

# NUTRITION AND METABOLISM

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*These lecture notes accompany my lectures on pathophysiology in the study module "Nutrition and Digestion" at the Medical University of Innsbruck. 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.*

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Just to stay alive, and still without any activities, we already need a lot of energy: about 1 kcal (0.9 for women, 1.0 for men) per kg body weight and hour. A particular energy guzzler is our brain, which, while only making up 2% of our body weight, consumes about 18% of our basal metabolic rate, regardless of whether we use it in any meaningful way. The remainder of our energy expenditure at rest goes to the liver (26%), muscles (26%), heart (9%), kidneys (7%) and what's left (14%). Apart from this fuel that we burn, we need defined "building blocks and tools" for our body, and we have to get rid of unnecessary or even toxic molecules. In short, we cannot live without massive exchange of substances with our environment. Exchange entails risks. Our gastrointestinal tract and liver are responsible for managing this task while minimizing the risks.

Simply put, the strategy is the following: Keep complex stuff outside. Break it down to "generally accepted building blocks". Import only those secure building blocks. Alas, we have to make many exceptions. For one, we depend on some highly complex molecules that we have to import for lack of the ability to synthesize them ourselves, e. g., vitamin B12. We need special tools for that, like the special services required to move old master paintings across geographical and political barriers. On the other hand, we have to recycle in large quantities some substances previously secreted into the gut, e. g., bile acids and bile salts. Due to their diversity, we have to apply relatively unspecific transport mechanisms, sometimes resulting in unwitting co-absorption of dangerous material. Finally, to guard our borders, we need to give our defense system an idea of the dangers outside, as well as a chance to learn how to tell them apart from the numerous harmless food antigens and benign bacterial symbionts that we need to tolerate. To do that, we open channels via M cells to take in small random samples of complex structures from outside. Although we monitor these samples closely, the process is sometimes used against us to slip in pathogenic stowaways.

## 1. EVOLUTIONARY ASPECTS

What is healthy food? This question touches everybody and is able to raise high emotions. Food fads come and go, their respective supporters fighting each other with gusto. The habit of

consuming high-fat meat or meat products like briny ham or sausages on a daily basis, common in affluent parts of the world, certainly isn't healthy. However, the opposite, a purely vegetarian or vegan diet, also would lead to malnutrition. At least vitamin B12 must be supplemented; In addition, there are a number of potentially critical nutrients, especially in certain life situations where there is an increased need: in babies, children, adolescents, pregnant women and breastfeeding women. These include protein in terms of quantity and amino acid composition, long-chain n-3 fatty acids, iron, zinc, vitamin D, calcium and iodine. It is not uncommon for such deficiency symptoms to be found in the children of vegan parents: with the best of intentions, they assume that the form of nutrition that has proven itself for them for years must also be good for the kids. If attention is paid to potentially critical nutrients and in case these are supplemented if necessary, a vegetarian lifestyle is quite healthy: Vegetarians have fewer heart attacks, fewer strokes and they suffer less frequently from type 2 diabetes mellitus, high blood pressure or some types of cancer.

In this debate, it is helpful to keep an eye on our evolutionary origins. While modern *Homo sapiens* lives all over the world, this is a fairly recent development. For all we know from analyses of mitochondrial DNA and Y chromosomes, all humans originating from Europe, Asia, Australia and the Americas are descendants of a fairly small group of people who emigrated out of Africa and the Middle East between 70,000 and 60,000 years ago. In evolutionary terms, this time is too short to accommodate a great deal of genetic adaptation. In other words, to a large extent we are still the product of selective forces active in Paleolithic Africa. We are optimized for Paleolithic conditions.

While it is impossible to reconstruct the menu of that time, a few aspects seem very likely. Most of the time, food was in short supply. Selective pressure favored people with "thrifty alleles" who were able to form reserves, fat deposits, efficiently during occasional times of plenty to help them survive long periods of penury. Meat of wild game was desirable, but hard to obtain. For the majority of their nutrition, people relied on plants, meaning roots, tubers, nuts and in wetter places, fruits. Carbohydrates generally made a smaller contribution to the diet. Most of the time, these were of the complex type, as mixtures of starch and fiber. "Sweet" simple sugars like glucose or fructose were rarely available, restricted, e. g., to berries in fall or wild honey. Milk and cereals, products of the Neolithic (agricultural) revolution starting 10,000 years ago, were absent. Compared to our average Western diet, food intake was thus likely high in protein and fibers, moderate in fat and low in carbohydrates, especially far lower in short-chain carbohydrates. Gathering enough food meant daily hiking and hard physical labor. In the African sun, it was important to conserve water. For osmotic reasons, this implied conserving salt, which was sparse except at the coast.

Archeological evidence tells us that the agricultural revolution had a strongly negative impact on human health. People suffered from malnutrition, were smaller, had bad teeth and had a lower life expectancy.

These considerations are intended to illustrate conditions of importance for our nutrition and health. Expressly, I **don't** want to imply that we should eat a Paleolithic diet today, although I believe that would be healthy. Firstly, for the large majority of us, this is simply impossible. Paleolithic meat was qualitatively different from today's meat: wild game contains far less fat than our fatstock. Secondly, in view of the calories required, grains, corn and rice are irreplaceable today. In a world with eight billion people, we have to behave differently than our Paleolithic ancestors, who numbered a few hundred thousands at most. The factory farming that

would be necessary to provide so many people with meat on a regular basis is neither ethical nor justifiable in terms of climate considerations.

What probably comes closest to the diet of our Palaeolithic African ancestors today is the "Mediterranean diet". To the regret of (probably not only) the writer of these lines, pasta, lasagne and crème brûlée are not meant. During investigations after World War II, it was noticed that the people on the mediterranean island Crete were particularly healthy and grew particularly old. This rural, physically hard working, certainly not prosperous population ate a diet rich in vegetables, salads, fruit, fish and olive oil, complemented by a little chicken and mutton and hardly any sugar apart from honey.

We urgently need to develop a form of nutrition that integrates health and ethical concerns, while at the same time being fair and economically feasible on a global scale. But this is not my topic. My goal here is to make the health-related aspects of nutrition understandable.

## 2. OVEREATING

Today, the thrifty Paleolithic genetic endowment is less than optimal for people living in conditions of ample food supply. Our sedentary daily routines exacerbate this mismatch. Regarding food availability, we live in a constant feast. If we do not consciously counteract this, we accumulate too much fat, one of our most pressing health challenges.

What is too much? Most commonly, we assess body weight using the BMI (body mass index, body weight in kg divided by the square of height in meters): the normal range for women is 19-24, and for men, it is 20-25. Values above these ranges are considered overweight, above 30, obese. We use BMI routinely because it is easy to determine, but it does not take into consideration the distribution of weight between fat and lean body mass: A BMI of 30 has a different significance for a female hammer thrower than it does for a woman who hardly exercises. Other factors that may contribute to increased weight like bone density, bone diameter, shoulder width and hip width also remain out of consideration. Additionally, for tall men, higher BMIs are less informative. A simple and useful measurement due to the importance of visceral fat is determining waist circumference, which only requires a measuring tape. Recommended values are below 80 cm for women and below 94 cm for men; values over 88 cm for women and 102 cm for men indicate significantly increased health risks. When related to height (waist-to-height ratio), it is in some respects more informative than the BMI. For adults up to 40 years old, a value of <0.5 is recommended, and for those over 50 years old, <0.6. A more informative assessment, which however requires appropriate equipment, is provided by bioelectrical impedance analysis. This method estimates the distribution between fat and lean body mass and relates it to age and height. Accurate methods for determining body fat percentage –underwater densitometry/air displacement plethysmography, DEXA, <sup>40</sup>K<sup>+</sup> counting, magnetic resonance imaging– require too much labor, can be burdensome for frail patients, and are also too costly for routine use.

Fat depots don't simply act like an inert pantry. The larger these deposits become, the more they develop an inflammatory component, although we do not yet understand fully why. In any case, there is immigration and activation of macrophages, which release their cocktail of IL-1 $\beta$ , IL-6 and TNF $\alpha$ . We'll see in a moment that this has a negative effect on insulin sensitivity.

The accumulation of fat starts to have toxic effects. Remember that toxicity is a function of the dose. There is increasing evidence that the moderate increase in free fatty acid (FFA) concentrations seen in obesity causes metabolic problems at two levels: insulin-sensitive target tissues and insulin-producing  $\beta$ -cells.

### **Insulin resistance in target tissues: Liver, adipose tissue, skeletal muscle**

Free fatty acids that are not "burned" accumulate in the cytosol of liver, skeletal muscle and adipose tissue and are reincorporated into triacylglycerols. In this process, levels of the intermediate diacylglycerol increase, too. Diacylglycerol is implanted into the cell membrane, functioning as a second messenger that recruits and activates protein kinase C (PKC). PKC $\epsilon$  phosphorylates the insulin receptor on threonine 1160 and thus inhibits the tyrosine kinase function of the receptor, reducing its signaling efficiency. In addition, active PKC and other serine/threonine kinases phosphorylate insulin receptor substrate 1 (IRS-1) at inhibitory sites, reducing its ability to transmit the insulin signal into the muscle cell (to avoid confusion: in contrast, activated insulin receptor phosphorylates IRS-1 on tyrosines, which is required for signal transduction). In healthy individuals, muscle and fat cells respond to insulin by inserting more units of glucose transporter 4 (GLUT4,  $K_M$  5mM) into the plasma membrane. If insulin signal transduction is compromised by the fatty acid effect, lower amounts of glucose are transported into these large volume tissues and blood glucose remains higher. Of course, all of our cells consume glucose all the time. Yet, they take up glucose via transporters GLUT1 and GLUT3 ( $K_M$  1mM), which are running at capacity at normal glucose levels ( $>4.5\text{mM} = 80\text{ mg/dl}$ ) anyway. Liver cells and pancreatic  $\beta$ -cells take up glucose via GLUT2 ( $K_M$  15-20mM). The higher  $K_M$  means more glucose is transported into these cells at higher blood glucose levels. In the liver, surplus glucose is metabolized to fatty acids, increasing the problem. Recall that GLUT1, GLUT3 and GLUT2 do not respond to insulin.

In an independent mechanism, saturated fatty acids like lauric acid (C12) or palmitic acid (C16) have been shown to act as partial agonists on TLR4 (the LPS receptor) and TLR2. Via this mechanism, increased plasma levels of FFA activate inflammatory pathways that also lead to reduced insulin-sensitivity of liver, muscle and adipose tissue. For example, IL-6 or TNF $\alpha$  released from adipose tissue induce SOCS (suppressor of cytokine signaling) proteins in liver cells. SOCS proteins bind and mask phosphorylated tyrosines on IRS-1, interfering with signal transduction and inducing proteasomal degradation of IRS-1.

Regular aerobic exercise enhances expression of enzymes required for fatty acid metabolism and increases the mass of mitochondria. That way, regular exercise leads to more efficient metabolism of fatty acids, and thus to an increase in insulin sensitivity.

### **Insulin resistance: Pancreatic $\beta$ -cells**

With target tissues less responsive to insulin, plasma glucose concentrations are slightly increased. Glucose enters pancreatic  $\beta$ -cells via glucose transporter 2 (GLUT2,  $K_M$  15-20 mM) in proportion to its blood concentration, stimulating the production of ATP. ATP binds to an ATP-sensitive  $K^+$  channel: the higher the ATP concentration, the lower the channel's open probability. The  $K^+$  channel contributes to membrane resting potential. Closing the channel elevates the membrane potential to the threshold of voltage-dependent  $Ca^{2+}$  channels. Influx of  $Ca^{2+}$  then leads to exocytosis of insulin-filled vesicles.

**Pharmacology cross reference:** The ATP-sensitive K<sup>+</sup> channel (subunit ABCC8 or SUR1, sulfonyl urea receptor 1) is the target for sulfonylureas. Binding of sulfonylureas promotes closure of the channel, thereby enhancing insulin secretion.

Increased blood glucose concentrations (which in healthy individuals range between 4 and 8 mM, or between 72 and 144 mg/dl) thus stimulate insulin synthesis and release. Beta cells have an enormous capacity to synthesize insulin; up to 50% of the total protein synthesized in the pancreas may be proinsulin. Yet over time, constant overproduction of proinsulin puts the endoplasmic reticulum under stress, and unfolded or misfolded proteins accumulate. This problem not only concerns insulin, but also a protein hormone called IAPP (islet amyloid polypeptide) or amylin, which is co-regulated as well as co-expressed and secreted with insulin. There are standard operating procedures for any cells in this situation: ER stress activates the unfolded protein response (UPR), which consists of several steps. First, general protein synthesis is inhibited. This reduces the output of insulin. Second, chaperone synthesis specifically is increased to aid in the folding of newly synthesized protein. Third, misfolded proteins are exported from the ER to the cytosol and broken down in the proteasome. If all this doesn't bring relief, eventually, programmed cell death is triggered. In addition, in this particular case, conglomerates of misfolded IAPP show a tendency for prion-like proliferation and are particularly toxic. Over time, this process reduces pancreatic  $\beta$ -cell capacity, leading from insulin resistance to overt diabetes mellitus type 2.

While we don't yet understand the exact chain of causation, the term "metabolic syndrome" was coined to describe the total of observed changes. Insulin resistance, via diabetes mellitus type 2, causes a broad spectrum of morbidity, including atherosclerosis, coronary artery disease, stroke, diabetic nephropathy, retinopathy and polyneuropathy.

These problems do not affect all of us to the same extent. In the genetic lottery, some of us have drawn more, others fewer thrifty alleles. In one study, an individual with 104 out of 194 thrifty gene variants was on average 11 kg heavier than someone with only 78.

### **What is a thrifty gene? One example: FTO gene and brown adipose tissue**

Life is not fair: Some people can eat a lot without adding weight, most people can't. Some people never seem to be cold; relaxedly, they sit around with shortsleeves while others try to pull the zippers of their jackets a little higher, freezing. Could it be that some people just "burn" more food?

Until a few years ago, it was thought that brown adipose tissue (BAT) only existed in babies. Usually, fat is white, but patches of specialized fat cells containing a high load of mitochondria appear beige or brown. These cells use their mitochondria to burn fat or glucose to produce heat. Normally, mitochondria use the energy of a proton gradient to produce ATP, like a mill would use the energy of a creek for grinding grain. If the miller uncoupled the cog wheels of his mill from the grindstone, the now faster turning wheels would produce only heat, not work. Similarly, by expression of uncoupling protein 1 (UCP1 or thermogenin), protons are diverted from driving ATP synthase (the grindstone) by being allowed to trickle back through the inner mitochondrial membrane. This way, beige or brown fat cells are able to uncouple fat burning from mitochondrial ATP production, burning a lot of fat for heat. The process is stimulated by a cooperation of sympathetic activation with thyroid hormone. Activation of  $\beta_3$ -adrenergic

receptors induces iodothyronine deiodinase type II, a selenoprotein converting thyroxine (T4) to the more active triiodothyronine (T3). T3 activates the thyroid hormone receptor, which sits at the promoter of the UCP1 gene.

Then, via the technique of <sup>18</sup>F-fluorodeoxyglucose PET-CT, it was recognized that beige or brown fat also existed in adults, in small patches in the anterior neck and in the thorax. Initially, it could only be identified in lean subjects or if the test subjects were exposed to cold. Over time, it was established that short-term exposure to cold (hours) increases BAT activity, long-term exposure (weeks) also expands BAT volume. With sufficiently harsh cold exposure, BAT can be demonstrated in almost everyone. Yet, lean people tend to have more BAT.

Independently from these developments, genome-wide association studies were carried out to identify genetic variants contributing to obesity. Repeatedly, a gene of unknown function, now designated *fat mass and obesity associated* (FTO) came out at the top. However, the mechanism by which certain allelic variants of this gene contributed to obesity remained unclear. Eventually, it was recognized as a DNA maintenance enzyme taking off methyl groups from thymidine, but that did not help to understand its relation to obesity.

Finally, in 2015, an unexpected model was proposed: the feature of the FTO gene contributing to obesity is not its encoded protein, but rather a binding site in intron 1 for a gene-regulatory protein which acts on genes further down the chromosome. Somewhere between exons 1 and 2 of the FTO gene on chromosome 16, a single nucleotide polymorphism (SNP rs1421085) means some of us carry a C (let's call it "close-fisted C"), some of us carry a T ("thermo T"). Of course, we have two of these chromosomes, so our genotype may be CC, CT or TT. To keep things simple, let's just consider CC and TT genotypes (CT should be somewhere in between).

1. You are a thermo-TT type? Then you are lucky, because you burn off fat more easily. A complex chain of events makes that happen. A gene-regulatory protein, ARID5B, binds to the DNA at the spot of thermo-T. It acts as repressor, inhibiting transcription of two genes further down the DNA, IRX3 and IRX5. These two genes encode proteins that are gene repressors in their own right, acting as switches between brown and white in the development of new fat cells. Thermo-T represses these repressors, allowing the development of higher amounts of BAT. If you are TT, you burn off a higher proportion of ingested calories for heat. Maybe you are the short-sleeved-in-the-cold-type! BUT if you had lived in the Paleolithic, you would have been less lucky. Constantly burning off more calories, you might have been among the first who starved in a prolonged famine.
2. You are a close-fisted-CC type? Then you are excellently equipped for a Paleolithic environment. Thrifty-C prevents binding of the ARID5B repressor protein. Down the line, IRX3 and IRX5 are expressed more strongly, preventing the browning program in new adipocytes. Lots of white adipose tissue is formed, allowing you to build fat stores in good times. Better chances to survive the next famine!

So, now we understand **one** thrifty gene. But there are more than four hundred of them, and each one contributes only a small fraction to the overall variance in body mass index observed. Depending on the stack of alleles we got from our parents in the grand genetic lottery, we are more energy-thrifty or more short-sleeved, and as yet there is nothing we can do about that. Those enviable individuals who missed out on thrifty alleles have no problem staying slim and trim; for the majority of us, maintaining weight requires a conscious effort.

An additional factor promoting overweight, which may be regarded as part of the Paleolithic program, is the fact that foods rich in sugar and fats (Yummy! Chocolate!) trigger the same reward pathways as certain drugs like cocaine. With limited availability of calorie-dense foods, this behavior-guiding mechanism was useful in times past; today, it is a problem. Food manufacturers and retailers, who make a profit from it, exploit this weakness with seductive advertising to the best of their ability.

### **The contribution of our microbiota**

Another aspect in which individuals differ is their complement of gut bacteria, collectively known as the gut flora in the past, but more commonly referred to as the microbiota today. Originally a plural term, I align with its general use as a singular noun. The microbiota of obese individuals distinctly differs from that of lean people, though it is less clear what is cause and what is effect. The human gut is inhabited by a complex community of bacteria and archaea, drawn from more than 1000 species. Each one of us hosts over  $10^{13}$  such cells from at least 160 species. About 60 species are found in more than 90% of all Europeans. Based on animal studies, it has been reported that the quantitative ratio of two phyla, Firmicutes (e.g., *Clostridium*, *Lactobacillus*) and Bacteroidetes (e.g., *Bacteroides*, *Xylanibacter*), correlates with obesity: Firmicutes predominate in obese individuals, while Bacteroidetes are more prevalent in lean ones. However, meta-analyses of human studies have not consistently shown this association.

Several factors influence the composition of the microbiota, with diet being the most significant.

Hypotheses in this field are derived from experiments based on germ-free mice. Germ-free mice are born and reared without exposure to any live microbes. These mice can subsequently be colonized with selected microbial species or "fecal transplants" from mice or humans to test whether this colonization leads to measurable physiological or pathological effects. Germ-free mice are protected from obesity and metabolic syndrome, but adiposity is substantially increased when they are colonized with conventional microbiota. Mice colonized with microbiota from obese mice or obese humans, containing, e. g., the archaeon *Methanobrevibacter smithii*, gain more weight than mice colonized from lean subjects. How is this possible? Microbiota may promote adiposity in four ways:

1. By increased energy harvest from food. In principle, we are familiar with this concept: think of ruminating cattle. A cow by itself is no more able to digest grass than we are, but it gets help from bacteria and archaea in its specialized digestive tract. Non-digestible carbohydrate components of grass, like cellulose or xylans, are fermented in its compartmentalized stomach with the help of microbes, enabling the cow to use a large part of the energy contained. In mice or humans, this process is far less efficient, as most microbes inhabit the colon, which is located downstream of the small intestines where energy-absorption takes place. Yet, short-chain fatty acids (acetate, propionate, butyrate) produced by fermentation of otherwise indigestible carbohydrates may be taken up even in the colon, enhancing energy extraction from food.
2. By directly adjusting physiological parameters of the host organism. Apart from being taken up as a form of energy, certain short-chain fatty acids activate G protein-coupled receptors Gpr41 and Gpr43. Activation of Gpr41 results in changes in secretion of PYY (described

below), activation of Gpr41 in changes in secretion of glucagon-like peptide 1 by intestinal L cells. A second example: gut epithelial cells of germ-free mice produce considerable amounts of fasting-induced adipose factor (fiap or ANGPTL4). Fiaf, a glycoprotein, inhibits lipoprotein lipase (LPL), which is responsible for the uptake of fatty acids from chylomicrons and VLDL into adipose tissue. More fiaf therefore means less adiposity. Colonization of germ-free mice with conventional microbiota suppresses expression of fiaf in intestinal cells, causing mice to grow fatter. Yet, if germ-free mice are specifically colonized with *Lactobacillus paracasei*, fiaf levels remain elevated.

3. By instigating inflammatory reactivity in the host. Interactions between genetic host factors, diet and microbiota may increase gut permeability. With higher permeability, traces of microbiota-derived lipopolysaccharides and microbial DNA reach the liver via the portal vein, activating Toll-like receptors 4 and 9, respectively. This pro-inflammatory effect may promote visceral adiposity, non-alcoholic fatty liver disease and insulin resistance.
4. By influencing food preferences generated in the CNS through vagal afferents triggered by microbial molecules.

To what extent these findings apply to humans is being investigated. Long-known phenomena, such as the healthful effects of dietary fiber, may be understood by their impact on the microbiota. For example, in patients with diabetes mellitus type 2, a defined fiber-rich diet promoted microbiota species that caused increased glucagon-like peptide production and decreased HbA1c levels. In any case, when thinking about our propensity to accumulate weight, we have to consider not only our genetic programming, but also our microbiota and the microbiota-selecting properties of our diet.

While the time for evolutionary adaptation was short since leaving Africa, changes in selective pressure have left their mark in some populations. We have indications that at the time food shortage reversed into its opposite for the first time, populations suffered massive bouts of morbidity. In parts of Europe, this started during Roman times, when progress in agriculture and well-organized transport systems led to massive improvements in the average food situation. Probably, many people died from diabetes mellitus and related conditions at a young age. In these societies, counter selection happened **against** thrifty alleles. Today, problems due to obesity and diabetes mellitus type 2 are most pronounced in those populations who encounter caloric plenty for the first time.

In principle, losing weight is straightforward: take in fewer calories than you spend. Yet, we are hardwired to hate the condition of negative energy balance. Soon, energy saving mechanisms kick in that make us passive and grumpy, let us freeze, let our thoughts constantly revolve around food and make us prone to shamelessly lie to ourselves. Therefore, few people are able to lose weight by just eating less. Successfully losing weight usually requires sweeping adaptations in our daily behavioral and social routines, including diet and drinking habits as well as the level of our physical activity. Aerobic exercise counters the energy saving mode.

Rather for our society's obsession with looks than for real health concerns, losing weight is high on our list of priorities. Yet, far too frequently, people are enticed to pay good money for bad advice or products of questionable value. In the following, I therefore provide a (free!) evidence-based list of recommendations on how to lose weight in a sustainable way. Many aspects are explained in more detail further down these lecture notes.



## How to lose weight sustainably

Above all, **body weight is a result of energy balance. Losing weight simply means taking in fewer calories than you spend.** There's no diet, no trick, no magic around that.

### The input side:

1. Resolve to eat a little less than you need for a long time. Most people are ill-served by rigorous short-term diets. They punish themselves for a few weeks, longing for the day it's finally over and setting themselves up to yo-yo back.
2. Hold yourself accountable. A long-term, realistic plan with achievable goals helps to attain them: losing one pound (0.5 kg) per month is feasible and supports the necessary changes in lifestyle habits.
3. Accept being a little hungry part of the day. As long as you take up calories from a meal, insulin predominates and parts of these calories are converted to fat. Once the process reverses, sympathetic activation and glucagon predominate, and you get hungry again. Hunger is the signal to Palaeolithic WoMan to go foraging again: first the exercise, then the next meal. As long as you are hungry, you burn fat. People who claim you can lose weight without being hungry usually are after your money.
4. Breakfast calories are more likely to be consumed, dinner calories are more likely to be stored: distribute daily calories in favor of breakfast! It's not necessary to skip a meal, but if you want to do that anyway, it's best to skip dinner. In order to prevent insulin – which inhibits lipolysis – from being constantly active, don't snack between meals and don't eat anything overnight (e.g., 8/16 interval fasting).
5. Drink water and tea. Forgo soft drinks and cut back on alcohol. Both contain plenty of "empty calories". Soft drinks contain lots of carbohydrates: fructose, glucose, sucrose. Alcohol is metabolized to acetyl-CoA and reductive equivalents: the building blocks for fatty acid synthesis. By drinking alcohol, we synthesize fat instead of burning it.
6. Cook-it-yourself: this gives you more control over what you eat.
7. Serve smaller portions, use smaller plates to counter the portion-size effect.
8. Eat unprocessed foods: fruit, vegetables, legumes, salads, lean meat, fish. Processed foods tend to be more energy-dense, i.e., contain too much fat and simple sugars. Plus, they frequently contain too much salt, trans-fats and other ingredients you are not aware of.
9. Eat protein-rich foods. By stimulating PYY, a protein-rich meal helps to delay the return of hunger compared to isocaloric alternatives. Protein-rich foods include lean meat, fish, and eggs. Vegetarians can resort to tofu, seeds (pumpkin, sunflower) and nuts.
10. Prefer low glycemic index foods. This matches well with the previous two points: unprocessed and protein rich foods tend to have a lower glycemic index. Cut down on sweets, sugar, chocolate, pastries. For breakfast, replace processed cereals or white bread with whole-grain muesli containing fresh fruits.

### The spending side:

11. Increase your daily energy spending: walk or cycle to work instead of driving. Take the stairs, not the elevator. Use a pedometer and try to increase your average daily step count.
12. Exercise not only consumes energy immediately, it also increases the basal metabolic rate for many hours afterwards (excess post-exercise oxygen consumption). 150 minutes per

week are recommended, intensive minutes count double and have a stronger afterburn effect. Vigorous walking, jogging, cycling, swimming, cross-country-skiing, dancing – anything will do.

13. Keep room temperature and clothing on the cool side. Let your body spend more energy on its own heating via adaptive thermogenesis by brown adipose tissue and muscle.

In order to lose weight, eating (too) little is far more important than doing a lot of exercise: you can't outrun a bad diet. Careful investigation of the energy balance (using the gold standard doubly labeled water method) of the hunter-gatherer Hadza population in Tanzania led to an unexpected finding: These people, who move a lot every day in search of food, consume only a little more calories than us desk people. If many calories are needed for exercise, the body obviously finds savings elsewhere. We know this from the positive health effects of sports: if we exercise regularly, our average heart rate drops, our average blood pressure drops; all of that saves energy.

Intuitively, our idea of losing weight looks something like this: for a certain period, we eat less until we reach our weight goal. Then, we return to eating "normally", meaning about as much as before. Unfortunately, this concept is mistaken, for two reasons:

1. Our body's energy expenditure is a function of its mass. If you have trouble believing this, try hauling around a twenty pound backpack for a few days. After shedding pounds successfully, our body requires fewer calories. Example: A 22 year old female, 5' 5", 154 lbs (BMI 25.7), decides to lose weight. After an arduous struggle and many setbacks, she loses 22 pounds. Her "new" 132 lbs-body requires only 2170 kcal per day, while her "old" 154 lbs-body was able to consume 2340 kcal without adding weight. From this alone, she needs to consume 170 kcal less than before to prevent regaining weight.
2. Yet, actual daily caloric allowance is even lower. Our body "memorizes" our elevated initial weight and tries to claw its way back up. Once a new maximum weight has been reached for a longer period of time, it is stored as the new target value, which the organism defends tooth and nail. In persons who had lost more than 20 pounds and who succeeded in keeping most of it off for at least a year it was found that their body still interpreted the lower weight as something of a deficit: satiety hormones leptin and PYY stayed reduced, hunger hormone ghrelin stayed high and the test persons reported an elevated sense of hunger. Careful measurements showed that people had lower total energy expenditure under these conditions than comparable control persons who had not lost weight. This is partly because their muscles are more energy-efficient in everyday life, as they tend to gravitate toward sparse Type I fibers (slow-twitch, myosin heavy chain I) and away from glycolytic Type II fibers (fast twitch, myosin heavy chain IIa and IIx).

In our example, at 132 lbs the young woman's body would consume 2170 kcal only if she had never exceeded that weight. Yet, her "post-154 lbs" 132 lbs-body remains in energy conservation mode, consuming less. So far, this additional reduction in energy expenditure has been quantified in few probands, and individual values varied widely. Therefore, it is not possible to pinpoint any exact number in our example, but it seems reasonable to assume a further reduction between 100 and 400 kcal. For how long? Unfortunately, we do not know that yet. For the few individuals assessed in this regard, the energy-saving mode was still being active six years later. It is difficult to find suitable subjects for such studies, since many people manage to lose weight temporarily, but very few succeed in maintaining the reduced weight for years. Those who manage to do so are usually very active in sports, as exercise is able to counteract the energy-saving mode to a certain extent.

Altogether, this puts the young woman's actual steady-state daily caloric requirement somewhere between 1770 and 2070. In other words: if she wants to keep those pounds off, she needs to consume 270-570 kcal or about half a meal less than what she would consider "normal", in the face of an increased sensation of hunger. Now we understand why it's so difficult to lose weight and keep it off. In conclusion, it is a lot easier to prevent overweight than to correct it. Preventing obesity and smoking are unspectacular and unrewarding (the "prevention paradox"), but are among the most effective medical tasks.

### **"Detox?", "Cleansing?"**

When these words are used, it usually means that we should buy a service or product, usually in connection with fasting. Incidentally, fasting itself is not expensive and can be very useful in a moderate form, e.g. as intermittent fasting.

During fasting, sugar, fats and amino acids, although constantly required, are no longer absorbed. We will consider sugar and fat later, in the liver section. But how do our cells deal with the lack of amino acids? Cellular proteins have different half-lives, and we need to resynthesize most of them continuously to keep cells functioning.

We all passed our first serious fasting period long ago, as newborns. With the cutting of the umbilical cord, we not only ran out of oxygen, which forced us to take painful first breaths. Gone was the effortless delivery of nutrients. Unfortunately, it took a few days for our mom's breast milk to come in. For us that meant: fasting and shrinking! How did we make ends meet? The secret is autophagy. In order to continue synthesizing proteins, our cells melted down large gulps of cytosolic protein soup and digested it into amino acids. Technically, this works by forming double-membrane balloons around areas of cytosol, which then fuse with lysosomes, recycling the proteins into amino acids. It was an emergency measure, but it kept our cells alive and with that, our organism. It took a week or two for us to get back to our birth weight, a milestone our mothers noted with satisfaction.

Even when we fast as adults, we trigger this non-selective autophagy. If important amino acids become scarce in the cell, this slows down the mTOR (mechanistic target of rapamycin) complex 1. This activates autophagy and in turn slows down many cellular functions. In many species, probably also in humans, lifelong austerity regarding energy prolongs lifespan. Whether and if so, to what extent this has anything to do with non-selective autophagy remains unclear for the time being.

In contrast to non-selective autophagy, there is also selective autophagy, which serves to break down bacteria that have invaded the cytosol or cell organelles that are no longer needed. This process is not initiated by fasting, but by the appearance of particulate matter that should be discarded and that is recognized by cytosolic receptors. Selective autophagy would be most comparable to detoxification, but we cannot control it from the outside.

In short: If detox is advertised to you, better reduce your food intake privately. That saves you a lot of money.

### 3. HIGH INTAKE OF SALT

Our genetic makeup prepares us better to cope with a lack of salt than with a glut of salt, like it prepares us better for a restriction in calories than for an abundance of them. While we have an extremely efficient sodium saving system in the form of the aldosterone system, our mechanisms to get rid of superfluous sodium are less refined, as sodium overflow did not exist during evolution of modern *Homo sapiens*. While evolution primed us to crave salt, we now have cheap salt within reach all the time. Together, these two conditions cause general overconsumption of sodium. With our unsophisticated elimination system, more sodium leads to an increase in fluid volume for osmotic reasons. At the margin, increased fluid volume in the closed system of our extracellular space results in increased pressure. Only with this elevation in blood pressure, we are able to excrete more sodium, as renal excretion is proportional to tubular flow rate (pressure natriuresis). Thus, our high intake of salt promotes hypertension. The [DASH eating plan](#) (Dietary Approaches to Stop Hypertension) addresses this issue and generally constitutes a useful guide to healthy nutrition.

### 4. MALNUTRITION

#### **Malnutrition due to Eating Disorders: Anorexia and Bulimia Nervosa**

"Ab crack," "bikini bridge," "hip cleavage," "ribcage bragging," "six-pack," "thigh brow," "thigh gap," to "Toblerone tunnel" – If you're familiar with all these terms, you may have taken a wrong turn onto 'Instagram' territory – which in this case might actually be better described as 'Instagrief' territory, as it may lead to distorted body perceptions. Others, incredulous, tap their heads: 'Tik-Tock, Tik-Tock' in disbelief. Social media, especially among young individuals still seeking their identity, amplifies the pressure to conform to fabricated, fantastical ideals. Eating disorders most commonly affect young women, but they are also seen in other age groups and men. A key characteristic is a body image disturbance – an altered perception of one's own body. While outsiders see emaciated underweight individuals, the sufferers perceive themselves as "too fat." This highlights once again how our CNS constructs our individual "world" and interprets sensory perceptions using this model. As long as the world models of people interacting overlap significantly, this discrepancy remains unnoticed. It becomes more baffling when the divergence is too large, as in the case of anorexia nervosa.

Physical consequences are severe to life-threatening. The metabolism shifts into an extreme energy-saving mode. Those affected constantly feel cold, partly due to low heat production and partly due to the lack of insulating subcutaneous fat. Blood pressure and heart rate are reduced, often accompanied by QT prolongation, increasing the risk of cardiac arrhythmias. Hypokalemia, triggered by abuse of laxatives and diuretics, and alkalosis due to vomiting, heightens the risk of rhythm disturbances and, along with fluid loss, kidney failure. The bone marrow, lacking materials, produces too few blood cells: anemia, leukopenia, thrombocytopenia. Blood tests also reveal hypoglycemia and other electrolyte imbalances that further increase the risk of heart arrhythmias. Osteoporosis due to protein deficiency raises the risk of fractures. Thyroid and sex hormones are diminished, leading to amenorrhea in women and loss of potency in men. Hair becomes thin, brittle, and falls out; bulimia destroys teeth through acid exposure. Therapeutic efforts must target the distorted world model and body image disturbance.

## Malnutrition Due to Resource Scarcity

In painful contrast to the opulence experienced in part of the world (approximately 400 million people suffer from Type 2 diabetes, and over 1 billion are affected by obesity), hunger is one of the most pervasive medical problems. About 800 million people are malnourished. According to the Food and Agriculture Organization of the United Nations (FAO), every five seconds, a child starves to death.

A large part of this hunger is man-made. This is true for its increase by climate change, but also for the economic system we use to produce and distribute food.

[Three examples:

1. For budgeting reasons, both the French wheat farmer and the owner of a bakery chain in Austria have an interest in securing their wheat price six months ahead. In principle, they could mutually agree to a shipment at a certain point in time at a certain price. A contract of this type is termed commodity future.

In real life, neither farmer nor baker has the time to look for a partner. They rely on an intermediary. What actually happens looks more like this: the farmer pays a certain amount to a broker for the right to sell his wheat until a certain date at a predetermined price. We call that a put option. Likewise, the bakery owner pays the broker for the right to buy an amount of wheat until a certain date at a predetermined price: he buys a call option. An option is a contract involving the trade of a right. The paying partner acquires the right, the partner who gets the money incurs the obligation to fulfill the underlying transaction. The price of the option is a kind of insurance premium to secure a wheat price in the future. As the term option implies, the holder has no obligation to do the deal: if the market price of wheat at harvest is higher than the option strike price, the farmer is free to sell to the highest bidder. Unexercised options expire worthlessly, with the seller keeping the premium. Rights and obligations may be traded, leading to an options exchange. Hedge funds, banks, insurance companies with not the least direct interest in wheat buy and sell these options if they expect to make a profit. A second type of exchange-tradable commodity forward transactions is futures. In contrast to options, a futures contract obligates both parties to fulfill the deal.

Most participants in commodity options and futures markets are neither producers nor end users. They are only interested in profit margins and will capitalize on any opportunity to raise them. For example, if the Russian government bans wheat exports in response to a harvest-threatening drought, well-connected market participants will notice this fact a little earlier than the Austrian baker or other end users. In the relatively small group of market participants from large finance companies, there is no need for explicit arrangements, it's clear to everybody how to profit from the situation: from this moment on, there will be considerable restraint regarding commitments to ship wheat. If an end user insists on securing a shipment, he has to pay up. In other words, the call option premium goes up in addition to an increase in the strike price. While a tightening of the wheat market is an entirely negative event from the perspective of food supply, it allows some market participants to make large profits. The expected crop shortfall in Russia by itself would have made wheat more expensive, but the options trade additionally amplifies price increases. Until expiration of the call options he owns, the Austrian baker is protected from price increases, but the next call he buys will be considerably more expensive. When the price of bread goes up again, we consumers are irritated. Yet, for the more than 1.2

billion people who have to survive on less than €1 per day, the resulting price increases quickly become life-threatening.

Is there any solution? It might be beneficial to restrict access to food commodity exchanges. Legal regulation might grant access to food commodity trading for producers, end users, and, as far as necessary, brokers, but prevent speculation in agricultural commodities detached from actual use. Regulations along these lines have not even been attempted so far and would probably have to be enacted against determined lobbying efforts by the financial services industry.

2. Subsidies for agriculture in wealthy countries: Europe subsidizes its agriculture using public funds. This is necessary to stabilize and secure local food production, ensuring Europe does not become overly dependent on imports. Import dependence becomes dangerous when international crises or supply chain issues interrupt external supplies. Moreover, the agriculturally shaped landscape is also part of European cultural identity. However, a side effect of subsidization in good times is an excess of agricultural products, which are then sold at prices that undercut local prices in countries like those in Africa: The average African farmer cannot produce nearly as efficiently to begin with and also receives no subsidies. If they cannot sell their produce due to competition from cheaper imports, they have no choice but to reduce or cease production. Should the imports fail or become more expensive, local production cannot be quickly ramped up again.

3. Another factor tending to raise food prices is the production of biofuel. E5 designates gasoline containing 5% ethanol, which is produced by fermenting wheat, corn or sugar beets. While it is possible to use, e. g., sugar cane from wetlands instead, preventing direct competition for agricultural land, today's actual share of wheat and corn in global biofuel production is high.

Has any of this any relation to medicine? If people die from it unnecessarily: yes!]

As a result of poverty, people survive on very restricted diets, essentially maize or rice. While this may satisfy caloric requirements, it fails to supply many essential nutrients, over time inescapably leading to illness and, potentially, death.

Given a reliable source of carbohydrates, our organism is able to produce energy. It is also able to resynthesize other complex carbohydrates and most lipids. The problems start with the proteins. Of the twenty amino acids, we lack the ability to synthesize eight –children even more-, making it necessary to take them up from outside. In maize or rice, several of these are present in inadequate amounts. Essentially, our proteins are chains of amino acids: one missing link is enough to disrupt the chain. In the extremely restricted diets of the poor in the developing world, the limiting amino acid is usually lysine, followed by tryptophan.

### **Kwashiorkor**

As soon as one amino acid becomes limiting, the organism cannot produce enough protein molecules. This becomes visible first for those proteins that have to be produced in high copy numbers: muscle proteins and albumin. Albumin is necessary to maintain oncotic pressure and a lack of albumin results in pedal edema and pot belly by ascites. In a seeming paradox, the liver tends to be enlarged by fatty infiltrates, as lipids fail to be shipped to the periphery for lack of apoproteins required to assemble VLDL. Thinning hair of reduced pigmentation and

dermatitis are further typical signs. Affected children and adults are apathetic and highly susceptible to infections, as an effective immune response depends on the production of antibodies and cells. The term "Kwashiorkor" originates from the Ghanese Ga language, meaning "the sickness the baby gets when the new baby comes". As the older child is weaned from the mother's breast, the balanced amino acid composition of breast milk is replaced by the lopsided one of high-starch crops like maize. Of course, these restricted diets invariably entail additional nutritional deficiencies, regarding, e. g., iron, vitamin B12 and niacin, and tend to contain high levels of contaminating aflatoxin, all of which contribute to the disease.

## 5. CENTRAL REGULATION OF APPETITE

Appetite and eating behavior are regulated in the hypothalamus, with much of the activity concentrated in the arcuate nucleus. Here, information on energy supply converges via afferent neuronal and hormonal (e. g., leptin, ghrelin) signals.

### **Portion size effect**

Food intake increases with portion size. Give the average person a large plate with more than she/he can eat, and she will eat a given amount. Give the same person an even larger portion, and she will eat more than the first time around. Although we are far from understanding complex brain functions like this, the portion size effect is probably part of the program to increase reserves during times of plenty.

**Practical implications:** when trying to lose or to maintain weight, use smaller portions/plates. Avoid those bathtub-size popcorn bowls.

### **Leptin**

Leptin (from the Greek word *leptos*=thin) acts as an input to the central nervous system reflecting the prevailing food situation. In response, the CNS reacts with meaningful adaptations of eating behavior, reproductive functions and bone metabolism (*anorexia nervosa*, for example, combines decreased leptin levels, amenorrhea and osteoporosis).

The signal protein leptin is almost exclusively secreted by adipocytes (an *adipokine*). Its long term plasma level is proportional to the size of an individual's fat storage. Around this level, leptin levels oscillate diurnally dependent on food intake, with a minimum before breakfast and a maximum late in the evening. Longer phases of the day without food intake ("post-absorption phases", e.g. in the second half of the night) are associated with a drop in leptin: if liver glycogen decreases, the associated drop in plasma glucose and insulin leads to a reduction in leptin levels. The CNS reacts to this with a feeling of hunger and an activation of the "stress axis" CRH-ACTH-cortisol. Elevated cortisol combined with low insulin activates gluconeogenesis, stabilizing blood sugar levels. If the period without food intake lasts longer than a day ("hunger phase" or fasting, although the feeling of hunger then disappears), liver glycogen is depleted, leptin levels are halved and the body switches into an energy saving mode, which we will discuss later when we deal with the liver.

Leptin and other signaling molecules affect neurons in the arcuate nucleus in the hypothalamus, adjacent to the floor of the 3rd ventricle. This is possible because the arcuate nucleus is adjacent to the median eminence, which is one of the circumventricular organs positioned outside the blood-brain barrier. Decreased leptin levels lead to a strong feeling of hunger, leptin levels in the normal range to satisfaction ("satiety") with increased willingness to be active and to spend energy. Further increases of leptin levels by obesity remain without significant additional effect. For some time, leptin was hoped to be the answer to the obesity epidemic. Indeed, a very small percentage of obese people have leptin deficiency. However, the overwhelming majority of obese individuals were found to be leptin resistant, much like type 2 diabetics are insulin resistant, meaning high leptin concentrations do not reduce their appetite.

### **"Hunger" is generated in the hypothalamus**

The sensation of satiety is generated by a combination of signals. Central cooperation between leptin and insulin plays an important role. Both leptin and insulin stimulate "anorexigenic" (appetite-reducing) POMC-expressing neurons in the arcuate nucleus. These neurons release a POMC (pro-opiomelanocortin) cleavage product,  $\alpha$ -MSH ( $\alpha$ -melanocyte stimulating hormone), at their synapses.  $\alpha$ -MSH stimulates certain melanocortin receptors (MC4R) on "satiety" neurons, resulting in increased metabolic rates and diminished food intake. About 4% of patients with severe early-onset obesity have mutations in melanocortin receptors. POMC neurons also synthesize CART (protein encoded by cocaine-amphetamine related transcript), which also promotes satiety and activity. Incidentally, POMC neurons are also stimulated by nicotine, explaining the lower average body mass index of smokers as well as their tendency to gain weight after quitting.

**Pharmacology cross reference:** For some time, drugs from the amphetamine family were broadly used as appetite suppressants. In Europe, this practice was discontinued when it became clear that their dependency-promoting properties and other unwanted effects could not be dissociated. Drugs from the amphetamine family work by increasing serotonin concentrations in the synaptic cleft. POMC neurons are activated via serotonin 5-HT<sub>2C</sub> receptors. The simultaneous activation of 5-HT<sub>2B</sub> receptor by some of these drugs, however, promoted fibrosis of heart valves and pulmonary artery wall, and repeatedly led to valve problems and pulmonary hypertension. The 5-HT<sub>2C</sub> agonist lorcaserin was approved in the US until 2020, but not in the EU, to aid weight loss. Likewise, the combination of phentermine (amphetamine derivative)/topiramate (antiepileptic) is approved in the USA, but not in the EU. Only the combination **bupropion/naltrexone** is approved in the EU, which showed a weight loss of only 3-5% compared to placebo. Bupropion from the amphetamine group as a single substance is indicated to stop smoking and to treat severe depression, while naltrexone, an opioid receptor antagonist, is used to prevent relapse from former opioid and alcohol dependence. Naltrexone inhibits  $\beta$ -endorphin-mediated autoinhibition of POMC neurons. Side effects of both drugs include appetite suppression, which has been declared the main effect in the combination product. Very common side effects include nausea, vomiting, constipation, and abdominal pain, which makes appetite suppression seem plausible. In clinical testing, 24% of subjects discontinued treatment due to an adverse event.

In contrast, leptin inhibits a second group of neurons in the arcuate nucleus that release Agouti-related peptide (AgRP) and neuropeptide Y (NPY). These counteract the POMC-expressing



neurons. AgRP acts antagonistic at the MC4R. The AgRP "hunger neurons" promote hunger, food intake, and build-up of fat deposits while slowing the readiness for activity and thermogenesis in brown adipose tissue. A simple, but impressive experiment to personally experience your AgRP neurons in full swing: don't eat anything for 24 hours and observe yourself: torpid, freezing, ill-humoured.

But then the reward: a good meal! In addition to leptin, the prospect of imminent food intake induces the CNS to dampen the activity of AgRP neurons. The CNS is an organ to project the future: sitting in front of steamy plates, our satisfaction already starts to increase and hunger begins to fade; we do not have to suffer from nagging hunger until leptin and insulin finally rise after eating.

What is hunger? Hunger is an initially undirected feeling of displeasure generated by AgRP neurons. We try to avoid this unpleasant mental state and learn early in life which behaviors – food intake – silence the AgRP neurons. Babies and small children show their feeling of displeasure very directly. They can not help themselves yet, but have no difficulty triggering the necessary behavior in their parents.

Sensory influences (the steaming plate) reduce the activity of the AgRP, but a second phase of early attenuation is caused by signals from the intestine. This probably has the following background: if we were to eat until glucose, leptin, and insulin are high enough to shut down AgRP neurons, we would overshoot the target, as the gut at that point in time contains a large amount of food still awaiting absorption. So, early on, the gut reports an estimate about the amount of food that can be expected to be absorbed soon; and would you please be so kind to lift that hungry feeling right now? This occurs via signal peptides such as PYY and GLP-1, which report on the one hand via afferent neurons of the autonomic nervous system (vagal nerve to *nucleus tractus solitarii*) and on the other hand directly to the CNS.

### **Peptide YY (PYY)**

PYY is released by mucosal neuroendocrine L cells in the distal gut in response to intake of food. PYY levels increase within 15 minutes, long before nutrients have reached the L cells, and remain elevated for up to six hours. Comparing isocaloric meals high in either carbohydrates or protein or fat, respectively, meals high in protein lead to the highest plasma levels of PYY. In addition to peripheral effects, like reducing gastrointestinal motility and secretion, PYY promotes satiety. It specifically activates Y2-receptors on hypothalamic neurons, again resulting in activation of the anorectic POMC-expressing neurons in the arcuate nucleus. In addition to PYY, cholecystokinin chips in to promote postprandial satiety.

**Practical implications:** A protein-rich meal helps to delay the return of hunger, making it easier to stick to a diet.

On the one hand, protein is needed every day; on the other hand, it cannot be stored. Protein intake could therefore be a useful parameter for regulating food intake. The "**protein leverage hypothesis**" postulates that you eat until you have absorbed enough protein. So, according to this hypothesis, if you eat food rich in carbohydrates and fat, you will continue to have an appetite until you reach the required amount of protein.

## Glucagon-like peptide-1 (GLP-1)

A second hormone released by intestinal L-cells is GLP-1. This 31-amino acid peptide has a plasma half-life of just 2 minutes, as it is rapidly cleaved by the protease DPP4 (Dipeptidyl Peptidase 4). DPP4 is expressed on most cells as a transmembrane protein CD26, but it is also secreted in a soluble form. It cleaves dipeptides (X-Proline or X-Alanine) from the N-terminus of polypeptides. GLP-1 enhances glucose-dependent insulin secretion by  $\beta$ -cells in the pancreas, increases  $\beta$ -cell mass, and inhibits glucagon release. It delays gastric emptying and enhances the feeling of satiety centrally; patients report that their pangs of hunger are absent and that food no longer dominates their thoughts as before. The neurons targeted by GLP-1 are not only located in the hypothalamus but also in other brain regions, including the *area postrema* (a circumventricular organ at the caudal end of the fourth ventricle, dorsal to the *nucleus tractus solitarius*).

### Pharmacology cross reference:

Two classes of diabetes drugs are used to increase GLP-1-like activity:

- GLP-1 agonists with longer half-lives, such as liraglutide, semaglutide
- DPP4 inhibitors like sitagliptin

Because they target the same pathway, it does not make sense to combine them.

In addition, **liraglutide** and the even more effective semaglutide are approved to support weight loss in obesity.

**Semaglutide** led to a 17% weight loss after just over a year when injected subcutaneously once a week. As a lifestyle drug among moderately overweight individuals eager to lose weight, it sparked such demand that it became scarce for its intended patients. Moreover, it introduced new first-world problems like "Ozempic face": wrinkles and sagging cheeks due to diminished 'filler', making one appear older. Tragically, this leads to a need for another round of facelifts. Semaglutide is identical to GLP-1 in 29 of 31 amino acids. A replacement of alanine at the second position prevents cleavage by DPP4 and the addition of a fatty acid increases albumin binding. GLP-1 agonists must be gradually introduced to avoid severe nausea and vomiting. Two dosages have been introduced: the lower dosage, Ozempic, targets Type 2 diabetics. At a higher dosage, Wegovy is approved for aiding weight loss. Semaglutide must be carefully titrated up due to frequent unpleasant gastrointestinal side effects: at the highest dosage, nausea in 44%, vomiting in 24%, diarrhea in 30%, abdominal pain in 20%, and constipation in 20%. GLP-1 agonists are a goldmine for manufacturers: they require continuous medication, otherwise, the weight returns. As peptides, the -glutides must be injected, right? In principle, yes, but surprisingly there is also an oral version: in a higher dosage with a special galenic formulation, Semaglutide in pill form—Rybelsus—is approved for Type 2 diabetes mellitus. It is combined with a lipid-like substance abbreviated as SNAC. Both are transported transcytotically through the stomach mucosa in vesicles right from the stomach. However, absorption is not very efficient. Therefore, this formulation requires about ten times as much Semaglutide as the injectable forms, which is counterproductive given the current high demand for Semaglutide.

An elegant approach would be to activate the GLP-1 receptor with an artificial small agonist. **Orforglipron**, a non-peptide activator of the GLP-1 receptor in development, can be taken

orally and achieved a weight reduction of about 15% after 36 weeks in a Phase 2 study. The pill for obesity is thus slowly rolling our way.

**Tirzepatide** (Mounjaro) is a dual receptor agonist ("twincretin"), a non-natural "fusion peptide" that activates both the GLP-1 receptor and the GIP (glucose-dependent insulintropic peptide) receptor. GIP is released from K cells of the duodenum after food intake. It enhances insulin secretion and has numerous other effects, including in the central nervous system (CNS) and in bones. Tirzepatide, 10 or 15 mg once weekly subcutaneously, led to a weight loss of about 20% after 72 weeks; it also reduced blood pressure starting from the first half-year of the medication, thus likely independent of the weight loss.

If dual receptor agonism yields such good results, why not take this approach a step further? The triple receptor agonist **Retatrutid** activates GLP-1, GIP, and glucagon receptors ("triple G-agonist") and is in advanced clinical trials. Activation of the glucagon receptor might seem counterproductive at first: glucagon, which helps us during periods we have nothing to eat, raises blood sugar. However, glucagon also increases lipolysis, which is highly desirable in obesity. In a Phase 2 study, there was a weight loss of up to 24% compared to 2% for placebo. Particularly attractive is the observation that liver fat decreased in a high percentage of those treated.

Unfortunately, weight loss during therapy with GLP-1 agonists results not only in a reduction of fat but also, to a lesser extent, in a decrease in muscle mass. Therefore, it is all the more important for individuals to change their lifestyle and counteract this undesired effect through physical training. Acute pancreatitis, ileus and gallstone formation occur more frequently with GLP-1 agonists than with placebo.

## **Ghrelin**

Ghrelin is considered the main counter player of leptin. It is produced during fasting by distinct cells within the mucosal layer of the stomach, especially in the fundus, and in the pancreas. Sleeve gastrectomy, a bariatric surgery procedure, removes a large part of the gastric fundus, thereby strongly reducing ghrelin production.

Ghrelin might sound like the name of a malevolent hunger sprite, but more prosaically, it is simply an acronym for "growth hormone release-inducing," as it also plays a role in the release of growth hormone. It is a peptide composed of 28 amino acids. Its receptor is expressed by central neurons in the arcuate nucleus as well as by peripheral afferent vagus neurons. Ghrelin increases feelings of hunger and appetite and is suppressed by physical exercise. In summary, ghrelin contributes to both an increase in body weight and growth in height.

## 6. DIGESTION AND ABSORPTION OF CARBOHYDRATES

Simple sugars and refined sugars (sucrose) in the form of chocolate, biscuits, confectionery etc. have great appeal to most of us. Supermarkets increase their sales by positioning them by the shelf where we queue up in front of the checkout. Sweets stimulate our CNS reward center. This was a useful mechanism for our ancestors in Palaeolithic Africa. They seldom encountered anything sweet. Occasionally, they robbed a wild beehive. Seasonally, they may have indulged

in berries. Think of grapes: They contain half glucose and half fructose, which need not be digested at all, only absorbed. They trigger an enormous insulin response and are therefore ideal for building up fat stores for bad times. Today, in contrast, in our situation of constant plentiful availability, our penchant for sugar leads to obesity, metabolic syndrome and DM2.

The majority of natural carbohydrates are ingested in the form of starch. Starch consists of layered, water-insoluble granules with an average of 20-30% amylose and 70-80% amylopectin. Cereal grains contain about 75% starch by weight, rice 90%, potatoes, which contain a lot of water, 15%.

Amylose consists of linear chains of  $\alpha$ -1,4-glycosidically linked glucose units – 1000-2000 in cereals, 2000-4500 in potatoes.

Basically, amylopectin has the same structure as our glycogen:  $\alpha$ -1,4-glycosidically linked glucose units plus  $\alpha$ -1,6-glycosidic branches. However, an amylopectin macromolecule is much larger than a glycogen molecule and about 100 times the size of amylose. Like the spokes in a folded umbrella, the chains lie close together, forming a semi-crystalline structure.

Due to the water insolubility of starch granules, we have a hard time digesting them: the  $\alpha$ -amylase can only nibble a little on the surface of the particle, the starch granule behaves like roughage. This is the case, for example, when we eat raw food. Therefore, it was a brilliant idea of a particularly intelligent ancestor, whose name has unfortunately been forgotten, to make use of fire and an equally brilliant idea from WilMa Firestone (WMF) to develop the cooking pot. When we heat the raw food in water to make stew, we break open the starch granules and make their glucose chains accessible to  $\alpha$ -amylase. Using this trick, we get a multiple of the nutritional value from the same plants. Unfortunately, to some extent, this process is reversible. If the hydrogenated starch is left cool for a certain period of time, the semi-crystalline structure reforms. We call this "retrogradation". Retrogradation makes our bread stale: it loses water and becomes harder. This happens particularly quickly around 0°C, which is why baked goods should never be stored in the refrigerator. We can delay this hardening effect by using baking additives: fats, emulsifiers and enzymes. Again, amylases are particularly useful, breaking down starch before the baking process, and lipases, which split fatty acids from fats and thus produce emulsifiers again.

How well we can digest starch depends on how the food is prepared. Starch parts that cannot be digested in the upper small intestine are called resistant starch (RS):

RS1 – starch physically locked away, as in unprocessed grains

RS2 – starch grains structurally not accessible to amylase, as in raw food

RS3 – starch no longer accessible to amylase due to retrogradation

RS4 – starch degradation products that are secondarily cross-linked such as resistant dextrins

RS5 – starches complexed with lipids

The fact that part of the starch we ingest may be resistant to digestion does not mean it is useless, not even in terms of its energy content. Resistant starch reaches the large intestine, serving as food for many bacteria, which, for example, metabolize it to short-chain fatty acids such as butyrate. Short chain fatty acids are absorbed in the large intestine; in addition, they directly nourish our enterocytes there.

Digestion of amylose and amylopectin starts with the enzyme  $\alpha$ -amylase, which is present in saliva and pancreatic secretion. In between, in the stomach, carbohydrate digestion is paused,

as  $\alpha$ -amylase is inactivated by gastric acid.  $\alpha$ -Amylase is an endoenzyme able to break internal  $\alpha$ -1,4-glycosidic linkages between glucose units, but not terminal ones or linkages adjacent to  $\alpha$ -1,6-linkages. Therefore,  $\alpha$ -amylase digestion does not result in monosaccharides, but rather in packs of two (maltose) or three glucose units (maltotriose) and  $\alpha$ -limit dextrins, containing  $\alpha$ -1,6-branch points and adjacent linkages.

Carbohydrate digestion is completed by three brush border enzymes: maltase (glucoamylase), lactase and sucrase-isomaltase. Maltase cleaves maltose, maltotriose and longer 1,4-linked glucose polymers. Lactase breaks the lactose disaccharide into its galactose and glucose monosaccharide units. Sucrase-isomaltase in fact consists of two enzymes directly attached to each other. The sucrase moiety splits the sucrose disaccharide (the sugar from the sugar bowl) into its glucose and fructose units. Only the isomaltase moiety is able to break the  $\alpha$ -1,6-glycosidic bonds of limit dextrins; it is also able to cleave  $\alpha$ -1,4-linkages.

Finally, the resulting monosaccharides are absorbed into enterocytes with the help of transporters. Glucose import is driven by a  $\text{Na}^+$  electrochemical gradient. One glucose unit and two sodium ions are cotransported by the  $\text{Na}^+$ -coupled glucose transporter (SGLT1). Thus, moderate dehydration should be treated with a combination of electrolytes and glucose or carbohydrates, as the added glucose increases the rate of  $\text{Na}^+$  absorption. SGLT1 is able to ferry glucose as well as galactose into the enterocyte, but not fructose, which forms a five-membered ring. Fructose is absorbed via GLUT5. At the basolateral side, all three monosaccharides exit the cell via GLUT2.

## **Glycemic index**

Eventually, the majority of all food carbohydrates appear in the blood in the form of glucose. Depending on food composition, the rise in plasma glucose concentration may be fast, producing a spike, or more protracted. To express this property in the form of a simple number, the glycemic index was created. The glycemic index (GI) of a food is defined as the area under the curve of plasma glucose concentration over two hours in response to an amount of the food containing 50g carbohydrates, in relation to that of a pure 50g glucose meal, times 100. Thus, glucose has a glycemic index of 100; "high glycemic index foods" like white bread, potatoes, most white rice varieties, most processed breakfast cereals are around 70 or higher; "low glycemic index foods" like whole grains, pure dairy products or most fruits or vegetables are around 55 or less. (Caution: in some, mostly US, tables, a standard of white bread instead of glucose is used, resulting in different values). GI is attributed great importance in many popular diet systems, including the Glyx diet, South Beach Diet, etc. Is that warranted?

Intake of high glycemic index foods causes a postprandial spike in glucose concentration, followed by a commensurate spike in insulin secretion. During the following hours, this promotes carbohydrate oxidation at the expense of fat oxidation, while surplus glucose is converted to fat. In controlled experiments in animals, e. g. in rats, high-GI-fed animals developed more body fat and insulin resistance compared with control animals fed a low-GI diet of otherwise identical composition, including identical total carbohydrate content.

There is considerable disagreement in the literature as to the extent to which this concept applies to humans. Experiments in humans cannot be done with the same rigor. Necessarily, changes in GI influence fiber content, texture, sugar content and therefore palatability of foods. We all tend to eat more of a food if it tastes good. At its core, obesity is foremost the result of a positive

caloric balance; if at all, the glycemic index plays a role secondary to that. Still, in the absence of definitive evidence, to be on the safe side, it seems like a good idea to emphasize low-GI foods in nutrition.

**Practical implications:** For breakfast, consider replacing white bread with jam or industrial cereals with whole-grain muesli containing yoghurt, fresh fruits and nuts.

### **Lactose intolerance**

Lactase is one of the disaccharidases located in the brush border of the enterocyte. It breaks the lactose disaccharide into glucose and galactose. While the enzyme is expressed at high levels in babies, expression levels decline with age. Therefore, adults worldwide tend to suffer from lactose intolerance. Exceptions are descendants of populations who traditionally relied on dairy farming, like in northern Europe. Here, nutrition exerted a selective force over thousands of years, favoring those individuals who were able to maintain lactase expression longer than others. This ability is based on a single SNP 14,000 bases upstream of the lactase gene: the position is normally occupied by a C, while in descendants of northern European dairy farmers a T creates a binding site for the transcription factor Oct1. This addition of an upstream transcription factor sustains persistent expression of the lactase gene. In other words, lactose "intolerance" is the norm, lactase persistence the exception. This explains why lactose "intolerance" is the norm in people of African or Asian descent, but rarely occurs in Scandinavia. Within Europe, lactose intolerance increases from north to south; In the German-speaking countries about 15% are affected, in the Mediterranean countries, percentages are much higher.

Disaccharides cannot be taken up by enterocytes. Due to the osmotic effect of uncleaved lactose, as well as by secondary changes in bacterial colonization, affected individuals suffer from flatulence and diarrhea in response to intake of lactose-containing food (milk, ice cream, cheese, chocolate,...). As colonic bacteria produce  $H_2$  when metabolizing lactose, breath  $H_2$  in response to an oral dose of lactose may be measured to test for lactase deficiency.

### **Fructose-malabsorption**

The capacity of fructose transporter GLUT5 is limited and varies between individuals. If fructose intake exceeds absorptive capacity, remaining intestinal fructose causes diarrhea and flatulence by osmotic effects and bacterial metabolism. Foods rich in fructose include the sugar in our sugar bowl, fruit, honey and corn syrup-containing industrial foods. A high percentage of people with European roots suffer from fructose malabsorption. Fructose malabsorption is not to be confused with the rare *hereditary fructose intolerance* (HFI). The enzyme missing in HFI, aldolase B, is required to break fructose into two three-carbon-molecules in the liver.

### **Fructose controversy**

The fructose content of the Western diet is high, due to high uptake of sucrose, the use of high fructose corn syrup in industrial-manufactured food and the use of fructose in soft drinks. Following absorption, fructose is quantitatively extracted and metabolized by the liver. This

requires a lot of ATP, which is hydrolyzed to AMP, part of which is degraded to uric acid. High fructose consumption seems to moderately increase the risk of gout. Following metabolism in the liver, part of the fructose load is released as glucose and lactate, part used for lipogenesis, increasing the risk of non-alcoholic fatty liver disease. In rodents, high fructose feeding causes insulin resistance and there is a long controversy to what extent that may also be true for the actual levels of fructose consumption in humans.

A meal leading to an increase in blood glucose causes pancreatic beta cells to secrete insulin. A comparable load of fructose releases far less insulin: due to "fructose-vacuating" by the liver, the low plasma levels of fructose (<0.5 mM, compared to 5.5 mM = 100 mg/dl for glucose) allow only small amounts to enter beta cells via GLUT2. Recall that insulin, together with leptin, acts as a satiating signal in the hypothalamus. A person consuming fructose is therefore likely to feel less satiated than a person consuming an isocaloric amount of glucose. Indeed, food craving was shown to be stronger in the same individuals after ingestion of a fructose-containing than after a glucose-containing soft drink. Fructose may therefore accentuate our more general problem of overfeeding with simple sugars resulting in weight gain, lipotoxicity and insulin resistance.

### **Acrylamide**

Acrylamide is formed during roasting or baking starchy foods such as chips, biscuits, french fries or coffee. It is absorbed in the intestines. In the liver, with the help of CYP2E1, glycidamide is formed, which forms DNA adducts and thus has a mutagenic effect. Whether the levels of acrylamide in the diet are critical is controversial. In controversial cases, we may usually assume that any potential effect will at least not be very large.

## **7. IRON**

Iron is a limiting resource for many biological systems. Humans and animals need iron to handle oxygen, in hemoglobin and myoglobin as well as in cytochrome enzymes. About 2 billion people, more than a quarter of the world population, are iron deficient; about 500 million suffer from manifest iron deficiency anemia. Iron deficiency without anemia can lead to non-specific CNS symptoms (fatigue, lack of concentration, headaches, restless legs syndrome, other sleep disorders, delays in mental and motor development in children), skin symptoms (angular cheilitis, hair loss) or palpitations. Fungi and bacteria need iron as well, and go to great lengths to obtain it. One way our body limits infections is by depriving these microorganisms of iron.

Dietary iron is primarily absorbed in the duodenum. It comes in two forms: still integrated in heme or free. The majority of heme iron stems from myoglobin in red meat. It is absorbed *en bloc* with the heme group by a special transport mechanism, a process which is more efficient than absorption of free iron. Heme iron is only released within the cell. Intestinal nonheme iron may be ferric ( $\text{Fe}^{3+}$ ) or ferrous ( $\text{Fe}^{2+}$ ).  $\text{Fe}^{3+}$  is not soluble at pH values above 3; it forms stable complexes with many anions and is not readily absorbed.  $\text{Fe}^{2+}$  is soluble up to pH 8; it is cotransported with protons by the divalent metal transporter (DMT1). Nutritional aspects: 15%-35% of heme iron is absorbed, largely independent of the composition of the diet. In contrast, only 5-12% of non-heme iron is absorbed, and this absorption is frequently further reduced by dietary factors. Iron-binding phytates, e.g. in cereals, tannins, e.g. in green tea, black tea and coffee, or the alkaline pH of dairy products reduce this percentage. For this reason, vegans and

vegetarians are more likely to become iron deficient, especially in situations of increased need such as in children, pregnant women, breastfeeding women and women with heavy menstrual bleeding. What to eat for plant-based iron? – Legumes (soybeans, lentils, chickpeas), oilseeds (pumpkin seeds, sunflower seeds), nuts (almonds, walnuts), spinach (yes, don't mention it, still true), black molasses in a time window separate from tea or coffee. Lemon juice (ascorbic acid, acid) improves absorption, proton pump inhibitors massively worsen it.

**Zinc insert:** Phytic acid, which is generally found in plant foods, but is particularly abundant in cereals, not only binds iron but also zinc, thus interfering with absorption of both metals. From the outset, plant food contains less zinc than animal-based food. This makes zinc a potentially critical trace element in a purely plant-based diet. Zinc is necessary for the correct conformation of many proteins (e.g. zinc fingers in steroid hormone receptors) and for the enzymatic function of most metalloproteases (e.g. collagenase). Zinc deficiency can lead to growth disorders and developmental delays in children, poorer resistance to infections, anorexia, psychiatric disorders, dermatoses, brittle nails, hair loss and reduced male fertility.

**Iron** is exported from the duodenal enterocyte into the blood by the solute carrier ferroportin, an iron exporter expressed by all cells.  $\text{Fe}^{2+}$  is dangerous due to its ability to generate hydroxyl radicals. On export, it is therefore oxidized to  $\text{Fe}^{3+}$  with the help of copper-containing hephaestin.

In the circulation,  $\text{Fe}^{3+}$  is then ferried around by transferrin. Blood plasma, too, contains a copper-containing enzyme able to convert  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ , ceruloplasmin.

If cellular iron levels are low, uptake from blood is increased via an elegant mechanism. The mRNA for the transferrin receptor contains several iron response elements (IRE) in its 3' untranslated region. IREs form characteristic stem-loop structures. At low cellular iron levels, these structures are bound by specialized proteins termed IRE binding proteins (IRE-BPs) or iron regulatory proteins (IRPs). By binding the stem-loop structures, the proteins protect the mRNA from being degraded by RNases, resulting in high expression levels of the transferrin receptor and high iron uptake. Once iron levels have been restored,  $\text{Fe}^{2+}$  binds to iron regulatory proteins, thereby modifying their structure in a way that they cannot bind to IREs anymore. This destabilizes the transferrin receptor mRNA, leading to lower expression and lower  $\text{Fe}^{2+}$  uptake from the circulation.

Iron response elements are also used the other way round: in the ferritin mRNA, a single element is present in the 5' untranslated region, in front of the translation initiation codon. At low intracellular  $\text{Fe}^{2+}$  levels, the IRE is bound by IRE-BP and translation is blocked; high  $\text{Fe}^{2+}$  levels remove the IRE-BP, allowing vigorous translation of the mRNA and high-level expression of this  $\text{Fe}^{2+}$  storage protein. Ferritin storage of excess iron occurs mainly in the liver. Although most of the ferritin is within cells, plasma levels, which are in equilibrium with intracellular stores, may be used to monitor iron reserves.

Combined, the mechanisms described up to this point would regulate intracellular iron levels, but would over time still result in iron overload by continuous enteric uptake. To prevent that, the liver is the starting point of an additional negative feedback loop. Extracellular iron levels are sensed by hepatocytes by a protein complex including the transferrin receptor and transmembrane protein HFE ("high Fe"). Adequate iron levels lead to induction and secretion of hepcidin, a 25 amino acid-peptide. Hepcidin causes ferroportin internalization and degradation, blocking iron export from all cells. So, if serum iron is adequate, ferroportin is



blocked in all cells, including duodenal enterocytes. As intracellular iron in duodenal cells accumulates, uptake from the intestinal lumen decreases.

As we have [already seen](#) in our occupation with the immune system, hepcidin is also induced by inflammation via a second regulatory mechanism. IL-6 and other inflammatory cytokines induce an acute phase response in the liver, which includes secretion of hepcidin. As we saw before, hepcidin blocks the export of iron from cells. In this case, the cells most affected are macrophages, which contain high levels of iron from erythrophagocytosis, the constant breakdown of aging erythrocytes. In addition, ferroportin expression in macrophages is down-regulated by inflammatory cytokines and directly by TLR4-activation. Together, these mechanisms sequester the iron in the reticuloendothelial system and deprive the infecting microorganisms from required iron. During an acute infection, this artificial "internal iron deficiency" does not result in negative consequences. However, in chronic infection, it may cause anemia in the face of abundant reserves of iron in the reticuloendothelial system. This iron, is not made available to erythroid progenitors. Similar mechanisms also lead to anemia in malignant diseases. The term "anemia of chronic disease" encompasses both causes.

Therefore, the serum concentrations of iron and transferrin saturation are reduced in both iron deficiency anemia and in anemia of chronic inflammation. Ferritin is low in iron deficiency anemia but normal or increased in anemia of chronic disease. However, since the two forms of anemia often occur together, the ferritin value is not a reliable diagnostic tool. If ferritin is low, iron deficiency is a fact. Pure iron deficiency anemia usually responds well to iron supplementation. If there is a combination of iron deficiency with anemia of chronic disease, supplementation does not help: in this case, oral iron is not absorbed and intravenously administered iron is stored in the liver macrophages, so that it is not available for erythropoiesis.

## **Hemochromatosis**

Iron overload has toxic effects. Overload may be the result of frequent transfusions or of certain forms of hemolytic anemia; alternatively, it may be the manifestation of a common inherited disorder, hemochromatosis. The majority of hemochromatosis patients carry mutations in their HFE ("high Fe") genes, typically Cys282Tyr. In the absence of normal HFE, hepcidin expression by hepatocytes is inadequate, resulting in unchecked iron uptake from the intestine. For many years, iron accumulates without causing symptoms. Once buffering mechanisms are overwhelmed, iron overload leads to cirrhosis of the liver, diabetes mellitus, arthritis and a bronze pigmentation of the skin. Via hepcidin, total body iron is normally regulated between 2 and 6 g. In hemochromatosis, this amount grows by 0.5-1 g per year, sometimes reaching a total of 50 g or more. In males, symptoms usually don't start until they reach their forties; females are not affected due to their monthly period. Therapy is mediocrally simple, but effective: phlebotomy until the iron overload is corrected. Originating in Northern Europe only 1200-1400 years ago, the C282Y mutation is surprisingly common in people with European roots: about 10% are heterozygous, 1 person in 200-400 homozygous. Even among male homozygotes, only a part develops clinical symptoms. The frequency of this recent mutation suggests some selective advantage. Affected women may have an easier time compensating their monthly losses of iron. Macrophages, which are iron-depleted in hemochromatosis, in this state may be better able to kill off infecting intracellular bacteria such as *Mycobacterium tuberculosis* or *Salmonella typhimurium*.

## 8. FOLATE

Folate is present in many vegetables, e. g., in spinach, lettuce, broccoli and beans, in liver and liver products and, as aficionados never fail to emphasize, in beer. In its tetrahydrofolate (THF) form, it is essential for nucleotide biosynthesis (purines and thymidine) and with that, for all rapidly proliferating cells. Furthermore, it is required for additional 1-carbon transfers in many biosynthetic pathways. A typical example is the synthesis of methionine from homocysteine. homocysteine is toxic; its concentration is kept low by this complex reaction with the help of THF and B12. Folate deficiency is the most common cause of hyperhomocysteinemia and therefore a cardiovascular and neurodegenerative risk factor. Depending on the specific reaction, the coenzyme is active in one of several forms: for example, synthesis of dTMP from dUMP requires 5,10-methylene-THF, while methionine synthesis is dependent on N<sup>5</sup>-methyl-THF.

From natural sources, folate is taken up mainly in polyglutamate form. In the duodenum, the conjugated glutamates are taken off one by one by a brush border peptidase, except for the last one. Folate is then absorbed by exchange against a hydroxyl ion (OH<sup>-</sup>). It is then transported to the liver, where it is reduced first to dihydrofolate (DHF) and then to its active form THF by the enzyme dihydrofolate reductase (DHFR). From there, it is "loaded" with a 1-carbon group from the side chain of serine.

Folate deficiency results in problems in all rapidly proliferating tissues. Clinical symptoms usually develop in the form of anemia. As the number of erythrocytes produced is too low, in a compensatory mechanism they are at least stuffed with as much hemoglobin as possible, resulting in megaloblastic anemia. However, their increased size makes it harder for them to squeeze through the meshwork of the reticuloendothelial system, expediting their break down in the spleen.

Cell proliferation is especially high during the embryonic and fetal periods, and folate deficiency may lead to neural tube defects like *spina bifida*. Therefore, pregnant women are routinely substituted with folate. As neural tube defects originate very early in pregnancy, it would be advisable for women to take folate even before conception.

## 9. VITAMIN B12

One of the most complex "small" molecules of our body is vitamin B12, or cobalamin. So far, we know of only two reactions for which B12 is required: the synthesis of methionine and the "debranching" of methylmalonate, an intermediate product in the breakdown of odd-numbered fatty acids and amino acids. In the synthesis of methionine, B12 cooperates with N<sup>5</sup>-methyl-THF, from which it accepts the methyl group that is then transferred to the sulfur atom of homocystein. Vitamin B12 is synthesized only by certain bacteria, yet required by an enormous food chain, ranging from many other bacteria to all animals. As it is not present in vegetables of fruit, we take it up in the form of animal products: meat, fish, eggs and, to a limited extent, dairy products. B12 deficiency is therefore the main problem of vegan nutrition, ahead of iron deficiency and others. It is not a problem if you take B12 produced with the help of certain bacteria as a dietary supplement. The molecule is too complicated to produce it by chemical synthesis.

Vitamin B12 is absorbed in the ileum only and has to be protected from digestion until it arrives there. In the acidic environment of the stomach, it is bound by haptocorrin (or "R-protein"), produced by salivary and gastric glands. In addition to acid, parietal cells produce intrinsic factor (IF), a glycoprotein which initially cannot bind B12 at the acidic pH in the stomach. In the duodenum, haptocorrin is digested by pancreatic proteases, and IF takes over. The B12:IF complex is highly resistant to the digestive onslaught in duodenum and jejunum. On arrival in the ileum, the complex is specifically bound and taken up into the enterocyte by receptor-dependent endocytosis. The process absolutely requires IF; free cobalamin is neither bound nor absorbed. In the enterocyte, the complex dissociates, and B12 is taken over by the protein transcobalamin, which is also instrumental in exocytosis and transport to the periphery and to the liver. A large pool of B12 is constantly subjected to enterohepatic circulation: excreted with bile and reabsorbed in the ileum.

### **Pernicious anemia**

A problem in any one of the many systems required for its absorption leads to vitamin B12 deficiency. A strictly vegetarian or vegan diet contains inadequate amounts of vitamin B12. Another frequent cause of deficiency is an autoimmune reaction against parietal cells, which produce acid and intrinsic factor. The liver stores one or two years' worth of vitamin B12, meaning symptoms of deficiency develop very gradually. The symptoms are those of folate deficiency. Lack of vitamin B12 blocks all of the body's folate reserves in the N<sup>5</sup>-methyl-THF form, leading to a secondary shortage of 5,10-methylene-THF (the reaction from 5,10-methylene-THF to N<sup>5</sup>-methyl-THF is irreversible; THF can only be regenerated if the methyl group is taken over by B12 to synthesize methionine). Yet, 5,10-methylene-THF is absolutely required to produce dTMP. Another deficient derivative, N<sup>10</sup>-formyl-THF, is required for *de novo* synthesis of purines. Hence, B12 deficiency results in the same megaloblastic anemia as folate deficiency.

Additional symptoms are a distinctive glossitis and neurological problems, starting with peripheral polyneuropathy. Still later, the CNS is affected with weakness, ataxia, memory impairment and depression. Weakness and ataxia are the result of demyelination of the dorsal columns of the spinal cord a syndrome termed funicular myelosis. The pathophysiological mechanism has not been clarified in detail here; elevated homocysteine levels may be a contributing factor.

## **10. SELENIUM**

Let us take a look at Selenium as just one example of important micronutrients. Selenium is in the same group of the periodic table as oxygen and sulfur, meaning it is quite reactive and eager to take over electrons. At the same time, its readiness to give electrons back is higher than that of halogens. Selenium is essential for vital redox reactions, like the detoxification of H<sub>2</sub>O<sub>2</sub> by glutathione peroxidase, and for the activation and inactivation of thyroid hormone by deiodination. It is inserted into proteins in the form of a special rare amino acid, selenocystein, which carries a selenium atom at the position of the usual sulfur (or oxygen in the parallel structure of serine). In the genetic code, there is no dedicated codon for selenocystein. Instead, one of the three stop codons, UGA, can be interpreted as a selenocystein codon with the help of a specific translation factor. This factor binds to a signal loop structure in the 3'-UTR of the mRNA encoding the enzyme in question.

In most places, there is no need to worry about selenium. However, low levels of natural selenium in the ground and thus in food are found in certain defined regions, notably in China. There, selenium deficiency may lead to Keshan disease, which is characterized by congestive cardiomyopathy that may be fatal.

## 11. FATTY ACIDS

Digestion and absorption of fats is covered below. Here, we will only consider the nutritional debate on fatty acids.

Depending on their source, fats contain a range of different fatty acids. **Saturated fatty acids**, incorporated in triglycerides, form the majority of our own body fat, and the same is true for pigs, cattle and sheep. Thus, meat and dairy products from these animals contain mainly saturated fats. Examples are palmitic acid (16:0) and stearic acid (18:0; the first number indicates the number of C atoms, the second number those of the double bonds). Palm oil, the least expensive dietary fat, contains about 40% palmitic acid and 40% monounsaturated oleic acid. **Unsaturated fatty acids** contain at least one double bond. Our body is able to synthesize monounsaturated fatty acids, like oleic acid (18:1), but not polyunsaturated fatty acids (PUFA). PUFA are synthesized by plants, and we have to take up at least two of them, linoleic acid (18:2) and  $\alpha$ -linolenic acid (18:3), from which we can synthesize the others. These two are therefore called essential fatty acids. PUFA are particularly found in certain vegetable oils. Oily fish are rich in long-chain n-3 PUFAs, which are originally synthesized in marine algae and accumulated via the food chain.

Docosahexaenoic acid is important for brain and eye development and function; it is concentrated in the phospholipids of cell membranes in the brain and retina. DHA accounts for 15-20% of all fatty acids in the frontal cortex. Since vegans do not eat fish, DHA is a potentially critical nutrient for them, especially for pregnant and breastfeeding women and their children. One way out is to obtain DHA at the source, before the fish, from microalgae cultures and take it in in the form of microalgae oil. Mice fed a diet lacking in long-chain n-3 PUFAs display increased signs of anxiety. There is also evidence in humans that a diet low in n-3 PUFAs during pregnancy is correlated with reduced emotional stability in the early childhood years.

There is no doubt that the mixture of fatty acids we consume with our diet affects our health. Most of what we know comes from nutritional studies, where the human body is treated as a black box: nutritional input is correlated with output in the form of health parameters of cohorts or large populations. The molecular mechanisms behind these correlations remain largely in the dark. Numerous hypotheses have been put forward but remain insufficiently tested.

Discussions on fatty acids have centered on two topics: n-3/n-6 and trans fatty acids.

### **n-3/n-6 polyunsaturated fatty acids**

Depending on the position of the double bond nearest to the methyl end of the carbon chain, the majority of polyunsaturated fatty acids fall into one of two main groups:

- n-3 (sometimes referred to as omega-3) PUFA, like  $\alpha$ -linolenic acid (18:3), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6). Oily fish is rich in n-3 fatty acids. The only oilseed that contains predominantly  $\alpha$ -linolenic acid is linseed or common flax, which has been cultivated in Europe since the Neolithic period. Linseed oil oxidizes very easily, hardens soon and was therefore used as the last, preserving varnish layer for oil paintings (hence the word *vernissage*).
- n-6 PUFA, like linoleic acid (18:2) and arachidonic acid (20:4). A number of vegetable oils contain predominantly linoleic acid: sunflower oil, corn oil, soybean oil, safflower oil and walnut oil.

Greenland Inuit were found to have very low levels of coronary heart disease despite a fat-rich diet derived almost exclusively from animal sources. As their intake of long-chain n-3 PUFA from fish was very high, a causal link was proposed. Cohort studies and randomized clinical intervention studies using fish oil supplementation have led to contradictory results, but the weight of the data seem to support a decrease in coronary heart disease with higher intake of n-3 PUFA. The mechanism remains unclear. On the other hand, positive effects on arteriosclerosis were also described for n-6 PUFA. Two aspects are probably important:

1. that we absorb sufficient PUFA in relation to saturated fatty acids
2. that the PUFA are in a favorable ratio n-3: n-6. A ratio of at least 1:5 is recommended; in our western diet, however, the actual ratio is around 1:15.

### Trans fatty acids

Unsaturated fatty acids exist in either of two configurations: cis or trans. Cis means, the carbon chains sit at the same side of the bond axis, producing a kink in the fatty acid. This kink makes a bunch of cis-acids awkward to stack (they literally "don't stack up"), while rod-straight saturated acids and nearly-straight trans-unsaturated acids are stacked easily. In other words: saturated and trans-unsaturated fatty acids, and the lipids containing them, stick together and have relatively high melting points, whereas cis-acids have a low melting point. For example, C-18 saturated stearic acid has a melting point of 69°C, mono-unsaturated trans acid elaidic acid one of 45°C, while the respective cis-acid, oleic acid, has a melting point of just 17°C. Thus, at body temperature of 37°C, complex lipids containing cis-unsaturated fatty acids tend to be more fluid than their saturated or trans-unsaturated counterparts, which tend to stick together. Lower fluidity, however, cannot explain the observed negative effects of trans fatty acids, since our organism contains plenty of saturated fatty acids which are even less fluid. By their kink, trans fatty acids have been proposed to block enzymes, interfering with metabolization of other fatty acids. While there is yet no agreement on exact mechanisms, trans fats are associated with increased risk for arteriosclerosis, coronary heart disease and stroke. In addition, trans-fats have been reported to raise "bad" LDL cholesterol and to lower "good" HDL cholesterol.

So, we'd like more cis-fats and less trans-fats. Where do they come from, anyway?

**Cis fats** represent the bulk of vegetable fats, which, due to their low melting point, form liquid oils after extraction. Examples are olive oil (75% oleic acid, 10% linoleic acid, 15% saturated), canola oil (60% oleic acid, 20% linoleic acid, 10%  $\alpha$ -linolenic acid, 7% saturated, up to 4% trans fats through the refining process), corn oil (50% linoleic acid, 30% oleic acid, 15% saturated), soybean oil (55% linoleic acid, 25 oleic acid, 10% saturated) and sunflower oil (65%

linoleic acid, 20% oleic acid, 10% saturated). A cultivar to reduce linoleic acid, high oleic acid sunflower oil (80% oleic acid, 10% linoleic acid, 10% saturated) approaches the composition of olive oil. Linseed oil is the only vegetable oil that mainly contains n-3 fatty acids (63%  $\alpha$ -linolenic acid, 15% linoleic acid, 15% oleic acid, 7% saturated). It is particularly sensitive to oxygen, oxidizes easily and quickly becomes bitter. Tip: Buy in small quantities, use generously as a salad oil and use up quickly! Percentages given are only rough indications, as there is considerable batch-to-batch variability.

**Trans fats** occur naturally in ruminants such as cattle and sheep as a small percentage of their total fat. For example, butter (70% saturated, 20-25% oleic acid) may contain up to 4% of trans-fats. Therefore, pre-industrial humans consumed low amounts of trans-fats. This changed with industrial food production. While plant oil is cheap to produce, it oxidizes (turns rancid) more quickly, it is difficult to transport and you cannot spread it on your bread. So, ways to harden plant oil (i.e., increase its melting point) were developed, which relied on partial hydrogenation. The idea is to add hydrogen back to unsaturated fatty acids, converting part of them to saturated acids and reducing the number of double bonds in the rest. However, this process has the setback of switching a considerable percentage of fatty acids into the trans configuration. The process results in much larger amounts of trans fatty acids than occur naturally. In essence, we convert good dietary fats to bad ones. These partially hydrogenated fats rich in trans-fats displaced natural solid fats in many areas, especially in industrially produced baked goods, snacks, fast foods and restaurant staples.

For a long time, the adverse medical effects of trans fats remained underappreciated. Later, however, growing health awareness led to legislative changes in many countries, which greatly reduced the permissible proportion of trans fats in industrially produced food and in restaurant dishes. Oils with a higher proportion of polyunsaturated fatty acids should not be used for roasting and deep-frying, since, at the high temperatures reached, that would again lead to formation of trans-fatty acids and oxidation products. Particularly bad would be repeated heating. On the other hand, clarified butter, olive oil and high oleic sunflower oil are well suited for roasting and deep-frying. In boiling and stewing, there is no need to worry, as in these cases temperatures do not exceed 100°C.

To reduce cardiovascular disease, the WHO has recommended since 2018 that no more than 10% of the daily calorie requirement be in the form of saturated fats and no more than 1% in the form of trans fats. Large epidemiological studies have shown that substituting carbohydrate calories with fat calories leads to reduced mortality as long as unsaturated fats are involved. Polyunsaturated fatty acids reduce mortality more than monounsaturated fatty acids. However, substituting saturated fats for carbohydrates increases mortality, while substituting trans fats leads to a large increase in mortality.

## 12. FOOD PROTEINS; BEGINNING OF PROTEIN DIGESTION IN THE STOMACH

How do we meet our food protein needs? Foods of animal origin contain the "right" mix of amino acids. In addition to ethical and climate change concerns, however, this solution also has a nutritional snag: meat, dairy products, eggs, as we have just seen, contain the "wrong", namely predominantly saturated, fatty acids, as well as significant amounts of cholesterol.

Plant-based foods contain the "right" fats but the "wrong" mix of amino acids. We have already become aware of the relative lack of lysine in grains and rice; tryptophan is limiting in corn, legumes have a reduced content of sulfur-containing amino acids, etc.

The traditional, "Paleolithic" solution to this problem is to eat less meat and more vegetables. This is also recommended for children with their high need for balanced protein, iron, B12 and Ca<sup>2+</sup>.

If you want to completely avoid animal products, it's important to mix plant-based protein sources. Legumes (such as soybeans, beans, peas, lentils, chickpeas, and peanuts), oilseeds (like pumpkin seeds and sunflower seeds), and nuts (such as almonds, walnuts, and hazelnuts) contain relatively high amounts of protein. Soybeans/ edamame/ tofu are particularly well-suited as a base due to their relatively optimal amino acid profile. However, their low content of sulfur-containing amino acids (methionine and cysteine) must be compensated for with other plant sources such as grains. The strategy is quite simple: combine legumes with grains. Rice with beans is a staple meal that has proven effective in less affluent parts of the world.

Mothers who follow a vegan or vegetarian diet and wish to extend this to their babies should breastfeed their infants. This automatically ensures that the babies receive sufficient protein with the correct amino acid composition (and usually enough docosahexaenoic acid). If breastfeeding is not possible or desired, the only alternative is soy-based infant formula. This formula is fortified with methionine, as well as iron and zinc, because it contains phytic acid, which, as mentioned, binds iron and zinc. A small area of uncertainty arises from the fact that this infant formula contains soy isoflavones (genistein, daidzein, and glycitein), which act as phytoestrogens. Potential negative effects later in the lives of these children cannot yet be conclusively ruled out.

In psychological experiments involving interpersonal negotiations in role-playing games, it has been shown that tolerance/punishment readiness can be influenced by the composition of a previous meal, particularly its protein content. Tyrosine is a precursor of dopamine and noradrenaline, and tryptophan is a precursor of serotonin. A very hypothetical, probably oversimplified explanation for the observed "influence on free will" would therefore be the availability of these neurotransmitter precursors in the blood. Solving difficult problems over a shared meal is certainly a recommended approach.

Many people worry about not consuming enough protein and therefore turn to processed products like protein shakes or protein bars. This is entirely unnecessary and even counterproductive. The recommended daily allowance for adults is 0.8 grams per kilogram of body weight, which means for our standard 70 kg individual, 56 grams of protein per day. This recommended daily allowance takes into account the normal distribution of protein needs in the population: it determines the mean and adds twice the standard deviation. With this method, 97.5% of people consume MORE protein than they need at 0.8 g/kg body weight. That leaves 2.5%—primarily muscular, heavily built men—who may need a bit more, but not much more. Most forms of diet easily meet this protein requirement without ever having to think about it. If one consumes a reasonably varied diet sufficient in calories to maintain stable weight, one automatically consumes enough protein, whether vegan, flexitarian, or otherwise. In fact, most of us consume twice the recommended daily amount. Excess protein is not storable and does not lead to more muscle but is converted to fat. Additionally, the increase in nitrogen from metabolized amino acids burdens the kidneys. However, the protein requirement per kg

increases for elderly people who metabolize protein less efficiently. To maintain their muscle and bone mass, they need regular resistance training and 1.2-1.5 g of protein per kg of body weight.

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Digestion of food proteins is initiated by the joint action of acid and pepsinogen in the gastric "food mixer". In the stomach, little happens in the way of carbohydrate or fat digestion.

Proteins are pretty hard to "crack", as their folding protects them from protease attack. The first cut inside the chain of amino acids is the most difficult one. Once that has been made, the fragments tend to lose part of their protective folding structure and may be broken down more easily. Very high proton concentrations (stomach pH can fall to about 1) denature (unfold) parts of proteins, thereby enabling these critical first cuts. Proteases are categorized by the chemical group used to break a peptide bond: the main families are serine, cysteine, aspartic and metalloproteases. The ability of an endopeptidase to cleave a peptide bond depends on the amino acid sequence of the substrate protein. The stomach's main protease, pepsin, is an aspartate protease cutting next to nonpolar amino acids, especially bonds following phenylalanine. Due to these sequence limitations, a set of different proteases is required to degrade proteins efficiently.

The components of gastric juice are secreted by numerous mucosal glands, which contain different types of cells. Mucous neck cells produce protective mucus, chief cells secrete pepsinogen, (neuro)endocrine cells release signaling molecules. Antral G cells, e.g., produce gastrin, while D cell-produced somatostatin inhibits gastrin release. Stem cells and their early progeny, transit amplifying cells, maintain a cell population subject to rapid turnover. Acid is produced by parietal cells.

Upon stimulation, parietal cells are able to increase their secretory surface by a factor of 50-100 by fusing tubulovesicular membranes into their "canalicular" apical membrane. Acid is secreted by an  $H^+/K^+$ -ATPase pumping protons into the lumen in exchange for  $K^+$ . The  $K^+$  recycles back into the lumen via  $K^+$  channels, followed by  $Cl^-$ . To generate protons, the parietal cell takes up  $CO_2$  and  $H_2O$ , converting them to  $H^+$  and  $HCO_3^-$  with the help of carbonic anhydrase.  $HCO_3^-$  exits the cell across the basolateral membrane in exchange for  $Cl^-$ . The net result is secretion of HCl into the gastric lumen, a process that is activated by two types of stimuli: acetylcholine from vagal neurons and gastrin from antral G cells. These stimuli activate parietal cells both directly and indirectly, via activation of histamine-secreting neuroendocrine cells below the epithelium, so-called ECL (enterochromaffine-like) cells. Parietal cells receive the indirect signal via H2 histamine receptors.

**Pharmacology cross reference:** Proton pump inhibitors like omeprazole bind covalently to cysteine at the cytosolic part of the  $H^+/K^+$ -ATPase, inactivating it irreversibly. The effect is sustained until new pumps are resynthesized, which takes more than a day. Histamine H2-receptor antagonists like ranitidine, which inhibit acid production to a lower extent, have been largely replaced by proton pump inhibitors.

Pepsinogen is the product of chief cells. It is secreted in response to a range of neuronal and hormonal stimuli, most importantly acetylcholine from vagal neurons. Pepsinogen is activated by acid. At low pH, it spontaneously splits into an N-terminal peptide and active pepsin. Efficient spontaneous cleavage occurs only below pH3, followed by a positive feedback



mechanism in which activated pepsin cleaves more pepsinogen. Pepsin is an endopeptidase with a pH optimum between 1.8 and 3.5; above that range, pepsin is inactive. Pepsin digestion results in large peptide fragments, called peptones. Peptones in turn activate G cells in the stomach's antrum to secrete gastrin, as well as I cells in the duodenal and jejunal epithelium to secrete cholecystokinin.

To summarize the role of stomach acid: acid helps breaking peptide bonds; at very low pH, there is a steep increase in spontaneous hydrolysis. In addition, acid causes proteins to denature, making them accessible to proteases. Finally, stomach acid kills the majority of all bacteria, protecting us from infections. All these benefits stand in marked contrast to one major disadvantage: of course, hydrochloric acid would have the same aggressive effects on the epithelial lining of the stomach. Thus, to protect epithelial cells, a constantly renewed mucus gel layer of neutral pH is absolutely required.

Mucus-producing cells sit at the surface of the mucosa, as well as in the pit and at the neck of gastric glands. Mucus consists of long sulfated carbohydrate chains bound to tetramers of the glycoprotein mucin. Due to their strongly polar nature, these proteoglycans are always wrapped in a cloud of hydration. This semi-solid mucus gel layer is alkalinized by  $\text{HCO}_3^-$ , thus forming a neutralizing barrier which protects gastric epithelial cells. Rising like a moving stairway, the mucus gel layer is constantly renewed from below and digested from above.  $\text{HCO}_3^-$  produced by mucous cells remains captured in the gel layer meshwork.; active pepsin is excluded. The few pepsin molecules that make it into the meshwork are rapidly inactivated by the rising pH gradient. Production of  $\text{HCO}_3^-$  and mucus depend on locally produced prostaglandin E (PGE), which is synthesized via cyclooxygenase-1 (COX-1).

**Pharmacology cross reference:** In case we intend to administer drugs via the oral route, gastric acid is frequently a challenge regarding galenic formulation. Some pharmaceuticals would be inactivated by gastric acid; others might damage the gastric mucosa. One example is proton drug inhibitor omeprazole proper, which in the stomach needs to be protected from acid. One option is to coat tablets with an acid-resistant layer. However, that may cause the pills to remain in the stomach much longer than intended. After all, the stomach is a mixing machine with the task of keeping protein-rich food in an acidic environment for a long time. To prevent peristalsis from pressing out too much chymus prematurely, the pylorus contracts in a way that only particles of less than 2 mm diameter may pass. Paradoxically, larger particles are retained in the stomach until it is nearly empty. Only then, the pylorus slackens, allowing the last larger particles to pass into the duodenum. If the patient eats several times a day, that may cause a so-called monolithic gastro-resistant tablet to remain in the stomach for multiple hours, being released and starting its (or their, with a 3x1 drug regimen) intended pharmaceutical action only late during the night. The problem may be solved by MUPS (multi-unit pellet system), where gastro-resistant pellets with diameters below the critical 2 mm are consolidated into a larger tablet. In the stomach, the pill disintegrates into pellets which are able to pass the pylorus individually. Expensive!

### 13. GASTRITIS AND GASTRIC/DUODENAL ULCER

The hallmark of gastritis is superficial epithelial erosions due to a reduction of the protective barrier. A peptic ulcer, a deeper, localized lesion in the gastric or duodenal mucosa, has an extremely hard time to heal under the constant barrage of acid and pepsin. Until proton pump

inhibitors became available and before *Helicobacter*-infection was recognized as a curable cause of ulcers, frequently the only way out was to surgically resect the larger part of the stomach.

*Helicobacter pylori* is unusually resistant to acid. It preferentially colonizes the mucosa of the antrum, away from the fundus region where acidity is highest because parietal cells are most prominent. It moves towards the more neutral end of an acid gradient, uses proteases to digest its way into the mucus and attaches to gastric epithelial cells. By cleaving urea, it produces its own private  $\text{HCO}_3^-$ -buffer plus  $\text{NH}_4^+$ . Proteases as well as  $\text{NH}_4^+$  have toxic effects on adjacent epithelial cells. Only some strains of *H. pylori* are pathogenic: they contain a genome section termed cag-pathogenicity island (cag stands for cytotoxin-associated gene). Encoded genes enable the bacterium to inject peptidoglycan components into mucosa cells, where these are recognized by NOD-like receptors. The resultant inflammatory reaction breaks up the epithelial barrier, further increasing the bacterium's chances of survival. The changes lead to an increase in production of gastrin, boosting acid production overall. While only some of those infected develop symptoms, *H. pylori* causes the majority of gastric and duodenal ulcers, with NSAIDs a distant second at less than 20%. Ten to twenty percent of those infected develop an ulcer over their lifetime. Infection may be assessed via the carbon urea breath test, in which  $^{13}\text{C}$ - or  $^{14}\text{C}$ -labeled urea is broken down to radioactive  $\text{CO}_2$ . A drink containing urea is consumed, exhaled  $^{13}\text{C}$ - $\text{CO}_2$  is measured.

**Pharmacology cross reference:** Non-steroidal anti-inflammatory drugs (NSAIDs, like aspirin and ibuprofen) and glucocorticoids, the two drug families prescribed most frequently to combat inflammation, inhibit synthesis of prostaglandin E via different mechanisms. As an unwanted side effect, this also happens in gastrointestinal mucosa, reducing secretion of  $\text{HCO}_3^-$  and diminishing production of the mucus gel layer. As a result, formation of ulcers and gastrointestinal bleeding are among the dreaded complications of long term anti-inflammatory therapy. Proton pump inhibitors are used routinely to counter this effect. Unfortunately, as its name implies, gastric protection protects only the stomach, but not the intestines, while negative effects of NSAIDs affect the mucosa of the entire gastrointestinal tract. While COX-1 is constitutively expressed in all cell types, COX-2, which is induced in macrophages and other inflammatory cells, produces the majority of prostaglandins in inflammation. To reduce unwanted effects, COX-2 inhibitors were developed, which turned out to be only a partial success: COX-2 inhibitors don't upset the gastrointestinal tract as much yet were found to cause a higher rate of atherothrombotic complications.

#### 14. PROTEIN DIGESTION AND ABSORPTION

From the stomach, small batches of chyme (semiliquid food mass) are released into the duodenum by the pylorus (gatekeeper). The mixture is acidic and contains the products of pepsin digestion, peptones. Acidic pH stimulates S cells in the duodenal epithelium to produce secretin, which stimulates pancreatic duct cells to produce  $\text{HCO}_3^-$ . Peptones stimulate I-cells in the duodenal and jejunal epithelium to secrete cholecystikinin (CCK). CCK mediates satiety and induces the release of bile from the gallbladder and stimulates the exocrine pancreas. Together, secretin and CCK cause the release of the pancreatic cocktail of digestive enzymes in a considerable volume of alkaline secretion, neutralizing the chyme.

In the duodenum, protein digestion is continued by a series of pancreatic enzymes with optimal operating pH in the slightly alkaline range. Once more, these are secreted as inactive proenzymes: trypsinogen, chymotrypsinogen, proelastase, proprotease E and carboxypeptidases A and B. A brush border enzyme, enteropeptidase (enterokinase), cleaves trypsinogen into active trypsin and a terminal peptide. Trypsin proceeds to activate additional trypsinogen molecules as well as the other proteases and peptidases. Trypsin, a serine protease, breaks peptide bonds following positively charged amino acids lysine and arginine. Closely related chymotrypsin prefers bonds following phenylalanine, tyrosine, tryptophane and methionine. The two carboxypeptidases are exopeptidases, nibbling amino acids one by one from the C-terminus. Altogether, protein digestion results in single amino acids (30%) and small peptides of a few amino acids (70%).

The following two steps in protein digestion are accomplished by enterocytes via a number of brush border peptidases and cytosolic peptidases. Brush border exopeptidases further break down peptides, and carriers for tetra-, tri- and dipeptides (PepT1) as well as for single amino acids cotransport the material into the cell together with either protons or sodium. In the cytosol of the enterocyte, tetra-, tri- and dipeptides are broken down into single amino acids, which are then released into the portal blood at the basolateral side.

The mechanisms described above serve solely to take up amino acids. In addition, but only to a small extent, larger peptides and complex proteins are ferried across the epithelial barrier. This is the main responsibility of M cells, specialized cells in the intestinal epithelium covering Peyer's patches (please see [lecture notes on immunology](#)). This enables the immune system to survey the contents of the intestine.

## 15. PANCREATITIS

Pancreatic juice is, even more than gastric juice, a biochemical bomb. Normally, this bomb is secured by multiple safety mechanisms. Proteases are being produced as inactive precursors. Packaged in secretory granules, these are kept away from critical cell components. As an additional precaution, pancreatic trypsin inhibitor is co-packaged into the granules, buffering up to 10% of accidentally activated trypsin. The granule contents are kept at a low pH, at which pancreatic enzymes remain inactive.

In spite of all these precautions, occasionally, the enzymatic cascade is triggered inside the pancreas, promptly resulting in its self-digestion. The most frequent triggers are gallstones blocking the papilla of Vater and liberal consumption of alcohol. The chain of causation between these triggers and acute pancreatitis is incompletely understood. If gall backs up due to concrements, its components damage pancreatic cell membranes, increasing their permeability. Sometimes, a stone may form sort of a valve, letting in activated enzymes from the duodenal lumen. Intake of alcohol may stimulate secretion to such an extent that lipase from backed-up pancreatic juice starts to damage cell membranes. In contrast to proteases, lipase is already secreted in an active form.

Some genetic polymorphisms may predispose for pancreatitis, which thus may run in families. Pancreatic trypsinogen is the product of several distinct genes. By their charge, trypsinogen variants may be separated by electrophoresis. A specific mutation, Arg122His, of the main variant, cationic trypsinogen (Gene: PRSS1 for *protease, serine, 1*), makes activated trypsin

resistant to breakdown by other trypsin molecules. As a result, this form of trypsin stays active much longer, predisposing the individual for pancreatitis. As one mutated allele is sufficient, the predisposition is inherited as a dominant trait. Mutations in trypsin inhibitor (Gene: SPINK1 for *Serine Protease INhibitor, Kazal-type 1*) interfere with its ability to buffer autocatalytically activated trypsin. SPINK1 mutations are the basis of autosomal recessive familial pancreatitis.

Acute pancreatitis is a life-threatening disease. Once pancreatic self-digestion is triggered, the full cocktail of activated enzymes enters the circulation, activating inflammatory cells and damaging endothelial cells in distant organs. Potential outcomes include disseminated intravascular coagulation, hemolysis, shock, acute renal failure and acute respiratory distress syndrome (ARDS).

Chronic pancreatitis can, over time, lead to exocrine pancreatic insufficiency, resulting in malnutrition and symptoms such as abdominal pain, diarrhea, and fatty stools. If untreated, severe osteoporosis can develop. The elastase in pancreatic secretions is very stable compared to other proteases; a significant portion of it is excreted in the stool and can be measured for diagnostic purposes. A reduction in the amount of elastase excreted indicates exocrine pancreatic insufficiency. In such cases, pancreatic enzymes must be supplemented orally.

## 16. CYSTIC FIBROSIS

Cystic fibrosis is the most common life-threatening genetic disease in people of European origin, affecting about 1 in 2000. The autosomal recessive disease obtained its name from the destruction of pancreatic tissue, which originally resulted in severe malnutrition and early death due to the deficiency of pancreatic enzymes. Since the development of oral enzyme replacement, the major cause of morbidity is pulmonary disease. A thickened mucous layer inhibits ciliary clearance and forms an ideal substrate for infections with *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Cystic fibrosis is the result of mutations in the CF gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is required to produce the  $\text{HCO}_3^-$ -rich secretion of pancreatic fluid. While CFTR is a  $\text{Cl}^-$ -transporter at the apical membrane, ductal  $\text{Cl}^-$  is required for exchange with  $\text{HCO}_3^-$  present within the ductal cell. CFTR consists of two membrane-spanning domains with adjacent ATP-binding sites, separated by a large regulatory cytoplasmic loop containing multiple PKA and PKC phosphorylation sites. Secretin action at the ductal cell activates PKA via cAMP, and parasympathetic stimulation by acetylcholine activates PKC. Thus, digestive stimuli activate  $\text{HCO}_3^-$ -rich secretion by phosphorylation of CFTR.

One mutation,  $\Delta\text{F508}$ , causes about two thirds of all cases of CF. The deletion of phenylalanine 508 alters the structure of the protein in a way that it is broken down prematurely and does not reach the apical membrane. Many different other mutations, altogether more than 1000, each present in only a tiny fraction of patients, have been described to result in partial or total loss of CFTR function. CFTR is required in many epithelial cell types. Thus, its lack in CF patients may cause a host of medical problems, including meconium ileus in babies, sinusitis, infertility, diabetes and biliary cirrhosis.

**Pharmacology cross reference:** In patients with a homozygous  $\Delta F508$  mutation, the combination of Lumacaftor/Ivacaftor (tablet, Orkambi®) is able to relieve symptoms: Lumacaftor has chaperone activity, so that a small proportion of CFTR proteins fold correctly and reach the membrane, where Ivacaftor increases open probability.

## SOME NON-INFECTIOUS INTESTINAL DISEASES

### 17. CELIAC DISEASE

Celiac disease, also known as gluten-sensitive enteropathy or celiac sprue, is an enteropathy triggered by certain proteins in wheat, rye or barley in genetically predisposed individuals. Oats are tolerated by most patients but tend to be cross-contaminated with other grains. Maize and rice are no problem. With a prevalence of 0.5 to 1%, celiac disease is quite common in Caucasians, while it is less frequent in other populations. Remember, grains have only been an essential part of human nutrition for about 10,000 years. Grain protein nomenclature is somewhat mystifying to non-experts. To enable germination, grains contain carbohydrates (in the form of starch) and amino acids (in the form of storage proteins). Starch and some of the proteins are water-soluble. The mix of non-water-soluble proteins is summarily called gluten (from the Latin word *gluten*, for glue). Generally, the alcohol-soluble subfraction of gluten proteins is called prolamins, as they are rich in prolines and glutamines. Prolamins have different names in specific grains. In wheat, these prolamins are called gliadins, in barley, hordeins and in rye, secalins. With wheat our predominant grain, gliadins, which come in several forms ( $\alpha/\beta$ ,  $\gamma$  and  $\omega$ ), usually are the pathogenic agents of celiac disease.

As our digestive proteases do not cleave peptide bonds next to prolines and glutamines efficiently, gliadins are resistant to enzymatic degradation in the intestine. The digestive process thus results in typical peptides of e. g., 20 -35 amino acids, which in sensitive persons are able to activate immune mechanisms in two ways.

Gliadin peptides have direct effects on enterocytes. Only a minority of these mechanisms are known in detail. Some of the gliadin peptides have an affinity to chemokine receptor CXCR3 on the apical membrane of enterocytes. Activation of CXCR3 results in secretion of the protein zonulin (pre-haptoglobin 2), which makes tight junctions more permeable, allowing gliadin peptides to cross the epithelial barrier. In addition, CXCR3-expressing lymphocytes may be attracted.

In addition, gliadin peptides cause enterocytes to secrete IL-15 and to express MICA, a MHC class I-like protein induced by various forms of cell stress, on their cell membrane. IL-15 causes intraepithelial lymphocytes to express the MICA-receptor NKG2D. Via this non-adaptive mechanism, the lymphocytes proceed to kill the epithelial cells, making the intestinal wall even more permeable and over time reducing the numbers of mature epithelial cells (villous atrophy).

In a second mechanism, the ubiquitously expressed enzyme tissue transglutaminase (tTG) converts glutamine residues to glutamic acid, and the resulting negatively charged gliadin fragments are preferentially presented in specific MHC II molecules, variants of HLA-DQ2 (heterodimers from DQA1\*05 and DQB1\*02 alleles) or HLA-DQ8 (heterodimers from DQA1\*03 and DQB1\*0302 alleles). Note that these are very common alleles, so their presence

is in no way predictive of celiac disease, but other DQ-types seem unable to do the same job. Antigen-presenting cells (APC) presenting deamidated gliadin peptides on DQ activate naïve CD4-positive T cells. CD4<sup>+</sup> T cells mainly differentiate into TH1 cells, which produce IFN $\gamma$ . By activating macrophages and other cell types, this results in a chronic inflammatory state of the intestinal wall. As these mechanisms are cellular, they may be characterized as a [type IV hypersensitivity reaction](#).

These mechanisms result in loss of mature enterocytes, which can be only insufficiently compensated by increased proliferation of transit amplifying cells in crypts. Accordingly, the diagnostic duodenal or jejunal biopsy shows villous atrophy, crypt hyperplasia and an increase in intraepithelial lymphocytes.

Before biopsy, useful serologic tests include IgA antibodies against tissue transglutaminase and antibodies against deamidated gliadin. Antibodies are a byproduct, rather than a disease mechanism. Usually, tTG-specific T helper cells are not found, and should not be found, as tTG is a self-molecule. The antibodies are generated when B cells expressing a tTG-binding B cell receptor internalize a complex of tTG attached to a gliadin peptide. Presenting the gliadin peptide on MHC DQ, the B cell gets help from a gliadin-specific T cell to produce a tTG-specific autoantibody. In this case, T cells against one component of a complex of macromolecules help to produce an autoantibody against another component of that complex. We have already seen an analogous mechanism when [considering conjugate vaccines](#).

Celiac disease may start at any age and at any intensity, with many atypical presentations. The lack of mature enterocytes leads to nutritional deficits, e. g., regarding iron and vitamins. In a full-fledged form, symptoms may include abdominal distention, chronic diarrhea, weight loss or failure to thrive.

The only effective treatment is a life-long gluten-free diet.

Some people have neither celiac disease nor a classic allergy to wheat components, but still do not tolerate wheat-based foods well. In such cases, ATI (amylase trypsin inhibitors, gluten protein components) and FODMAPS (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) are discussed. The idea is that FODMAPS may be more difficult for some individuals to digest and/or ATI components of gluten delay the digestion process. In both cases, this would lead to increased feeding of intestinal bacteria with corresponding intestinal symptoms.

FODMAPs include high lactose and fructose content for previously discussed reasons, but also sorbitol, fructans, and galactooligosaccharides. Therefore, a relatively high content of FODMAPs is found in numerous everyday foods that most of us consume without problems. These include dairy products like milk, yogurt, ice cream, and soft cheese; grain-based products such as pasta and bread; and many legumes, including beans and lentils. Some vegetables – leeks, onions, garlic, cauliflower, asparagus – and some fruits – apples, pears, cherries, peaches, plums – also contain high levels of FODMAPs. Additionally, honey (fructose) and certain nuts, such as pistachios and cashews, are high in FODMAPs. Consequently, a low-FODMAP diet is very restrictive, nutritionally problematic, and generally considered as a last resort. Foods with low FODMAP content include eggs, hard cheese, unprocessed meat, fish, tofu, rice, other vegetables – potatoes, carrots, eggplants, bell peppers, cucumbers, lettuce – and other fruits – citrus fruits, kiwis, cantaloupes – as well as other nuts – peanuts, walnuts. If a low-FODMAP diet does not improve symptoms after 2-4 weeks, indicating that FODMAPs were not the cause,

it should be discontinued. If the diet does bring noticeable improvement, it is advisable not to stick strictly to it indefinitely but to gradually reintroduce other foods to achieve a personalized, tolerable dietary composition.

## 18. INFLAMMATORY BOWEL DISEASE

Inappropriate immune activation in the intestinal mucosa is the hallmark of inflammatory bowel disease (IBD), which comprises **Crohn's disease** and **ulcerative colitis**. While showing many similarities, the two diseases differ in affected sites and in the depth of penetration of the inflammatory process. Ulcerative colitis is limited to the colon and rectum, and affects only mucosa and submucosa; perforations are rare. Crohn's disease, in contrast, may affect any part of the gastrointestinal tract. Frequently, it involves several intestinal segments at the same time, e.g., terminal ileum and rectum, in an inflammatory process that is typically transmural.

Inflammatory bowel syndrome seems to involve an aberrant immune response against intestinal microbiota. In this sense, it is not an autoimmune disease, which would require a self-antigen. On the other hand, we could also regard our gut microbiota as part of our "greater self", as we are tolerized against it under normal circumstances. In IBD, something in this tolerization progress has gone wrong.

This seems to be facilitated by certain alleles of polymorphic loci. In many IBD patients, the epithelial barrier seems to be weakened:

1. In a connection that is not yet sufficiently understood, tight junctions between epithelial cells are more permeable in patients with specific NOD2 alleles. Recall that NOD2 is an intracellular pattern recognition receptor for PAMPs from bacterial cell walls. Also, remember that *H. pylori* actively abuses this mechanism to loosen the stomach wall.
2. Patients with specific alleles of other genes produce lower levels of defensins, antibacterial peptides secreted into the intestinal lumen by, e.g., cryptal Paneth cells.
3. Still other patients have an altered IL-23 receptor. IL-23 is an IL-12-related proinflammatory cytokine that stimulates proliferation and activity of TH17 and NK cells.
4. The intestinal barrier function can also be impaired by inflammatory damage to cell membranes. As we have seen, long-chain polyunsaturated fatty acids are particularly susceptible to oxidative changes by reactive oxygen species (ROS). These membrane fatty acids are protected by the selenoenzyme GPX4 (glutathione peroxidase 4). People with genetically weaker GPX4 function not only have an increased tendency to develop intestinal inflammation via this mechanism, they also have changes in intestinal microbiota, although the exact connections have yet to be researched. For these patients, and these patients only, it seems therefore advisable to reduce their consumption of PUFA.

In summary, more than 250 polymorphic loci associated with the disease were identified, some of which have in common that they make the penetration of increased bacterial material into the intestinal wall seem plausible, others that they promote excessive reactions of the immune system. Reacting to these bacterial components, dendritic cells activate naïve T cells to become TH17 and TH1 cells. Recall that TH17 cells enhance early non-specific immune mechanisms, especially recruitment of neutrophils. Abundant neutrophils are typical for IBD, infiltrating and destroying crypts in the form of crypt abscesses. Macrophages are activated directly by bacterial PAMPs, as well as by TH1 cells via IFN $\gamma$ . TNF $\alpha$  secreted by macrophages and TH1 cells

contributes to tissue damage via protease induction, and proteases in turn damage tight junctions. In summary, innate and adaptive immune mechanisms increase epithelial permeability in a vicious circle.

**Pharmacology cross reference:** **Anti-TNF $\alpha$  therapy** is beneficial in treating fistulating Crohn's disease. **Ustekinumab**, an antibody against both IL-12 and IL-23, is approved for Crohn's disease and ulcerative colitis.

Environmental factors have an influence, too. Populations less exposed to certain infectious diseases have been found to suffer more from IBD, a phenomenon also observed with respect to allergic diseases. The hygiene hypothesis postulates a requirement for infections to establish normal tolerance levels. In animal models, helminth infection is able to prevent or reduce IBD development. Compatible with this hypothesis, IBD incidence worldwide is on the rise, and prevalence in Europe, North America and Australia is higher than in other parts of the world.

Hyper reaction from the immune system is also seen in extra-intestinal manifestations, including erythema nodosum, migratory polyarthritis, sacroileitis, ankylosing spondylitis, uveitis and cholangitis.

Symptoms vary widely, depending on location and intensity. Crohn's disease begins in most patients with moderate diarrhea, abdominal pain and fever, sometimes mimicking acute appendicitis. Colitis ulcerosa, with its involvement of distal colon and rectum, typically comes with lower abdominal cramps and diarrhea with bloody and mucous constituents. In both forms, the disease tends to be chronic, starting at a young age, with attacks that come and go, and may require surgery.

## 19. HEPATIC PATHOPHYSIOLOGY

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 reject 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.

The following section briefly discusses the functions of the liver, from which the clinical consequences of dysfunction can usually be derived.

**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 triglycerides 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 feel 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 energy is 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,  $\beta$ -hydroxybutyrate and acetoacetate, from the ever-increasing flood of fatty acids. This happens because the liver via  $\beta$ -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 acetyl-CoAs lined up in this traffic jam, every two are fused, detached from CoA and released into the blood; mainly in the form of  $\beta$ -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  $\text{NH}_4^+$  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 presence of very high blood sugar levels.

### **Diet: carbohydrates or fat?**

**Ketogenic diet.** Typically, the protein content in most diets is around 16-18%; for almost everybody, it falls within the range of 10-35%. A protein content over 35% is not advisable for several reasons. Notably, consuming higher amounts of protein puts increased strain on our

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 short to medium terms, such a diet can be effective in reducing weight. What about the long term?

**Long-term ketogenic diet?** We have long known that the ketogenic diet has a positive effect on certain forms of epilepsy. But what about the general effects of a ketogenic diet? Unfortunately, the data on how healthy or unhealthy different distributions of the macronutrients carbohydrates, fats, and proteins are is very inadequate. Ideally, we would like randomized controlled trials spanning thirty years or more with tens of thousands of participants, where people consistently adhere to a specified macronutrient distribution, meticulously documented and controlled, all while ensuring all micronutrient requirements (minerals, vitamins, trace elements) are met, coupled with detailed documentation of their health outcomes. Aside from the fact that such a study is not feasible: Would you participate in such a study? No sweets for thirty years because you were randomized into 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 plenty of uncertainties. Self-reported food items were then converted into a macronutrient distribution using standard conversions – how many calories from carbohydrates, fats, and proteins does a serving of leeks, a jam sandwich, or a pizza contain? It was then assumed that these dietary habits did not change significantly over the years of the study. 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 countries with a focus in 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 their 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 statement from PURE on this issue is: A higher fat content in the diet is not, as long assumed, associated with increased mortality but with reduced mortality. However, this statement only applies to carbohydrate percentages between "medium" (lowest quintile: 46% of energy requirements) to high (highest quintile: 77%); lower carbohydrate percentages were not represented. For the represented spectrum portion, the finding 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.

**GLUT1 Deficiency Syndrome** is a very rare genetic deficiency of GLUT1 that primarily manifests in the CNS. The neurons receive insufficient energy, leading to seizures in affected children and, over time, developmental disorders. What alternative fuel can be supplied to the neurons? Ketone bodies, which must be provided at all times within a certain concentration range. This requires an extraordinarily strict, purely ketogenic diet for life. Those affected can only consume minimal amounts of absorbable carbohydrates because the liver halts ketone body production as soon as blood sugar levels rise slightly.

**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  $\beta$ -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  $\beta$ -cells, causing release of a little more insulin. Insulin inhibits glucagon secretion in the neighboring  $\alpha$  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  $\text{NAD}^+$  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), acid-base stabilization  
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 ( $\text{NH}_3$ ) or rather, at physiological pH, the ionized ammonium ion ( $\text{NH}_4^+$ ). 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  $\text{NH}_4^+$ , 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  $\text{CO}_2$  and water.  $\text{CO}_2$  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  $\text{HCO}_3^-$  as  $\text{NH}_4^+$

(net, as actual metabolization is much more complicated). Consumption of 100 g/d of protein results in production of approximately 1000 mmol  $\text{HCO}_3^-$  and 1000 mmol  $\text{NH}_4^+$  per day. From an acid-base perspective,  $\text{NH}_4^+$  could spare a proton while  $\text{HCO}_3^-$  could take one up. Yet, with a pKa of 9.2,  $\text{NH}_4^+$  is quite unwilling to release its proton at the physiological pH of 7.4.  $\text{HCO}_3^-$ , on the other hand, readily accepts protons, followed by elimination as  $\text{CO}_2$ , so that the net process would result in alkalization.

In alarm, we almost choke on our scrambled eggs-  $\text{NH}_4^+$  is toxic,  $\text{HCO}_3^-$  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, unsuspecting 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  $\text{NH}_2$ -groups per  $\text{C}=\text{O}$  unit. Um, then how does that square with our acid-base balance? If we transmogrify  $\text{NH}_4^+$  into a  $\text{NH}_2$ -group, that leaves an  $\text{H}^+$  on the table; OK,  $\text{HCO}_3^-$  is missing one, so those two may cancel each other out, but what about the second  $\text{NH}_4^+$ ? 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  $\text{NH}_4^+$ , 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  \$\text{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  $\text{NH}_4^+$ ; that would be far too toxic. We need a secure acid/ammonium tanker: glutamine. From this tanker, the kidney retrieves  $\text{NH}_4^+$  and excretes it. Each  $\text{NH}_4^+$  excreted by the kidney needs not be neutralized by  $\text{HCO}_3^-$  and thus saves  $\text{HCO}_3^-$  (one or one-half, depending on how one looks at it).

Consequently, in the liver we have two options to deal with  $\text{NH}_4^+$ :

1. We use it to neutralize  $\text{HCO}_3^-$  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  $\text{HCO}_3^-$ , option 2 saves  $\text{HCO}_3^-$ . 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  $\text{HCO}_3^-$  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  $\text{NH}_4^+$ , which is then fused with  $\text{HCO}_3^-$  and an ATP-derived phosphate to carbamoylphosphate. Carbamoylphosphate is fed into the urea cycle. Activity of liver glutaminase depends directly on pH: lower pH  $\rightarrow$  less carbamoylphosphate  $\rightarrow$  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  $\rightarrow$  more glutamine that is dispatched to the kidney. It makes sense that this enzyme is predominantly expressed near the center of the liver lobule:  $\text{NH}_4^+$  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  $-\text{OH}$ , 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 metabolism.
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 metabolism. 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 PPAR $\alpha$ . 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.

\*

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<sub>2</sub> 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<sup>+</sup> 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<sup>®</sup>-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 metabolism. 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<sup>®</sup>). 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, 7 $\alpha$ -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 7 $\alpha$ -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 7 $\alpha$ -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<sup>+</sup>-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<sup>+</sup>-driven Na<sup>+</sup>-H<sup>+</sup>-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  $\text{Ca}^{2+}$  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  $\text{COO}^-$ -groups. Via binding to  $\text{Ca}^{2+}$ , these double  $\text{COO}^-$ -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  $\text{Ca}^{2+}$  by citrate or EDTA). In the absence of the second  $\text{COO}^-$ , the factors remain soluble and never meet, further impairing blood coagulation. Vitamin K is also required for  $\text{Ca}^{2+}$ -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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