NUTRITION AND DIGESTION

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

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Just to keep living, and without any special activities, we already need lots of energy: about 30 kcal (126 kJ) per kg body weight per day. Apart from this fuel, which we burn, we need "building blocks and tools" for our body and we have to get rid of unnecessary or toxic molecules. So, we cannot live without massive exchange with our environment. Exchange entails risks. To get the job done while minimizing these risks is to a large extent the responsibility of the gastrointestinal tract and the liver.

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. In addition, 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 normal harmless antigens that need to be tolerated. To do that, we open channels 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 dangerous material.

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. But neither is the opposite, a purely vegetarian or vegan diet, for lack of B12 and usable iron, which need to be substituted.

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 to function under those 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 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. Milk and cereals, products of the neolithic (agricultural) revolution starting 10,000 years ago, were absent. Average plant:animal subsistence ratio has been estimated around 65:35. Compared to our average Western diet, food intake was likely high in protein and fibers, moderate in fat and low in carbohydrates. To feed oneself required lots of movement and hard manual 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.

These condiderations 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. Firstly, for the large majority of us, this is simply impossible. In view of the calories required, grains, corn and rice are irreplaceable. Paleolithic meat was qualitatively different from today's meat: wild game contains much less fat than our fatstock. Secondly, in a world with seven billion people, we have to behave differently than our ancestors, who numbered a few hundred thousands at most. Intensive mass-animal farming on the scale required to provide meat frequently for so many people seems ethically impossible. We urgently need to develop a form of nutrition that integrates health and ethical concerns and is fair and economically feasible on a global level. But this is not my topic. Here, I concern myself only with aspects of nutrition relevant for human health. For practical reasons, I assume the present situation in affluent countries, where meat and other animal source foods are available in every grocery shop.

2. OVEREATING

Today, this thrifty genetic endowment is less than optimal for people living in conditions of ample food supply. The imbalance is accentuated by a pervasive lack of physical activity and exercise. Concerning the availability of food, we live in a continuing feast. If we fail to make a stand against the constant lure of food, we accumulate fat deposits until they start to be toxic. Remember that toxicity is a function of the dose. There is accumulating evidence that the moderate increase in free fatty acids (FFA) seen in obesity causes metabolic problems at two levels: insulin-producing cells and insulin-sensitive target tissues.

FFA induce production of IL-1β, IL-6 and IL-8 in human islets. IL-1β expression is enhanced by an autostimulatory process, and the production of insulin deteriorates under the influence of IL-1β. Bockade of IL-1β function with the IL-1 receptor antagonist anakinra improved β-cell function and reduced levels of glycated hemoglobin.

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 cause activation of inflammatory pathways, resulting in reduced insulin-sensitivity of liver, muscle and adipose tissue.

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. 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.

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 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, grumpy, cold, craving for food and ready 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 body's attempts to enter energy conservation mode.

More for our society's obsession with appearance than due to 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. Make a long-term plan. One pound (0.5 kg) a month is reasonable.
3. Accept being a little hungry part of the day. As long as you take up calories from a meal, insulin predominates and part of these calories are converted to fat. Once the process reverses, glucagon predominates, and you get hungry again. Hunger is the signal to Paleolithic 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. Have breakfast, have lunch. It's not necessary to skip a meal, but if you want to, skip dinner. Don't eat too much in between. You don't want insulin to be active all day.
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 musli 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. Build up a routine to have some form, any form of aerobic exercise two, better three times a week for at least 30 minutes. This increases metabolism for many hours. 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 muscle and brown-adipose-tissue.

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. 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. 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 quanitified in few probands, and invididual 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.

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. For how many years? We don't yet know. The research necessary to determine the duration of this enery conservation mode remains to be done. Let's hope it's not for the rest of our lives.

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. Prevention of weight gain, as well as prevention of smoking, are mundane yet highly effective medical goals.

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

In painful contrast to the opulence experienced in part of the world, hunger is one of the most pervasive medical problems. About 1 billion people are malnourished. According to Jean Ziegler, working for the United Nations, mortality due to malnutrition accounted for 58% of total mortality in the world in 2006. 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 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 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 at breakfast and a maximum late in the evening. In addition, changes in food situation cause temporary divergence. Leptin levels decrease following a few hungry days, and increase after a period of feasting. Leptin acts on numerous tissues, but its main target is thought to be the brain. Leptin is able to cross the blood brain barrier, affecting the autonomic nervous system via hypothalamic centers. A fall in leptin causes the sensation of hunger; an increase, a feeling of satiety. 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.

In promoting the feeling of satiety, leptin cooperates with insulin, which has an analogous effect in the CNS. Both leptin and insulin stimulate "anorexigenic" (appetite-reducing) POMC-expressing neurons in the arcuate nucleus. These neurons release a POMC (pro-opiomelanocortin) cleavage product, α-MSH (α-melanocyte stimulating hormone), at their synapses. α-MSH stimulates melanocortin receptors 3 and 4 (MC3R and MC4R), resulting in increased metabolic rates and diminished food intake. About 4% of patients with severe early-onset obesity have mutations in MC3R or MC4R. POMC neurons also synthesize CART (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 the tendency to gain weight after quitting.

A second population of neurons in the arcuate nuclues counteracts the POMC-expressing neurons, promoting a feeling of hunger, food intake, weight gain, white-fat storage and reducing brown fat thermogenesis. This second "orexigenic" population expresses Neuropeptide Y (NPY) and agouti-related protein (AgRP). Levels of NPY and AgRP are increased by negative energy balance, as elicited by fasting, and decreased by leptin.

Pharmacology cross reference: For some time, drugs from the amphetamine family were broadly used as appetite suppressants. This was discontinued when it became clear that their dependency-promoting properties and other unwanted effects could not be dissociated.

Peptide YY (PYY)

PYY is released by mucosal neuroendocrine L cells in the distal gut in response to intake of food. Comparing isocaloric meals high in carbohydrates, 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.

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

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. Ghrelin (acronym for *growth hormone release inducing*) is a 28 amino acid peptide with a linked octanol group. Ghrelin activates its receptor, GHSR (GH secretagogue receptor), which is expressed in the hypothalamic nucleus arcuatus as well by afferent neurons of the vagus nerve. Ghrelin stimulates appetite and, in addition, seems to be involved in the stimulation of growth hormone release. In summary, ghrelin contributes to the accumulation of body mass and linear growth.

6. DIGESTION AND ABSORPTION OF CARBOHYDRATES

The better part of nutritional carbohydrates is taken up in the form of starch. Starch digestion starts with the enzyme α-amylase, which is present in saliva and pancreatic juice. In between, in the stomach, carbohydrate digestion is paused, as α-amylase is inactivated by gastric acid. α-Amylase is an endoenzyme able to break internal α-1,4-glycosidic linkages between glucose units, but not terminal ones or linkages adjacent to α-1,6-linkages. Therefore, α-amylase digestion does not result in monosaccharides, but rather in packs of two (maltose) or three glucose units (maltotriose) and α-limit dextrins, containing α-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 are 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 α-1,6-glycosidic bonds of limit dextrins; it is also able to cleave α-1,4-linkages.

Finally, the resulting monosaccharides are absorbed into enterocytes with the help of transporters. Glucose import is driven by a Na^+ electrochemical gradient. One glucose unit and two sodium ions are cotransported by the 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 Na^+ absorption. SGLT1 is able to ferry glucose as well as galactose into the enterocyte, but not fructose, which forms a fivemembered 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 rices, 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 what extent this concept is valid in humans. Experiments in humans cannot be done with the same rigor. Necessarily, changes in GI influence fibre 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 processed cereals or white bread with whole-grain musli containing yoghurt and fresh fruits.

Lactase deficiency

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. Hence, while lactose intolerance is the norm in people of African or Asian descent, it is rare in Scandinavia. In Europe, about 15% of adults are affected.

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 metabolization. Foods rich in fructose include 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 hydrolized to AMP, part of which is degraded to uric acid. High fructose consumption seems to moderately increase the risk of gout. Following metabolization in the liver, part of the fructose load is released as glucose and lactate, part used for lipogenesis. 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. While it is possible that fructose accentuates hepatic insulin resistance, that is probably just one aspect of our more general problem of overfeeding with simple sugars resulting in weight gain, lipotoxicity and insulin resistance.

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. 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 (Fe^{3+}) or ferrous (Fe^{2+}). Fe^{3+} is not soluble at pH values above 3; it forms stable complexes with many anions and is not readily absorbed. Fe^{2+} is soluble up to pH 8; it is cotransported with protons by the divalent metal transporter (DMT1). In all, only 10%-15% of dietary iron is absorbed. Iron-binding tannins from tea or the alkaline pH of dairy products further reduce this percentage.

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

In the circulation, Fe^{3+} is then ferried around by transferrin. Blood plasma, too, contains a copper-containing enzyme able to convert Fe^{2+} to 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, 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 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 Fe^{2+} levels, the IRE is bound by IRE-BP and translation is blocked; high Fe^{2+} levels remove the IRE-BP, allowing vigorous translation of the mRNA and high-level expression of this 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. Uptake of iron via the transferrin receptor leads, via activation of transmembrane protein HFE ("high Fe"), 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.

In a second regulatory mechanism, hepcidin is also induced by inflammation. 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. The iron, although present in macrophages, is not available to erythroid progenitors.

Therefore, the serum concentration 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, but keep in mind that the two forms of anemia may coincide.

Hemochromatosis

Iron overload has toxic effects. Overload may be the result of frequent transfusions or of certain forms of hemolytic anemia; alternatively, it may 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 medievally 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, like that of methionine. 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^5-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^-). 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^5-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.

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 und 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^5-methyl-THF form, leading to a secondary shortage of 5,10-methylene-THF (the reaction from 5,10-methylene-THF to N^5-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^{10}-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 demyelinization of the dorsal columns of the spinal cord, a syndrome termed funicular myelosis. The details of its pathophysiological mechanism have not been resolved.

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 und 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_2O_2 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 in the lecture notes on liver function. 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). **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 to survive, linoleic acid (18:2) and α-linolenic acid (18:3), from which we can synthesise the others. These are therefore called essential fatty acids. Certain plant oils are therefore rich in PUFA, e. g., sunflower oil or corn oil. Fatty fish contain ample PUFA, too, which are originally synthesized by algae and taken up via the food chain.

The mix of fatty acids taken in certainly plays a role in human 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 α-linolenic acid (18:3), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6). Fatty fish is rich in n-3 fatty acids.

- n-6 PUFA, like linoleic acid (18:2) and arachidonic acid (20:4).

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 clincal 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. One hypothesis is that n-6 arachidonic acid, which is the starting point for the synthesis of proinflammatory mediators and thromboxane, competes with n-3 PUFA for the same enzymes; more n-3 PUFA would thus mean less vessel wall inflammation and platelet aggregation. Potential beneficial effects of n-3 PUFA have also been discussed in neurological diseases, depression and cancer.

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, e. g., in the wall of coronary arteries. These less-fluid lipids 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 form the majority of many plant fats, which, due to their low melting point, form liquid oils after extraction. Examples are olive oil (72 % oleic acid, 13% saturated), canola oil (61% oleic acid, 7% saturated, 4% trans fats), corn oil (60% linoleic acid, 25% oleic acid, 15% saturated) and sunflower oil (67% linoleic acid, 20% oleic acid, 12% saturated). 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 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 (increase its melting point) were developed, which mainly 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 nutritional fats to bad fats. These partially hydrogenated fats rich in trans-fats have 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. During the last few years, growing awareness led to widespread legislative efforts to strongly reduce trans fats in industrially produced food components.

12. PROTEIN DIGESTION STARTS IN THE STOMACH

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 a 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 luminal part of the H^+/K^+-ATPase, inactivating it irreversibly. The effect is sustained until pumps have been replaced by resynthesis, 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 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 alkalized by 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. 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 HCO_3^- and mucus depend on locally produced prostaglandin E (PGE), which is synthesized via cyclooxygenase-1 (COX-1).

13. GASTRITIS AND GASTRIC/DUODENAL ULCER

The hallmark of gastritis are 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. While only part of those infected develop symptoms, H. pylori causes the majority of gastric und duodenal ulcers, with NSAIDs a distant second at less than 20%. The exact mechanism is not yet entirely clear. Probably, pathogenesis evolves along these lines: Infection causes low intensity-inflammation of the antral mucosa, which in some of the affected grows strong enough to reduce somatostatin production of D cells. This results in an increase in production of gastrin, which in turn sends acid secretion into overdrive.

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 gastric and duodenal mucosa, reducing secretion of 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. While COX-1 is constitutively expressed in all cell types, COX-2 , which is induced in macrophages and other inflammatory cells, is responsible for the pro-inflammatory effects of prostaglandins. This led to development of COX-2 inhibitors, 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 HCO_3^- Peptones stimulate I-cells in the duodenal epithelium to secrete cholecystokinin (CCK). CCK induces the release of bile from the gallbladder and stimulates the exocrine pancreas. Together, secretin and CKK 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).

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 HCO_3^--rich secretion of pancreatic fluid. While CFTR is a Cl^--transporter at the apical membrane, ductal Cl^- is required for exchange with 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 HCO_3^-–rich secretion by phosphorylation of CFTR.

One mutation, ΔF508, 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, each of which present in only a small percentage of patients, have been described to result in partial of 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

FAT DIGESTION is discussed in the lecture notes on liver (dys-)function.

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. Note that cereal grains entered human nutrition only 15,000 years ago.

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 are 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 (α/β, γ and ω), usually are the pathogenetic 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+ T cells mainly differentiate into TH1 cells, which produce IFNγ. 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.

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.

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. 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. Another group of patients seem to produce lower amounts of defensins, anti-bacterial peptides secreted into the intestinal lumen by, e. g., cryptal Paneth cells. In summary, several polymorphic loci may contribute to the fact that more bacterial material than normal permeates the intestinal wall.

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 Crohn's disease, infiltrating and destroying crypts in the form of crypt abscesses. Macrophages are activated directly by bacterial PAMPs, as well as by TH1 cells via IFNγ. TNFα 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-TNFα therapy is beneficial in treating fistulating Crohn's disease.

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.

Hyperreaction 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.

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