAH

http://www.chemie.fu-berlin.de/chemistry/bio/aminoacid/gif/phe.gif
Alkaptonuria a tyrosinemia 1 treatment

nitison (NTBC)

Tyrosine \[\text{Tyrosine-transaminase} \rightarrow \text{p-Hydroxyphenylpyruvate} \]

Homogentisate

Acetoacetate, Fumarate, 4-Fumarylacetocetate, 4-Maleylacetoacetate
Gaucher disease

Lysosomal storage disorder

Deficiency of glucocerebrosidase (acid beta glucosidase)

Accumulation of glucosylceramide preferentially in cells of macrophage origin (Gaucher cells)

Multisystem disorder

Hepatomegaly, splenomegaly, bone disease, trombocytopenia, anemia, lung infiltration

In type 2 and 3 Gaucher disease: CNS disease

Clinical variability, chronic progression
Type 1: chronic non-neuronopathic
Type 2: acute neuronopathic
Type 3: chronic neuronopathic
Substrate inhibition therapy

Mutant enzymes have residual activities

N-butyldeoxyjirimycin (Zavesca)
Inhibitor of glucosylceramide synthase
Gaucher disease, GM1 gangliosidosis
Substrate reduction therapy for Gaucher disease

Inhibition of synthesis of glycosphingolipids

Miglustat (OGT 918, SC-48334, N-butyldeoxynojirimycin) orally active iminosugar

Inhibits glucosylceramide synthase and synthesis of glycosphingolipids

Effect of reduced influx rate on steady state substrate concentration under the conditions of Conzelmann/Sandhoff model
Mean leucocyte $\text{GM}_1$ values fell by 38.5% over 12 months in these patients ($p < 0.05$).
Lysosomal transporters deficiencies

Cystinosis – cystinosin deficiency
renal disease with Fanconi syndrome
renal failure – renal transplantation
corneal crystals, photophobia
growth retardation
hypothyroidism
normal intelligence

ocular form

Sialuria – sialin deficiency
Adult cystinosis

B  Untreated cystinotic lysosome

C  Cysteamine-treated cystinotic lysosome

Cystine

Cysteamine

Mixed disulfide of cysteine and cysteamine

Cysteine

Cysteamine
Cystinosis

G

H

cystin

cysteamin
Figure 4. Renal Function in Patients with Cystinosis Treated with Cysteamine and in Untreated Patients, According to Age.
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

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   c) Supplementation of alternative substrates

3. Lowering toxic effects of metabolites
   a) Removal of toxic metabolites
   b) Blocking toxic effects

4. Stimulation of residual activity
   a) Treatment with coenzymes
   b) Pharmacological chaperones

4. Supplying deficient enzyme
   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Type I Glycogen Storage Disease (Glucose 6-Phosphatase Deficiency, von Gierke Disease)

Excessive accumulation of glycogen in liver, kidney and intestinal mucosa

Patients usually present in infancy with hepatomegaly and/or hypoglycaemic seizures, hyperlactacidemia after a short fast

Gout, hyperlipidemia (hypertriglyceridemia), skin xanthomas

Doll-like face, thin extremities, short stature, protuberant abdomen (hepatomegaly), inflammatory bowel disease

Fibrosis, liver adenomas - cave: malignant transformation, Atherosclerosis

Fasting tolerance improves with age, long-term complications

Treatment: frequent feeding, nocturnal nasogastric drips in infancy, uncooked cornstarch, liver transplantation

Autosomal recessive, overall incidence is 1:10000, frequent in Ashkenazi
The diagnosis is based on clinical presentation, abnormal blood/plasma concentrations of glucose, lactate, uric acid, triglycerides, and lipids, and molecular genetic testing.
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

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   a) Substitution of deficient products
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   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Fatty acids and mitochondria

1. Long-chain fatty acid → fatty acid
2. Fatty acyl-CoA
3. Fatty acyl-carnitine
4. Fatty acyl-CoA → acetyl-CoA → (HMG-CoA) → ketones
5. CO₂

PLASMA  CYTOPLASM
Mitochondrial-beta oxidation of fatty acids

1. Dehydrogenation

\[
\begin{align*}
R-\text{CoA} & \xrightarrow{\text{FAD}, \text{FADH}_2} \quad R-\text{trans-}\Delta^2-\text{Enoyl-CoA} \\
\text{Acyl-CoA-Dehydrogenase} & \\
\end{align*}
\]

2. Hydration

\[
\begin{align*}
R-\text{trans-}\Delta^2-\text{Enoyl-CoA} & \xleftrightarrow{+\text{H}_2\text{O}, -\text{H}_2\text{O}} \quad R-\text{L-3-Hydroxyacyl-CoA} \\
\text{Enoyl-CoA-Hydratase} & \\
\end{align*}
\]

3. Dehydrogenation

\[
\begin{align*}
R-\text{L-3-Hydroxyacyl-CoA} & \xrightarrow{\text{NAD}^+, +\text{H}^+, \text{NADH}} \quad R-\text{3-Ketoacyl-CoA} \\
\text{Hydroxyacyl-CoA-Dehydrogenase} & \\
\end{align*}
\]

4. Thiolysis

\[
\begin{align*}
R-\text{3-Ketoacyl-CoA} & \xrightarrow{+\text{CoA-SH}, \text{Thiolase}} \quad R-\text{Acyl-CoA} + \text{H}_3\text{C}\text{-CoA} \\
\text{3-Ketoacyl-CoA} & \\
\end{align*}
\]
MCT treatment in long-chain fatty acid oxidation defects and carnitine cycle deficits

Enzymes of β-oxidation specific for long, medium, short chain fatty acids

Medium chain fatty-acids enter mitochondria directly (not via carnitine cycle)

Treatment with medium-chain triacylglycerols (MCT oil)

VLCAD deficiency (very-long-chain acyl-CoA dehydogenase): Hypoketonemic hypoglycemia, cardiomyopathy, muscle weakness, severe metabolic decompensation with coma
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
   a) Substitution of deficient products
   b) Increasing the load of the substrate
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4. Supplying deficient enzyme
   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Acute toxic encephalopathy
- organic acidurias,
- maple sirup urine disease
- urea cycle disorders

Removal of toxic metabolites
Ammonia > 400 umol/l, insufficient effect of conservative therapy
Leucine > 1500 umol/l
...

Hemodialysis
Hemofiltration

Exchange transfusion
Peritoneal dialysis
Alternative pathways for waste nitrogen synthesis in hyperammonemia

**Phenylbutyrate** → phenylacetylglutamine – 2 N per molecule
**Benzoate** → hippurate – 1 N per molecule
Alternative pathways for removal of toxic metabolites

Isovaleric aciduria

Treatment with glycine $\rightarrow$ isovalerylglycine

Organic acidurias

Carnitine $\rightarrow$ carnitine conjugates
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
   a) Substitution of deficient products
   b) Increasing the load of the substrate
   c) Supplementation of alternative substrates

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   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Biotin - water-soluble vitamin from B complex, Coenzyme in four carboxylases
Apoenzymes are inactive without biotin

- pyruvate carboxylase - gluconeogenesis, mitochondria
- propionyl CoA carboxylase - amino-acid catabolism, mitochondria
- β-methylcrotonyl CoA carboxylase - amino-acid catabolism, mitochondria
- acetyl CoA carboxylase - biosynthesis of fatty acids, cytosol

Biotin is derived from the diet and possibly also from the synthetic activity of gastrointestinal microflora.
raw eggs decrease the bioavailability of biotin (avidin in egg-white binds biotin)

**Biotinidase deficiency**
Severe : less than 10% of biotinidase activity
Partial: 10%-30% of biotinidase activity

**Holocarboxylase synthetase deficiency**
Fig. 2. A, B. Areas of hyperintensity (T2-weighted MRI) in the medulla, periaqueductal grey matter (A) and cervical and upper thoracic spinal cord (B). C, D. After 14 months treatment MRI abnormalities have disappeared.
## Frequency of Clinical and Biochemical Features in Children with Biotinidase Deficiency

<table>
<thead>
<tr>
<th>Percentage of Affected Children</th>
<th>Symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 50</td>
<td>Alopecia, Development, Hypotonia, Ketolactic acidosis, Organic aciduria, Seizures, Skin rash/skin infection</td>
</tr>
<tr>
<td>25±50</td>
<td>Ataxia, Conjunctivitis, Hearing loss, Lethargy, Mild hyperammonemia, Tachypnea/apnea/breathing problems, Visual abnormalities: loss of vision/optic atrophy</td>
</tr>
<tr>
<td>10±25</td>
<td>Coma, Feeding difficulties/vomiting/diarrhea, Fungal infections, Hepatomegaly, Speech problems, Splenomegaly</td>
</tr>
</tbody>
</table>

According to MMBIB, Ch.156
Disorders in which were described cofactor-responsive variants

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Cofactor</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylmalonic acidemia</td>
<td>hydroxycobalamin</td>
<td>some</td>
</tr>
<tr>
<td>Biotinidase deficiency</td>
<td>biotin</td>
<td>all</td>
</tr>
<tr>
<td>Holocarboxylase synthase</td>
<td>biotin</td>
<td>majority</td>
</tr>
<tr>
<td>Glutaric aciduria type I</td>
<td>riboflavin</td>
<td>rare</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBS deficiency</td>
<td>pyridoxin</td>
<td>about 50%</td>
</tr>
<tr>
<td>CblC deficiency</td>
<td></td>
<td>frequent</td>
</tr>
<tr>
<td>MTHFR deficiency</td>
<td>folic acid</td>
<td>rare</td>
</tr>
<tr>
<td>Maple sirup urine disease</td>
<td>thiamin</td>
<td>rare</td>
</tr>
<tr>
<td>Respiratory chain deficiencies</td>
<td>ubichinone</td>
<td>unconfirmed</td>
</tr>
<tr>
<td>Propionic acidemia</td>
<td>biotin</td>
<td>probably never</td>
</tr>
<tr>
<td>HPA – biopterin disorders</td>
<td>tetrahydrobiopterine</td>
<td>all</td>
</tr>
<tr>
<td>HPA – PAH deficiency</td>
<td>tetrahydrobiopterine</td>
<td>rare in classic PKU</td>
</tr>
<tr>
<td>Ornithineaminotransferase</td>
<td>pyridoxin</td>
<td>rare</td>
</tr>
</tbody>
</table>

According to Walter, Wraith in Fernandes et al (eds), Inborn metabolic diseases, diagnosis and treatment, 4 th ed., Chapter 5
Fig. 156-6 Two children with biotinidase deficiency shown before and after biotin treatment. A. Child with biotinidase deficiency at 2 years and 9 months of age with alopecia and periorbital and perioral rash, before biotin therapy. B. Same child after 4 months of biotin therapy. (From Thoene et al.\textsuperscript{261} Used by permission of New England Journal of Medicine.) C. Child with biotinidase deficiency at 10 months of age, before biotin therapy. D. Same child at 30 months of age, after 20 months of biotin therapy.
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
   a) Substitution of deficient products
   b) Increasing the load of the substrate
   c) Supplementation of alternative substrates

3. Lowering toxic effects of metabolites
   a) Removal of toxic metabolites
   b) Blocking toxic effects

4. Stimulation of residual activity
   a) Treatment with coenzymes
   b) Pharmacological chaperones

4. Supplying deficient enzyme
   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Misfolded proteins

Damage

Functional protein
Residual function

Accumulation
Loss-of-function

Toxic conformation
Chaperone sequestration
Aggregation
Gain-of-function

Semi unfolded protein

Gene variation

Degradation
Loss-of-function

Nascent protein

Pharmacological chaperones for lysosomal disorders

Misfolded proteins are degraded by ERAD (ER-associated degradation)
Pharmacological chaperones: exogenous molecules that assist folding of the mutant protein
Residual activity of mutant proteins can be rescued

Fabry disease
Gaucher disease
Pharmacological chaperones for lysosomal storage disorders

Often analogs of the substrate
Competitive inhibitors
Bind to the active site and „stabilize“ the enzyme
In vitro effect

Fabry, Gaucher - clinical testing

Pharmacological chaperones in LSDs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme deficiency</th>
<th>Chaperone(s)</th>
<th>Other available therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabry disease</td>
<td>GLA</td>
<td>DGJ, galactose, 1-DGJ-lysine, galactostatin bisulphite</td>
<td>ERT</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>GBA</td>
<td>IFG, NB-DNJ, DNJ, NOV, 2,5-anhydro-2,5-imino-α-glucitol</td>
<td>ERT, SRT</td>
</tr>
<tr>
<td>(G_m) gangliosidosis</td>
<td>GLB1</td>
<td>NOEV</td>
<td>—</td>
</tr>
<tr>
<td>(G_m) gangliosidosis</td>
<td>HEXA</td>
<td>Pyrimethamine</td>
<td>—</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>GAA</td>
<td>DNJ, NB-DNJ</td>
<td>ERT</td>
</tr>
</tbody>
</table>

NOV: \(N\)-octyl-beta-valienamine.
65. Identification of Ambroxol as a potential enzyme enhancement-agent for Gaucher disease

Don Mahuran a, Gustavo Maegawa a, Michael Tropak a, Justin Butner a, Gregory Kornhaber b, Brigitte Rigat a, Joe Clarke a, a Hospital For Sick Children, Toronto, ON, Canada, b ExSAR Corporation, Canada

Gaucher disease, currently treated by enzyme replacement therapy (ERT), is caused by a deficiency of lysosomal beta-glucosidase (GCase). The disadvantages of ERT include its high cost, its ineffectiveness in treating the CNS or other organ compartments and the unfolded protein response in cells. Small molecule-based enzyme enhancement therapy (EET) is a promising approach that can potentially be used alone or in combination with ERT to address these deficiencies. Clinical trials of isofagomine, an inhibitor of GCase (IC\textsubscript{50} \sim 0.04 \, \mu M), as an EET-agent are being initiated. In order to accelerate the process of obtaining IND-status for new EET-agents we have screened the NINDS library of FDA-approved drugs for compounds that inhibit and/or stabilize the target enzyme towards heat denaturation. Using GCase as the target we identified Ambroxol, an expectorant, as a candidate EET-agent. Despite Ambroxol being only a weak inhibitor of GCase, IC\textsubscript{50} = 27 \, \mu M, at higher concentrations it compared favorably with isofagomine in its ability to rescue mutant N370S GCase in patient cells. However, it was not as effective at rescuing F213I GCase. Hydrogen–Deuterium exchange Mass Spectrometry (H/D-Ex) was used to compare the regions in GCase that were stabilized by these compounds. Isofagomine has been shown through co-crystallization to stabilize a loop structure at GCase352–357. H/D-Ex confirmed these data, while Ambroxol was ineffective at stabilizing this loop. However, both compounds were equally effective in stabilizing another region, GCase283–288, indicating its importance as a target for EET-agents.
High-throughput screening (HTS) of small molecules

Screening of libraries of small molecules for biological effects
  e.g. pharmacological chaperones

Assay: (absorbance, ELISA, fluorescence, microscopy, etc.). Miniature format – microtitre plates, automation

Libraries – hundreds of thousands of compounds

Libraries of approved drugs – e.g. NINDS etc.
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
   a) Substitution of deficient products
   b) Increasing the load of the substrate
   c) Supplementation of alternative substrates

3. Lowering toxic effects of metabolites
   a) Removal of toxic metabolites
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   b) Pharmacological chaperones

4. Supplying deficient enzyme
   a) Bone marrow transplantation
   b) Organ transplantations
   c) Enzyme supplementation therapy
   d) Gene therapy
Bone marrow transplantation

Haematopoetic stem cell transfer

Pro:
In contrast to enzyme replacement therapy can influence CNS disease

Con:
High morbidity and mortality

Lysosomal disorders
Mucopolysacharidosis I
  - Modifies natural course of the disease
  - Early treatment can prevent neurological disease
  - Residual disease
Other MPS disorders
MPS III – no improvement of neurological progression
Other lysosomal disorders

Peroxisomal disorders
X-ALD
Treatment strategies

1. Reduction of load into the affected metabolic pathway
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. Correction of product deficiency
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   c) Enzyme supplementation therapy
   d) Gene therapy
Organ transplantations

Liver transplantation

Glycogen storage disorders
Urea cycle disorders
Organic acidurias

Often successful, often high mortality

Kidney transplantation

In disorders leading to irreversible kidney failure

Cystinosis
Hyperoxaluria type I
Fabry disease

Heart transplantation

Fabry disease
Treatment strategies

1. **Reduction of load into the affected metabolic pathway**
   a) Dietary restriction
   b) Inhibition of enzymes in the metabolic pathway proximal to the metabolic block

2. **Correction of product deficiency**
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3. **Lowering toxic effects of metabolites**
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4. **Stimulation of residual activity**
   a) Treatment with coenzymes
   b) Pharmacological chaperones

4. **Supplying deficient enzyme**
   a) Bone marrow transplantation
   b) Organ transplantations
   c) **Enzyme supplementation therapy**
   d) Gene therapy
Sorting of proteins containing MP6 signal

protein-M6P-M6PR → lysosome

protein-M6P → protein

protein → Secretion pathway

cis-Golgi

mannose-6-phosphate
Gaucher disease

Lysosomal storage disorder

Deficiency of glucocerebrosidase (acid beta glucosidase)

Accumulation of glucosylceramide preferentially in cells of macrophage origin (Gaucher cells)

Multisystem disorder

Hepatomegaly, splenomegaly, bone disease, trombocytopenia, anemia, lung infiltration

In type 2 and 3 Gaucher disease: CNS disease

Clinical variability, chronic progression
Type 1: chronic non-neuronopathic
Type 2: acute neuronopathic
Type 3: chronic neuronopathic
Enzyme supplementation therapy in Gaucher disease

Receptor-mediated endocytosis

Macrophage targeted glucocerebrosidase - treatment with exoglycosidases

Mannose receptor (macrophages, endothelia, liver)

Regular infusions

Originally glucocerebrosidase isolated from human placentas (Ceredase, Genzyme)

Recombinant enzyme

Cerezyme (Genzyme) – Cho cells

Does not cross haematoencephalic barrier

High costs
Enzyme supplementation therapy

Supplementation of deficient enzyme in regular infusions

Gaucher disease (glucocerebrosidase)
Fabry disease (alpha galactosidase A)
Pompe disease (acid alpha glucosidase)
MPS I (alpha iduronidase)
MPS II (alpha iduronate sulfatase)
MPS VI, Maroteaux-Lamy (arylsulfatase B)
Niemann-Pick disease B (acid sphingomyelinase)
MPS IVA, Morquio A, ...

Production of recombinant enzymes
Genzyme, TKT, Biomarin, Shire, Inotech, ...
Treatment strategies

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   d) Gene therapy
Gene therapy

(Exception: Adenosine deaminase deficiency )
X-linked adrenoleukodystrophy

X-linked disease, ALDP: Xq28

ABC half-transporter ALDP: functions as a homodimer and accepts acyl-CoA esters

Cerebral X-ALD - a rapidly progressive intensely inflammatory myelinopathy that may involve autoimmune mechanisms.

Adrenomyeloneuropathy is a noninflammatory distal axonopathy involving mainly the spinal cord long tracts and to a lesser extent peripheral nerves

Addison disease
Asymptomatic
Heterozygous females

Biochemical defect at the level of long-chain acyl-CoA synthetase - elevated levels of very-long chain fatty acids
Hallmarks of X-ALD neuropathology
AGE OF ONSET OF NEUROLOGICAL SYMPTOMS OF CEREBRAL FORMS OF ADRENOLEUKODYSTROPHY AND ADRENOMYELONEUROPATHY

CEREBRAL
ALD

AMN

NUMBER OF PATIENTS

0  5  10  15  20  25  30  35  40  45  50

AGE IN YEARS

0  5  10  15  20  25  30  35  40  45  50  55  60  65  70
**Lorenzo's oil**

A 4 : 1 mixture of glyceryl trioleate and glyceryl trierucate

Normalizes the levels of VLCFA in the plasma of X-ALD patients.

Possibly partially effective in prevention of progression in patients without neurological symptoms/adrenomyeloneuropathy

Developed by Augusto Odone

![Lorenzo's oil image]

- **Erucic acid**
  - Chemical structure:
  - Developed by Hugo Moser

- **Oleic acid**
  - Chemical structure:
Survival for cerebral X-linked adrenoleukodystrophy following hematopoietic cell transplantation.

(A): Kaplan–Meier estimate of survival for cerebral X-linked adrenoleukodystrophy following related and unrelated donor hematopoietic cell transplantation.

(B) Kaplan–Meier estimate of survival for cerebral X-linked adrenoleukodystrophy following hematopoietic cell transplantation by neurological deficit and MRI severity.

Expert Review of Neurotherapeutics 2008, Vol. 8, No. 9, Pages 1367-1379