# PATHOPHYSIOLOGY OF THE LIVER

**Arno Helmberg**

*These lecture notes accompany my lectures on liver function and dysfunction in the study module "Nutrition and Digestion" at Innsbruck Medical University. The English version serves two purposes: as a learning aid for international students and to encourage German-speaking students to familiarize themselves with medical English; the lectures are delivered in German. The translation from the original German version is my own; I am afraid it will occasionally sound appalling to native English speakers, but it should at least be intelligible.*

*Version 6.0 e* 
*Pdf- version of http://www.helmberg.at/liver-dysfunction.htm*


To maintain its integrity, our organism carefully guards its borders at all times. Yet, we could not survive without massive exchange with the outside world. Exchange comes with considerable risks: carefully maintained balances might be disturbed, and toxic substances might wreak havoc in the body. The "port authority" dealing with these complex challenges is the liver.

A huge resorptive area –the intestinal epithelia—is required for taking up sufficient amounts of nutrients. Intestinal epithelial cells ("dock workers") are good at moving stuff. Making them also check and metabolize all this stuff probably would negatively affect their efficiency. Accordingly, these functions are performed later, after all the blood coming from this huge surface area has been funneled through the portal vein into the liver. This implies two consecutive capillary exchange systems: one in the intestinal wall, the second, with low remaining blood pressure, in the liver. The exchange of huge amounts of molecules between hepatocytes and blood is facilitated by fenestration of the endothelial cells lining wide sinusoids, allowing plasma to enter the perisinusoidal space, also known as space of Disse. In this slow-flow compartment, blood plasma comes into direct contact with the hepatocytes' microvilli, which are packed with transport proteins. The perisinusoidal space is also home to specialized pericytes, hepatic stellate cells or Ito cells, which store vitamin A and are able to synthesize components of the extracellular matrix. In healthy liver tissue, extracellular matrix is very subtle to minimize diffusion distances. Two additional cell types, both part of the innate immune system, are found within the sinusoids, in direct contact with the blood: a large number of macrophages, called Kupffer cells, and Pit cells, a type of natural killer (NK) cells.

As an import manager, the liver also has to be able to rejects imports, i. e. send molecules back to the intestine. This is made possible by the biliary system, the smallest branches of which start as plasma membrane-bounded canaliculi between two hepatocytes. The three-dimensional polygonal meshwork of canaliculi opens into terminal bile ductules that have their own epithelium.

The importance of liver functioning is illustrated by situations where it suddenly stops, such as death cap poisoning. Amanitin, the main poison, is a cyclical octapeptide with a structure stable enough to withstand cooking. It binds and inactivates the 140 kDa subunit of RNA

polymerase II, blocking all gene expression leading to proteins. This results in cessation of liver function, hepatocyte necrosis and in most cases, death of the patient after 48 to 72 hours.

In the following, individual liver functions will be discussed separately, making the different symptoms of liver dysfunction easily understandable.

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| FUNCTION: | Homeostasis of energy metabolism |
| DYSFUNCTION: | Fatigue, weakness, disorders of lipid metabolism, non-alcoholic fatty liver disease, dyslipoproteinemia |

Meals, after which lots of nutrients are resorbed by the small intestine, alternate with times between meals or even fasting phases. Yet, most cells need energy all the time. The liver has important buffering functions to maintain a continuous supply of energy sources in the blood.

**Following meals**, large amounts of glucose are taken up by the small intestine, part of which enters the hepatocytes via the insulin-independent transporter GLUT2. Glucose as such cannot be stored, but it can be polymerized to glycogen in the liver and in skeletal muscle. The liver can store up to 10% of its weight in glycogen (100-120g). However, the amount of energy that can be stored this way is restricted: for their hydroxyl groups, glucose units are very hydrophilic: 1 g of glycogen binds 2.7 g of water. Hence, this form of energy storage carries too much dead weight and volume to be efficient. Surplus glucose is thus metabolized to fatty acids via acetyl-CoA. Fatty acids are combined with glycerol, and the resulting triglicerides for the most part are released into the blood in the form of VLDL (very low density lipoprotein). Yet, too frequent or high intake of nutritional carbohydrates over time causes fat to accumulate in hepatocytes. This results in functional impairment of the cells, starting with insulin resistance, a condition termed non-alcoholic fatty liver disease (NAFLD). The third task of the liver during this phase is to take up remnants of chylomicrons, sort out the complex mix of lipids contained (e. g., lipid-soluble vitamins or xenobiotics) and to recombine and release the remaining lipids as VLDL. Remember that lipids are the only segment of nutrients that go past the liver via lymphatic vessels. From there, enterocyte-produced chylomicrons enter the blood stream via thoracic duct and venous angle. Lipoprotein lipase (LPL), which is anchored to endothelial plasma membranes, helps to move triglyceride components from chylomicrons into fat and muscle cells; only chylomicron remnants are taken up by the liver.

**Between meals**, or more precisely after completion of intestinal nutrient uptake, energy stores are drawn upon to maintain levels of energy sources in the blood. Fatty acids are available in virtually unlimited quantities from fat depots, but not all cells are able to handle fatty acids. For some cell types (CNS, erythrocytes), glucose is a *sine qua non*. Muscle glycogen is not available for these purposes, as myocytes use it themselves, metabolizing part of it to lactate. A continuous level of blood glucose is maintained by first breaking down liver glycogen. As soon as this source is exhausted, hepatocytes start to synthesize fresh glucose, using first the available lactate and later amino acids ("gluconeogenesis"). During fasting phases, the liver also provides short-chain fatty acid derivatives, acetoacetate and β-hydroxy-butyrate, together known as ketone bodies. While the CNS cannot use fatty acids, it can substitute part of its normal glucose consumption by these ketone bodies.

We tend to view the hormone **insulin** only from the perspective of the resorption phase following nutrient intake: the high glucose levels characteristic of this phase enhance insulin secretion, and insulin in turn helps to move all that glucose into skeletal muscle and fat cells. Yet, insulin remains important between meals, e. g., over night. During this phase, systemic insulin levels are too low to increase glucose uptake into cells. Yet, these low levels continue to limit gluconeogenesis in the liver. As insulin is released into portal blood, concentration in the liver is higher than elsewhere. In addition, insulin inhibits release of glucagon in pancreatic islets. The liver has an enormous capacity for gluconeogenesis, which is driven by glucagon. The actual rate of gluconeogenesis depends on the **glucagon/insulin ratio**. Thus, glucose production is constantly throttled by insulin in a feedback inhibition loop. In the absence of insulin-dependent throttling, the liver would generate glucose at much higher rates. In people with metabolic syndrome, due to insulin resistance of the liver, gluconeogenesis is insufficiently restricted. The result is glucose overproduction between meals, as illustrated by elevated levels of fasting blood sugar in the morning. This explains a fact that is otherwise counter-intuitive: why should glucose be elevated in a person who is hungry after many hours without food? Bottom line: between meals, it's not like the liver laboriously scrapes together some sugar. Rather, gluconeogenesis is a bountiful source of glucose in need of constant throttling by insulin.

FUNCTION: Amino acid metabolism, nitrogen excretion (urea synthesis)
DYSFUNCTION: Hepatic encephalopathy, acid-base instability

Amino acids, derived from muscle protein, are the main source of material for gluconeogenesis. This solves the problem of glucose supply in fasting, but there are snags. For starters, if you transform amino acids to glucose, you are left with the amino groups. A hydrolyzed amino group is ammonia ($NH_3$) or rather, at physiological pH, the ionized ammonium ion ($NH_4^+$). Surplus systemic ammonium is only inefficiently eliminated via the kidneys. Elevated levels are toxic, especially for cells of the CNS.

Disposal of nitrogen also affects **acid-base balance**, which we addressed in renal pathophysiology. There, we anticipated a critical decision made by the liver: the percentages of nitrogen disposed of as urea and $NH_4^+$, respectively. We now take a closer look at this regulation.

As usual, while having breakfast, we muse about pathophysiology. Apart from the nitrogen problem, metabolizing proteins for energy also has more complex acid-base implications. "Burning" carbohydrates and fats (bread and butter) results in $CO_2$ and water. $CO_2$ is a potential acid, but gets eliminated via the lungs. "What about proteins?", we wonder, while enjoying our eggs.

Most amino acids are neutral: they contain two ionized groups of opposite charge, a carboxy- and an amino-group. When metabolized, they give rise to the same amount of $HCO_3^-$ as $NH_4^+$ (net, as actual metabolization is much more complicated). Consumption of 100 g/d of protein results in production of approximately 1000 mmol $HCO_3^-$ and 1000 mmol $NH_4^+$ per day. From an acid-base perspective, $NH_4^+$ could spare a proton while $HCO_3^-$ could take one up. Yet, with a pKa of 9.2, $NH_4^+$ is quite unwilling to release its proton at the physiological pH of

7.4. $HCO_3^-$, on the other hand, readily accepts protons, followed by elimination as $CO_2$, so that the net process would result in alkalization.

In alarm, we almost choke on our scrambled eggs- $NH_4^+$ is toxic, $HCO_3^-$ is alkaline: something must be done! The simplest and most logical idea would be to fuse the two into some garbage molecule. Bingo! That's exactly what the urea cycle is about. Compared to the two rioters, urea is extraordinarily good-natured: nonreactive, nontoxic, unsuspicious from an acid-base perspective, nitrogen-condensing. A little hard to excrete, maybe, but we trust our kidneys will come up with something.

Nitrogen-condensing? Yes, because urea contains two NH2-groups per C=O unit. Um, then how does that square with our acid-base balance? If we transmogrify $NH_4^+$ into a NH2-group, that leaves an H+ on the table; OK, $HCO_3^-$ is missing one, so those two may cancel each other out, but what about the second $NH_4^+$? When incorporating that one, a proton is most certainly left over! (A caveat: If we don't intend to become biochemists, we better leave it at that. Technically, as always, it is more complicated: the second amino group has its origin not directly in $NH_4^+$, but is donated by aspartic acid. We could try to follow stoichiometry from reaction to reaction, but ultimately, the fact remains that protons are left over. At this point, we become aware of another spine-crawling sensation-) Alarm! Have we been putting out the fire with gasoline? One minute ago, we were whirling into alkalization, now we are sinking into an acid swamp! What are we to do, renounce proteins completely? Limit ourselves to sugar and fat? Pure chocolate diet?

Then –phew!- tension falls off: we remember that our kidneys are quite proficient in excreting acid- what is more, they even excrete it preferentially in the form of $NH_4^+$! Now, the only thing we need to see to is that all these acid equivalents are not sent from the liver to the kidneys as $NH_4^+$; that would be far too toxic. We need a secure acid/ammonium tanker: glutamine. From this tanker, the kidney retrieves $NH_4^+$ and excretes it. Each $NH_4^+$ excreted by the kidney needs not be neutralized by $HCO_3^-$ and thus saves $HCO_3^-$ (one or one-half, depending on how one looks at it).

Consequently, in the liver we have two options to deal with $NH_4^+$:

1. We use it to neutralize $HCO_3^-$ generated by breaking down amino acids and put it into urea
2. We put it into the glutamine tanker to ship it to the kidneys, where it is excreted

Option 1 consumes $HCO_3^-$, option 2 saves $HCO_3^-$. Now, if we succeed in controlling the ratio of these two options intelligently, we escape both forms of potential acid-base catastrophe originated by burning amino acids for energy.

Lo and behold, the ratio between the two options turns out to be controlled by pH- that's as intelligent as it gets! A small dip in pH reduces urea production, yet enhances glutamine synthesis. With a lower part consumed and a higher proportion saved, the remaining $HCO_3^-$ counters the upcoming tendency to acidosis. Conversely, an increase in pH has mirror-inverted results.

[No learning content- Exclusively for our biochemistry aficionados:

The reduction in urea synthesis rate by dipping pH is mediated by the enzyme glutaminase. In mitochondria of periportal cells, glutaminase provides $NH_4^+$, which is then fused with $HCO_3^-$ and an ATP-derived phosphate to carbamoylphosphate. Carbamoylphosphate is fed into the urea cycle. Activity of liver glutaminase depends directly on pH: lower pH → less carbamoylphosphate → less urea per unit time.

Increased loading of the glutamine tanker is simply and directly mediated by glutamine synthetase, which is controlled by pH in the reverse mode: lower pH → more glutamine that is dispatched to the kidney. It makes sense that this enzyme is predominantly expressed near the center of the liver lobule: $NH_4^+$ generated in the lobule's periphery and not incorporated into urea drifts downstream and needs to be loaded onto the glutamine tanker in its non-toxic form.]

FUNCTION: "Filtering" particulate matter from portal blood
DYSFUNCTION: Increased susceptibility to infections

Kupffer cells constitute more than 80% of the body's resident macrophages. They are very efficient in phagocytosing particulate matter from portal blood. "Particulate matter" includes aging red blood cells, but also bacteria that are swept in from the intestines. Kupffer cells express a large range of receptors for pathogen-associated molecular patterns (PAMPs). By recognizing, phagocytosing and inactivating pathogens, Kupffer cells are an important component of the inborn defense system against infections (see section on macrophages in immunology lecture notes).

FUNCTION: Elimination of unwanted or questionable lipophilic molecules (biotransformation, cytochrome P450 oxidases)
DYSFUNCTION: Toxicity, depending on specific molecule

Intestinal epithelia are not picky. They absorb many substances that are potentially noxious or at least of questionable value. Consequently, it is up to the liver to get rid of them; not an easy task, especially for lipophilic molecules, as everything that leaves our body is more or less aqueous (as opposed to lipophilic, anyway). The hepatocyte's solution is biotransformation, a mechanism consisting of two steps. In a first step, a reactive group (a "handle"), like –OH, is introduced into the molecule. Most frequently, this is accomplished by the cytochrome P450 enzyme system. In a second step, a hydrophilic molecule (e. g., glucuronic acid, sulfate, glutathione) is conjugated to the handle. Usually, the entire conjugate is then hydrophilic enough to be excreted via the bile, sometimes even via the kidneys.

**Cytochrome P450 enzymes** contain heme as a prosthetic group, with a central coordinated Fe atom that makes redox reactions possible. (The name P450 is derived from "pigment with an absorption maximum at 450 nm", from the original method to measure these enzymes following saturation with CO). The human genome contains about 50 genes for this type of enzyme, the majority of which are expressed in hepatocytes. They are designated by enzyme family (numbers), subfamily (letters) and individual gene, e. g., CYP3A4, CYP2D6, CYP2C19, CYP2E1. Human individuals differ with respect to the range of their cytochrome P450 activities for several reasons:

1. Many of these genes are polymorphic. In other words, different individuals have slightly different versions of CYP genes encoding slightly different enzyme variants. Gene multiplicity and diversity are thought to be the result of evolutionary selection in response to local differences in plant alkaloids, depending on prevailing edible plants in specific regions of the world.
2. Some of these enzymes show gender-specific expression, e. g., CYP2B13, CYP3A16 and CYP4A12 (Rinn et al., Dev. Cell 6: 791-800, 2004). For example, CYP3A16 is expressed only in females, but not in males. This may lead to gender-specific differences in drug metabolization.
3. For many of these enzymes, heavy use by substrates results in induction by transcriptional feedback mechanisms. Therefore, even persons with identical genes may have differing cytochrome P450 expression levels depending on their living conditions/personal history.
4. For some CYPs, expression levels change with age. For example, breast-fed infants from coffee-drinking mothers are quite sensitive to the caffeine taken up, as they hardly express the CYP enzyme required for caffeine metabolization. Thus, stressed mothers may get more rest when cutting back on coffee themselves.

While biotransformation is all about reducing risks, it inherently also entails risks: a substance that is entirely harmless by itself may inadvertently be converted to something more dangerous. A classical example is aflatoxin B1. Aflatoxin is produced by *Aspergillus flavus*, a fungus contaminating peanuts, pistachios, corn etc., if stored at other than cool and dry conditions. The fungus-produced molecule by itself is inactive when taken up with food. Yet, the hepatic cytochrome P450 enzymes oxidize it to a highly reactive metabolite. Aflatoxin-epoxide forms DNA adducts, promoting mutations and over time, hepatocellular carcinoma.

Necessarily, the cytochrome P450 system impacts on pharmaceuticals. On the one hand, many drugs lose activity due to metabolization. With many oral drugs, a so-called first pass effect is observed: some drugs are extracted form portal blood and metabolized so efficiently that it is hard to reach useful blood levels.

On the other hand, toxicity may result from metabolization. Metabolization rates may in turn be modified by other CYP substrates. A medically relevant example for this type of **interactions** is the metabolization of alcohol and acetaminophen.

**1. Alcohol:** Ethanol is mainly oxidized to acetaldehyde by the enzyme alcohol dehydrogenase (ADH). In addition, chronic intake of alcohol induces CYP2E1, which catalyzes the same reaction. Even if induced strongly, the capacity of CYP2E1 remains small compared to that of ADH, leaving intact the cap on metabolization rate at 0.11-0.12 g/kg body weight per hour (in round terms, 0.1 ‰ per hour). Produced at this rate, acetaldehyde already acts slightly cytotoxic; it is further oxidized to acetate by the enzyme aldehyde dehydrogenase. Acetate is then activated to Acetyl-CoA. In both oxidation steps, reductive equivalents are produced. Metabolization of alcohol thus yields Acetyl-CoA plus reductive equivalents, which both can be used to produce ATP via citric acid cycle and respiratory chain. Surplus material is simply used for fatty acid synthesis. This pathway explains the two main forms of pathology directly caused by alcohol: alcoholic hepatitis and fatty liver. With ongoing alcohol consumption, both of these can over time result in cirrhosis.

Two alleles leading to more rapid accumulation of acetaldehyde are frequent in people from South East Asia. ADH allele ADH1B*Arg47His causes more rapid oxidation of ethanol to acetaldehyde. In contrast, aldehyde dehydrogenase allele ALDH2*2- (Glu504Lys) metabolizes acetaldehyde more slowly than normal. In both cases, alcohol intake quickly results in uncomfortable symptoms including flushing (facial redness, dip in blood pressure), nausea and headaches. In Europe, the genetic basis of alcohol metabolization is more homogeneous.

Hepatic metabolization of alcohol in relation to body weight is equal in females and males. Still, the female organism is more sensitive: intake of the same quantity of alcohol results in higher blood alcohol concentrations. This is not only due to females' lower average weight: alcohol mainly distributes in the aqueous phase of the body, a fraction that is smaller in women than in men. A second difference is an ADH-isoenzyme expressed in gastric mucosa, which is less active in women. Since at least 20% of alcohol is taken up via the gastric mucosa, a larger fraction of this percentage reaches the blood in women. Statistically, the probability of liver cirrhosis in women increases from a daily alcohol consumption of 20g; in men, this threshold is in the region of 40-50 g/day. In men, daily intake of 70-80 g/day usually results in cirrhosis.

Alcohol content of beverages is not given in g, but in % by volume: beer around 5%, meaning 50 ml per liter; wine around 12%, that is 120 ml/l. To calculate g from ml, the density of alcohol (about 0.8 g/ml) has to be taken into account, which is lower than that of water (1 g/ml). Thus, the number of ml times 0.8 equals the amount of alcohol in g: 0.5 l of beer contain 20 g of alcohol, 0.25 l of wine, about 24 g. Daily intake of the equivalent of 4 (Continental) beers or one bottle of wine (0.75 l) is beyond the cirrhosis threshold, even in men.

Blood alcohol level after a number of drinks can be roughly estimated according to Widmark: blood alcohol concentration equals the quantity of ingested alcohol (in g), divided by the person's body weight (in kg) times the estimated fraction representing its aqueous phase (about 0.6 for women and 0.7 for men). The formula yields g alcohol per kg distribution volume, a part of which is blood, and therefore blood alcohol concentration in per mill (g alcohol per 1000 g of blood; 1 per mill is a tenth of one percent). Usually, this estimate exceeds measured values by 10-30%, as part of the ingested alcohol is already metabolized during mucosal passage, and part is excreted (urine, respiration) or metabolized in the liver while drinking and resorption are still going on. For more accurate results, alternative methods for estimating blood alcohol content factor in additional variables such as individual height/weight relations or age. For a rough estimate of blood alcohol content a few hours after alcohol consumption, 0.11 to 0.12 ‰ (0.011 to 0.012 %) per hour are subtracted from the starting value.

**2. Acetaminophen** (called Paracetamol in German): At recommended dosage, virtually all of acetaminophen is sulfated and glucuronidated in the liver; only negligible amounts are metabolized by CYP2E1 to a highly reactive intermediate, NAPQI (N-acetyl-p-benzo-quinone-imine). NAPQI is toxic, as it has the ability to covalently bind to cellular macromolecules. To detox NAPQI and other intermediates, cells produce a certain amount of glutathione, a small, amino acid-based molecule containing an –SH group. With its many

electron pairs, the sulfur atom reacts with NAPQI and similar molecules very efficiently, making glutathione a protective shield for cellular macromolecules.

At toxic dosage, acetaminophen first saturates sulfation and glucuronidation pathways, leaving more acetaminophen to be metabolized to NAPQI by CYP2E1. Over time, all available glutathione is consumed, after which NAPQI acts directly toxic. In case of CYP2E1 induction by chronic alcohol consumption, the fraction of acetaminophen metabolized to NAPQI is much larger to begin with; in other words, the threshold for acetaminophen toxicity is much lower (under these circumstances, cases of liver toxicity by 5-6 tablets à 500 mg per day have been described).

These considerations illustrate the problem caused by cytochrome P450 enzymes in pharmacotherapy. If effective dose and toxicity of many drugs are influenced by CYPs, but CYP configurations vary between individuals, many drugs are bound to act differently in different individuals. A few examples: CYP2D6 is instrumental in breaking down antidepressants, antipsychotics and some beta blockers, but required to activate the opioid tramadol (Tramal®-drops) to its active form. CYP2D6 comes in many allelic variants: some individuals carry defective alleles, others, especially from Ethiopia or Saudi Arabia, carry multiple-copy alleles. Consequently, in persons with high CYP2D6 activity, many drugs used to treat depression or acute psychosis won't work due to rapid metabolization. Conversely, identical doses of the very same drugs may be toxic in persons with low or missing CYP2D6 activity, while in these persons, Tramal drops won't be effective in relieving pain.

In principle, critical allelic CYP variants may be diagnosed to identify persons at risk. One diagnostic test relying on oligonucleotide DNA array technology was introduced in 2004 (AmpliChip CYP450®). It analyzes CYP2D6 and CYP2C19 genes for known polymorphisms so that enzyme activities may be extrapolated. Over time, improvements in high throughput-sequencing should further facilitate determination of an individual's cytochrome P450 status.

To appreciate the full potential for complications, however, it has to be kept in mind that these genetic differences are superseded by differences in expression levels due to enzyme induction, gender and age, as well as by competitive inhibition if drugs or food constituents are metabolized by the same cytochrome P450 enzyme. E. g., naringenin, a constituent of grape fruit juice, inhibits CYP3A4 and other CYPs, thereby increasing bioavailability of many drugs, including statins.

FUNCTION: Inactivation of steroid hormones
DYSFUNCTION: Gynecomastia, testicular atrophy, changes in body hair pattern

The method of conjugating lipophilic substances with hydrophilic moieties to facilitate elimination is also used for endogenous molecules, e. g., for steroid hormones or bilirubin. This way, steroid hormones are inactivated and excreted. Chronic liver insufficiency in men causes estrogen, which is produced at low rates, to accumulate, causing the above-mentioned symptoms.

FUNCTION: Elimination of bilirubin
DYSFUNCTION: Jaundice

Bilirubin is a porphyrin metabolite. Its primary source is the heme group of hemoglobin; a small fraction stems from coenzymes in respiratory chain and cytochrome P450 enzymes. Bilirubin has to be efficiently eliminated, as it is toxic at moderately elevated concentrations. Several transport systems facilitate bilirubin uptake into hepatocytes, making problems at this step unlikely: organic anion transporter protein 1 (OATP-1), bilitranslocase etc. Once within the cell, bilirubin is conjugated with glucuronic acid by UDP-glucuronyl transferase (UGT). Conjugated bilirubin is then pumped against a steep concentration gradient into the canaliculus by canalicular multispecific organic anion transporter (cMOAT, also known as MRP2= mdr related protein 2); this step requires ATP. In case a transport problem arises at this latter step, both conjugated and unconjugated bilirubin levels rise, making bilirubin detectable (and visible) in urine.

Genetic deficiencies concerning bilirubin elimination include Gilbert-Meulengracht syndrome (very frequent and harmless), Crigler-Najjar syndromes type I and II (all three of them UGT dysfunctions of different intensities), Dubin-Johnson syndrome (defective cMOAT) and Rotor syndrome (extremely rare and not yet tied to a specific gene; unclear whether fundamentally different from Dubin-Johnson syndrome).

FUNCTION: Elimination of cholesterol
DYSFUNCTION: Hypercholesterolemia, dyslipoproteinemia

Our organism is able to synthesize cholesterol, but by and large unable to break its core structure down- hence the need for excretion. This is done in two ways: either directly or by conversion to bile acids. 30-60% of secreted biliary cholesterol is reabsorbed, the rest is eliminated. In cholestasis, an atypical cholesterol-rich lipoprotein appears in the blood, lipoprotein-X.

FUNCTION: (Bile secretion- in parentheses, as this is no value in itself)
DYSFUNCTION: Cholestasis, cholelithiasis

Irrespective of its cause, a stoppage or marked reduction of bile flow is referred to as **cholestasis**. As there are different transport systems for different bile components, the term cholestasis is used for a range of situations, from an impairment concerning all bile components –as in mechanical obstruction-- to one restricted to bile acids. Cardinal symptom is itch, caused by a systemic increase in bile acids.

Starting from cholesterol, hepatocytes synthesize bile acids by adding hydroxyl groups and shortening and oxidizing the side chain to a COOH group. This process yields the primary bile acids cholic acid and chenodeoxycholic acid, which are frequently conjugated with either taurine, glycine, sulfate or glucuronate. In the intestine, a fraction of these bile acids is dehydroxylated by bacterial enzymes to form the secondary acids deoxycholic and lithocholic acid. Unconjugated bile "acids" are very weak acids, while conjugated forms, which have lower pKa, are mostly ionized and are therefore referred to as bile salts. Bile salts and -acids

are subject to enterohepatic recirculation: the entire pool is recirculated 5-10 times a day. This requires efficient transport proteins at both sides of the hepatocyte, which have to accommodate all these various forms and thus cannot be terribly specific. Consequently, they are also able to transport other molecules such as certain drugs or complex toxins. Transport from portal blood into the hepatocyte is facilitated by the $Na^+$-powered Na-taurocholate cotransporting polypeptide (NTCP). A second transporter, organic anion transport protein 1 (OATP-1), exchanges $Cl^-$ for ionized bile salts (OATP-1 has also been shown to transport amanitin into hepatocytes). Protonated bile acids can enter the hepatocyte by non-ionic diffusion. In hepatocytes, bile salts are buffered by binding proteins. They are then actively secreted into the canaliculus against a 100- to 1000-fold concentration gradient by the ATP-driven bile salt export pump (BSEP). For sulfated and glucuronidated forms, cMOAT is used, too. Defective BSEP alleles cause familial cholestasis syndromes of varying intensities.

Apart from problems caused elsewhere in the body –generalized pruritus, fat maldigestion, increase in cholesterol levels, jaundice etc.--, cholestasis also feeds back onto the performance of hepatocytes themselves. Bile salts are pretty toxic molecules to begin with. Increasing their cellular levels leads to atypical, fetal bile salts with additional hydroxylations in wrong positions. These are even more toxic, further increasing cholestasis. Generally, many different conditions are able to cause cholestasis: cholestasis is a logical result of acute hepatitis; it may be a relatively isolated adverse reaction to some drugs (e. g., competitive BSEP inhibition by steroids, ciclosporin A, rifampicin) or it may be induced mechanically by gallstones or tumors.

The frequent occurrence of **gallstones** is not surprising seen the small window of solubility for lipophilic molecules in a largely aqueous transport medium. Typical ranges for biliary molecules other than water would be around 67% bile salts and 22% phospholipids, both of which are required to keep 4% of cholesterol and fractions of a percent of bilirubin-diglucuronide in solution. As soon as cholesterol or bilirubin exceed certain thresholds, bile salts and phospholipids fail to keep them soluble in the form of micelles and stones start to nucleate. Small crystals can still be transported into the intestine; slightly larger conglomerates are dangerous, as they can get stuck at bottlenecks of the biliary duct system. Large stones may completely fill the gallbladder without causing symptoms. Cholesterol "stones" are the most frequent type; if dark Ca-bilirubinate predominates, we speak of pigment stones.

FUNCTION: Fat digestion
DYSFUNCTION: Steatorrhea, vitamin deficiencies ADEK

Bile is a form of liquid soap required to emulgate nutritional fat. Active substances are bile acids and phospholipids. Triglycerides constitute more than 90% of fat in food, forming fairly large liquid droplets at the body temperature around 37°C. Lipases, protein enzymes better soluble in the aqueous phase than in lipids, can only be active at the surface of these droplets. To digest fat efficiently, it is thus necessary to massively increase this boundary surface, which can only be done by addition of large amounts of surface-active bile acids and phospholipids. In the intestine, these biliary fats make up two to four times the amount of fats from food. Lipases cleave larger, more neutral lipid molecules like triglycerides, cholesterol ester or lecithin into smaller, relatively more water-soluble fragments such as fatty acids,

monoglycerides, cholesterol or lysolecithin, which in turn line up at the boundary layer and contribute to increasing total surface area. By this continuous redistribution in favor of surface-active fragments, fat droplets shrink over time from large, multilamellar vesicles to small vesicles with a single double membrane and further to tiny mixed micelles, with a single layer of surface-active lipids surrounding fewer and fewer neutral lipids. These tiny structures are able to diffuse into the mucus layer at the enterocyte surface that is continuously acidified by the $Na^+$-driven $Na^+$-$H^+$-antiporter, until the micelles practically bump into the apical brush border of the cells (otherwise, contents of the small intestine are alkaline). At this low pH, fatty acids are protonated, facilitating non-ionic diffusion into and through the cell membrane. Other lipids enter the cell by diffusion, too; in addition, uptake of some of them is probably facilitated by membrane transport proteins. Within the cell, lipids are reassembled, combined to chylomicrons and set free at the basolateral side of the enterocytes. From the extracellular fluid, they reach the blood via the lymph stream at the venous angle, bypassing the liver. In case too little bile reaches the intestine, fat from food is insufficiently digested and absorbed; most of it is eliminated in a light-colored, voluminous form of diarrhea termed steatorrhea.

Over time, this condition may cause a deficiency of lipid-soluble vitamins. Most prominently, impaired coagulation, a common occurrence in liver insufficiency, is aggravated by vitamin K deficiency (explained in the next section). Compared to vitamin K, deficiencies of other lipid-soluble vitamins are of minor importance. Vitamin A is stored in hepatic stellate (Ito) cells. In the blood, it is transported via retinol-binding protein that is itself synthesized in the liver. Low levels of vitamin A may cause impaired vision in the dark ("night-blindness"). Vitamin D is either taken up with food, or produced within the body from 7-dehydrocholesterol with the help of ultraviolet light (UV). In both cases, the inactive precursor has to be activated by two sequential hydroxylation steps. The first is performed in hepatocytes by cytochrome P450 hydroxylation at position 25. The second is done in the kidney under tight regulation by parathyroid hormone. Lack of vitamin D over time lowers $Ca^{2+}$ reserves of the body, leading to insufficient mineralization of bone. Finally, vitamin E has antioxidant function. No defined symptoms of a vitamin E deficiency are known.

FUNCTION: Synthesis of plasma proteins (albumin, clotting factors, acute phase proteins, transferrin, etc.)
DYSFUNCTION: -Hypoproteinemia/ edema/ ascites
-Clotting problems (coagulopathy)

The majority of plasma proteins is synthesized and secreted by the liver. Therefore, chronic liver dysfunction results in reduced plasma protein concentrations. Albumin, accounting for 60% of total plasma protein, is instrumental in maintaining oncotic pressure, necessary to reabsorb interstitial fluid into the venous leg of capillary vessels. Low albumin levels cause fluid to accumulate interstitially, while blood volume tends to be low. As chronic liver dysfunction is frequently associated with cirrhosis and portal hypertension, the combination of increased portal filtration pressure with reduced oncotic pressure frequently results in pronounced ascites.

Acute phase proteins like C-reactive protein (CRP) or mannan-binding lectin (MBL) contribute to defense against infections. More information may be found in the lecture notes on immunology.

Clotting factors are adversely affected by hepatic insufficiency via two mechanisms. In addition to a general shortfall in protein synthesis, specific factors lose biological activity due to vitamin K deficiency. Vitamin K is required to add additional carboxyl groups to the second to last-carbon atom of glutamic acid residues of clotting factors II, VII, IX and X, producing two adjacent $COO^-$-groups. Via binding to $Ca^{2+}$, these double $COO^-$-groups anchor the respective factor to the phospholipid membranes of aggregating thrombocytes (remember that one way to prevent clotting of a blood sample is to remove $Ca^{2+}$ by citrate or EDTA). In the absence of the second $COO^-$, the factors remain soluble and never meet, further impairing blood coagulation. Vitamin K is also required for $Ca^{2+}$-binding proteins in bone, e. g., osteocalcin. Of course, in vitamin K deficiency, reduced blood clotting causes symptoms far earlier than problems with bone mineralization.

**Pharmacology cross reference:** Derivatives of coumarin (acenocoumarol/Sintrom®, phenprocoumon/Marcoumar®) are vitamin K antagonists, inducing artificial vitamin K deficiency with the purpose to inhibit clotting activity (e. g., following pulmonary embolism). In case these drugs are discontinued, e. g., to allow dental work or surgery, it takes considerable time until sufficient amounts of biologically active factors are resynthesized.

FUNCTION: Monitoring intestinal import in a low-pressure capillary system
DYSFUNCTION: Portal hypertension

In events causing pronounced loss of hepatocytes (e. g., viral hepatitis, alcohol, sustained cholestasis) the liver's attempts to regenerate lead to secondary remodeling, coarsening the organ's delicate architecture. In addition, hepatic stellate (Ito) cells are activated to increase production of extracellular matrix, e. g., collagen and proteoglykans, causing fibrosis. The increase in diffusion distances, combined with reduced endothelial fenestration, impair the exchange of material between hepatocytes and blood plasma. Destruction of normal liver architecture reduces the total cross section of all portal blood vessels, resulting in portal hypertension (imagine a clogged filter). In turn, portal hypertension causes hypersplenism (sequestration and breakdown of blood cells in the spleen), portocaval anastomoses (esophageal and gastric varices, hemorrhoids, caput medusae), and ascites. Via portocaval anastomoses, blood coming from the intestine is shunted directly into the systemic circulation, avoiding the liver with its filtering and detox mechanisms. Esophageal varices may rupture, leading to life-threatening bleeding episodes that are extremely hard to stop.

**Hepatorenal syndrome** is renal failure due to underperfusion, secondary to liver dysfunction. Two mechanisms are thought to contribute to renal underfilling: the reduction in effective blood volume by reduced oncotic pressure and the dilation of blood vessels in the splanchnic circulation "stealing" blood from the systemic circulation. The decrease in effective blood volume sensed by the juxtaglomerular apparatus permanently activates the renin-angiotensin-aldosterone system, with secondary hyperaldosteronism in turn aggravating ascites by retention of sodium and water.

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