The Biochemistry of Adrenoleukodystrophy (ALD & AMN)

Paul Watkins MD, PhD
Kennedy Krieger Institute &
Johns Hopkins University
Baltimore, MD
Part 1
Fatty acid biochemistry
Some basics...

- **What is Biochemistry?**
  - The chemistry of biological systems
  - The chemistry of life
- Need to know a little bit of biology...
- Need to know a little bit of chemistry...
Chemical Elements

- Living things are composed of several chemical building blocks, called “elements”
- The most abundant elements are:
  - Carbon (C)
  - Hydrogen (H)
  - Oxygen (O)
- Other important elements include:
  - Nitrogen (N)
  - Sulfur (S)
  - Phosphorus (P)
Chemical Bonds

- Elements can link together by forming chemical “bonds”

- Substances containing elements linked together by chemical bonds are called “Compounds”

- There are rules that must be followed:
  - Carbon (C) can bond to 4 other elements
  - Hydrogen (H) can only make 1 bond
  - Oxygen (O) can make 2 bonds
  - Nitrogen (N) bonds to 3 other elements
Chemical compounds: some examples

Water (H₂O)  H-O-H

Ammonia (NH₃)  H-N-H

Oxygen (O₂)  O=O

In oxygen, there are 2 bonds between the Os. This is a stronger “double bond”

Methane (CH₄)  H-C-H
Biochemical Problem in ALD

- Too much **Very Long Chain Fatty Acid** (VLCFA)

- Buildup of VLCFA is in all parts of the body, especially:
  - Brain & spinal cord
  - Adrenal Glands

VLCFA’s in blood plasma allows diagnosis of ALD by blood test
So what is a Fatty Acid?

- A Fatty acid is also a chemical compound

- Made up of 3 chemical elements
  - Carbon (C)
  - Hydrogen (H)
  - Oxygen (O)
Fatty Acids

- “Chains” of $C$ and $H$ form the “fatty” part of a fatty acid:

```
H H H H H H H H H H H H H
H-C-C-C-C-C-C-C-C-C-C-C-C-C
H H H H H H H H H H H H H
```

- Fatty Acids can have a chain that is...
  - **Short**: 2-4 $C$’s
  - **Medium**: 6-10 $C$’s
  - **Long**: 12-20 $C$’s
  - **Very Long**: 22-? $C$’s
Fatty Acids

- C, H, and O bond together to form the “acid” part of a fatty acid:

\[
\begin{array}{c}
\text{O} \\
\text{\text{\text{-C-O-H}}}
\end{array}
\]

- Notice that while C is bonded to the O on the right by a single line (bond), it is bonded to the O above by a “double bond”
Fatty Acids

Short-chain FA: Acetic acid (vinegar)

- H-C-C-O-H
- H

Long-chain FA: Palmitic acid (C16:0)

- H-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-O-H

- H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H

Very long-chain FA: Cerotic acid (C26:0)

- H-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-C-O-H

- H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H H

- (about 50% longer than long-chain FAs)

May 2020: This presentation is intended for personal use. Please do not share or repost unless you are granted permission from Dr. Watkins and the ULF. office@ulf.org
What does the body do with Fatty Acids?

Like carbohydrates (sugars), fatty acids are used as a "fuel" for the body:

- Breakdown of fatty acids → Energy + Heat + C, H, and O that are recycled

- The energy released from FA breakdown is stored as ATP, which is used to drive muscle contraction, heart pumping, breathing, brain function, and many other processes.
Popular misconception:

Fatty acid molecules (including VLCFA) are freely floating around in our bloodstream, cells, and organs

Reality:

99% of fatty acids are found in the more complex molecules shown in the next few slides
Triglycerides

The fat on a piece of meat (or on us!)

Triglycerides are made up of 3 fatty acids attached to glycerol (glycerin)
Phospholipids

The molecules that make up the membranes that surround all our cells and tissues

Fatty acids are also the main components of phospholipids
Phospholipids

The most abundant phospholipid in membranes is called “Phosphatidyl choline”, or PC.

If we remove one of the fatty acids from PC, we get “Lyso-PC”, or LPC.

When that one fatty acid is the VLCFA containing 26 carbons, we have “C26-LPC”, which is the compound measured in newborn screening.
Sphingolipids and Glycolipids

Also found in membranes, especially in myelin membranes, and necessary for normal brain function.
VLCFAs

- VLCFAs - important part of brain cell membrane lipids, especially myelin

- Like other fatty acids, VLCFAs are normal components of the body and are necessary for life.
VLCFAs - where do they come from?

- VLCFAs come from:
  - Diet (= “intake”)
  - Elongation of shorter fatty acids (= “synthesis”)
What do VLCFAs and Goldilocks have in common?

The balance between “just right” and “too little” or “too much”

Intake + synthesis  \[\rightleftharpoons\]  Excretion + breakdown

too much  \[\rightleftharpoons\]  too little
VLCFAs (contd.)

What are the likely causes of excess VLCFA in ALD?:

- Body makes too much
- Body cannot remove excess

Early studies showed that the process which degrades VLCFAs ("β-oxidation") was defective in cells from ALD patients.
Fatty acid breakdown (β-oxidation): removal of 2 carbons at a time

* Four chemical reactions

May 2020: This presentation is intended for personal use. Please do not share or repost unless you are granted permission from Dr. Watkins and the ULF. office@ulf.org
More recent studies indicate that VLCFA synthesis (elongation of long-chain FAs, or LCFA) is also increased in cells from ALD patients.
VLCFA Synthesis

Dietary LCFAs

16

18

20

22

24

26
A brief summary...

- Fatty acids are chemical compounds made of C, H, and O.
- VLCFA are ~50% longer than LCFA that are abundant in our normal diet.
- The elevated VLCFA levels in ALD come from both increased synthesis and decreased degradation.
- How does this relate to the ALD gene and protein?
Part 2
Genes and Proteins
Genes and proteins

- All ALD patients have a “mutation” in their ALD gene

- What does that mean????
A view through the microscope...
Chromosomes
The X-Chromosome

The ALD gene is found at the tip of the longer arm (q) of the X-chromosome.
DNA

- Chromosomes are mainly DNA
- The “Double helix” of DNA is made up of 4 chemicals called “nucleotides” or “bases”:

  - Adenine (A)
  - Cytosine (C)
  - Guanine (G)
  - Thymine (T)

- A single strand of DNA is millions of bases long

Pairing of T on one strand with A on the other, or C with G, causes the double helix to form
DNA: a blueprint for making proteins

- DNA is made up of the 4 bases: A, C, G, and T
- The order, or “sequence”, of As, Cs, Gs, and Ts on a strand of DNA is really the “instructions” for making proteins
- A GENE is a piece of DNA that has the instructions for making one specific protein
From DNA to protein:

- Proteins, like DNA, are long chains of chemicals.
- While DNA is a chain of bases A, T, C, and G, proteins are chains of **AMINO ACIDS**.
- There are 20 different amino acids.
From DNA to protein...

Since DNA has only 4 bases, but there are 20 different amino acids, how does the DNA code work?

- A group of 3 bases in the DNA sequence is called a “codon” that specifies *one specific* amino acid

- There are also “stop” signals in the code

- “Messenger RNA” or mRNA serves as an intermediate in between DNA and protein
mRNA

- mRNA has a single strand
- It faithfully copies the base sequence of one of the DNA strands
- In RNA, the base Uracil is used instead of Thymine
From mRNA to protein...

The ALD protein has 745 amino acids!

How many bases are needed to encode this protein?
The ALD gene

- Known to be near the tip of the long arm of the X-chromosome
- “Discovered” in 1993 by Drs. Aubourg, Mandel and coworkers:
  - Location pinpointed more accurately
  - Sequence of As, Cs, Gs, and Ts (bases) determined
  - The “instructions” for making the ALD protein require 2238* bases!

*For math geeks who know that $745 \times 3 = 2235$ and not 2238, we have to add 3 more bases for the stop signal.
The ALD gene

- All ALD patients have a “mutation” in their ALD gene:
  - An *incorrect* base (for example, a “T” where an “A” should be)
  - One or more bases *missing*
  - One or more *extra* bases
The ALD protein

- When there is a mutation in the ALD gene, what happens to the ALD protein?
- Several possibilities:
  - A single incorrect amino acid gets placed in the protein (may or may not affect function)
  - The amino acids are correct up to a point, but then after that they are all incorrect (definitely affects function)
  - The amino acids are correct up to a point, but then we run into a premature "stop" signal (definitely affects function)
What does the ALD protein do?

- We suspected that the ALD protein would be one of the enzymes known to participate in the breakdown or synthesis of VLCFA.
What does the ALD protein do?

- When the sequence of amino acids in the ALD protein was compared to the sequences of all other known proteins, there was no similarity to fatty acid synthesis or breakdown enzymes!
- The ALD protein was most similar to a large family of “transporter proteins”
Transporters:

Getting something from one side of a membrane...
...to another
Where is the ALD protein found inside cells?

The ALD protein is located in the membrane surrounding “peroxisomes”

What are peroxisomes?
Cells and their internal structures

Smaller compartments within cells are called “organelles”
Some of these are:

- Nucleus
- Peroxisomes
- Mitochondria
- Endoplasmic reticulum
- Lysosomes
- Cell membrane
- Nucleus
- Peroxisomes
Cells and their internal structures

- Biochemical processes take place within specific organelles
- Often, the internal location of a biochemical process is important for understanding how disease results
- Degradation (breakdown) of VLCFA occurs in peroxisomes
- Synthesis of VLCFA via elongation of LCFA occurs in the endoplasmic reticulum
Recent work shows that the ALD protein transports VLCFA into peroxisomes, where VLCFA breakdown occurs.
The previous slide isn’t entirely accurate!

All fatty acids, including VLCFA, are usually attached to a “helper molecule” called Coenzyme A, or CoA for short, to be degraded by β-oxidation, elongated, or transported across the peroxisome membrane.

CoA is included on the next 2 slides...
Putting it all together - Normal cells

- Normal cells
- Breakdown of excess ALD Protein
- VLCFA synthesis (elongation)
- LCFA-CoA
- VLCFA-CoA
- Breakdown of excess

May 2020: This presentation is intended for personal use. Please do not share or repost unless you are granted permission from Dr. Watkins and the ULF. office@ulf.org
VLCFA accumulate and are used as building blocks to make complex lipids (triglycerides, phospholipids, etc.).

Putting it all together - ALD cells

VLCFA synthesis (elongation)

May 2020: This presentation is intended for personal use. Please do not share or repost unless you are granted permission from Dr. Watkins and the ULF.
office@ulf.org
Part 2 summary...

- Chromosomes are structures in the nucleus of cells that are made up of thousands of genes.
- A gene is a piece of DNA containing "instructions" for making one specific protein.
- If the gene has a mistake in its DNA, the protein that it codes for will often be defective.
- The ALD gene encodes the ALD Protein (ALDP).
- ALDP is now thought to transport VLCFA into peroxisomes where degradation takes place.
A final summary...

- Knowing something about the biochemistry of ALD allows us to perform tests that are useful in the diagnosis and prevention of ALD.

- Knowing something about ALD biochemistry has also facilitated development of therapies such as Lorenzo’s oil, bone marrow transplantation, and gene therapy.

- Knowing something about the biochemistry of ALD also allows us to search for drugs that may, in the future, be beneficial for treatment.