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.

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 collected and funneled via 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 (*Amanita phalloides*) 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.

FUNCTION:	Homeostasis of energy metabolism
DYSFUNCTION:	Fatigue, weakness, disorders of lipid metabolism, non-alcoholic fatty liver disease, insulin resistance, metabolic syndrome

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, and gluconeogenesis is therefore inhibited: in hepatocytes, insulin causes phosphorylation and breakdown of the transcription factor Foxo1. Otherwise, Foxo1 drives the transcription of enzymes involved in gluconeogenesis (PEPCK, glucose-6-phosphatase). Part of the glucose absorbed in the intestine is transported into the hepatocytes with the help of the insulin-independent transporter GLUT2 (KM 15-20 mM). Glucose as such cannot be stored, but it can be polymerized to glycogen in the liver and in skeletal muscle. The liver is able to store up to 10% of its weight in glycogen (100-120g). However, the amount of energy that can be stored in this way is limited: 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. Increased concentrations of free fatty acids and complex fats result 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 tapped under control of glucagon and sympathetic activity (plus growth hormone during the night) 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 tissues (CNS, erythrocytes, renal medulla), 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.

At the same time, **gluconeogenesis**, the synthesis of new glucose from lactate, glycerol or amino acids, increases. This is regulated via two pathways:

1. Falling insulin removes its previous inhibition of the Foxo1-dependent transcription of enzymes of gluconeogenesis in the hepatocytes.
2. Falling blood sugar and insulin levels inhibit the release of leptin by fat cells. The CNS reacts to this drop in leptin with an activation of the "stress axis" CRH-ACTH-cortisol (typical high level of cortisol in the early morning hours after overnight fast). Cortisol, in cooperation with the now low insulin levels, triggers lipolysis in adipose tissue. Free fatty acids (non-esterified fatty acids, NEFA) and glycerol mobilized from fat cells rise in the blood and reach the liver. Hepatocytes break down these fatty acids into acetyl-CoA; Acetyl-CoA allosterically activates the enzyme pyruvate carboxylase, which catalyses the first reaction of gluconeogenesis (to oxaloacetate). Lipolysis in adipose tissue thus causes increased gluconeogenesis via increased turnover of liver pyruvate carboxylase.

In addition to a limited amount of glycerol and lactate, the starting material required for gluconeogenesis is primarily amino acids, which cortisol mobilizes from muscles and bones. Of muscles and bones? But don't we need to protect those? Pfff... we have such vast amounts of protein in our muscles and bones that we can easily "borrow" this tiny fraction for a short period of time for gluconeogenesis. Through ingenious amino acid metabolism in the muscle cell, the muscles supply mainly alanine to the liver as the standard energy carrier.
Keep in mind that both pathways require very low levels of insulin. If the insulin level rises slightly, the rate of gluconeogenesis decreases. Let's also remember that insulin blocks lipolysis. So if we nibble on a sweet in the evening in front of the telly or head hungry for the fridge at night, we prevent the lipolysis planned for this time or head hungrily for the fridge at night, we prevent the lipolysis planned for this time; we reactivate the absorption phase and synthesize fat instead of breaking it down.

Pharmacology cross-reference: Pharmacological doses of **glucocorticoids** stimulate gluconeogenesis, causing a sharp increase in blood sugar. With prolonged use, they have a peripherally catabolic effect – we use amino acids, which we take from bones and muscles, as material for gluconeogenesis – so that osteoporosis and loss of muscle mass occurs.

Fasting phase. If the phase without food intake or with insufficient food intake lasts longer than a day, the liver glycogen is used up. In addition, the delivery of alanine from the muscle and thus the rate of gluconeogenesis decreases: blood sugar drops from around 90 to around 70 mg/dl (5 to 4 mM). It is not yet sufficiently clear how this regulation takes place, it is in any case organized by the CNS. Leptin level is halved, TSH decreases even more, with the resulting reduction in thyroid hormone, the overall energy consumption is also reduced: e.g., mitochondrial energy production in the liver is put on the back burner. You start to fell cold, blood circulation in the skin decreases, in particular hands and feet feel icy. This reduces heat losses to the outside and thus saves on ATP heating costs. When resting, cardiovascular enery ist saved, too: the heart beats a little more slowly, blood pressure drops a little. While the body was able to take out an amino acid loan at the skeletal muscles in the post-absorption phase for a short time without any inhibition or danger, it now has to change its strategy: it cannot cannibalize the muscles in the long term, as they continue to be needed for searching for food, etc.

At the same time, the liver also produces so-called ketone bodies, β-hydroxybutyrate and acetoacetate, from the ever-increasing flood of fatty acids. This happens because the liver via β-oxidation breaks down more fatty acids to acetyl-CoA than it can feed into the citric acid cycle. To do that, it would require more oxaloacetate ("fats burn in the flame of carbohydrates"), which is now also consumed for gluconeogenesis and thus becomes limiting. From the pool of actyl-CoAs lined up in this traffic jam, every two are fused, detached from CoA and released into the blood; mainly in the form of β-hydroxybutyrate, with a smaller portion as acetoacetate. Both are medium-strong acids, so they release protons, resulting in a ketoacidotic metabolic state. The renal cortex now gets involved into gluconeogenesis, contributing up to a third of the now reduced synthesis rate: Recall that the proximal tubule combines the excretion of acid in the form of $NH4^+$ with gluconeogenesis. The heart and the cortex of the kidney prefer ketone bodies to glucose anyway; heavy energy consumer CNS, which cannot do anything with fatty acids, after a while gets the hang of ketone bodies, too, over time preferentially metabolizing ketone bodies (up to 75% of the energy requirement) over glucose. That way, a lot of glucose can be saved by our energy-hungry brain during longer periods of caloric deficiency. In turn, that saves amino acids: protein consumption drops from around 75g per day to 20g. Without this adaptation, the muscles would be broken down far too quickly during prolonged periods of hunger. So, basically, ketosis is a trick to feed the CNS from fat stores: the adipose tissue releases fatty acids, which are converted by the liver into ketone bodies, which in turn become the staple food of the CNS. In addition, increased fatty acid concentrations cause resistance to the already very low insulin in the typical insulin-controlled tissues muscle, adipose tissue and liver, so that a larger proportion of glucose, which is only sparsely produced anyway, remains for the CNS, erys and renal medulla.

It is possible, then, that **insulin resistance** induced by elevated free fatty acid concentrations, which we have long been regarding as an exclusively pathological phenomenon, is a physiological component of an emergency program that enabled our ancestors to survive long periods of starvation with very low levels of blood sugar. Sustained food abundance has never occurred in human evolution. Today this mechanism is our undoing. When we are severely overweight with metabolic syndrome, we have the opposite nutritional situation - plentiful instead of meager - but also an increased concentration of free fatty acids, which now leads to insulin resistance in the presense of very high blood sugar levels.

Diet: carbohydrates or fat?

Ketogenic diet. The percentage of protein in most people's diets ranges from 10-35%. A proportion beyond 35% is not advisable, for several reasons. For one, the more protein we eat, the more stress we put on out kidneys. We can split the rest between carbohydrates and fat. A common recommendation is 45-65% carbohydrates, 20-35% fat. A ketogenic low-carb diet reduces the carbohydrate content extremely in favor of the fat content. In some aspects, this imitates the fasting phase. Due to greatly reduced insulin release, this type of diet, in combination with caloric restriction, can help to reduce weight. After a transition period characterized by the loss of considerable amounts of water (as glycogen stores are depleted; looks great on the scale: "Wow, finally a diet that works, I've lost 2 kg already!"), the body adapts surprisingly well to this change in food supply. Some populations, such as the Inuit, systematically nourished themselves this way due to a lack of carbohydrates. For a short to medium term, such a diet can indeed help to reduce weight. What about the long term?

Long-term ketogenic diet? How healthy or unhealthy are different distributions of our three energy carriers, carbohydrates, fat and protein? Unfortunately, the data on this question is very insufficient. Ideally, we would like to see randomized controlled trials over thirty years and more with tens of thousands of participants, in which people constantly, with precise documentation and control, adhere to a specified distribution of macronutrients, of course while complying with all requirements for micronutrients (minerals, vitamins, trace elements). All this with meticulous documentation how their health develops. Apart from the fact that this is not possible: Would you take part in such a study? No sweets for thirty years because you were randomized to the keto group? For these reasons, there are very few studies that offer reasonably reliable data on our question. In these studies, the participants were asked by questionnaire at the beginning, at most once again later, about their eating habits. This type of self-reporting already introduces uncertainty. Self-reported food items were then converted into a macronutrient distribution using standard conversions – how many calories from carbohydrates, fat and protein are in a serving of leeks? a bread with jam? a pizza? It was then assumed that these dietary habits did not change significantly during study years. As you can see, these data contain numerous levels of uncertainty. In my opinion, the most meaningful data come from three prospective studies: PREDIMED (Spain), PURE (18 states with a focus on Asia and South America) and ARIC (4 communities in the USA):

1. PREDIMED is the only randomized interventional study of the three. In Spain, three groups of subjects at cardiovascular risk were compared. All three were on a Mediterranean diet. The first group was encouraged to supplement there nutrition with ample extra virgin olive oil; the second, to supplement it instead with a daily allocation of nuts (almonds, hazelnuts and walnuts, rich in polyunsaturated fatty acids; oil or nuts, respectively, were provided). The third group was asked to try to reduce fat – which automatically means increased carbohydrates – and received small non-food gifts instead of oil/nuts. The composite endpoint included myocardial infarction, stroke, death from cardiovascular causes. Result: the fat-reduced group had a clearly increased risk compared to the two groups that had consumed more vegetable fats.
2. The main message of PURE in this regard: a higher proportion of fat in the diet was not associated with increased, as had long been assumed, but rather with reduced mortality. Importantly, however, this statement only applied to carbohydrate percentages between "medium" (lowest quintile: 46% of energy requirements) to high (highest quintile: 77%). The result for this part of the spectrum was: the more carbohydrates, the higher the mortality.
3. Only the ARIC study included a proportion of subjects consuming less than 46% carbohydrates. A U-shaped mortality curve emerged with a minimum at a carbohydrate content of 50-55%; above and below, mortality increased. The subjects with the lowest carbohydrate intake therefore satisfied their energy requirements mainly with fat. For the majority of probands in this group, this meant high intakes of saturated animal fat and a significant increase in mortality. Only a small part of the probands consumed primarily vegetable - unsaturated - fats and proteins. This part showed reduced mortality, but the proportion was too small to draw firm conclusions.

What do we learn from this? All three studies argue against a low-fat diet, which has been heralded as ideal for decades. PREDIMED and PURE do not provide any information about a carbohydrate-reduced diet; ARIC finds a minimum of mortality at 50-55% carbohydrate content, below which mortality increased again. So far we do not have reliable data on ketogenic nutrition. In the ARIC study, carbohydrate intake did not reach the low levels required for a ketogenic diet. However, one can extrapolate from the ARIC data that in the long run, a ketogenic diet is probably all the less healthy if energy intake is mainly based on animal fats

and proteins. Uncertainty remains about a plant-based ketogenic diet. We cannot exclude that a plant-based ketogenic diet MIGHT be healthy, but so far we lack the data. A plant-based ketogenic diet is not easy to implement in daily practice (pure peanut butter, anyone? Tofu dumplings in olive oil?).

Isn't that a contradiction to the expectation that long-term food restriction will have a life-prolonging effect in humans, as it does in many animal species? Isn't it also a ketogenic diet if you live in a hypocaloric state for years? No: a hypocaloric diet is not fasting. If you eat a hypocaloric diet that is balanced in terms of macronutrients, ketosis does not develop, since you consume carbohydrates regularly and the body does not have to switch to predominantly burning fat.

Type 1 diabetes mellitus. Before insulin became available as a drug, DM1 was a death sentence. The absence of insulin induces the ketoacidotic state of starvation. In front of full pots and with sky-high blood sugar, the children died of "internal starvation". They shed muscle and substance until it was no longer compatible with life.

Control of gluconeogenesis: 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 by inserting units of GLUT4 (K_M 5 mM) into the membrane. Yet, insulin remains important between meals, e. g., over night.

At the low post-absorption glucose levels (80-100 mg/dl, equivalent to 4.5-5.5 mM), critical tissues like the central nervous system are continuously supplied with glucose via GLUT1 and GLUT3. With their K_M of 1 mM, these transporters always work in the saturation range. In contrast, little glucose enters β-cells of the pancreatic islets, due to the much higher threshold of GLUT2 (K_M 15-20 mM). If the blood sugar level is slightly increased by gluconeogenesis, proportionally a little more glucose enters β-cells, causing release of a little more insulin. Insulin inhibits glucagon secretion in the neighboring α cells; less glucagon and more insulin enter the venules draining into the portal vein. In this situation, systemic insulin levels remain too low to promote uptake of glucose into muscle or adipose tissue. However, since insulin is secreted into the portal blood, its concentration in the liver is higher than in the rest of the body; this insulin in combination with reduced glucagon limits gluconeogenesis. Gluconeogenesis is thus constantly throttled by an insulin feedback 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.

Pharmacology cross reference: Metformin inhibits gluconeogenesis, making it the premier drug in type 2 diabetes mellitus. Over time, several mechanisms have been proposed to explain this effect. Among those, most convincing seems a metformin effect on a mitochondrial redox enzyme, which leads to increased NADH and diminished NAD^+ concentrations in the cytosol of the hepatocyte. As a result, less lactate can be transformed into pyruvate which is required

for gluconeogenesis. This also explains a tendency to lactic acidosis. As we will see shortly, we observe a similar effect when metabolizing alcohol, making the metformin + alcohol combination particularly problematic.

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 study material- 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 (please 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, the presence of substrates leads to increased expression. Therefore, even persons with identical genes may have differing cytochrome P450 expression levels depending on their living conditions/personal history. Mechanism? Please see next section!

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.

Enzyme induction. The detoxification system is not rigid but capable of adapting to varying demands. We often absorb small amounts of all sorts of toxins as we navigate a world where everyone is grappling to defend themselves and their ecological niche against others. Consider two examples that we don't usually take up via natural pathways but that are of medical interest:

- The Pacific yew (*Taxus brevifolia*) protects itself from being misused as chow by synthesizing a substance that interferes with the function of microtubules and, with that, the cell division spindle. We call this poison paclitaxel and use it as a drug in cancer therapy, e.g. against breast cancer.
- The soil-borne bacterium *Amycolatopsis rifamycinica* keeps competitors at bay by synthesizing a compound that interferes with the function of DNA-dependent RNA polymerase from other bacteria. We call this substance rifampicin and use it as an antibiotic against bacterial infections, e.g. against tuberculosis.

The two substances are complex and lipophilic. In hepatocytes, they both bind to a member of the family of nuclear receptors, the pregnane-X- receptor (PXR). Nuclear receptors are our sensors for lipophilic molecules from both inside the body and outside, sensors that help us to adapt gene expression to changed needs. The PXR then binds to response elements in the promoter of CYP3A4 and other CYPs, increasing their expression. In addition, PXR induces conjugating enzymes for the second step, e.g. glutathion S transferase, as well as membrane transporters for discharge of the conjugate into the bile canaliculus. Both substances are metabolised by CYP3A4. Via this mechanism, paclitaxel and rifampicin soon accelerate their own degradation while also affecting the breakdown of other drugs. Progesterone, too, induces the proteins of this detoxification mechanism via binding to the pregnane X receptor, so that pregnant women metabolize drugs differently; something that we obviously can not or do not want to test. An analogous way to react against foreign ("xenobiotics") or endogenous lipophilic substances works via another nuclear receptor, the constitutive androstane receptor (CAR). CAR binds other ligands than PXR and activates a different range of cytochrome P450 oxidases.

Parenthesis: Xenohormones (endocrine disruptors). All of us are currently participating in a large-scale food experiment: We are taking in substances that mankind has never encountered before. According to Paracelsus' *dosis facit venenum*, we hope that nothing will happen as long as we keep the dose small. Substances that work in low concentrations by binding to high-affinity receptors could spit us into the soup. In particular, lipophilic substances that are easily absorbed, often accumulate in adipose tissue and bind to nuclear receptors are under discussion. We express 48 different nuclear receptors; for the majority of them, the natural ligand is still unknown. Since many nuclear receptors are expressed in almost all body cells and each affect the expression of hundreds of genes, a theoretical impact assessment is impossible. Temporal windows of particular sensitivity (e.g. embryogenesis) and epigenetic effects on later generations are conceivable. Some examples from a long list of substances discussed:

- **Bisphenol A** (BPA) has weak estrogenic effects and other poorly understood effects. For example, an increase in T helper cells in the spleen was reported, which could have an impact on the incidence of allergies. BPA is found in plastic soda bottles, the liners of food and beverage cans, plastic toys and kids' shoes. It was used in baby bottles until 2011, and on

thermal paper from receipts until 2020. In 2023, based on new data, the European Food Safety Administration recommended reducing the BPA-TDI (tolerable daily intake) by a factor of 20,000 (from 4 micrograms/kg body weight and day to 0.2 nanograms).

- **Phthalates** have an estrogen effect. They are used as plasticizers in PVC, also in medical cables and hoses, and in cosmetics.
- **Atrazine** alters ovarian hormone production. It is widely used as a herbicide in the US but has been banned in the EU since 2003.
- **Perfluorooctanoic acid** (PFOA) binds to estrogen receptor and PPARα. Because of its oil- and water-repellent properties, PFOA is used in the manufacture of many plastics, e.g. non-stick coatings for frying pans and outdoor clothing. It is practically non-degradable, accumulates in fatty tissue and can be detected in almost every individual today. There are hints that it may promote the development of kidney and testicular cancer. It has been banned in the EU since 2020.

It is possible, albeit difficult, to reduce potential risks without understanding them in detail: the principle is to minimize contact between food and plastics. This is especially true when they are heated (microwave, frying pans, fast food containers,…). No canned foods. In addition, buying organic reduces potential risks from herbicides and pesticides.

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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.

Let's take a closer look at the effects of these systems 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: a large fraction is extracted from portal blood and metabolized so efficiently that it is sometimes hard to reach useful blood levels.

On the other hand, also in drugs, metabolization may result in toxicity, a process that may be enhanced by metabolism of other molecules. A medically relevant case in point 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 uses O_2 and NADPH to produce acetaldehyde. This second pathway generates free radicals and interferes with NADPH-dependent regeneration of glutathion. 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, NADH + H^+ is produced. Metabolization of alcohol thus yields Acetyl-CoA plus NADH, both of which can be used to produce ATP via citric acid cycle and respiratory chain. Surplus material is simply used for fatty acid synthesis. By drinking alcohol, we synthesize fat instead

of breaking it down. The pathway explains the two main forms of pathology directly caused by alcohol: alcoholic hepatitis and fatty liver. With ongoing alcohol consumption, both of these may over time result in cirrhosis. NADH not only inhibits fatty acid oxidation. As we have seen with metformin, high concentrations of NADH also inhibit gluconeogenesis by preventing the oxidation of lactate to pyruvate. In fact, NADH will cause the reaction to reverse. This way, too much alcohol may lead to hypoglycemia and lactic acidosis. Type 2 diabetics who consumed alcohol the night before often marvel about their "excellent" blood sugar levels the following morning.

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. Another example is the platelet inhibitor clopidogrel, which is only converted into its active metabolite by CYP2C19. In 10-15% of patients, CYP2C19 is less active due to allelic variants, so that therapeutic platelet inhibition is not achieved.

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, organic anion transporter proteins (OATPs), facilitate bilirubin uptake into hepatocytes, making problems at this step unlikely. 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, systematic designation ABCC2); 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, combined deficiency of OATPs required for uptake of bilirubin from sinusoidal blood).

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 at positions 7 and 12 and shortening and oxidizing the side chain to a COOH group. Production is subject to negative feedback: expression of the rate-limiting enzyme, 7α-hydroxylase, is reduced when enough bile acids are present. Bile acids in the hepatocyte bind to the farnesoid X receptor (FXR), another member of the nuclear receptor family that suppresses the expression of the 7α-hydroxylase gene (CYP7A1). De novo synthesis yields the primary bile acids cholic acid and chenodeoxycholic acid, which are frequently conjugated with either taurine, glycine, sulfate or glucuronate. In the intestine, bacteria partially remove the 7α-hydroxyl group, forming 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, SLC10A1). In addition, the family of organic anion transport proteins (OATPs, encoded by genes SLCO1A2, SLCO1B1 and SLCO1B3) transport ionized bile salts into the cell (OATPs have also been shown to transport the death cap's poison, 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, ABCB11). For sulfated and glucuronidated forms, cMOAT is used, too. If the bile acid concentration in the hepatocyte increases, the farnesoid X receptor induces BSEP, so that more bile acids can be secreted. Defective BSEP alleles cause familial cholestasis syndromes of varying intensities.

Pharmacology cross reference: OATP1B1 (gene: SLCO1B1) also transports statins from the blood into the hepatocytes; their main site of action but also where they are metabolized and excreted. A common allele – 18-28% heterozygous, 2-3% homozygous in a European genetic background –, T521C with the amino acid exchange valine 174 to alanine, shows a greatly reduced transport rate for statins, so that statin plasma concentrations are increased in these individuals, with a concomitant increase in the risk of myopathy. This effect is not equal for all statins; it is more pronounced for simvastatin than for others. Other drugs taken up via OATP1B1 amplify this effect; these include amiodarone and cyclosporine.

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.

Pharmacology cross reference: The transmembrane protein NPC1L1 (Niemann-Pick C1-Like 1) is involved in the transport of cholesterol from the intestinal lumen into the enterocytes. **Ezetimibe** can block this transport, preventing uptake of dietary cholesterol as well as re-uptake of cholesterol secreted via the bile. It thus reduces LDL cholesterol levels, but this effect is not on par with that of statins.
Orlistat covalently and irreversibly blocks lipases in the gastrointestinal lumen. It is approved in the EU for weight loss. Fats cannot be absorbed unless they are broken down by lipases. This kind of treatment enforces dietary discipline, since even a relatively modest dietary fat content leads to explosive diarrhea with fatty stools.

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, phenprocoumon) 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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