What are essential fatty acids (EFAs)?

Our body can make most of the fats it needs. However, there are certain fats that are required for specific functions, but cannot be made in the body. Therefore, they must be obtained from dietary sources. These fats are called essential fatty acids (EFAs). Specifically, these fats are linoleic acid (chemical structure: 18 carbon atoms with 2 double bonds) and alpha-linolenic acid (chemical structure: 18 carbon atoms with 3 double bonds) and can be found in plant oils (linoleic acid: sunflower and corn oils; alpha-linolenic acid: linseed and soybean oils). Linoleic and alpha-linolenic acids are the ‘parent’ EFAs and can be converted further in the body to other long-chain fats (usually containing 18-22 carbon atoms) which are highly unsaturated (more than 3 double bonds). These EFA metabolites are often referred to as long-chain polyunsaturated fatty acids (LCPUFAs). Directly and indirectly, LCPUFAs perform specific immune and nervous system functions within the body.

What can happen if the body does not have enough EFAs and LCPUFAs?

In general, EFA and LCPUFA deficiency in the body can result in several consequences such as skin lesions, hair loss, infertility, growth retardation, and nervous system dysfunction such as numbness, leg pain, impaired vision and reduced learning capacity. There are also many degenerative changes in major organs like the liver and the kidneys. Some researchers believe that low levels of EFAs and LCPUFAs can lead to heart disease and cancer because of the role of these fatty acids in providing a properly functioning immune system.

Patients with liver disease have low levels of EFAs in the body.

Pediatric patients with liver disease requiring liver transplantation usually have low levels of EFAs in the body, which can be disadvantageous to their condition. The cause(s) of low EFA levels in these patients is (are) not known, but may be related to one or more of the following factors:

1. Increased dietary EFAs in feces and constant dietary intake (malabsorption)
2. Conversion of EFAs into other fats or used for energy (changes in metabolism)
3. A shift in the amount of EFAs and LCPUFAs between body tissues (redistribution)

Malabsorption of dietary EFAs is thought to be largely responsible for low EFA levels in the body since patients with liver disease usually have strongly reduced amounts of bile in their
Summary for non-biologists

intestine. Bile is a mixture of fats and organic salts that is made in the liver and released in the intestine during a meal. Bile assists in the absorption of dietary fat due to its emulsifying property and to its ability to contribute specific components for the packaging of fat from the intestinal cell to the blood.

In addition to malabsorption of EFAs, patients with liver disease may have changes in their fat metabolism. Since the liver is the organ where a majority of fat metabolism takes place, patients with damaged liver cells may not be able to efficiently convert EFAs to LCPUFAs. Additionally, these patients may use EFAs and LCPUFAs for energy due to increased energy needs or for making potent hormone-like substances called prostaglandins, which are produced in a number of tissues in response to a variety of stimuli.

So, changes in absorption and/or metabolism in liver disease patients can largely contribute to low levels of EFAs and LCPUFAs in their body.

**The primary aim of this thesis:**

Eventually, we want to develop a (dietary) treatment for patients with liver disease to correct their low levels of EFAs and LCPUFAs. In order to do this, we attempted to understand why they have low levels of EFAs and LCPUFAs in their blood. Due to the fact that they have disturbed bile secretion into the intestine and that their liver cells are damaged, it would seem likely that changes in absorption and metabolism of EFAs and LCPUFAs could be affected in patients with liver disease. In our experiments, we focused primarily on how the lack of bile in the intestine affects the absorption and metabolism of the major dietary EFA, linoleic acid. We did this by using animals and patients known to have changes in either the amount of bile they have in the intestine or in the composition of bile. If we would find that EFAs are poorly absorbed in the absence of bile, then it would be helpful to design fats that are easily absorbed in bile-deficient conditions. If we would find that EFAs are poorly metabolized in the absence of bile, then a mere increase in the oral intake of EFAs and LCPUFAs seems helpful.

**Major findings of experiments performed in this thesis:**

1. In our rat model in which all bile was continuously collected outside of the body (called **bile diversion**), we found that linoleic acid levels in the blood were decreased. We checked if this decrease was due to malabsorption of linoleic acid (because there is no bile in the intestine), but it was not. These rats actually ate more food, which completely compensated for the amount of dietary linoleic acid lost in the feces. So, when you subtract how much linoleic acid was in their feces compared to how much they ate, they were actually ‘absorbing’ the same (‘net’) amount of linoleic acid as control rats. We also measured the metabolism of linoleic acid to one of its LCPUFAs in the blood. Our findings show that bile-diverted rats seem to have a more rapid conversion of linoleic acid to its LCPUFA. This change could be responsible for the decreased levels of linoleic acid in blood.
2. We used another rat model in which no bile was allowed to enter the intestine. However, in this model, the passage where bile enters the intestine was tied, blocking the release of bile into the intestine. Under these circumstances, the bile components normally secreted into bile will accumulate in the liver and in the blood, giving the skin and eyes a yellow ('jaundiced') look. This condition (called bile-duct ligation) is similar to what happens in pediatric patients with end-stage liver disease. Again, we studied linoleic acid absorption and metabolism in these rats. Our findings were rather different from what we found in the bile-diverted rats. Indeed, these rats had a high amount of linoleic acid in their feces, or increased malabsorption of dietary linoleic acid. When we calculated the amount of linoleic acid in the feces compared to what they were eating, we found that they were ‘absorbing’ significantly less linoleic acid than normal rats. However, of all the different dietary fats, linoleic acid was absorbed to the greatest extent, suggesting that linoleic acid absorption is relatively preserved under these conditions. We also noticed that the appearance of linoleic acid in the blood was delayed in bile duct-ligated rats compared to control rats. So, in bile duct-ligated rats, the net absorption of linoleic acid is decreased; however, relative to other fatty acids, its absorption is preserved.

3. In the bile-diverted and bile duct-ligated rat, there is an absence of bile in the intestine. Using these models, we could study if the quantity of bile is important for linoleic acid absorption. In order to study the effect of the quality of bile, or its composition, on linoleic acid absorption, we used a different animal model. Specifically, we wanted to determine how important certain lipids in bile (called phospholipids) were for linoleic acid absorption. We proposed that phospholipids were important for EFA levels in the body because 1) they assist in the packaging of fat into large fat droplets (called chylomicrons) in the intestinal cell so that it can be transported to the blood and 2) their structure contains high amounts of EFAs and LCPUFAs. An attractive way to study the effect of biliary phospholipids on EFA levels in the body and on EFA absorption is to use an animal which has been genetically altered so that it produces bile without phospholipids. Presently, there are mice that produce phospholipid-free bile called mdr2 knockout mice. We did three separate studies in which we used different types of diets in order to study linoleic acid and dietary fat absorption. The types of diets used were as follows: low-fat (14 en%, ~50% linoleic acid), high-fat (35 en%, ~30% linoleic acid) and high-fat, EFA-deficient (35 en%, 3% linoleic acid). The levels of linoleic acid in the mdr2 knockout mice were similar to that in control mice, regardless of diet. On low- and high-fat diets, dietary fat absorption in mdr2 knockout mice was comparable with that of controls, and within the range of normal dietary fat absorption (>95%). Similar to bile-diverted and bile duct-ligated rats, the mdr2 knockout mice had a delayed, and even absent, appearance of fat in the blood after administration of a fat load. These findings suggest that phospholipids in bile are not necessary for linoleic acid levels in the body, or for linoleic acid absorption; however, their absence results in a delay in the plasma appearance of administered fats.
In the experiment in which we used the EFA-deficient diet, we found that EFA-deficient mice have decreased fat absorption (~70%). In contrast to what has been found in studies with EFA-deficient rats, EFA-deficient mice had an increased flow and secretion of bile components. This finding suggests that changes in bile in EFA deficiency are not responsible for fat absorption in this condition in mice. Mdr2 knockout mice on an EFA-deficient diet had an even lower fat absorption (~60%), which suggests that phospholipids in bile may at least partially protect against further fat malabsorption in EFA deficiency. In summary, studies with mdr2 knockout mice on different diets have revealed that phospholipids in bile are not necessary for the absorption of linoleic acid or for the levels of linoleic acid in the body.

4. Finally, we studied fat absorption in children with cystic fibrosis. Cystic fibrosis is a genetic disease which leads to malfunctioning of several major organs, such as the pancreas, lungs and even the liver. Many patients with cystic fibrosis malabsorb their dietary fat. To increase the amount the fat absorbed, most patients are given oral pancreatic enzymes since they do not produce their own. However, oral enzyme therapy does not completely correct malabsorption. They continue to absorb only 80-90% of their dietary fats. Efficient absorption of dietary EFAs and LCPUFAs is especially important in these patients because they already have low levels of these fats in their blood. In our study, we tried to investigate why they had continued malabsorption of dietary fat despite the pancreatic enzyme therapy. By giving them certain fats, we could distinguish which step in their absorption process was disturbed. We found that they had malabsorption of dietary fats because of problems with the solubilization step (in which bile plays a key role) in absorption. Fat malabsorption in cystic fibrosis patients can be eliminated by correcting the solubilization step in absorption, in addition to pancreatic enzyme therapy.

“Take home messages..”

The absorption of dietary fats requires many steps, including the transport of fat from the diet into the intestine cell, and the transport of fat from the intestine cell to the blood. Of all the dietary fats, the major dietary EFA, linoleic acid, seems to be transported relatively efficiently from the diet into the intestine cell in the absence of bile in the intestine, as determined by measuring diet intake and fecal fat excretion. However, the appearance of linoleic acid in the blood (so, the exit of fat from the intestine into the blood) is delayed significantly in bile-deficient animals. Specifically, this effect appears to be related to the absence of biliary phospholipids.

This conclusion strongly suggests that impaired EFA and LCPUFA status in hepatobiliary disorders can be alleviated by increasing their oral/enteral administration.