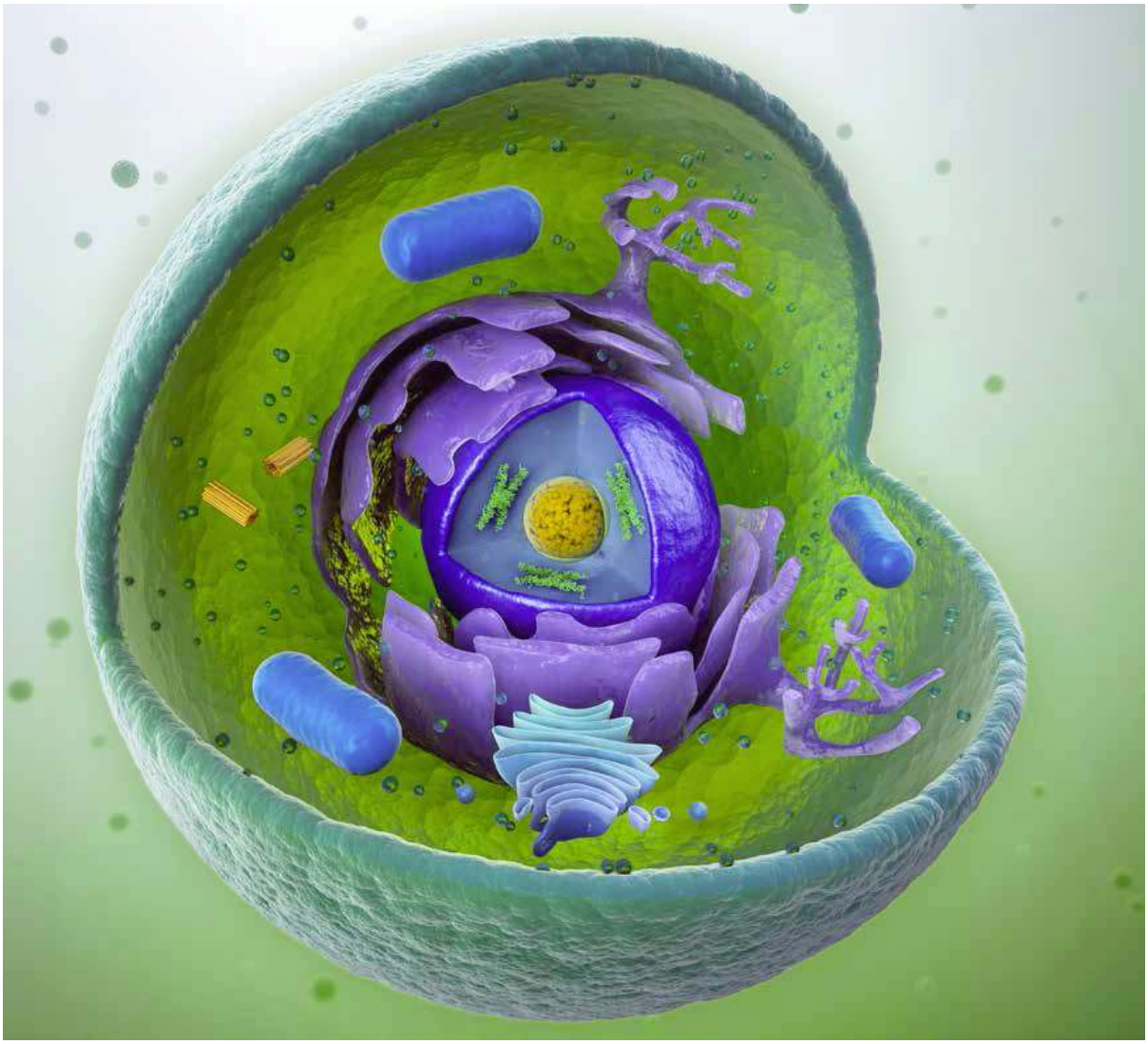
THE BIOCHEMISTRY OF PEROXISOMES

KARLIJN ELIAS



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The picture on the front page comes from "Experiment NL 2014", a publication of the Dutch Organization for Scientific Research with an overview of the best discoveries of that year

Explanation image:

It is a model of a cut-open cell with all organelles clearly visible. The peroxisomes are the small dark green spots.

High-school research paper

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Note of the translator: *major parts of this paper have been automatically translated by Microsoft Translator. I have quickly checked the results and corrected it when there was a clear misinterpretation. Yet, I am not a professional translator, so I found it difficult to maintain my daughter's writing style over the language barrier. Where I had to correct whole sections you can recognize some of my own writing style. When in doubt, feel free to check the original (in Dutch).*

I am also no medical professional, although I followed some biochemistry courses on academic level. The Dutch medical terms are checked by Kevin Berendse Msc, but it could be that in this version some of the medical and / or chemical terms have lost their meaning by a too literal translation.

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FOREWORD

My choice for the subject biochemistry of peroxisomes I made for the following reasons:

- I like the subjects' biology and chemistry.
- I find biochemistry quite interesting.
- I have a peroxisomal disease
- It seemed to me interesting to examine how a peroxisome works, so I better understand my disease and other diseases.

I want to thank my father for his guidance in my profile workpiece. I would also like to thank him for the interpretation of the scientific texts. Without his help there was no well understandable research paper!

I want to thank my mother for checking spelling and grammar errors in my profile workpiece. My mom has help my understanding of the subject by tested me by asking critical questions and to helping to articulate my findings. And also she was a great help in planning and structuring the task. Thanks to her I have finished my research paper on time.

I was too ambitious in approaching this problem. I wanted to research every aspect; introducing far too many research questions, which resulted in that I could not oversee everything. My parents have guided me along to a more focused research paper.

I would also like to thank my teacher, Mrs Molendijk, for answering the questions I had on the design of the research paper. I would also like to thank her for providing the molecule building sets, which I was allowed to borrow. Also, I would like

to thank her for her kind comments after reading the draft of this paper.

I want to thank Kevin Berendse Msc, researcher Pediatrics Neurology/Genetic Metabolic Diseases in the AMC, for supplying all the information material. I would also like to thank him for answering my detailed questions about the information I had found myself. In addition, I was very glad that I was allowed to work with him on the practical assignment of my research paper. And that he had prepared the trial so superbly.

I would like to thank Merel Ebberink PhD, researcher Pediatrics Neurology/Genetic Metabolic Diseases in the AMC, for the presentation she gave for me personally. I would also like to thank her for giving me a copy of her PhD thesis.

Professor Dr. B.T. Poll-Thé, Pediatric neurologist at the AMC, I know since my eighth. I thank her for providing the article with which I began my research. I'm glad she is so proud of me.
I hope she will continue to be my doctor.

INTRODUCTION

Peroxisomes are organelles within the cell from which very little is known. There is still a lot of research to be done on peroxisomes. It is important that knowledge of peroxisomes grows, because there can go many things wrong inside peroxisomes. If anything goes wrong, it is called a peroxisomal disease. Researching on peroxisomal diseases can help doctors to identify this disease as early as possible. Then they can treat patients with peroxisomal diseases better.

But in order to do that, one must first know how it works in a peroxisome. In other words: first the biochemistry of a peroxisome has to be understood. Only then one can hope to find effective treatments of peroxisomal diseases. That is why I have as main research question:

What are the biochemical effects of peroxisomes?

Not everyone knows what peroxisomes are. You do not learn about them at biology in high school. This is partly because little is known about the peroxisome. As a result, peroxisomes are quite unknown organelles. I knew not much of peroxisomes. That is why I have defined the following sub-question:

What are peroxisomes?

As I said before: If something goes wrong in a peroxisome, it can cause a peroxisomal disease. I knew only my own illness, but I knew nothing of other peroxisomal diseases. I wanted to get a better picture of the peroxisomal diseases out there. Or the clinical picture which may arise on the malfunctioning of a peroxisome. Therefore, my next sub-question is:

What is the clinical picture?

To know the peroxisomal disease the patient can encounter, one must first examine what and where something goes wrong inside the patient. But also one must know why it goes wrong. These questions can only be answered by watching how a peroxisome normally functions. That is why I have as next sub-question:

What are the biochemical activities of peroxisomes?

But how can I find out when a peroxisome work and why others do not? To which aspects do I look? To answer these questions, I have the following part question figured out:

How to show you that the peroxisomes still functions?

Reliable source of background knowledge

The research on this paper consists of a theoretical and a practical part. Most sources that I found for the theoretical part were pretty scientific. Information on this issue can be hard to find. And once found, it was difficult to extract from this articles the information needed for my paper. I have interpreted those academic sources by making from all these sources a summary in my own words. That was not an easy job. My parents helped which checking if I had made the correct interpretation. My research paper is based on 24 knowledge sources. Examples of these knowledge sources include wikipedia, internet sites, scientific articles, a PhD thesis and even a Dutch textbook on chemistry two centuries old.

Research paper content

In the following chapters explain each of the sub-questions one by one. In Chapter 1 the peroxisome is introduced. In Chapter 2 I describe the most common syndromes. In Chapter 3 I tell about three biochemical activities in the peroxisome. In Chapter 4 I give a short summary of the practical assignment. The full laboratory report is added as annex. In Chapter 5 I culminate all my collected knowledge about the sub-questions to answer my main resarch question.

PEROXISOMES

1.1. GENERAL INFORMATION

Peroxisomes are tiny organelles[1]. They have a round or oval shape. The average diameter is between 0.2 and 0.5 µm. The peroxisome is surrounded by a membrane. The space inside the membrane is called the peroxisomal matrix. The number of peroxisomes varies by body cell. For example, there are hundreds of peroxisomes in the liver cells, while there are dozens of peroxisomes in skin cells. The average life span of peroxisomes is around two days, but varies by body cell. In skin cells is the life about two days. In the liver cells this is shorter. Inside the peroxisome is among others hydrogen peroxide present. This is a toxic substance. Due to the presence of this agressive substance a persoxisoom can rather wear fast.

The difference between peroxisome peroxisomes and other organelles is that it has an extra thick membrane. The biochemical processes of the peroxisome are separated from the rest of the cell through the thick membrane of the peroxisome.

1.2. BUILDING BLOCKS OF A PEROXISOME

A peroxisome contains at least 63 proteins[2]. Proteins can be divided into two categories: proteins necessary for the construction of a peroxisome and proteins needed for its internal functioning. For example, the peroxisome contains hydrogen peroxide. That is an example of a substance that the peroxisome turns over with the enzyme catalase. Catalase is an enzyme involved in the internal functioning of a peroxisome.

[1] Prof. Dr. B.T. Poll-The en Prof. Dr. P.G. Barth, 'Van kleine dingen... over peroxisomen, onzichtbare fabriekjes met een grote betekenis', De specialist aan het woord, UMC Magazine 1999, p. 1 [2]
[2] Benno Beukema, 'Stofwisselingsziekten - Een inleiding in de aangeboren stoornissen van de stofwisseling', Nederlandstalige website [3]

Catalase is the enzyme I'm going to visualize in the experiment of Chapter 4. How the hydrogen peroxide is turned over I not go tell in this research paper. Instead, I will give another example of a decomposition process in the peroxisome, which is beta oxidation. This process is explained in Chapter 3.

Enzymes in General:

Enzymes are proteins. They build chemical substances on, or break they chemically off[3]. In the digestive system the enzymes break down substances into smaller substance, until they are small enough to be absorbed in our body cells. Enzymes work on specific substance only. They start a chemical reaction by binding to a substance. That substance is called the substrate. The connection between enzyme and substrate is called the enzyme-substrate complex. The substrate fits into a cavity of the folded enzyme. After a short time, the substrate molecule is divided in two pieces, and let it go by the enzyme. The enzyme is unchanged from the reaction. It can be used again for the same reaction.

Each enzyme has its own chemical reaction with one type of fabric: the substrate. The number of conversions that an enzyme can do in a certain time, i.e. the enzyme speed, depends on the factors temperature and acidity. At the minimum temperature the enzymes work very slowly. The higher the temperature, the faster the enzymes start to work. The faster the enzymes go to work, the more materials there can be hyphenated. At one point the enzymes reach an optimum temperature. Here are the most conversions per second. When the temperature gets higher, there are more conversions per second. So the enzymes seem to work here even faster. But when the temperature goes up also a number

[3] Dr. Hugo W. Moser en Dr. Stephan Kemp, 'X-linked Adrenoleukodystrophy Database', Nederlandstalige website [1]

of enzymes gradually break down. At the maximum temperature are all enzymes are broken. Enzymes also have an optimum acidity.

The peroxisome is itself also build from a collection of enzymes. Each enzyme has its own substrate, optimum temperature and optimum acidity. So, the peroxisome consists of proteins. Both the membrane as the interior contains proteins. The protiens in the interior are called matrix protiens. Those proteins are made outside the peroxisome. The task of the membrane proteins enzymes during the biogenesis is to get the matrix protiens inside. Biogenesis is the biological process by which organic material is formed, in this case the peroxisome. After the peroxisome biogenesis the task of the membrane enzymes is to get the substrate inside. A number of examples of substrates of the peroxisomes are: very-long-chain fatty acids, phytaanzuur, plasmalogenen, pipecolinezuur, oxalic acid, materials for the construction of DHA-AA and the forerunner of Cholic acid. With those substrates the peroxisome can do three things. The peroxisome can assemble, dismantle or convert the substances. In Chapter 3 I give an explanation on what happens with three of those substrates.

1.3. PEROXINS

Some of the proteins of the peroxisome are called peroxins[4]. Those protiens are encoded by the PEX genes in the DNA. For the designation of those peroxins one uses the tag PEX + number. There are fourteen different types in the human body known peroxins that cause a disease. Peroxins can work on a specific private task individually or in groups. Groups with the same task are called peroxins complexes. One of the tasks of

[4] Merel S. Ebberink, 'Molecular and genetic characterization of peroxisome biogenesis disorders', proefschrift Universiteit van Amsterdam (2010), p. 122-123 [23]

such a complex is: take care of the import of membrane proteins, which are needed for the construction of the peroxisome. Another task is that they allow for importing the matrix enzymes in the peroxisome. A third task of a complex of peroxins is the import and export of the substrate.

1.4. PEROXISOME BIOGENESIS

About the peroxisome biogenesis of the there are different theories[5]. There is much debate about how a peroxisome develops. Currently, there are two common theories about the origin of a peroxisome.

The first theory: biogenesis of the peroxisome organelle from the Endoplasmic Reticulum (also called ER). It begins with the formation of the peroxisomal membrane. The membrane proteins are imported into the membrane. That goes like this: the membrane proteins are made outside the peroxisome. Some proteins are built into the peroxisome membrane and support to get the other membrane and the matrix enzymes into the peroxisome. This theory is shown in Figure 1.

[5] Drs. Kevin Berendse, onderzoeker Pediatrics Neurology / Genetic Metabolic Diseases, AMC Amsterdam, persoonlijke communicatie [4]

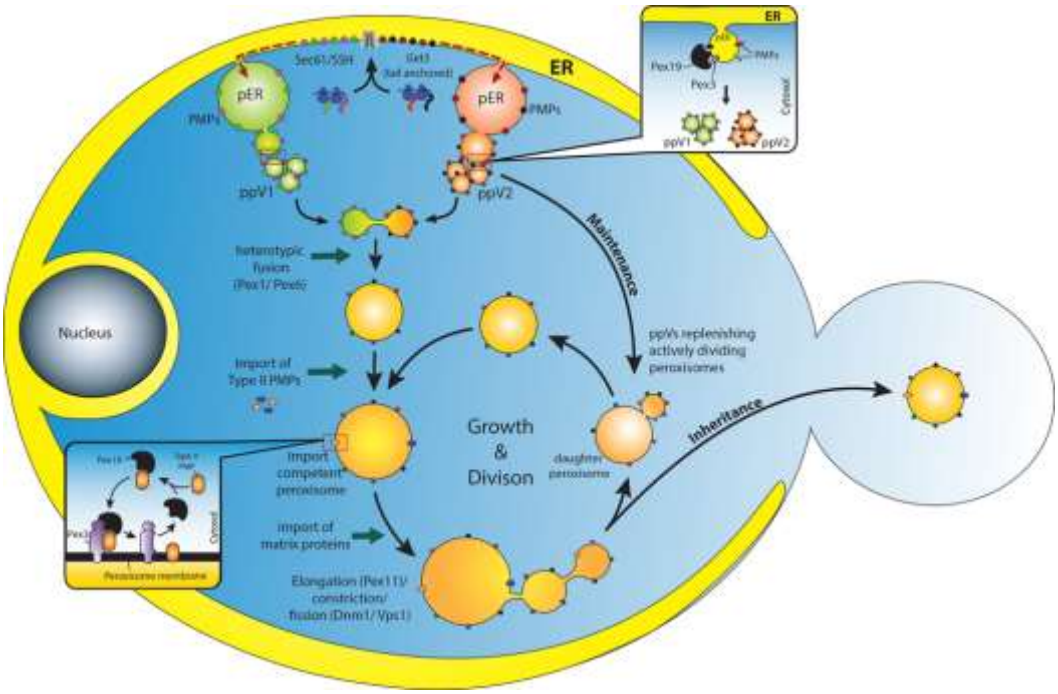


Figure 1: Peroxisome biogenesis from the Endoplasmatic Reticulum.
Source: drs. Kevin Berendse [4]

The second theory: from the peroxisome biogenesis itself. The peroxisome contains the PEX 11 protein. PEX 11 is responsable (partly) for the splitting of the peroxisome. So by the activity of PEX 11 (among others) new peroxisomes are created. This theory is shown in Figure 2.

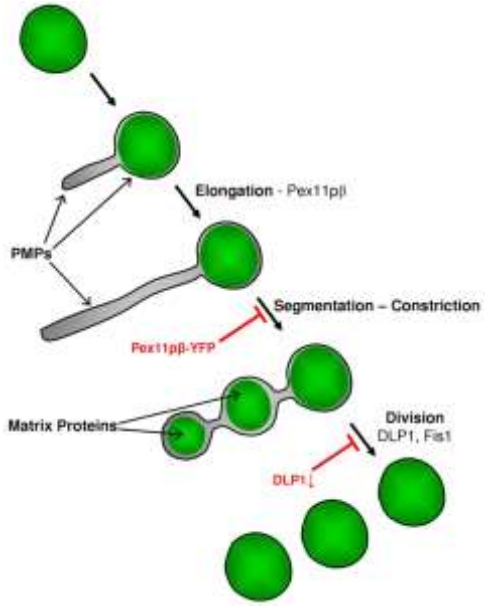


Figure 2: Biogenesis using among other PEX 11.
Source: drs. Kevin Berendse [4]

The just formed peroxisomes are basically empty organelles. To function they must be filled with membrane- and matrix proteins. Below, I'm going to tell you how membrane and matrix proteins, and the substrate of the peroxisome, are in im- and exported. As I already described in the previous paragraph, many of the peroxins are responsible for the import of membrane and matrix proteins. In Figure 3 is an overview of almost all proteins in the membrane can be found.

To import cell membrane proteins the complex PEX 3, PEX 16 and PEX 19 is needed. Outside the membrane, in the interior of the cell (cytosol), PEX 5 and PEX 7 are present. PEX 5 can recognize proteins, such as catalase, with a PTS1 signal. A PTS1 signal is a kind of postal code that this is a substrate for the peroxisome. The PTS1 signal is needed to pass a controller enzym in the membrane.The controller is also known as the Receptor. Receptors ensure that not all proteins can go in the peroxisome. There are two receptors: Receptor 1 and Receptor 2. There are two receptors, because there are two PEX proteins are that provide for the transport of proteins. They are also called PTS1 Receptor and PTS2 Receptor. When a substrate is bound to a Recepor, it is recognized by a PEX-protein. PEX 5 binds with the protein with a PTS1 signal so that the protein can go within the peroxisome. PEX 7 can recognize proteins with a PTS2 signal and binds it to itself. Bound to PEX 7 the protien can enter the peroxisome. In other words: the cooperation between PEX 5 and the PTS1 signal on one hand and PEX 7 and PTS2 signal on the other hand ensure the transport of proteins in the peroxisome.

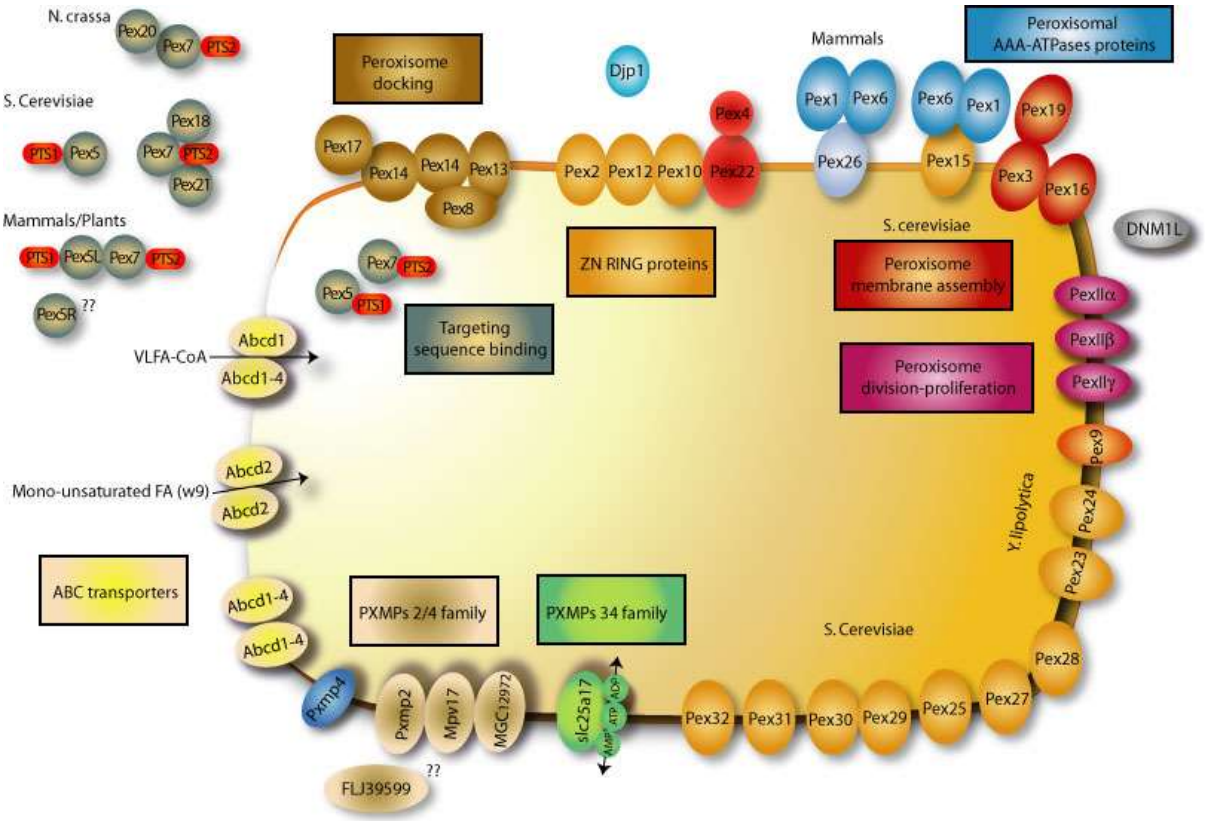


Figure 3: Pex and and other proteins of a peroxisome
Source: PeroxisomeDB 2.0 [25], Peroxisomal pathways for Homo Sapiens

PEX 5 and PEX 7 bind themselves hereafter to PEX 13 and PEX 14. These two peroxins sit in the membrane. Also PEX 2, PEX 10 and PEX 12 together form a complex. This complex ensures that a protein is brought back across the membrane. Finally the complex PEX 1, PEX 6 and PEX 26 ensure that the PEX 5 or PEX 7 protiens are brought back to the cytosol. Thus begins the cycle of insertion of protein in the peroxisome over and over again.

PEROXISOMAL DISEASES

2.1. INTRODUCTION

Peroxisomes are organelles with an important function[6]. If something goes wrong in a peroxisome, this manifests in a peroxisomal disease. There are several peroxisomal diseases, all with severe symptoms. In the next chapter I tell what there biochemical in a peroxisome goes wrong. In this chapter I tell about the family of peroxisomal disorders.

2.2. SYMPTOMS OF PEROXISOMAL DISEASES

Peroxisomal diseases are hereditary[7]. The formation of the peroxisomes and peroxisomal enzymes is encoded in the DNA, in the form of genes. There are fourteen known genes which are involved in the coding for the proteins embedded in the structure of the peroxisomal membrane. If there is a mutation in such peroxisomal membrane gene, the mutation leads to a peroxisomal disease. Those genes are called the PEX genes (see Chapter 1). Mutations are changes in the DNA code. Mutations in the PEX genes have the effect that peroxisomes are not working properly.

There are two types of peroxisomal defects. The first is that a particular matrix enzyme inside the peroxisome does not work. This is called peroxisomal enzyme deficiency. It could also be that the matrix enzymes work correctly, but that the membrane proteins do not work properly. This is called a peroxisomaal biogenesis defect (PBD).

[6]Prof. Dr. B.T. Poll-The en Prof. Dr. P.G. Barth, 'van kleine dingen... over peroxisomen, onzichtbare fabriekjes met een grote betekenis', De specialist aan het woord, UMC Magazine, 1999, blz 1 [2]
[7]Prof. Dr. B.T. Poll-The en Prof. Dr. P.G. Barth, 'van kleine dingen... over peroxisomen, onzichtbare fabriekjes met een grote betekenis', De specialist aan het woord, UMC Magazine, 1999, blz 4 [2]

A number of examples of peroxisomal diseases:

1. X-linked adrenoleukodystrophy (X-ALD)[8]. This is the most common peroxisomal disease. It is connected to an X chromosome. In the beginning these patients develop normally. Later they get bothered by symptoms as troubled hearing and seeing, paralysis, seizures and dementia. It is an example of a peroxisomal enzyme deficiency.
2. Neonatal adrenoleukodystrophy [9]. Children with this disease have no distinctive look. They can have seizures shortly after their birth. In the first year they develop normally. But then they go backwards. This disease is an example of a peroxisomaal biogenesis defect.
3. The infantile Refsum Syndrome[4]. Children with this disease have a distinctive look. They have a high forehead and a large fontanel. The fontanel is a part of the skull of young children that have not yet been paved. They have no or little ear lobes and an enlarged liver. They remain back in mental development. They have congenital retinal abnormalities, which manifest in bad sight. They also abnormalities in their hearing nerves, which result in hearing bad. The development of these children is much better than the children with the syndrome of Zellweger. They can reach puberty or maturity. This disease is an example of a peroxisomaal biogenesis defect.
4. The syndrome of Zellweger[10]. This is the most serious peroxisomal disease. These children are from birth very weak. They have liver disease making them often see yellow. They cannot see by congenital retinal

[8] Dr. Hugo W. Moser en Dr. Stephan Kemp, 'X-linked Adrenoleukodystrophy Database', Nederlandstalige website [1]
[9] Prof. Dr. B.T. Poll-The en Prof. Dr. P.G. Barth, 'van kleine dingen... over peroxisomen, onzichtbare fabriekjes met een grote betekenis', De specialist aan het woord, UMC Magazine, 1999, blz 4 [2]
[10] Prof. Dr. B.T. Poll-The en Prof. Dr. P.G. Barth, 'van kleine dingen... over peroxisomen, onzichtbare fabriekjes met een grote betekenis', De specialist aan het woord, UMC Magazine, 1999, blz 4 [2]

abnormalities, like patients with Refsum's syndrome. Also, they can not hear well by damage to the hearing nerves. They often have seizures. They look in appearance so special that a physician could recognize them directly. They usually not survive their first year. This disease is an example of a peroxisomaal biogenesis defect.

My own disease can be classified in the Zellweger spectrum. The difference between Zellweger spectrum and the syndrome of Zellweger is that a spectrum is a collection of peroxisomal disorders. Syndrome is a disease where the cause is not know (Meanwhile the cause is known, so ' syndrome ' is a historical name). A syndrome is also a number of clinical symptoms that occurs among a group of similar patients. That is how the term syndrome has to be interpreted in my case. The cause of my disease is known; a mutation prevents the PEX 1 protien to function correctly.

In addition to the very serious disease classified as the syndrome of Zellweger, there are also milder variants in the Zellweger spectrum, for example me. In my DNA it is genetic defect found, I have a PEX1 mutation. This is the most common mutation[11].

My hearing and vision is impaired, and I have to adjust my diet[12]. I may not eat nuts, fish and ruminant fats, because they contain phytanic acid. I cannot disassemble phytanic acid, resulting in accumulation of phytanic acid in my body. Accumulation of phytanic acid can lead to heart disease.

[11] Merel S. Ebberink, Molecular and genetic characterization of peroxisome biogenesis disorders, proefschrift Universiteit van Amsterdam (2010), [23], hoofdstuk 2

[12] Drs. Kevin Berendse, onderzoeker Pediatrics Neurology / Genetic Metabolic Diseases, AMC Amsterdam, persoonlijke communicatie [4]

I need to get the substance DHA-AA as diet supplement [13], because the peroxisome can make no DHA. DHA is important for the development of the nervous system and the retina. However, if I just get DHA, then the concentration of AA drops automatically. This is demonstrated by research[13]. That is why the supplement consists of both DHA and AA.

My body makes the intermediate of bile acids, but can't convert it into the final product[12]. In the meantime my body continues to produce the intermediate product. The intermediate product is a toxic bile acid. Since half a year I participate in a medical experiment where I get bile acid capsules to avoid my body to make toxic bile acids.

Phytanic acid, DHA and bile acid are the substances I will use in the next chapter as examples for the biochemical processes in a peroxisome.

[13] Drs. Kevin Berendse, onderzoeker Pediatrics Neurology / Genetic Metabolic Diseases, AMC Amsterdam, persoonlijke communicatie [4]

BIOCHEMISTRY
INSIDE THE PEROXISOME

3.1. INTRODUCTION

Many different biochemical activities take place in the peroxisome[14]. To describe all those biochemical activities would too much and too complicated for this research paper. That is why I will focus on a few examples of those activities.
The peroxisome provides:

- the partial synthesis of DHA-AA.
- the degradation of phytanic acid.
- the conversion of the precursor of cholic acid

In this chapter I'm going to work out those three examples of biochemical activities.

3.2. DHA-AA SYNTHESIS

3.2.1. OMEGA-3 FATTY ACIDS

DHA is an omega-3 fatty acid[15]. Omega-3 fatty acids are essential fatty acids, they are essential because the body can not create them. The abbreviation DHA stands for Docosa Hexaenoic Acid. DHA is a mono unsaturated fatty acid. It is a long-chain-fatty acid. It is common in seafood, such as fish and shellfish[16]. The human body can create DHA from the omega-3 fatty acid alpha-linolenic acid (ALA)[17]. The conversion of ALA is tricky; about 8% of ALA can be converted into EPA (an intermediate substance), and only 4% can be converted from

[14] Benno Beukema, 'Stofwisselingsziekten - Een inleiding in de aangeboren stoornissen van de stofwisseling', Nederlandstalige website [3]
[15] Wikipedia, Docosahexaeenzuur [8]
[16] Voedingscentrum Encyclopedie-Omega 3 [14]
[17] Wikipedia, Omega 3 fatty acids – list of omega 3 fatty acid [5]

EPA to DHA[18]. DHA is an important substance for the development of the nervous system. It is also an important substance for the development of the retina.

The elements carbon (C), hydrogen (H) and oxygen (O) are found in DHA and in other omega-fatty acids. Fatty acids are chains of hydrocarbons with an acid head. Hydrocarbon is a bond between carbon and hydrogen: C-H. There are two types of bonds between hydrocarbons: single bonds and double bonds. A single bond looks like this: C-C. A double bind looks like this: C = C. Substances with only single bonds are called saturated fats. Substances with one or more double bonds are unsaturated fats. The omega fatty acids are unsaturated substances with the first double bond *n* number of steps from the omega side (see Figure 4). There can be one or more double bonds in the fatty acid chain.

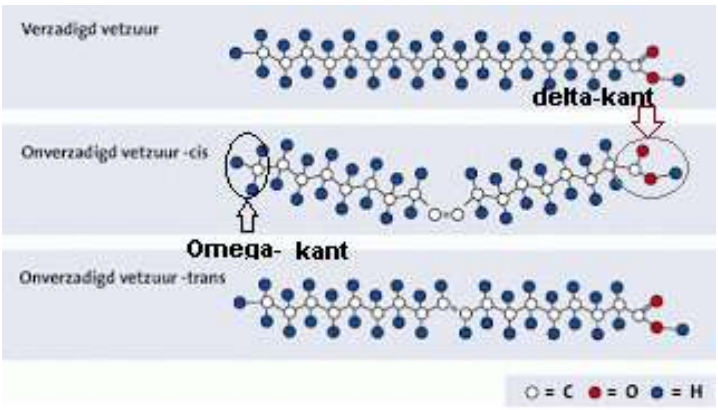


Figure 4: General structure formulas for saturated and unsaturated fatty acids. Source: Pure Voeding Blogspot [26], De chemische structuur van vet.

DHA consists of 22 carbon atoms, 32 hydrogen atoms and two oxygen atoms. DHA can also be written as $C_{22}H_{32}O_2$. This is called a chemical formula.

[18] J.T. Brenna, 'Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man', Curr Opin Clin Nutr Metab Care. 2002 Mar;5(2):127-32 [24]

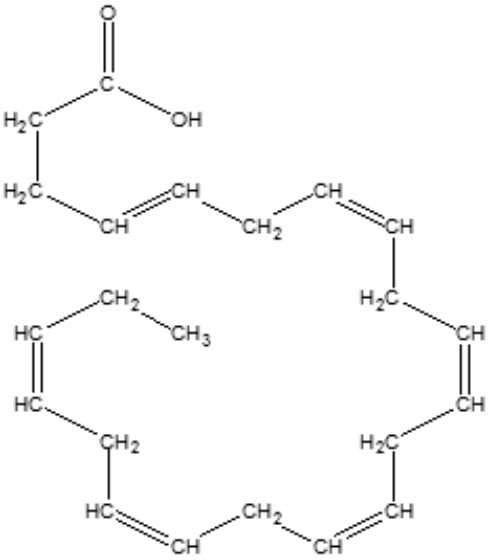


Figure 5: Structural formula DHA
Source: Wetenschappelijk informatie over visolie [16]

The 'acid' part of the DHA contains one carbon atom, two oxygen atoms and one hydrogen atom. This is called a Carbonyl group: COOH. This side of the fatty acid is called the delta side. The other side of the fatty acid (ends with CH_3) is called the omega side (see Figure 5). DHA is also noted in this way: C22: 6n-3. C22 represents the number of carbon atoms, 6 stands for a total of six double bonds, and n-3 indicates that the first double bound is at position 3 from an omega side.

3.2.2. OMEGA-6 FATTY ACID

AA is an omega-6 fatty acid[19]. Omega-6 fatty acids, like omega-3 fatty acids, are essential fatty acids. The abbreviation AA stands for Arachidonic Acid[20]. AA is a polyunsaturated fatty acid. It is found in animal fats and vegetable products. It is in the human body formed from linoleic acid and gamma-linolenic acid[21].

[19] Wikipedia, Arachidonzuur [9]
[20] Wikipedia, Omega 6 fatty acids – list of omega 6 fatty acid [6]
[21] Voedingscentrum Encyclopedie- Omega-6 [13]

AA consists of 20 carbon atoms, 32 hydrogen atoms and 2 oxygen atoms. The molecular formula of AA is $C_{20}H_{32}O_2$. It is a chain of carbon atoms with hydrogen atoms, like DHA.

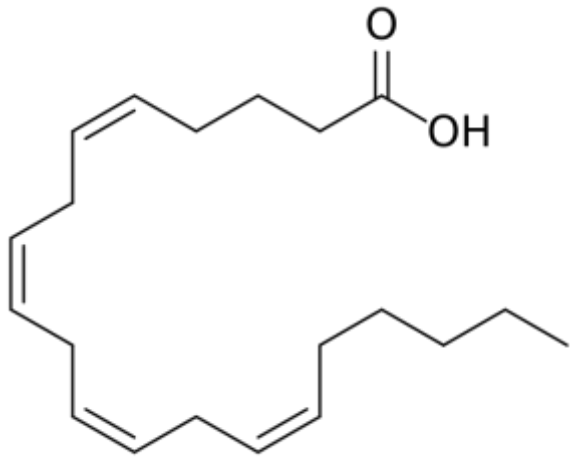


Figure 6: Structural formula AA
Source: Wikipedia, Omega 6 fatty acids [6]

AA has, like DHA, an 'acid' part that consists of one carbon atom, two oxygen atoms and one hydrogen atom: COOH. The other format for AA is C20: 4n-6. It has in total four double bonds and n-6 indicates that the first double bond is on position 6 from the omega side.

3.2.3. SYNTHESIS

The peroxisome is responsible for the partial assembly of DHA-AA[22]. Another word for assembly is synthesis. Most of the synthesis of fatty acids is done in the cytoplasm, outside the peroxisomes. In the cytoplasm fatty acids are made with a chain up to 16 carbon atoms[23]. Longer chains of fatty acids are built by other enzyme systems, such as those inside the peroxisome and endoplasmatic reticulum (ER).

[22] Prof. Dr. B.T. Poll-The en Prof. Dr. P.G. Barth, 'van kleine dingen... over peroxisomen, onzichtbare fabriekjes met een grote betekenis', De specialist aan het woord, UMC Magazine, 1999, blz 3 [2]
[23] Lubert Stryer, 'Biochemistry", Freeman New York 1988, H20: fatty acid metabolism) [17]

DHA Synthesis:

The substance alpha-linolenic acid gets in the ER an additional double bound[24]. This is called Δ6-desaturation. Δ6-desaturation means that at the sixth place from the delta side a single bond turns into a double bound[25]. The next step should have been a Δ5-desaturation. However, two double bounds can not exist directly next to each other. Therefore the fatty acid chain is extended with two carbon atoms at the delta side. This extension is called elongation. After this elongation the Δ5-desaturation could be applied. This chemical reaction is nearly equavalent to a Δ6-desaturation, with the only difference that the double bond is introduced at the fifth place from the delta side. The synthesis continues with two additional elongations, followed by the introduction of another double bound by Δ6-desaturation. The chain is now longer than the final product DHA, so two carbon atoms have to be cut off. This can only be done in the peroxisome. The cut off is performed by a bèta-oxidation at the delta side.

AA Synthesis:

The substance linolenic acid gets in the ER an additional double bound through Δ6-desaturation. This if followed by an elongation and a Δ5-desaturation, equivalent with the first synthesis steps of DHA.

On the next page the synthesis of the DHA-AA is shown schematically:

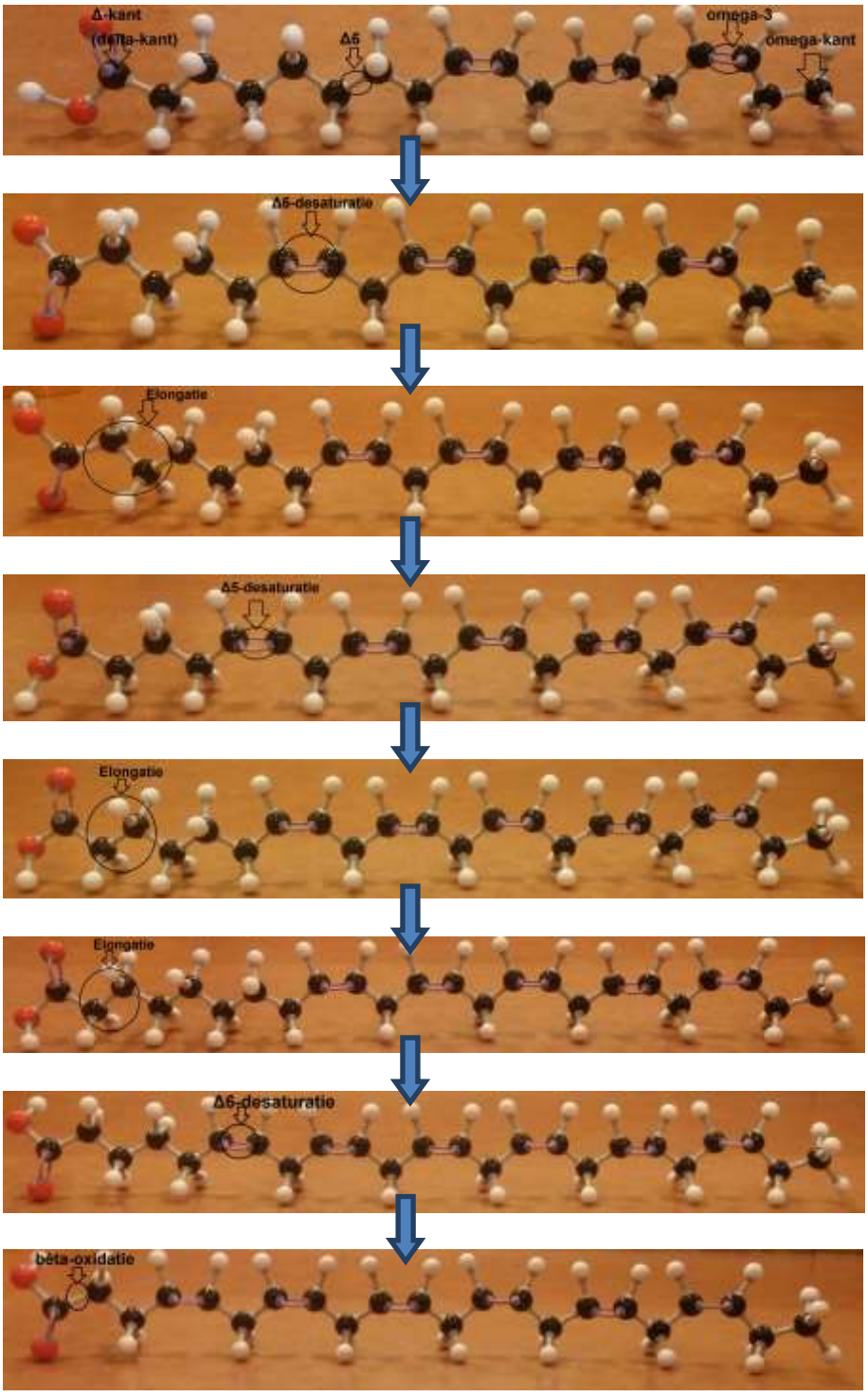
[24] Sacha Ferdinandusse, Simone Denis, Petra A. W. Mooijer, Zhongyi Zhang, Janardan K. Reddy, Arthur A. Spector, and Ronald J. A. Wanders, 'Identification of the peroxisomal β-oxidation enzymes involved in the biosynthesis of docosahexaenoic acid', Journal of Lipid Research, Volume 42 (2001) 1987- 1995 [20]
[25] Wikipedia, Desaturase [12]

DHA-AA Synthesis

DHA Synthesis	AA Synthesis
In the ER C18:3n-3 (ALA) Δ6-desaturation C18:4n-3 Elongation C20:4n-3 Δ5-desaturation C20:5n-3 Elongation C22:5n-3 Elongation C24:5n-3 Δ6-desaturatie C24:6n-3 **In the peroxisome** C24:6n-3 Bèta-oxidation C22:6n-3 (DHA)	**In the ER** C18:2n-6 (linolenic acid) Δ6-desaturation C18:3n-6 Elongation C20:3n-6 Δ5-desaturation C20:4n-6 (AA)

Table 1: DHA-AA Synthesis (Table is a summary of a corresponding table in [15])

DHA Synthesis:



step 1: ALA

step 2: C18:4n-3

step 3: C20:4n-3

step 4: C20:5n-3

step 5: C22:5n-3

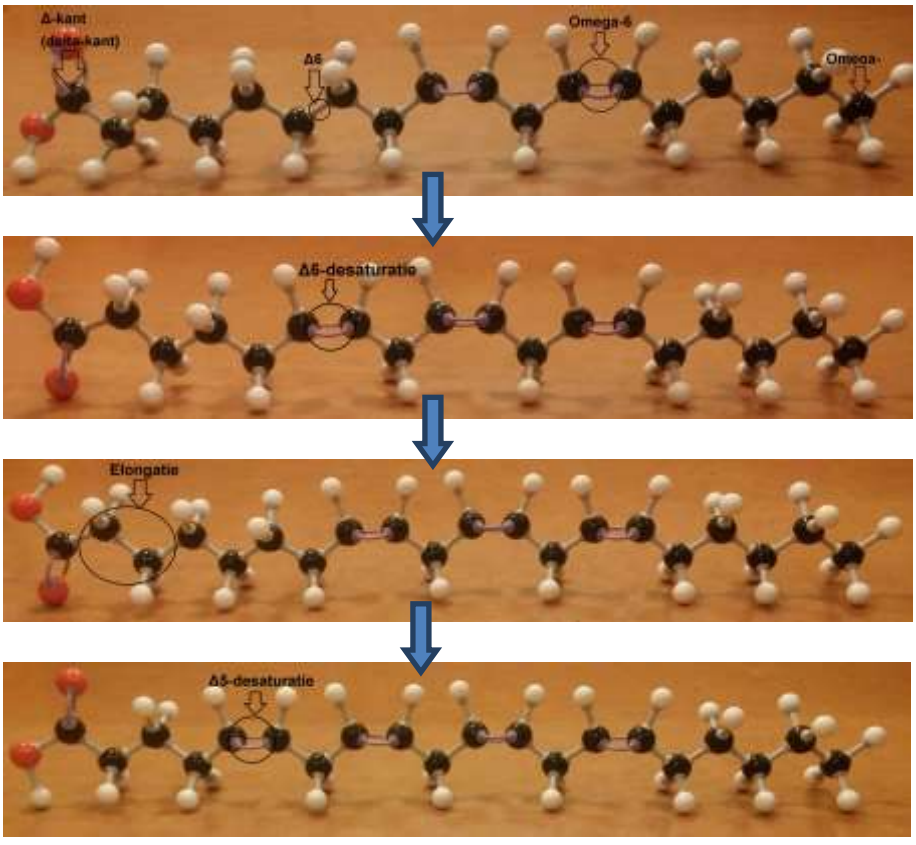
step 6: C24:5n-3

step 7: C24:6n-3

step 8: DHA

Figure 7: DHA synthesis visualised with moleculair construction sets.
Source: Karlijn Elias

AA Synthesis:



step 1: linolenic acid

step 2: C18:3n-6

step 3: C20:3n-6

step 4: AA

Figure 8: AA synthesis visualised with moleculair construction sets
Source: Karlijn Elias

The peroxisome has in the synthesis of DHA-AA an important function in the final step of making the product DHA.

3.3. PHYTANIC ACID DEGRADATION

3.3.1. DESCRIPTION

Phytanic acid is a branched fatty acid[26]. It occurs mainly in fish products, nuts and products of ruminants. It consists of 20 carbon atoms, 40 hydrogen atoms and 2 oxygen atoms. The molecular formula of phytanic acid is $C_{20}H_{40}O_2$. Phytanic acid has a chain of 16 carbon atoms with 4 methyl groups as branches. Methyl has the molecular formula CH_3.

[26] Wikipedia, Fytaanzuur [10]

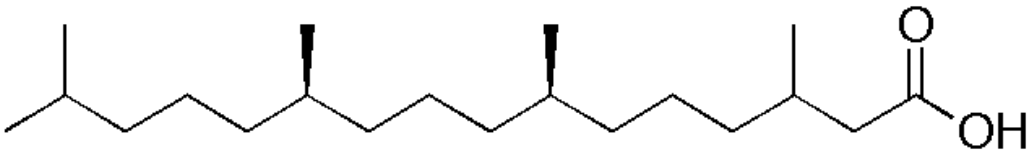


Figure 9: Structural formula phytanic acid
Bron: Wikipedia, Phytanic acid [7]

By the many methyl branches phytanic acid is hard to break down in the mitochondria. Instead the phytanic acid is disassembled in the peroxisome by means of alpha and bèta-oxidation. Another word for disassembly is degradation.

3.3.2. DEGRADATION PROCESS

To start the degradation process the substrate is first activated by a coupling to an enzyme, acyl-CoA synthetase (Coënzyme A, abbreviated to acyl-CoA)[27]. In Figure 10 acyl-CoA is indicated with a green atom, although in reality the enzyme is a large molecule. After the activation alfa-oxidation takes place. Alfa-oxidation is the proces by which fatty acids are shortened with one carbon atom by an oxidative decarboxylation. The result is carbon dioxide (CO_2) en pristanic acid (which consists of 19 carbon atoms). So, pristanic acid has one carbon atom less than phytanic acid, but the structure formula is nearly the same. Pristanic acid is also activated by acyl-CoA. After the activation the chain is shortened by applying bèta-oxidation four times. In some circumstances the effect of bèta-oxidation is the removal of 3 carbon atoms. The product of this reaction is propionyl-CoA. In other circumstances the effect of bèta-oxidation is the removal of 2 carbon atoms. The product of this reaction is acetyl-CoA. The products acetyl and propionyl can be converted into energy in the citric acid cycle.[28]

[27] Ronald J.A. Wanders, Jasper Komen, and Sacha Ferdinandusse, 'Phytanic acid metabolism in health and disease', Biochimica et Biophysica Acta. 1811 (2011) 498–507 [21]
[28] Lubert Stryer, 'Biochemistry', Freeman New York 1988, H20: fatty acid metabolism [17]

Schematic overview disassembly phytanic acid:

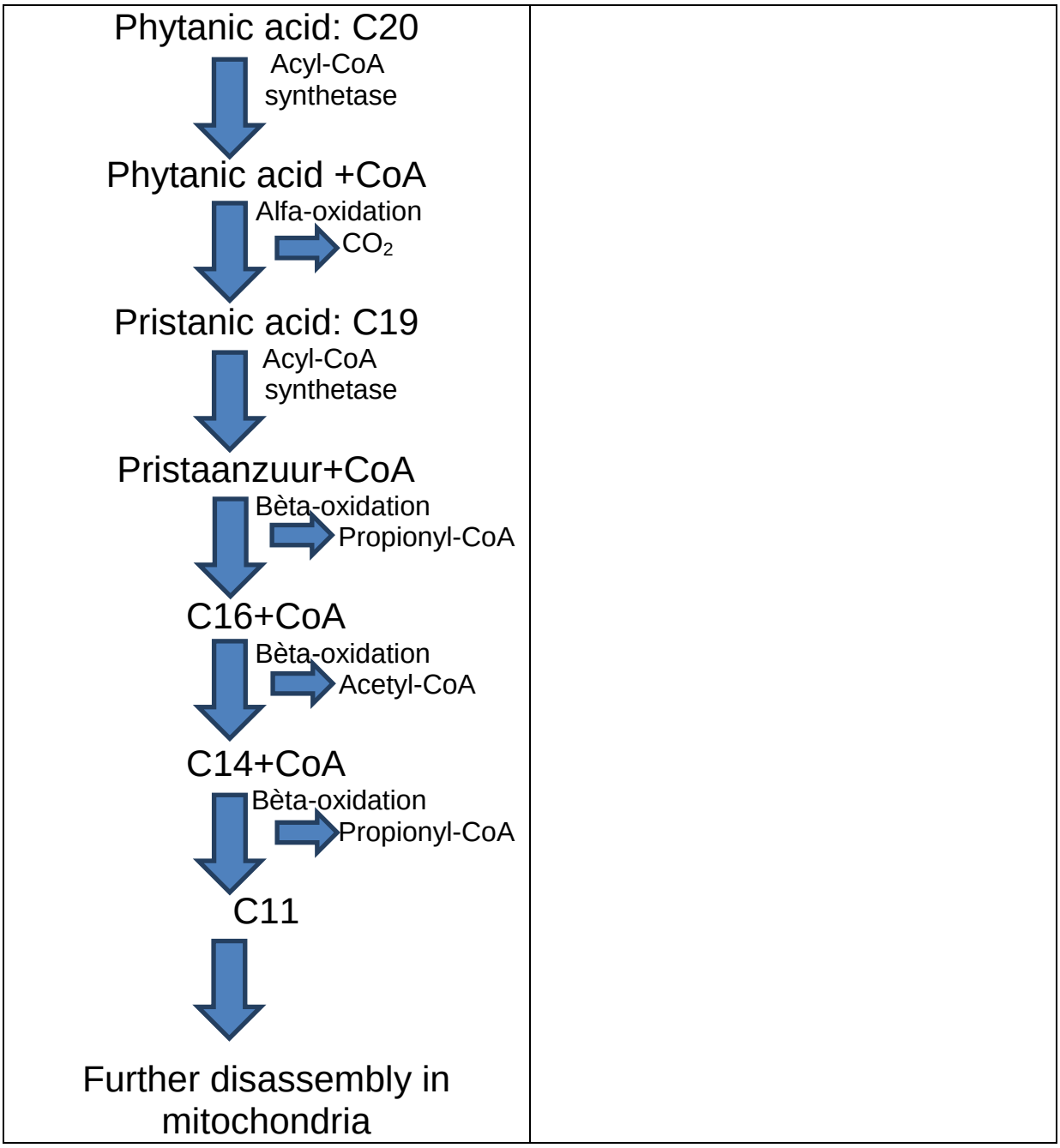
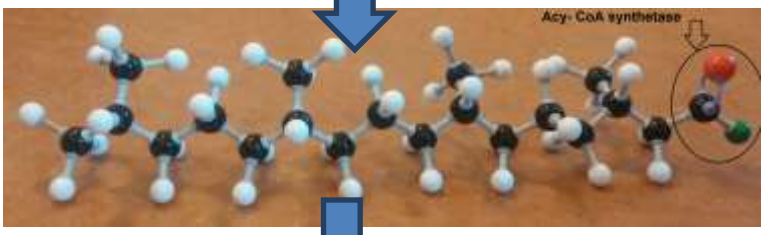


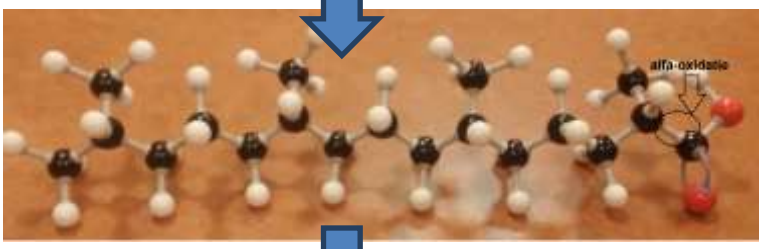
Table 2: phytanic acid degradation

Disassembly phytanic acid:

step 1: phytanic acid

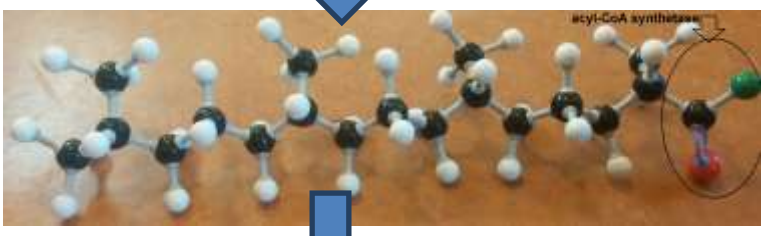


step 2: activated phytanic acid

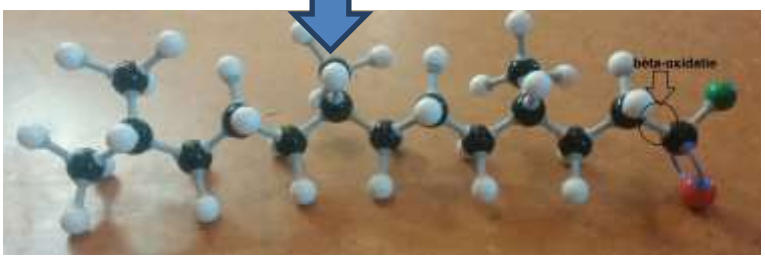


step 3: pristanic acid

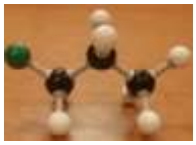
+ 



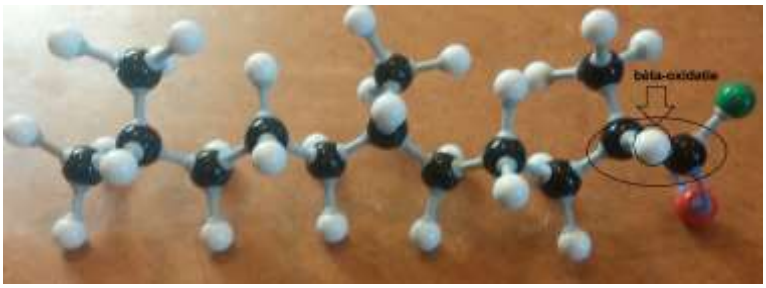
step 4: activated pristanic acid



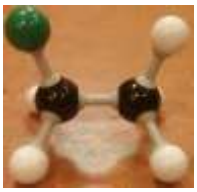
step 5: C16 + CoA

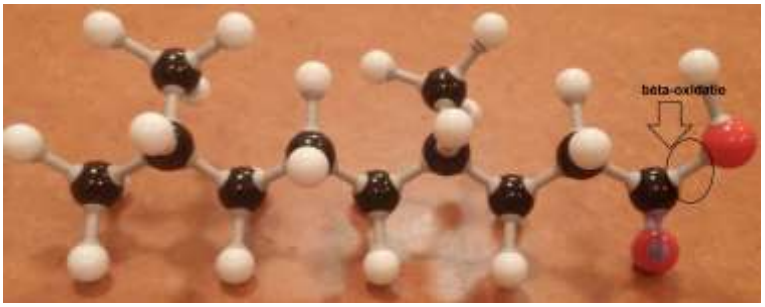
+ 

The next steps are illustrated on the next page

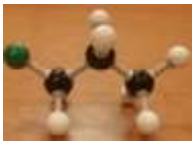


step 6: C14 + CoA

+ 



step 7: C11

+ 

Further degradation in mitochondria

Figure 10: Phytanic acid degradation visualised with moleculair construction sets
Source: Karlijn Elias

3.4. CHOLIC ACID CONVERSION

3.4.1. DESCRIPTION

Cholic acid is a primary bile acid[29]. Bile acids are substances that have an important role in the emulsification of water insoluble nutrients, such as fat. The molecular formula of cholic acid is $C_{24}H_{40}O_5$[30].

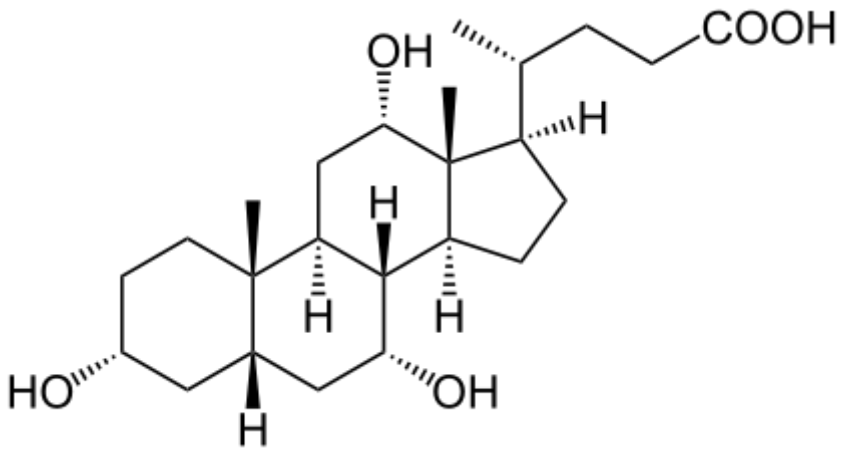


Figure 11: Structural formula cholic acid
Source: Wikipedia, Cholic acid [27]

3.4.2. CONVERSION PROCESS

The peroxisome converts the substance THCA (including 27 carbon atoms) to cholic acid[31]. THCA is an abbreviation of TriHydroxy-Cholestanoic Acid.

In the liver cholesterol is converted in five steps in THCA. THCA is then transported to the peroxisome can be converted in to in cholic acid. The process is started by activating THCA with CoA. The substrate THCA has, even after this activation, the wrong isomeric form. Isomers are compounds with the same molecular formula, but with one of the bindings mirrored. This is also called a stereoisomer. The enzyme racemase handles the

[29] Instituut voor Permanente Studie voor apothekers, Farmamozaïek – Cholzuur, Nederlandstalige website [18]
[30] A.P.N. Franchinont, 'Koolstof en Hare Verbindingen: Leiddraad bij de studie der soo gename organische chemie', Leiden, 1878 1e druk [19]
[31] Sacha Ferdinandusse and Sander M. Houten, 'Peroxisomes and bile acid biosynthesis', Biochimica et Biophysica Acta, 1763 (2006) 1427–1440 [22]

convesion of 25^{e} carbon bond in THCA of R-form to the S-shape. The R-and S-form are stereoisomers of THCA. In Figure 12 is this conversion is visualized. Please note the change in the red marked area where the H-atom and the methyl group take each other place. In this visualization a black triangle indicates that the group is located above the surface of the molecule structure. The dotted triangle indicates that the group is located below the surface of the molecule structure. This same format can also be seen in the visualization of phytanic acid in Figure 9.

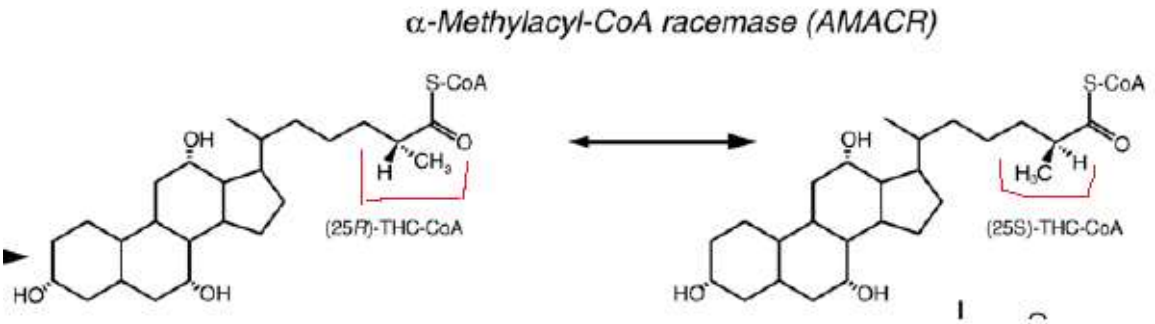


Figure 12: Conversion of R-form of THCA (structural formula left) to S-shape THCA (structural formula right)
Source: Sacha Ferdinandusse and Sander M. Houten, 'Peroxisomes and bile acid biosynthesis', Biochimica et Biophysica Acta, 1763 (2006) 1427–1440 [22]

Now that THCA is in the S-shape the carbon chain can be shorted by bèta-oxidation. In this case three carbon atoms are removed, the substrate is shortened from C27 to C24. The result is cholic acid. In this reaction also propionyl-CoA is created.

Conversion cholic acid

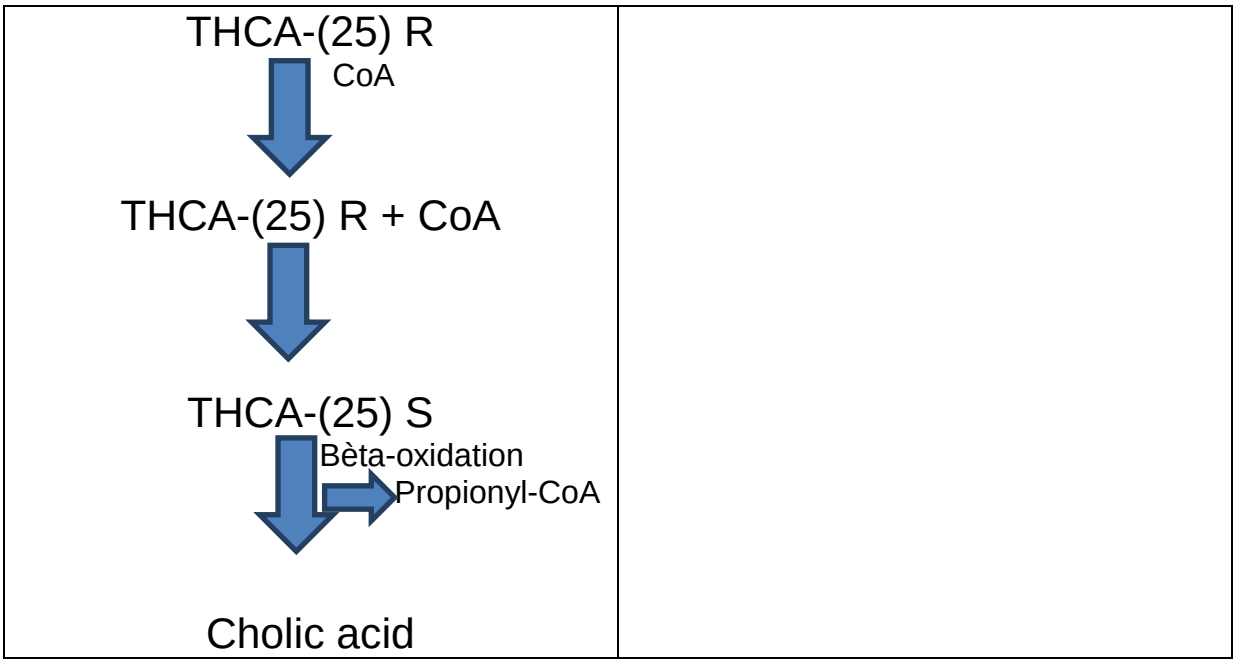
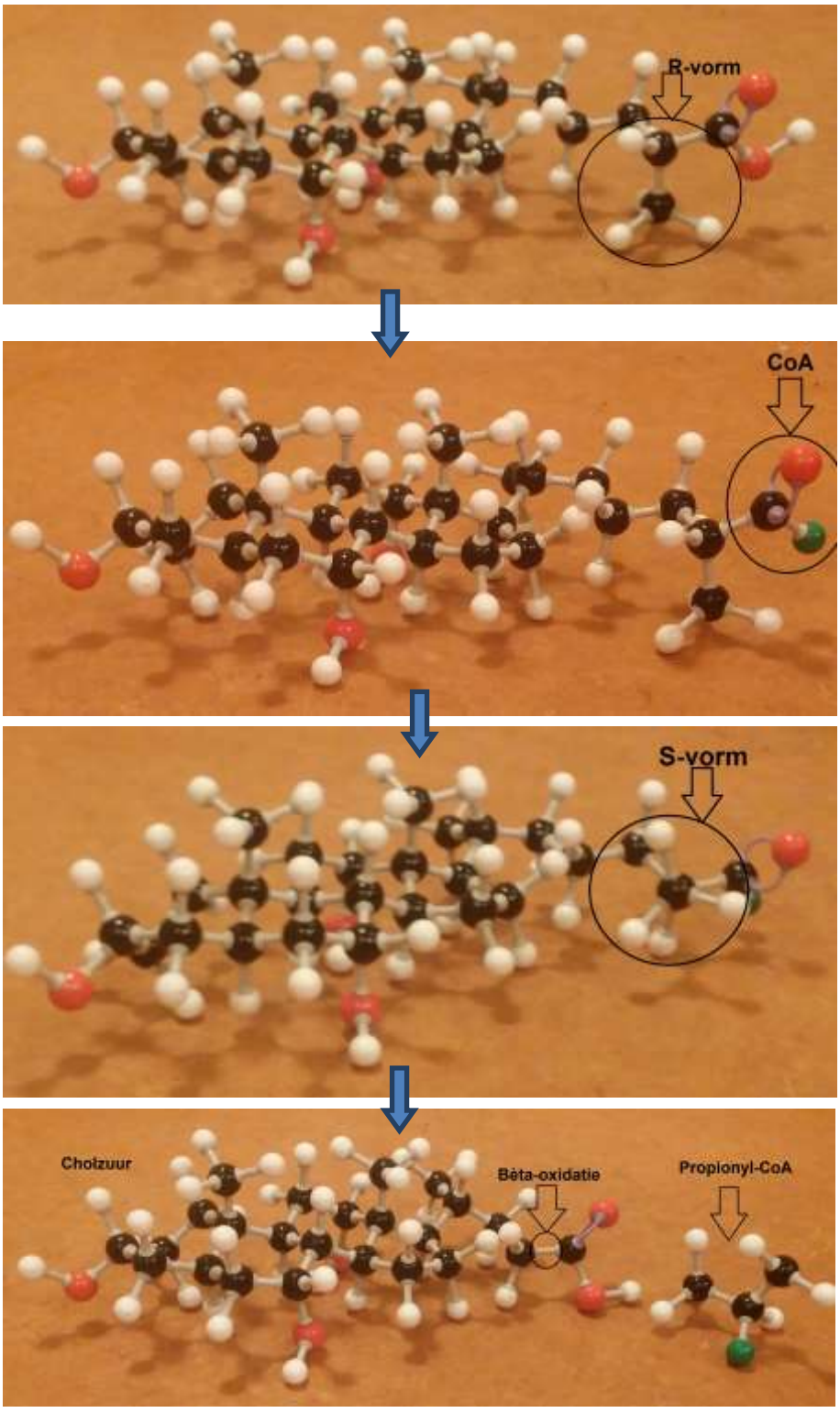


Table 3: Conversion cholic acid

Conversion cholic acid:



step 1: THCA-R

step 2: THCA-R+

step 3: THCA-S+

step 4: Cholzuur+ propionyl

Figure 13: Conversion cholic acid visualised with moleculair construction sets
Source: Karlijn Elias

3.5. THE RATIONAL BEHIND THIS THREE EXAMPLES

Inside a peroxisome many biochemical activities take place[32]. I have chosen to select three of those activities and to work their process out in detail. These are the reasons for my choice:

First of all, I think that to explain to all activities would be too much for a research paper at this level. The final version of the paper is 120 pages, including other biochemical activities would make it even thicker. Another example of synthesis could have been: the formation of plasmalogenen. Another example of degradation could have been the decomposition of pipecolinezuur. Another example of conversion and their by-products could have been the production of oxalic acid.

Secondly, the substances phytanic acid, cholic acid and DHA are precisely the adjustments in my own diet.

Finally: a defect in the peroxisome can go wrong in any of the three processes:

1. Synthesis
2. Degradation
3. Conversion

If something goes wrong in one of the this processes, this will manifest in a peroxisamal disease as introduced in Chapter 2. In this chapter I have given an example of each of the processes.

[32] Merel S. Ebberink, 'Molecular and genetic characterization of peroxisome biogenesis disorders', proefschrift Universiteit van Amsterdam (2010) [23]

This is the answer the question ' why these three activities? ':

The peroxisome has way too many and complicated activities. Also, the three substances in this chapter I have worked out are my diet adjustments. In addition, in the peroxisome something can go wrong at synthesis, degradation or conversion of substances. An example of each process is given.

DEMONSTRATION OF ACTIVITY IN A PEROXISOME (PRACTICAL ASSIGNMENT)

4.1. INTRODUCTION

On 17 October 2014 I went to a laboratory in the Academic Medical Centre in Amsterdam, to perform an experiment together with researcher Kevin Berendse. My research question was: ‘How to proof that peroxisomes are still perform biochemical activity?’. This research question I have answered by performing an experiment on living cells. In the annex the complete investigation report can be found. In this chapter I discuss the results and the conclusion of this experiment.

4.2. EXPERIMENTAL SETUP

Together with researcher Kevin Berendse we have looked what the procedure is to measure the activity of peroxisomes inside a cell.

In preparation Kevin Berendse has grown two cultures of human skins cells; one culture of a healthy person and one culture of a person with a PEX 1 mutation.

In Chapter 1, I have told that the enzymes and proteins can be divided into two groups. Group 1: enzymes and proteins which are active in the membrane of a peroxisome. Group 2: enzymes

and proteins which are active inside the matrix of the peroxisome.

Demonstration activity inside a peroxisome:

To demonstrate the function of the peroxisome, the enzyme catalase added to the skin cells. As I described in Chapter 1 catalase has a function in the peroxisome in converting hydrogen peroxide. In the healthy skin cells catalase is transported into the interior of the peroxisome. By inspecting the skin cells under a microscope (after immunofluorescence colouring of the culture) it can be seen that the peroxisomes of healthy persons have absorbed the catalase. In the skin cells of persons with a PEX 1 mutation the catalase is not absorbed, which indicates that the transport mechanisms through the membrane are not functioning. Catalase is still visible with immunofluorescence colouring, but the catalase is distributed over the cytosol and not concentrated in peroxisomes.

Demonstration of the presence of peroxisome

Catalase would also be distributed over the cytosol if no peroxisomes were present. To demonstrate the presence of peroxisomes, we have used a protein that is needed to build up the peroxisomaal membrane. This protein is called PMP70[33]. In healthy skin cells the protein is built into the peroxisomaal membrane. It binds to the protein complex of PEX 3, PEX 16 and PEX 19. After immunofluorescence colouring for this protein is clearly concentrated inside the peroxisomes. The other culture has mutation of PEX 1, which has nothing to do with the complex PEX 3, PEX 16 and PEX 19. After coloring the peroxisomes are also visible under the microscope. The

[33] Drs. Kevin Berendse, onderzoeker Pediatrics Neurology / Genetic Metabolic Diseases, AMC Amsterdam, persoonlijke communicatie [4]

peroxisomes are present, but there are significantly less than present inside a healthy person.

4.3. RESULTS AND CONCLUSION

As can be seen from the previous section; PMP70 indicates that peroxisomes are present.

With catalase show you that the peroxisome works. For the transport of catalase through the membrane the transport protein PEX 5 is required. PEX 5 leaves the peroxisome by binding with a protein complex in the membrane (PEX 1, PEX 6 and PEX 26). PEX 5 conveys the peroxisome in catalase by binding with another protein complex in the membrane (PEX 2 PEX, PEX 10 and 12). By a defect in one of the other PEX-proteins in the membrane PEX 5 can not do its work.

If PEX 5 can't do its work, then catalase may not be transported into the peroxisome. With catalase concentrated in the peroxisomes you can show that the peroxisome still work. When the catalyse enzyme can be found inside the peroxisome one can conclude that the transport through the membrane is still functional.

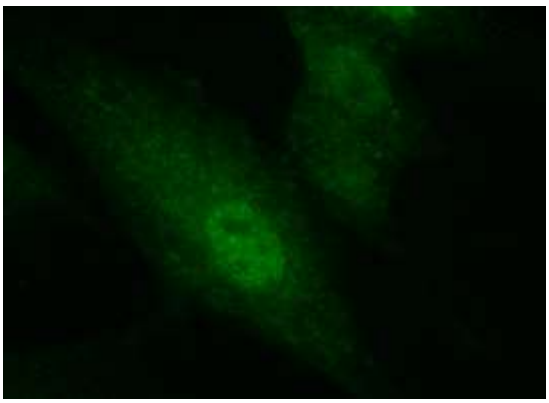
The prediction is that in a healthy person the peroxisomes will be visible with immunofluorescence colouring for both the catalase and PMP70 protiens. The prediction is that for a person with a PEX 1 mutation the peroxisomes will not visible with immunofluorescence colouring of the enzyme catalase. The transport through the membrane does not function in that case. The peroxisomes will be visible with immunofluorescence colouring of the protein PMP70. The peroxisomes are still built, even for a person with a PEX 1 mutation.

The immunofluorescence microscope was linked to a computer so that the images could be enlarged and I also could see the

cells well. We could also select and save pictures. Below is a table with the results, how the pictures should be intepreted. The pictures of the skin cells themselves are below the table.

	Catalase	*PMP70*
Healthy skin cell	*Peroxisomes visible (Photo 2)*	*Peroxisomes visible (Photo 4)*
PEX 1 mutation skin cell	*Peroxisomes not visible (Photo 1)*	*Peroxisomes visible, but fewer (Photo 3)*

Table 4: Resultats experiment



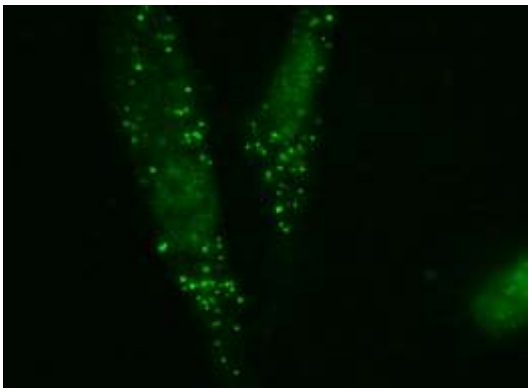
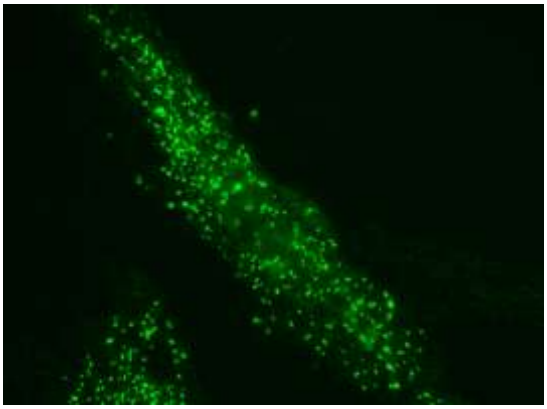


Photo 1: PEX 1 mutation peroxisome with catalase *Photo 3: PEX 1 mutation peroxisome with PMP70*



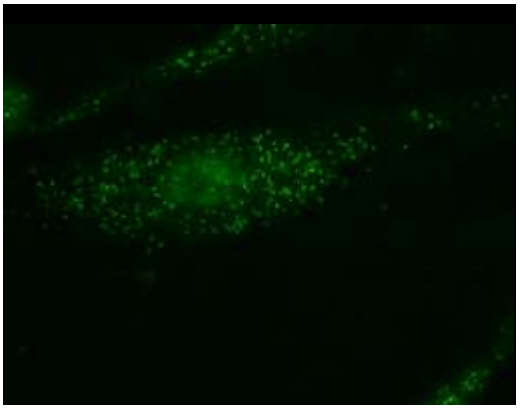


Photo 2: Healthy peroxisome with catalase *Photo 4: Healthy peroxisome with PMP70*

Interpertation results:

On Photo 1 two green, fuzzy ovals are visible; those are the outlines of two skin cells. In the lower cell is there a brighter circle visible; that is the cell nucleus. On Photo 2 there are lots of bright green specks in the ovals; those are the peroxisomes.

Catalase is active in these peroxisomes. On Photo 3 there are small green speckles, which are peroxisomes where PMP70 concentrates. There are clearly many more peroxisomes present on Photo 4, in the skin cells of a healthy person.

From this it can be concluded that the person with a PEX 1 mutation has peroxisomes, but they do not function well. There are also less peroxisomes compared to a healthy person.

So we can formulate the answer to the research question 'How to show that the peroxisomes still function? ':

By providing an enzyme that has a function inside the peroxisome, you can prove that the transport mechanism still works. With protein that has a function in the construction of the peroxisome, you can demonstrate that peroxisomes are present.

CONCLUSION

5.1. INTRODUCTION

In this research paper I have mainly sought to answer the research question:

What are the biochemical effects of peroxisomes?

In order to answer this research question, I answered four sub-questions:

1. What are peroxisomes?
2. What are peroxisomal diseases?
3. What are the biochemical activities of peroxisomes?
4. How to show that the peroxisomes still function?

5.2. SUB-QUESTIONS

5.2.1. SUB-QUESTION 1: WHAT ARE PEROXISOMES?

Peroxisomes are organelles that have an important function in synthesis, degredation and conversion of long-chain fatty acids.

5.2.2. SUB-QUESTION 2: WHAT ARE PEROXISOMAL DISEASES?

There are several peroxisomal defects. The manifestation of the disease is per defect different. But all defects have in common that that they lead to severe disability and/or premature death. Common problems have to do with the senses and the nervous system.

5.2.3. SUB-QUESTION 3: WHAT ARE THE BIOCHEMICAL ACTIVITIES OF PEROXISOMES?

1. Peroxisomes break down long-chain fatty acids by alpha and beta oxidation. Phytanic acid, for example, goes through a sequence of activation, alpha-oxidation and repeated beta-oxidation to be converted in a shorter fatty acid chain. The

shorter fatty acid chain can be further degradated in the mitochondria.

2. Peroxisomes can synthese long-chain fatty acids by providing the essential step of beta oxidation. An example of such long-chain fatty acids is DHA. The formation of this substance begins in the endoplasmatic reticulum. In the peroxisome is the last step in the synthesis is performed by shorten the forerunner by beta oxidation, which result in the final product DHA.

3. Peroxisomes can convert long-chain fatty acids by bèta-oxidation. An example of such long-chain fatty acids is cholic acid. Cholesterol is converted in the liver through a number of steps in THCA. THCA is a toxic precursor of Cholic acid. The peroxisome converts THCA through beta oxidation to the body usable cholic acid.

5.2.4. SUB-QUESTION 4: HOW TO SHOW THAT THE PEROXISOMES STILL FUNCTION?

There are proteins needed for the construction of the peroxisome and there are proteins necessary for the functioning of a peroxisome.

You can add an enzyme to the cytosol that has a function inside the peroxisome to show that the transportation through the membrame still functions. With protein needed for building the peroxisome, you can demonstrate that the peroxisomes are present.

In both cases you have to add a dye and to look with a fluorescence microscope to find the difference in pictures, to discover the peroxisomes and their biochemical activities.

5.3. MAIN RESEARCH QUESTION: WHAT ARE THE BIOCHEMICAL EFFECTS OF PEROXISOMES?

By building up long-chain-fatty acids, the peroxisomes make substances necessary for our body, such as DHA. By breakdown of long-chain fatty acids, such as phytanic acid, peroxisomes prevent accumulation of these substances in the body. Instead the fatty acids are converted into energy that we need. By conversion of long-chain fatty acids in substances that our body needs, the peroxisomes prevent toxic precursors of those substance do damage. One example of such substance is cholic acid.

So peroxisomes ensure production of essential fatty acids, preventing accumulation of long-chain fatty acids and prevent poisoning by intermediate products.

REFERENCES

[1] *Dr. Hugo W. Moser en Dr. Stephan Kemp, 'X-linked Adrenoleukodystrophy Database', website created July 1999, last visit October 2014, http://www.x-ald.nl/nederlands/, reliability score according to webdetective.nl: 29/35*

X-gebonden adrenoleukodystrofie (X-ALD) is de meest voorkomende erfelijke aandoening van de witte stof in het centraal zenuwstelsel. De ziekte komt voor bij 1 op de 17.000 pasgeborenen. X-ALD is een progressieve stofwisselingsziekte waarbij het myeline, het ruggenmerg, de perifere zenuwen, de bijnierschors en testis aangedaan raken. De ziekte wordt veroorzaakt door mutaties in het ABCD1 gen.

[2] *Prof. Dr. B.T. Poll-The en Prof. Dr. P.G. Barth, 'van kleine dingen... over peroxisomen', onzichtbare fabriekjes met een grote betekenis', De specialist aan het woord, UMC Magazine,1999*

Organellen zijn eigenlijk een soort fabriekjes in de cel, zoals peroxisomen, waar onder meer ingewikkelde vetmoleculen worden gemaakt en afgebroken. Die vetten dienen dan niet voor consumptie maar voor de vorming van bestanddelen waar het lichaam om allerlei redenen behoefte aan heeft, zoals het materiaal dat om de cellen heen zit, of dat in de cellen gedeelten van elkaar afschermt, membranen geheten.

[3] *Benno Beukema, 'Stofwisselingsziekten - Een inleiding in de aangeboren stoornissen van de stofwisseling', website created February 1998, last visit October 2014, http://home.kpn.nl/b1beukema/index.html, reliability score according to webdetective.nl: 25/35*

De aangeboren stofwisselingsziekten is de groep van aangeboren, recessief-erfelijke, meestal familiale ziekten, die zich in de regel op zeer jeugdige leeftijd openbaren, doch soms op rijpere leeftijd manifest worden; merendeels berusten zij op verminderde werking of afwezigheid van een bepaald enzym, waardoor een bepaalde fase van de stofwisseling wordt geremd of geblokkeerd, maar soms - c.q. tevens - berusten zij op een resorptiestoornis (resorptie: het proces waarbij vaste stoffen in het lichaam worden opgelost en in de lichaamsvloeistof opgenomen), of op een metabool moleculair structuurdefect in bepaalde eiwitten.

[4] *Drs. Kevin Berendse, onderzoeker Pediatrics Neurology / Genetic Metabolic Diseases, AMC Amsterdam, personal communication in period August-December 2014*

[5] *Wikipedia, Omega 3 fatty acids – list of omega 3 fatty acids, page created October 2001, last update October 2014, last visit October 2014 http://en.wikipedia.org/wiki/Omega-3_fatty_acid#List_of_omega-3_fatty_acids, reliability score according to webdetective.nl: 21/35*

Omega-3 fatty acids (also called ω-3 fatty acids or n-3 fatty acids are polyunsaturated fatty acids (PUFAs) with a double bond (C=C) at the third carbon atom from the end of the carbon chain. The fatty acids have two ends, the carboxylic acid (-COOH) end, which is considered the beginning of the chain, thus "alpha", and the methyl (CH3) end, which is considered the "tail" of the chain, thus "omega." The nomenclature of the fatty acid is taken from the location of the first double bond, counted from the methyl end, that is, the omega (ω-) or the n- end.

[6] *Wikipedia, Omega 6 fatty acids – list of omega 6 fatty acids, page created May 2004, last update October 2014, last visit October 2014, http://en.wikipedia.org/wiki/Omega-6_fatty_acid#List_of_omega.E2.88.926_fatty_acids, reliability score according to webdetective.nl: 21/35*

Omega-6 fatty acids (also referred to as ω-6 fatty acids or n-6 fatty acids) are a family of polyunsaturated fatty acids that have in common a final carbon-carbon double bond in the n-6 position, that is, the sixth bond, counting from the methyl end. Some medical research suggests that eating a lot of certain omega−6 fatty acids may lead to some diseases.

[7] *Wikipedia, Phytanic acid, page created May 2006, last update June 2013, last visit October 2014, http://en.wikipedia.org/wiki/Phytanic_acid, reliability score according to webdetective.nl: 21/35*

Phytanic acid (or 3,7,11,15-tetramethyl hexadecanoic acid) is a branched chain fatty acid that humans can obtain through the consumption of dairy products, ruminant animal fats, and certain fish. Western diets are estimated to provide 50-100 mg of phytanic acid per day. In a study conducted in Oxford, individuals who consumed meat had, on average, a 6.7-fold higher geometric mean plasma phytanic acid concentration than did vegans.

[8] *Wikipedia, Docosahexaeenzuur, page created May 2008, last update May 2013, last visit October 2014, http://nl.wikipedia.org/wiki/Docosahexaeenzuur, reliability score according to webdetective.nl: 21/35*

Docosahexaeenzuur (DHA) of 22:6(ω-3) is een polyonverzadigd vetzuur dat behoort tot de klasse van de omega 3-vetzuren. Chemisch gezien is DHA een carbonzuur met een keten van 22 koolstofatomen en zes cis-dubbele bindingen. De eerste dubbele binding staat op de derde koolstof, te beginnen met het einde van de keten (omega), vandaar omega 3.

[9] *Wikipedia, Arachidonzuur , page created March 2005, last update Auguss 2013, last visit October 2014, http://nl.wikipedia.org/wiki/Arachidonzuur, reliability score according to webdetective.nl: 21/35*

Arachidonzuur is een vetzuur dat in principe door het menselijk lichaam kan worden gevormd uit linolzuur of gamma-linoleenzuur, maar toch tot de essentiële vetzuren wordt gerekend. Het komt vooral voor in dierlijke vetten en is in plantaardige producten te vinden in pindaolie.

[10] *Wikipedia, Fytaanzuur, page created October 2006, last update August 2013, last visit October 2014, http://nl.wikipedia.org/wiki/Fytaanzuur, reliability score according to webdetective.nl: 21/35*

Fytaanzuur of 3,7,11,15-tetramethylhexadecaanzuur is een vertakt terpeenvetzuur dat voorkomt in onze dagelijkse voeding, vooral in zuivelproducten, visproducten en vlees van herkauwers.

[11] *Wikipedia, Galzuur, page created January 2009, last update August 2013, last visit October 2014, http://nl.wikipedia.org/wiki/Galzuur, reliability score according to webdetective.nl: 21/35*

Galzuren zijn onderdeel van de gal, dat de secretie van de lever bevat. Galzuren ontstaan door hydroxylatie en oxidatie van cholesterol. De galzuren worden in de ingewanden omgezet in galzouten door contact met een kation, zoals natrium. De belangrijkste galzouten zijn echter glycocholaat en taurocholaat, die een verbinding zijn van galzuur met respectievelijk glycine (een aminozuur) en taurine.

[12] Wikipedia, Desaturase, page created May 2006, last update March 2014, last visit October 2014, http://nl.wikipedia.org/wiki/Desaturase, reliability score according to webdetective.nl: 21/35

Desaturase is een aanduiding voor een groep enzymen die een elektron van een molecuul op een ander molecuul kunnen overbrengen. Omdat ze een molecuul oxideren en daarbij een ander reduceren, horen ze tot de familie van oxidoreductasen.

[13] Voedingscentrum Encyclopedie- Omega-6, last visit October 2014, www.voedingscentrum.nl, reliability score according to webdetective.nl: 13/35

Omega-6-vetzuren zijn meervoudig onverzadigde vetzuren. Het bekendste omega-6-vetzuur is linolzuur, dat vooral veel voorkomt in plantaardige oliën. Deze meervoudig onverzadigde vetzuren hebben een gunstig effect op het cholesterolgehalte en daarmee op het risico op hart- en vaatziekten.

[14] Voedingscentrum Encyclopedie-Omega 3, last visit October 2014, www.voedingscentrum.nl, reliability score according to webdetective.nl: 13/35

Omega-3-vetzuren (visvetzuren) zijn meervoudig onverzadigde vetzuren. De bekendste zijn alfa-linoleenzuur (ALA), eicosapentaeenzuur (EPA) en docosahexaeenzuur (DHA). Er zijn sterke aanwijzingen dat de inneming van voorgevormd EPA/DHA, zoals aanwezig in vette vis, extra bescherming geeft tegen hart- en vaatziekten.

[15] Carolien Makkink en Walter Gerrits, 'Omega-3-vetzuren sturen via voer', De Molenaar, jaargang 112, nummer 8, blz. 22-23, juni 2009

De mens kan de goed omega-3-vetzuren EPA en DHA zelf aanmaken uit alfa-linoleenzuur, maar in de praktijk gebeurt dit onvoldoende; dit heeft waarschijnlijk te maken met voedingspatroon. Een hoog gehalte aan linolzuur gecombineerd met een laag gehalte aan alfa-linoleenzuur vermindert de synthese van EPA en DHA.

[16] *Wetenschappelijk informatie over visolie*, *http://visolie-info.nl/vw_dha.php,* , *last visit October 2014, reliability score according to webdetective.nl: 12/35*

Visolie is een voedingssupplement. Het gebruik van visolie kan bijdragen aan een goede gezondheid, maar is niet bedoeld als medicijn. De werkzame bestanddelen in visolie zijn de essentiële vetzuren EPA en DHA. Deze vetzuren maken deel uit van de bekende Omega-3 vetzurengroep. Omega-3 vetzuren zijn meervoudig onverzadigde vetzuren.

[17] *Lubert Stryer, Biochemistry, Freeman New York 1988 (H20: fatty acid metabolism)*

Since its first edition in 1975, this extraordinary textbook has helped shape the way biochemistry is taught, offering exceptionally clear writing, innovative graphics, coverage of the latest research techniques and advances, and a signature emphasis on physiological and medical relevance. Those defining features are at the heart of this edition.

[18] *Instituut voor Permanente Studie voor apothekers, Farmamozaïek - Cholzuur, website created 1998, last visit October 2014, http://www.farmamozaiek.be/farmamozaiek/?q=taxonomy/term/2703, reliability score according to webdetective.nl: 19/35*

Galzuren hebben een belangrijke emulgerende functie voor water onoplosbare voedingsstoffen. Ze worden gevormd uit cholesterol in de lever. Cholzuur en chenodesoxycholzuur zijn primaire galzuren. Na conjugatie met taurine, glycine, zwavelzuur en glucuronzuur komen ze in de darm terecht. Desoxycholzuur en lithocholzuur zijn secundaire galzuren: de gastro-intestinale bacteriën liggen aan de basis van deze structuren. Ze vertegenwoordigen toch 20 tot 30% van de galzuren omdat ze via enterohepatische circulatie geabsorbeerd worden.

[19] *A.P.N. Franchinont, Koolstof en Hare Verbindingen: Leiddraad bij de studie der soo gename organische chemie, Leiden 1878 1^{e} druk*

Franchimont vond het leren van de eigenschappen en bereidingswijzen van chemische verbinding op zichzelf vrij nuteloos. Een degerlijke kennis draagt weinig bij tot de vorming van de student. Veel nuttiger is het volgens hem om het verband op te sporen tussen de samenstelling van de stoffen en hun eigenschappen.

[20] Sacha Ferdinandusse, Simone Denis, Petra A.W. Mooijer, Zhongyi Zhang, Janardan K. Reddy, Arthur A. Spector, and Ronald J. A. Wanders, Identification of the peroxisomal β-oxidation enzymes involved in the biosynthesis of docosahexaenoic acid, Journal of Lipid Research, Volume 42 (2001) 1987-1995

DHA (C22:6n-3) is an important PUFA implicated in a number of (patho)physiological processes. For a long time, the exact mechanism of DHA formation has remained unclear, but now it is known that it involves the production of tetracosahexaenoic acid (C24:6n-3) from dietary linolenic acid (C18:3n-3) via a series of elongation and desaturation reactions, followed by β-oxidation of C24:6n-3 to C22:6n-3. Although DHA is deficient in patients lacking peroxisomes, the intracellular site of retroconversion of C24:6n-3 has remained controversial. By making use of fibroblasts from patients with defined mitochondrial and peroxisomal fatty acid oxidation defects, we show in this article that peroxisomes, and not mitochondria, are involved in DHA formation by catalyzing the β-oxidation of C24:6n-3 to C22:6n-3. Additional studies of fibroblasts from patients with X-linked adrenoleukodystrophy, straight-chain acyl-CoA oxidase (SCOX) deficiency, d-bifunctional protein (DBP) deficiency, and rhizomelic chondrodysplasia punctata type 1, and of fibroblasts from l-bifunctional protein and sterol carrier protein X (SCPx) knockout mice, show that the main enzymes involved in β-oxidation of C24:6n-3 to C22:6n-3 are SCOX, DBP, and both 3-ketoacyl-CoA thiolase and SCPx.

[21] Ronald J.A. Wanders, Jasper Komen, and Sacha Ferdinandusse, Phytanic acid metabolism in health and disease, Biochimica et Biophysica Acta 1811 (2011) 498–507

Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) is a branched-chain fatty acid which cannot be betaoxidized due to the presence of the first methyl group at the 3-position. Instead, phytanic acid undergoes alpha-oxidation to produce pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) plus CO2. Pristanic acid is a 2-methyl branched-chain fatty acid which can undergo beta-oxidation via sequential cycles of betaoxidation in peroxisomes and mitochondria. The mechanism of alpha-oxidation has been resolved in recent years as reviewed in this paper, although some of the individual enzymatic steps remain to be identified. Furthermore, much has been learned in recent years about the permeability properties of the peroxisomal membrane with important consequences for the alpha-oxidation process. Finally, we present new data on the omega-oxidation of phytanic acid making use of a recently generated mouse model for Refsum disease in which the gene encoding phytanoyl-CoA 2-hydroxylase has been disrupted.

[22] Sacha Ferdinandusse, and Sander M. Houten, Peroxisomes and bile acid biosynthesis, Biochimica et Biophysica Acta 1763 (2006) 1427–1440

Peroxisomes play an important role in the biosynthesis of bile acids because a peroxisomal β-oxidation step is required for the formation of the mature C24-bile acids from C27-bile acid intermediates. In addition, de novo synthesized bile acids are conjugated within the peroxisome. In this review, we describe the current state of knowledge about all aspects of peroxisomal function in bile acid biosynthesis in health and disease. The peroxisomal enzymes involved in the synthesis of bile acids have been identified, and the metabolic and pathologic consequences of a deficiency of one of these enzymes are discussed, including the potential role of nuclear receptors therein.

[23] Merel S. Ebberink, 'Molecular and genetic characterization of peroxisome biogenesis disorders', PhD thesis, Universiteit van Amsterdam (2010)

Het menselijk lichaam is opgebouwd uit verschillende organen en elk orgaan is weer opgebouwd uit cellen. Iedere cel bevat verschillende organellen (compartimenten), elk met een eigen functie. Een cel bevat onder andere de volgende organellen 1) een kern, waar het erfelijke materiaal (DNA) is opgeslagen, 2) mitochondriën, die onder andere functioneren als een energiecentrale; Deze produceren energie om alle processen in het lichaam draaiende te houden, 3) lysosomen, die zorgen voor de afvalverwerking en 4) de 'mooiste' organellen: peroxisomen, waarin een aantal stoffen worden aangemaakt en andere stoffen juist worden afgebroken. Elke cel bevat een paar honderd van deze peroxisomen. Dit organel staat centraal in mijn proefschrift.

[24] JT Brenna, 'Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man'. Curr Opin Clin Nutr Metab Care. 2002 Mar;5(2):127-32.

Alpha-linolenic acid (18:3n-3) is the major n-3 (omega 3) fatty acid in the human diet. It is derived mainly from terrestrial plant consumption and it has long been thought that its major biochemical role is as the principal precursor for long chain polyunsaturated fatty acids, of which eicosapentaenoic (20:5n-3) and docosahexaenoic acid (22:6n-3) are the most prevalent. For infants, n-3 long chain polyunsaturated fatty acids are required for rapid growth of neural tissue in the perinatal period and a nutritional supply is particularly important for development of premature infants. For adults, n-3 long chain polyunsaturated fatty acid supplementation is implicated in improving a wide range of clinical pathologies involving cardiac, kidney, and neural tissues. Studies generally agree that whole body conversion of 18:3n-3 to 22:6n-3 is below 5% in humans, and depends on the concentration of n-6 fatty acids and long chain polyunsaturated fatty acids in the diet. Complete oxidation of dietary 18:3n-3 to CO2 accounts for about 25% of 18:3n-3 in the first 24 h, reaching 60% by 7 days. Much of the remaining 18:3n-3 serves as a source of acetate for synthesis of saturates and monounsaturates, with very little stored as 18:3n-3. In term and preterm infants, studies show wide variability in the plasma kinetics of 13C n-3 long chain polyunsaturated fatty acids after 13C-18:3n-3 dosing, suggesting wide variability among human infants in the development of biosynthetic capability to convert 18:3n-3 to 22:6n3. Tracer studies show that humans of all ages can perform the conversion of 18:3n-3 to 22:6n3. Further studies are required to establish quantitatively the partitioning of dietary 18:3n-3 among metabolic pathways and the

influence of other dietary components and of physiological states on these processes.

[25] *A. Schluter, A. Real-Chicharro, T. Gabaldon, F. Sanchez-Jimenez and A. Pujol (2010), 'PeroxisomeDB 2.0: an integrative view of the global peroxisomal metabolome'. Nucleic Acids Res, 38, D800-5.*

Peroxisomes are single-membrane subcellular organelles, present in most eukaryotic cells and organisms.
The Peroxisome fulfills essential metabolic functions in lipid metabolism, both catabolic (oxidation of pipecolic, phytanic and very-long chain fatty acids) and anabolic (synthesis of plasmalogens and bile acids). Moreover, the peroxisome plays a key role in free radical detoxification, differentiation, development and morphogenesis from human to yeast. Fatal disorders are related to defective peroxisomal function or biogenesis.
The aim of PEROXISOME database (PeroxisomeDB) is to gather, organise and integrate curated information on peroxisomal genes, their encoded proteins, their molecular function and metabolic pathway they belong to, and their related disorders. PeroxisomeDB contains the complete peroxisomal proteome of Homo sapiens (encoded by 85 genes) and Saccharomyces cerevisiae (encoded by 61 genes). Now, we have included 34 new organism genomes with the acquisition of 2426 new peroxisomal homolog proteins. PeroxisomeDB 2.0 integrates the peroxisomal metabolome of whole microbody familiy by the new incorporation of the glycosome proteomes of trypanosomatids and the glyoxysome proteome of Arabidopsis thaliana.

[26] *Pure voeding - Vetten, published October 2010, last visit December 2014, http://pure-voeding.blogspot.nl/, reliability score according to webdetective.nl: 10/35*

Het lichaam heeft elke dag vetten nodig. Vetten zijn een belangrijke bron van de vitamines A, D en E en essentiële vetzuren. Er zijn verschillende soorten vetten ... niet allemaal zijn ze even goed ....

[27] *Wikipedia, Cholic acid, page created October 2005, last update November 2014, last visit December 2014, http://en.wikipedia.org/wiki/Cholic_acid, reliability score according to webdetective.nl: 21/35*

Cholic acid, also known as 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid is a primary bile acid that is insoluble in water (soluble in alcohol and acetic acid), it is a white crystalline substance. Salts of cholic acid are called cholates. Cholic acid, along with chenodeoxycholic acid, is one of two major bile acids produced by the liver where it is synthesized from cholesterol. Of the two major bile acids, cholate derivatives represent approximately eighty percent of all bile acids. These derivatives are made from cholyl-CoA, which exchanges its CoA with either glycine, or taurine, yielding glycocholic and taurocholic acid respectively.

GLOSSARY

- *AA:* abbreviation of the omega-6 fatty acid with the original name (in English): Arachidonic Acid. In Dutch: Arachidonzuur.
- *ALA:* abbreviation of the omega-3-fatty acid with the original name (in English): Alpha Linolenic Acid. In Dutch Alfa-linoleenzuur.
- *Alfa-oxidation:* degradation process in the peroxisome where there is one carbon atom is taken away.
- *Bèta-oxidatie:* degradation process in the peroxisome where there is two or three carbon atoms are taken away.
- *Biochemical:* chemical processes in a living organism.
- *Biogenesis:* making organelles, cells and organisms.
- *Carbonyl group:* the acid part of a fatty acid that consists of two oxygen atoms, one carbon atom and one hydrogen atom.
- *Catalase:* an enzyme that breaks down hydrogen peroxide in the peroxisome.
- *Cholic acid:* primary bile acid.
- *Citric Acid Cycle:* a series of biochemical reactions in which energy is generated.
- *Cytosol:* fluid in the cytoplasm of a cell.
- *CoA:* Coënzym A dat vetzuren activeert.
- *Cytoplasma:* the contents of a cell, including the cytosol.
- *DHA:* abbreviation of the omega-3-fatty acid with the original name (in English): DocosaHexaenoic Acid. In Dutch: Docosahexaeenzuur.
- *Double bound:* a chemical bond between two atoms using from each atom with two electron pairs.
- *Elongation:* extending a fatty acid with two carbon atoms.

- *Emulsifying:* mixing water and fat with each other.
- *Mono-unsaturated fatty acid:* a fatty acid with one double bond.
- *Single bond:* a chemical bond between two atoms using from each atom a single electron pair.
- *Enzyme:* a protein in the body which allows for synthesis or degradation of a given substance.
- *Enzyme system:* a group of enzymes with a specific task in the body.
- *EPA:* abbreviation of the omega-3-fatty acid with the original name (in English): EicosaPentaenoic Acid. In Dutch: Eicosapentaeenzuur.
- *ER:* an organelle in a cell. Full name: Endoplasmic Reticulum.
- *Hydrocarbon:* a molecule with a binding between a carbon atom and a hydrogen atom.
- *Matrix:* contents of a cell or organelle.
- *Maximum temperatur* the maximum temperature where an enzyme a substance can function. The number of conversions that the enzyme does here is low.
- *Membrane:* a boundary of a cell or organelle and its surroundings.
- *Methyl group:* a side branch of a fatty acid that consists of one carbon atom and three hydrogen atoms.
- *Minimum temperature:* the minimum temperature where an enzyme a substance can build up or tear down. The number of conversions that the enzyme does here is low
- *Molecule formula:* a chemical formula that displays the number of elements of a substance.
- *Mutation:* a change of a gene in the DNA.
- *Optimum temperature:* the temperature where an enzyme does the most conversions.

- *Optimum acidity:* the acid level at which an enzyme value does most conversions.
- *Organelles:* particles in a cell that have a certain function in that cell.
- *Oxidative decarboxylatone:* organic reaction in which a carbon dioxide molecule is split off.
- *PBD:* abbreviation for Peroxisomal Biogenises Disorder. In Dutch: Peroxisomaal Biogenesis Defect.
- *Peroxins:* proteins of a peroxisome that are encoded at the PEX genes in the DNA.
- *PEX:* abbreviation for a peroxin.
- *PMP70:* a protein in the membrane of a peroxisome.
- *Phytanic acid:* a branched long-chain-fatty acid.
- *Stereo-isomer:* isomers that differ only in the three-dimensional orientations of their bindings.
- *Substrate:* a substance that is created or destructed by a particular enzyme.
- *Synthesis:* synonym for the word ‘create’.
- *THCA:* the forerunner of cholic acid. Full name: TriHydroxy-Cholestanoic Acid.
- *X-ALD:* abbreviation for the peroxisomal disease: X-linked Adrenoleukodystrophy.
- *Δ5-desaturase:* enzyme that performs Δ5-desaturation. This changes a single bond on the fifth place from the delta side into a double bond.
- *Δ6-desaturase:* enzyme that performs Δ6-desaturation. This changes a single bond on the sixth place from the delta side into a double bond.

ANNEXES

The original document (in Dutch) consists of a number of annexes which documented the progress of the research. The research has been performed mostly in the period May-December 2014. In total 120 hours is spent on this project.

When interested in one of the annexes, please try an automatic translator to translate that section from the original document: https://staff.fnwi.uva.nl/a.visser/home/ProfielWerkstukPeroxisomen.pdf