Intestinal Gene Expression Profiling and Fatty Acid Responses to a High-fat Diet

JONATHAN CEDERNAES
Dissertation presented at Uppsala University to be publicly examined in B22, Biomedicinskt centrum, Husargatan 3, UPPSALA, Thursday, April 18, 2013 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

**Abstract**


The gastrointestinal tract (GIT) regulates nutrient uptake, secretes hormones and has a crucial gut flora and enteric nervous system. Of relevance for these functions are the G protein-coupled receptors (GPCRs) and the solute carriers (SLCs). The Adhesion GPCR subfamily is known to mediate neural development and immune system functioning, whereas SLCs transport e.g. amino acids, fatty acids (FAs) and drugs over membranes. We aimed to comprehensively characterize Adhesion GPCR and SLC gene expression along the rat GIT. Using qPCR we measured expression of 78 SLCs as well as all 30 Adhesion GPCRs in a twelve-segment GIT model. 21 of the Adhesion GPCRs had a widespread (≥5 segments) or ubiquitous (≥11 segments) expression. Restricted expression patterns were characteristic for most group VII members. Of the SLCs, we found the majority (56 %) of these transcripts to be expressed in all GIT segments. SLCs were predominantly found in the absorption-responsible gut regions. Both Adhesion GPCRs and SLCs were widely expressed in the rat GIT, suggesting important roles. The distribution of Adhesion GPCRs defines them as a potential pharmacological target.

FAs constitute an important energy source and have been implicated in the worldwide obesity increase. FAs and their ratios – indices for activities of e.g. the desaturase enzymes SCD-1 (SCD-16, 16:1n-7/16:0), D6D (18:3n-6/18:2n-6) and D5D (20:4n-6/20:3n-6) – have been associated with e.g. overall mortality and BMI. We examined whether differences in FAs and their indices in five lipid fractions contributed to obesity susceptibility in rats fed a high fat diet (HFD), and the associations of desaturase indices between lipid fractions in animals on different diets. We found that on a HFD, obesity-prone (OP) rats had a higher SCD-16 index and a lower linoleic acid (LA) proportions in subcutaneous adipose tissue (SAT) than obesity-resistant rats. Desaturase indices were significantly correlated between many of the lipid fractions. The higher SCD-16 may indicate higher SCD-1 activity in SAT in OP rats, and combined with lower LA proportions may provide novel insights into HFD-induced obesity. The associations between desaturase indices show that plasma measurements can serve as proxies for some lipid fractions, but the correlations seem to be affected by diet and weight gain.

**Keywords:** Adhesion GPCR, delta-5 desaturase, delta-6 desaturase, desaturase index, Diet-induced obesity, estimated desaturase activity, fatty acid composition, gas chromatography, gastrointestinal tract, G-protein coupled receptor, high-fat diet, intestine, linoleic acid, liver, mRNA expression, palmitoleic acid, plasma, phospholipids, proximodistal, RT-qPCR, solute carrier, SCD-1, SCD-16, SCD-18, stearoyl-CoA desaturase, subcutaneous adipose tissue, subsection, triacylglycerols.

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To my beloved family:
My mother and father, my two
brothers, my grandparents and
Saatchi (for his ever-cheerful spirit)
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Additional publications


* equal contribution
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<tbody>
<tr>
<td>2-MAG</td>
<td>2-monoacylglycerol</td>
</tr>
<tr>
<td>7TM</td>
<td>Seven transmembrane</td>
</tr>
<tr>
<td>ALA</td>
<td>α-linolenic acid</td>
</tr>
<tr>
<td>BAI</td>
<td>Brain-specific angiogenesis inhibitor</td>
</tr>
<tr>
<td>BPA</td>
<td>Bisphenol A</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
</tr>
<tr>
<td>CAF</td>
<td>Cafeteria</td>
</tr>
<tr>
<td>CE</td>
<td>Cholesterol ester</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CM</td>
<td>Chylomicrons</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>D5D</td>
<td>Delta-5 desaturase</td>
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<tr>
<td>D6D</td>
<td>Delta-6 desaturase</td>
</tr>
<tr>
<td>DDE</td>
<td>Dichlorodiphenyldichloroethylene</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DIO</td>
<td>Diet-induced obesity</td>
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<tr>
<td>DNL</td>
<td>De novo lipogenesis</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>EI</td>
<td>Elongation index</td>
</tr>
<tr>
<td>EMR</td>
<td>EGF-like module containing mucin-like hormone receptor</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>GPS</td>
<td>GPCR proteolytic site</td>
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<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>FAS</td>
<td>Fatty acid synthase</td>
</tr>
<tr>
<td>FABP</td>
<td>Fatty acid binding protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HFD</td>
<td>High-fat diet</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LCFA</td>
<td>Long chain fatty acid</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acid</td>
</tr>
<tr>
<td>NSE</td>
<td>Neuron-specific enolase</td>
</tr>
<tr>
<td>OP</td>
<td>Obesity-prone</td>
</tr>
<tr>
<td>PCB</td>
<td>Polychlorinated biphenyl</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PL</td>
<td>Phospholipid</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptors</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>QALYs</td>
<td>Quality-adjusted life-years</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Quantitative reverse-transcriptase PCR</td>
</tr>
<tr>
<td>SD</td>
<td>Sprague Dawley</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
</tr>
<tr>
<td>SCD-1</td>
<td>Stearoyl-CoA desaturase 1</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-chained fatty acid</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acid</td>
</tr>
<tr>
<td>SLC</td>
<td>Solute carrier</td>
</tr>
<tr>
<td>SSB</td>
<td>Sugar-sweetened beverage</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 diabetes mellitus</td>
</tr>
<tr>
<td>VLCFA</td>
<td>Very-long chain fatty acid</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low-density lipoprotein</td>
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</table>
Introduction

Looking back, we humans, the end products of our genome, used to face a very different environment than that of today. Not only did the gastrointestinal tract not have to withstand the load of more than 50 kilograms of refined sugar each year\(^1\), but saturated fat intake was also substantially lower, as was the intake of sodium [1-3]. The only beverages consumed until around 12,000 years ago were, as far as we know, water and for a short time in life (i.e. as infants) milk [4]. At the same time, nutritious foods, high in dietary fiber, were more abundant, as they have been observed to still be in populations that have not adapted a modern lifestyle, e.g. in populations in New Guinea [3]. Replacing otherwise naturally occurring nutrients in our food, we now have food additives in the processed food that has become so common in today’s kitchens and a requirement for coping with the time constraints of our modern and stressful society.

Going further back into history, our ancestors gastrointestinal tracts certainly encountered far more bacteria, being ubiquitous in their food and earth’s environment (in the latter outnumbering the number of stars in our galaxy by a magnitude of six [5]), and possibly serving a beneficial role in immune system regulation by protecting them from both infections as well as autoimmune diseases [3]. As there were no caesarian sections, which shield the newborn baby from the natural and essential delivery of its first constituents of its vital gut flora, the absence of bacteria from the point of birth was not something to be reckoned with. Nor was our symbiosis with gut bacteria – influencing our metabolism, access to nutrients and our physical and mental health – disturbed later in life, by antibiotics that not only kill bacteria that cause disease, but which may also increase our risk of obesity.

Back in time, there were furthermore no pharmaceuticals; drugs engineered to hijack unknowing transport molecules in our intestines, thereby allowing entry into our bodies for treating or regulating blood clotting, blood pressure, pain, infections, cancerous growths, mood, satiety and a wealth of other con-

\(^1\) Per capita average refined sugar intake in the U.S. was 69.1 kg in 2000, up from 55.5 kg in 1970 [1]. This can be compared with the consumption trends reported for England, with just 6.8 kg in 1815, rising to 54.5 kg in 1970. Meanwhile, the consumption in Sweden was just 3.0 kg in 1850 but had already risen to 10 kg after 1885 [2]
ditions and disorders. Today, drugs are so common and some considered so beneficial for the good of society, that many of our elderly have a mind-bogglingly long list of them and that soon everyone might be eating daily concoctions to keep our bodies in continuous top shape and far away from hospital doors for as long as possibly conceivable.

The most recent changes to our diets and our lifestyles, occurring during the decades spanning the post-world war II era, have entailed a large increase in the availability of energy-dense, both from sugar and fat, highly palatable food, coupled with a general transition to sedentarism \[6, 7\]. A 24/7 modern society with increased stress has also led to a general decline in mental health and significantly impaired (both qualitatively and quantitatively) sleep. Among other things, this has resulted in a dramatic rise in the number of overweight and obese people.

By recent accounts, the global average body mass index (BMI) has increased by 0.4 kg/m\(^2\) per decade during the 1980-2008 period [8]. This has resulted in a staggering population of overweight and obese people, with the adult overweight (BMI ≥25 kg/m\(^2\)) population reaching 1.46 billion men and women in 2008. Expressed as relative numbers, this corresponds to 34.3% in the age-standardized prevalence of overweight people. Meanwhile, 13.8% or 297 million of the global population of women, and 9.8% or 205 million of the global population of men, were classified as being obese (BMI ≥30 kg/m\(^2\)) in 2008. Compared with the levels recorded for women and men in 1980, respectively 7.9% and 4.8%, this means that the prevalence of obesity in women and men has almost doubled in less than three decades.

The increase in obesity is not without consequences. It has increased the total weight that moves about our planet\(^2\) [9] – an unwanted increase in “mobile fat” that in itself demands energy for sustaining its very existence. This has exacerbated the already unsustainable strain on the resources and especially food energy demands of our planet and, if increasing obesity trends continue, could result in an energy demand increase corresponding to almost 500 million additional normal-weight people. The unprecedented increase in average body weight however already has considerable negative consequences for the individual bodies tasked with the strenuous burden of carrying our excess weight around.

\(^2\) According to Walpole et al.'s calculations, the extra weight or biomass produced by overweight or obese people in 2005 equaled 15 and 3.5 million tons, respectively [9]. The latter mass corresponded to how much 56 million people of average body mass would weigh. Not surprisingly, most of the excess biomass from obesity was found in North America, its contribution totaling roughly one third (34 %) of the obesity biomass, while the continent’s population in numbers only accounted for 6 % of the global population.
Diet-related chronic diseases in fact constitute the most common diseases in our modern society, affecting between half and two thirds of the adult human population [1]. While certainly no single dietary alteration (or diet alone for that matter) can be solely held responsible for the current prevalence of diet-related diseases, all of these dietary changes result in changed outcomes in the form of biochemical dietary indicators. These can be used as measures of the health of our bodies. One of these is fatty acid composition, which can be measured in many tissues, but most often in plasma, liver and adipose tissue. It has increasingly been found that such biochemical metabolites and ratios thereof are related to metabolic parameters and pathological conditions.

Notably, even under conditions favoring weight gain, such as access to a palatable diet high in energy-rich fat and/or sugar, not all individuals or animals become obese. Some instead appear resistant to the negative outcomes otherwise preordained by our modern lifestyle. Finding the reasons for this variability in susceptibility to a condition that affects a third of our global population will be crucial if we wish to continue extending our lifespan into additional healthy years of aging.

Furthering the understanding of how our modern living with our drastically altered environments and altered food habits affects our brains, our gastrointestinal tracts, our metabolism and our genome, which are still to adapt to our newfound lifestyles, is essential if we are to be able to halt and hopefully reverse diet-related morbidity and mortality in the form of conditions such as obesity, type 2 diabetes mellitus and cardiovascular disease, and to ease the strain of our planet so that it can cope with present as well as future human generations.
The Gastrointestinal Tract

Basic anatomy and physiology

The gastrointestinal tract (GIT) encompasses the mouth, oral cavity, esophagus, stomach, small intestine and colon, each with specific functions. The basic structure of the GIT is similar between mammals even though the gross morphology reveals apparent differences [10]. In contrast to humans, rodents have a substantial non-glandular stomach part that is used for digestion and storage of food, and is separated from its proper gastric portion. The latter portion is of glandular type and resembles that seen in e.g. humans, containing parietal, chief and cardiac cells. The stomach continues digestion initiated by enzymes secreted by the salivary glands (amylase and lipase), by adding acid, additional lipase and proteases that initiate protein breakdown [11, 12]. After initiation of digestion in the stomach, juices from the liver and pancreas that contain bile acids and pancreatic fluid, rich in bicarbonate, calcium and various enzymes (e.g. α-amylase, trypsin, carboxypeptidase and lipase) are released into the duodenum. These various secretions are regulated by neural (vagal, “cephalic”), hormonal (primarily secretin and cholecystokinin, CCK, but also leptin, ghrelin and peptide YY) and luminal (low pH, lipids and proteins) factors, and enables further digestion [10, 13]. Whereas the basal pancreatic bicarbonate secretion (i.e. between meals) is low in e.g. humans and dogs, rats (which lack a gallbladder) have a relatively higher basal secretion and instead show a comparatively lower increase in response to secretin, whereas all three species show weak secretory pancreatic bicarbonate responses to CCK or vagal stimulation [13].

Most of the absorption occurs in the small intestine, which for this purpose has an enormous surface area due to its folds, villi and microvilli extensions (each increasing the surface area by a factor of 3, 10 and 20, respectively) [10, 14]. Absorption in the small intestine is primarily carried out by cells called enterocytes (which are appropriately also called absorptive cells) [10, 14]. After absorption, food passes through the last part of the small intestine, ileum, into the large intestine, roughly 90-150 cm long in humans and only 9-11 cm long in rats [10]. The first part of the large intestine consists of the cecum, which is poorly defined in humans and considerably larger in other species [10, 15]. Water, electrolytes, minerals, and some nutrients and vitamins produced by bacterial fermentation processes, are absorbed in the co-
lon, which by far contains the largest proportion of GIT bacteria, also called the gut microbiome [10, 16, 17]. Whereas the composition of bacteria is quite similar in the large intestine between rats and humans, the former species has substantially more bacteria in regions proximal of the colon [10]. Finally, after passage through the GIT, the digestive remains are excreted as feces, which largely (up to 55%) consists of bacteria [18].

In light of the mentioned and numerous other documented differences in GIT anatomy, physiology and biochemistry between different laboratory animals and humans – including differences in pH, pancreatic secretions, mucus content, bacterial composition and intestinal membrane composition – it is evident that no laboratory animal that can replace studies in humans, as all of these factors can be important determinants for e.g. drug absorption mechanisms [10].

Redefining the role of the gastrointestinal tract:
getting the whole picture and not forgetting about the small inhabitants

The GIT has long been recognized for its obvious role for the intake, passage and disposal of e.g. nutrients, water, drugs and waste. In the past couple of decades, new crucial roles for the GIT have however been discovered, such as those involving the enteric nervous system (ENS), hormonal regulation and metabolism of drugs, immune system regulation and perhaps most excitingly those entailing the immense gut flora.

The enteric nervous system (ENS) of the GIT has approximately the same number of neurons as the spinal cord and has been called a “second brain” due to its complexity and its similarity to several features of the brain [19]. Although the ENS can function autonomously, thus lending further validity to its title as a sort of brain, the ENS is intimately linked to the central nervous system (CNS) – the so-called “brain-gut axis” [19, 20]. Through this axis, the ENS is bidirectionally involved in somatosensory regulation, and associations between stress and the severity and onset of GI disorders have been found [21]. Encompassing the ENS, enteroendocrine cells, the vagus and the CNS, the brain-gut axis is likely highly involved in regulating food intake, to a great extent by hormones that largely act on G-protein coupled receptors (GPCRs), such as the anorexigenic hormones cholecystokinin (CCK) and peptide YY (PYY), and the orexigenic hormones ghrelin and orexins A and B [19, 20, 22].

Sensation from three of our five classical senses, vision, taste and smell, occur via activation of GPCRs [22, 23]. In the GIT, taste starts at the tongue,
where the sensation of sweet, bitter and umami are detected by GPCRs [22].
Surprisingly, taste does not however stop at the oral cavity, but rather has been discovered to continue along the GIT, where classical taste and olfactory receptors have also been found and implicated in physiological functions such as hormonal release, transporter expression, absorption regulation and satiety signaling, thereby allowing the GIT to actively “taste” luminal contents in a complex manner that likely varies throughout the length of the GIT [22]. In response to activation of GPCRs, intestinal K cells secrete gastric inhibitory polypeptide (GIP), and intestinal L cells secrete the hormones glucagon-like peptide 1 (GLP-1) and PYY [22, 24]. GIP and GLP-1 participate in regulating insulin secretion in response to glucose [22], and there are already drugs targeting these molecules for treating type 2 diabetes [25]. More recent studies have also indicated that short-chained fatty acids (SCFAs) produced by the microbiome can induce the release of GLP-1 from cells in the colon, perhaps even through the gastrocolic reflex after the initiation of a meal [22, 26]. This raises the possibility of an even greater influence of our microbiome with the body’s energy metabolism and immune system [24]. The GIT is also the first site of metabolism, not only of nutrients but also of ingested drugs, as enterocytes contain drug-metabolizing enzymes from the Cytochrome P450 superfamily [27] that may be involved in important drug interactions [14, 28].

Furthermore, the largest component of the immune system is located in the GIT [29], which also serves as the main harbor for the large variety of microbes of the human body and which outnumber human cells and human genes at least by a factor of 10 and 100, respectively [30]. While the GIT of the fetus is sterile while in utero, this changes upon birth [31, 32]. At this time point the first batch of bacteria is delivered via contamination from regions along the birth canal, colonizing the GIT of the newborn and thereby establishing the so-called microbiome. In light of the sheer number of genes attributed to the microbiome, this has been lumped together with the human genome as the “metagenome” [33]. Furthermore, as the functions of the microbiome are so important, it has even been attributed the title of a metabolic “organ” [34].

Among their many functions, gut bacteria provide us humans with important metabolites with beneficial or detrimental effects, as well as nutrients as they are able to break down products that are non-absorbable and indigestible for the small intestine [35, 36]. They also serve as important gate keepers by preventing the passage of beneficial or pathogenic bacteria into our bodies [17, 37]. Our gut bacteria are increasingly being linked to conditions such as autoimmune disorders, cancer, psychiatric diseases and obesity [33, 38-41]. In 2006, Turnbaugh and his colleagues found that the microbiome of a phenotypically obese mouse was associated with a greater nutrient breakdown
capacity, and that transplantation of this microbiome to lean mice resulted in increased adiposity [33]. When mice free of gut bacteria from birth (“germ free”) are colonized with a normal microbiome, weight increases can be seen within 14 days, despite a decrease in food intake [34]. Delayed initial colonization of the GIT of the human fetus through cesarean section can possibly also affect early and later immune system function [31]. Additionally, the gut microbiome has been linked to colon cancer [42, 43], distal stomach cancer in the case of the infamous H. pylori [44], and possibly even stroke [36]. Compared to the interpersonal variation in the genetic code, there is a much larger variation in the composition of the gut microflora between two given individuals – even between genetically identical twins [45] – and this has been hypothesized to account for interindividual differences in e.g. nutrient uptake and drug metabolism [46].

In recent years there has been great interest in studies examining the potential benefit of adding “good” bacteria, known as probiotics, to both animals and humans. A recent study by McNulty et al. has uncovered that these bacteria may indeed alter carbohydrate and SCFA processing, as well as other metabolic pathways, and that these effects are produced with minimal or no alterations to the long-term composition of the human or mouse GI flora [47]. Probiotics have also been demonstrated to have potential cognitive effects. In a study by Diop et al., the authors demonstrated that probiotics can reduce stress-related symptoms from the GIT in humans [48]. Another study showed that some probiotics can reduce anxiety-related signs in rats and alleviate psychological distress in human subjects (as measured by the scales HSCL-90 and HADS), and decrease the urinary 24-h excretion of cortisol, a physiological hormone employed as an indicator of stress [49]. Other studies have examined the effects of probiotics on metabolism in both rodents and humans and found improvements in parameters such as the levels of LDL, triglycerides, plasma glucose and plasma fibrinogen, as well as reduced fat storage [50-59].

The gastrointestinal tract is often afflicted by chronic conditions such as inflammatory bowel disease (IBD) – which has also been linked to the gut flora [60] – and cancer. IBD has ominously but for unknown reasons increased in prevalence during the last decades, especially in Europe and the U.S. Notably, the increase was seen almost without exception in all studied countries and incidence rates seemed to peak in productive years (20-40 years of age) [61]. Colorectal cancer constitutes the third most common form of cancer and is more common in developed countries [62], whereas stomach and esophageal cancer are more common in developing countries and have a generally poor prognosis. Perhaps not surprisingly, diet has been implicated as an important causative factor in many cancers of the GIT [44, 63, 64].
To increase our understanding of the role that the GIT plays in these ranging states of physiology and pathology, it is essential to further study its molecular components. Important among these are the proteins mediating and regulating the interactions and transport of e.g. nutrients and drugs, between the lumen of the gut and the rest of the human body: membrane bound transporters and receptors.

There are cell lines available to study the expression of intestinal transporters and associated molecular transport, such as the Cacao-2 cell line. These cell lines have been important for studying uptake and efflux during drug development, but nevertheless, these may not represent the entire GI tract [65]. Even though these cell lines were originally derived from human colon adenocarcinoma, these cells actually display transport mechanisms similar to that in the small intestine. In cell culture, they are able to express typical small intestinal enzymes (e.g. lactase) and transporters (e.g. SLCs), secrete lipoprotein particles and form desmosomes, apical occluding junctions and an organized brush border with microvilli [65, 66]. In terms of their transporter gene expression profile, they approximate that of the jejunum and may therefore not be a suitable model for colonic gene expression [65]. In addition, relying on results from such isolated cultured cells leaves out the influence of many cells that occur in the natural in vivo setting, such as paneth cells, crypt cells and goblet cells [66].

Mapping gene localization and expression for all regions of the GIT is in light of the aforementioned important since the different segments indeed do have such varied physiology, molecular machinery and expressional profiles, while still being a part of the same organ system. Different molecules are absorbed or secreted in different parts of the GIT’s anatomical regions, such as in the proximal or distal parts of the duodenum or ileum. Examples include vitamin B12 and bile acid absorption, which occur in the distal ileum, and gut-derived satiety-regulating hormones such as ghrelin, CCK, peptide YY (PYY) and Oxyntomodulin [20, 67]. Common diseases such as inflammatory bowel diseases and cancers of the GIT are also known to more often affect certain regions of the GIT, and in some cases more frequently its proximal or distal areas. For example, Crohn’s disease more often affects the terminal ileum [68], possibly due to local microbial alterations [69]. Different types of e.g. gastric or colorectal cancers can occur either proximally or distally [70], and their proximodistal location may give them distinct gene expression patterns [71].

The importance of various regions of the GIT is exemplified by a closer examination of the appendix. Inflammation thereof, appendicitis, is an annoyance for many people, as it afflicts a tiny appendage (only 5-10 cm in
length (2-20 cm range); diameter of 5-10 mm), often affecting people of younger age – regardless of healthy or unhealthy lifestyle – for no apparent reason. Removing the appendix, i.e. an appendectomy, is typically performed without complications and often prophylactically (as it can reoccur and be life-threatening if left untreated). It constitutes the most commonly performed emergency general surgical procedure in the U.S. [72]. The procedure is in fact so common and generally uncomplicated, that the patient is nowadays often able to leave the hospital on the day of, or the day after, the surgery has taken place. As elegantly described in a recent book by biologist Robert Dunn, an appendectomy at least used to be something that surgeons or other doctors did not think twice about performing [73], believing that the appendix was something that we would have been better without in the first place (not an irrational thought as 12-23 % nevertheless have to remove it at some point in life [72]). Even Darwin remarked so³, believing that the appendix was a human remnant of the cecum of other apes, diminished in size through evolution as it no longer served any digestive purpose [74]. But surprisingly, gut bacteria, also small in size and “recently” rediscovered in their plethora of roles for human health and not only disease, also seem to be linked with the function of the appendix.

An interesting recent study confirms a theory that was developed by William Bollinger and Randy Parker at Duke University [75]. This theory holds that the role of the appendix is to serve as a reservoir of gut bacteria. This reservoir would come in handy in times of illness, specifically illnesses that afflicted the GIT and resulted in diarrhea or other conditions that disrupted or literally would wash away the normal gut flora.

The recent study referred to in the last paragraph does not seem to support the appendix as being just an evolutionary hiccup. This was evidenced by the fact that it seems to have evolved a minimum of 32 times, only having been lost less than seven times, in the 361 mammalian species that were examined [15]. Evolutionary appearance of the appendix did furthermore not seem to be correlated with any change in e.g. diet, social group size, activity pattern, fermentation strategy or anatomical features, although the latter were related to the size of the appendix. The fact that evolutionary gain outnumbered evolutionary loss of the appendix, indicated, as stated by the authors, that the role of the appendix remained of biological relevance. This role was concluded to be immunological, as a reservoir of the microbes that constitute the normal healthy gut flora, as previously hypothesized by Bollinger, Parker

³ In his book “The Descent of Man and Selection in Relation to Sex”, Darwin describes the Appendix in the following words: “That this appendage is a rudiment, we may infer from its small size … Not only is it useless, but it is sometimes the cause of death, of which fact I have lately heard two instances: this is due to small hard bodies, such as seeds, entering the passage, and causing inflammation.” [74]. At least the last part may be considered somewhat correct.
and others [75, 76]. This goes to show that even the tiniest structure may serve an important function in the GIT, emphasizing the importance of studying this organ system in its entirety, analogous to studies that stress the importance of being, or studies that in themselves are, hypothesis-generating rather than hypothesis-driven.
Adhesion GPCRs

Since the discovery of its first member Rhodopsin over a quarter of a century ago, it has been determined that GPCRs, or G-protein coupled receptors, represent one of the largest superfamilies of membrane bound proteins, the others being SLCs (see next section), voltage-gated ion channels and tyrosine kinase receptors [77]. There are more than 800 GPCR members, sorted into the five subfamilies: Rhodopsin, Secretin, Adhesion, Glutamate, Frizzled/Taste2, according to the GRAFS nomenclature system [78]. By far the largest of these receptor families is the Rhodopsin receptor family, with its approximately 670 proteins in humans [78]. The second largest GPCR family in humans in the Adhesion family, also referred to as the long N-terminal seven transmembrane receptors related to family B (LNB-7TM), which includes 30 members in rats and mice and 33 members in humans [79]. With the phylogenetic grouping of Adhesion GPCRs based on the 7TM regions, it became evident that there are seven groups [80].

GPCRs are characterized by having a transmembrane (TM) region consisting of seven $\alpha$-helices that span the plasma membrane (see Figure 1) [78, 81]. These form a receptor with a binding cavity for a ligand, but the extracellular segment may also be able to bind a ligand. GPCRs are involved in a high number of physiological functions, including development, neurotransmission, metabolism, reproduction, immune responses, and behavior. The signals that GPCRs convey and the ligands that activate these receptors vary greatly and can be endogenous – such as amines, peptides, proteins, lipids, nucleotides and neurotransmitters – as well as sensory, e.g. organic odorants, pheromones, tastes and photons [78, 81].

After binding of an extracellular ligand leading to GPCR activation, the receptors undergo changes in their conformation [22], initially small in their TM core, but larger on the intracellular side. This alters their communication with membrane-bound effector molecules, e.g. the G-proteins, which are then more readily bound to the GPCR on the inside of the cell membrane, and which ultimately results in cellular signaling networks and cellular responses [81]. The G-proteins coupled to GPCRs are heterotrimeric guanine nucleotide-binding proteins, consisting of three subunits ($\alpha$, $\beta$ and $\gamma$), which dissociate upon GPCR-mediated activation. After dissociation, depending on the type of $\alpha$ subunit, this subunit may continue cell signaling through ade-
nylate cyclase activation/inhibition (AC; increasing/decreasing intracellular cAMP), phospholipase stimulation (PLC; leading to diacylglycerol and inositol triphosphate generation) or Rho stimulation, another G-protein. The β and γ subunits form a Gβγ complex that can signal through AC, PLC, phosphatidylinositol 3-kinase gamma (PI3Kγ) and G-protein-regulated inwardly rectifying potassium channels.

The main feature of the Adhesion family is the long N terminus with a complex domain architecture which is thought to be highly glycosylated and form a rigid structure in the outer part of the protein. This extracellular portion contains the GPCR proteolytic site (GPS) and several various domains that can also be found in other proteins such as cadherin, epidermal growth factor, immunoglobulin, lectin, olfactomedin, thrombospondin and domains [80]. The GPS domain is referred to as an intracellular cleavage motif, pivotal for the protein transport from the endoplasmic reticulum to the membrane, while several other N terminal domains play important roles in the receptor-ligand binding as well as cell-to-cell and cell-to-matrix adhesion [82, 83]. Only a few members of Adhesion GPCRs have been demonstrated to interact with G-proteins [84, 85]. Distinguishing themselves even more from other GPCRs, Adhesion GPCRs are genomically complex, with each receptor having many isoforms [86], multiple alternatively-spliceable introns and large genomic sizes, making them difficult to study [87].

Established and potential roles of GPCRs in the GIT

GPCRs in the GIT are known to be involved in e.g. nutrient balancing, cancer, chemosensation, food intake regulation and regulation of the immune system [22, 88-93]. In some cases their gross expression patterns in the GIT have been established, but more subtle proximodistal and intercellular variations in expression and their biological functions have yet to be determined [94]. Dysfunction of Adhesion GPCRs is however already known to contribute to certain diseases affecting the GIT. For example, CD97 transcript overexpression has been associated with colorectal cancer [95], rectal adenocarcinoma recurrence and metastasis [96], and gastric carcinoma [97]. GPR56 has been found to be overexpressed in colon cancer, pancreatic cancer and esophageal cancer, and seems to be important for cancer cell adhesion [98, 99]. GPCRs from other families are also important for GIT physiology and pathology, where several GPCRs have been implicated in IBD pathogenesis and as potential drug targets for the conditions sorted under this umbrella term [100]. Furthermore, the GIT contains chemosensory enteroendocrine cells that can produce neuronal and hormonal signaling by either sensing luminal contents through GPCRs and secreting hormones that activate GPCRs in the GIT or throughout the body [22]. Many hormones that regu-
late energy metabolism and food intake are produced in the GIT and bind to GPCRs, and are currently being studied as potential pharmaceuticals to treat obesity and type-2 diabetes (T2DM). These hormones include GLP-1 (already available for treatment of T2DM), ghrelin, pancreatic polypeptide (PP) and peptide YY (PYY) [25]. The promising potential of these hormones partially stems from the fact that their levels are altered in e.g. obesity (lower levels of PP and PYY) and following weight loss induced by bariatric surgery (decreased ghrelin; increased GLP-1 and PYY) [25]. Finally, many cancer cells aberrantly overactivate or overexpress GPCRs, such as in colon cancer, in which e.g. EP1-EP4 have been linked to cancer growth [93] and GPR49 has been related to an increased incidence of primary tumors [101].

Although about 85% of Adhesion GPCRs are still orphans or lack biological characterization (further details about Adhesion GPCRs can be found in the review by Yona et al. [86]), some functions and roles have been attributed to these receptors. In addition to those described above for GPR56 and CD97, BAI1 is known to be involved in angiogenesis and in host responses to gram-negative bacteria [102, 103], whereas the EMR receptors have been shown to be involved in immune responses [104]. Crohn’s disease and ulcerative colitis are the two main IBDs affecting the GIT, and there is a growing body of evidence indicating that the onset of these pathologies may be related to an immune system deregulation by the ENS or the gut microbiota [41, 105]. Bearing in mind both the large body of evidence implicating GPCRs in normal GIT physiology and pathology, and the roles that Adhesion members have in e.g. immune regulation, it is not inconceivable that many of the Adhesion GPCRs will prove to have essential functions in the GIT, e.g. in the case of IBD pathogenesis and persistence, highlighting the importance of characterizing this receptor family in the GIT.
Solute carriers

Of the entire human proteome, membrane bound proteins account for more than a quarter (27%) [106]. Of these, solute carriers (SLCs) constitute the second largest superfamily. With its close to 400 members, SLCs make up the largest group of transporters, which also include other classes such as ion channels, water channels, ABC transporters and pumps [77]. A transporter that shares at least 20-25% of the amino acid sequence of the members of an SLC family is assigned as a member of that family [107]. SLCs are named using the abbreviation “SLC”, followed by the family number (e.g. SLC1), a separating letter (most often A), and finally the family member number (e.g. SLC1A7). In 2008, researchers at our research unit clustered 15 SLC sub-families into four main phylogenetic groups: α- β- γ- and δ-groups, the largest being the α-group which includes seven SLC families [77]. About 40% or 120 of the discovered SLCs remain orphans with unknown substrates and/or physiological function [77]. The majority of the SLCs are located in the plasma membrane, but apart from the nucleus, these transporters are also found in the membranes of synaptic vesicles, peroxisomes and mitochondria (see Figure 1).

SLCs function as passive or coupled transporters and exchangers, translocating molecules over the cell membrane, with passive transporters only translocating molecules along their concentration gradient, while exchangers often are driven by the cellular gradient of sodium. However, ATP does not actively drive SLCs. Nevertheless, counter-transport is indirectly derived via ATP-hydrolysis, driving gradient-producing active transporters. Nutrients such as sugars, amino acids and fatty acids, vitamins, neurotransmitters, nucleotides, inorganic ions, essential metals and drugs are among the diverse categories of substances transported by SLCs [77, 108].

The importance of e.g. amino acid transport across cell membranes can be exemplified by the sheer overrepresentation of such transporters among the SLCs, with over 60 known and perhaps in total as many as 100 possible amino acids transporters. This implies that such transport may have arisen multiple times throughout history, with a potential need for over-redundancy [77].
SLCs in the gastrointestinal tract

The importance of SLCs for the absorption of nutrients and pharmaceuticals in the gastrointestinal (GI) tract is well established. For example, the transporter SGLT1 (SLC5A1) utilizes secondary sodium-coupled transport to allow intestinal uptake of glucose and galactose [11, 12]. For each sugar molecule absorbed, two sodium ions are carried across the intestinal membrane and this transporter is able to generate a 10,000-fold gradient of glucose concentration across the luminal intestinal membrane. After glucose absorption, the monosaccharide exits through the other side of the enterocyte, its basolateral side, by employing GLUT2, another SLC transporter (SLC2A2). Uptake of the sugar fructose does not however rely on SGLT1, rather being carried into the enterocyte by another GLUT transporter, the passive GLUT5 transporter. Whereas GLUT5 is a weak glucose transporter, the facilitated diffusion transporter GLUT2 on the basolateral side enables both glucose and fructose to flow out of the enterocyte along their concentra-
tion gradients into the bloodstream for further energy utilization and metabolism. Uptake of peptides can instead be accomplished by PEPT1 (SLC15A1), a transporter that takes up all possible di- or tripeptides, including many peptidomimetics [109]. The transporter is so efficient that dipeptide solutions are more rapidly absorbed than single amino acids from the GIT lumen, and this also enables drugs employing PEPT1 transport, e.g. ACE-inhibitors (blood pressure-lowering) and aminoccephalosporins (antibiotics) to be rapidly and almost completely absorbed when taken orally.

Mutations in SLC genes have been linked to congenital chloride diarrhea [110], Hartnup’s disorder [111, 112], glucose galactose malabsorption [11] and lysinuric protein intolerance [113]. Moreover, expression levels of SLCs have been reported to be altered in inflammatory bowel disease (IBD) [114] and colon cancer [115, 116]. In IBD, Wojtal et al. found that of 15 studied SLC transporter mRNA transcripts, several were expressed at higher levels in the ileum and colon of IBD patients, e.g. PEPT1, the serotonin transporter (SERT; SLC6A4) and the equilibrative nucleoside transporter 1 (ENT1; SLC29A1) [114]. Meanwhile, other transcripts were found to be decreased in IBD compared with control subjects, including the carnitine transporter OCTN2 (SLC22A5). Intriguingly, the genes were also differentially expressed in IBD patients depending on whether inflammation was present or not, and most of the investigated genes had previously not been implicated in inflammation or IBD pathogenesis.

Glucose galactose malabsorption (GGM) is a very well-characterized disorder resulting from faulty membrane transporter function. The condition results in severe diarrhea that presents at a neonatal age and can result in death if not treated with water and electrolyte therapy [12]. The condition is alleviated if lactose (which is broken down into the following two sugars), glucose and galactose are removed from the diet. The two monosaccharides glucose and galactose are as described above absorbed by the SLC transporter SGLT1, and it was later indeed confirmed that mutations in the SGLT1 gene cause GGM [11].Interestingly, in Hartnup’s disorder and cystinuria, both conditions with mutations in specific SLC amino acid transporter genes (SLC6A19 in Hartnup’s disorder and SLC7A9 or SLC3A1 in cystinuria [111, 117]), the previously described PEPT1 transporter can ensure survival by allowing intestinal uptake of the afflicted amino acids as dipeptides [109], highlighting the aforementioned importance of a redundant amino acid transport system.

Linking SLCs and GPCRs in the GIT, it was known that expression of the SLC SGLT1 was regulated by concentrations of luminal monosaccharides. It was postulated based on current evidence that this regulation might involve a GPCR, able to sense sugar concentrations in the lumen of the GIT. Sweet
receptors of the T1R type of GPCRs have indeed been confirmed in the GIT at the mRNA and protein level [118], and were later shown to be necessary for regulation of SGLT1 mRNA and protein levels, and the absorption capability of glucose in mice [119]. Both SLCs and GPCRs have also been implicated with similar roles in the pathogenesis of IBD, where they may transduce signals generated by ligands of the gut microbiome, thereby maintaining intestinal homeostasis, microbiome-host relationships and normal immunity [100].
From high-fat and high-sugar diets to obesity

Fats in our diets provide us with approximately a third of our daily dietary energy supply [1]. Fats are however not only important as a dense storage medium and for generation of energy, but are also essential components of cell membranes and as precursors for a variety of molecules, while also being involved in e.g. the regulation of metabolism, immunity, food intake and circadian rhythms [120-124]. Fats consist of fatty acids (FAs), which most often are part of molecular compounds that have a high solubility in organic solvents [125]. These compounds are called lipids and comprise fats, fixed oils and waxes. FAs can exist as free molecules (non-esterified) in the body, but are more often bound by ester bonds to glycerol, cholesterol or phospholipids. These esterified FAs constitute over 90% of all FAs in human plasma [126]. The combination of an FA molecule (acyl group) that is linked to glycerol is per definition called an acylglycerol [1] and the majority of fats in our bodies and our diets occur as three FAs bound to one glycerol molecule, called triacylglycerols (TAGs) [1, 126].

Depending on their occurrence or number of double bonds, FAs can be divided into those that are saturated (SFA – no double bonds), monounsaturated (MUFA) and polyunsaturated (PUFA) [1, 125]. The typical western diet has an FA distribution where SFAs constitute a third to a half of all FAs, and PUFAs constitute one tenth to a third of the FAs [127]. The major dietary MUFA is the 18-chain oleic acid (OA, 18:1n-9), whereas the major PUFA is linoleic acid (LA, 18:2n-6). Of the PUFAs, there are two classes, n-3 and n-6 (also known as omega 3 and omega 6, respectively). These are named according to how far away the last double bond is from the terminal methyl end of the PUFA [128]. The n-6 LA and the n-3 α-linolenic acid (ALA, 18:3n-3) are two PUFAs which have to be supplied by the diet as mammals cannot produce these FAs endogenously – these FAs are therefore referred to as essential fatty acids (EFAs).

Fat – from the gastrointestinal tract to the fat depot

More than 90% of the fat in our diets occurs as TAGs, i.e. FAs esterified to all three hydroxyl groups of glycerol, but our diet also contains lipids in the
form of non-esterified fatty acids (NEFAs), cholesterol, cholesterol esters and phospholipids [12, 129]. Most of the fatty acids in the diet are long-chain fatty acids (LCFAs) of 12 or more carbon atoms (the most common ones being C16, C18 and C20 of carbon length 16-20) [12]. As dietary fats are insoluble in the water-rich environment of the GIT, the process of emulsification has to take place prior to continued fat digestion and absorption, which thereafter is extremely (>95%) efficient [12, 130]. While fat digestion is initiated in the stomach by lingual and gastric lipase, this process only contributes to approximately 15% of fat digestion, and is rather continued in the duodenum. Here bile acids, together with pancreatic enzymes such as pancreatic lipase, are secreted. Bile salts, cholesterol and phospholipids help stabilize the emulsified fats as smaller emulsion droplets with larger surface areas [12]. This produces an oil-water interface that increases the action of the lipases, breaking down the fat droplets from the outside towards their core. Pancreatic lipase thereby hydrolyzes dietary TAGs into FAs and 2-monoacylglycerol (2-MAG); cholesterol esterase splits dietary cholesterol into FAs and free cholesterol; and phospholipase A2 breaks down dietary phospholipids into FAs and lysophospholipids [12, 126]. After these enzymatic breakdown products together with bile salts have formed aggregates called micelles – structures that are soluble enough to travel past a physical barrier known as the unstirred water layer – the products are able to move to the brush border membrane of absorption cells, or enterocytes. However, short- (SCFAs) and medium-chain FAs (MCFAs) do not require micelles to reach the enterocyte.

SCFAs are naturally formed in the colon [123]. The process of SCFA formation is dependent on the fermentation process (anaerobic breakdown) of the colonic microbiota, which produces the 1-6 in carbon length SCFAs by fermenting oligosaccharides, polysaccharides, proteins, peptides and glycoprotein precursors. The major source for the fermentation is however resistant starches [123]. The fermentation primarily occurs in the cecum and proximal colon where there is a high number of bacteria and substrate availability. This process is also responsible for creating an acidic environment that is hostile to potential pathogens. SCFAs produced in the colon are rapidly absorbed either by diffusion [35, 123], or by employing transporters of the SLC 16 family, known as the monocarboxylate transporters (MCTs) [131, 132]. Metabolism of absorbed SCFAs primarily occurs at three different sites in the body: in the liver for gluconeogenesis, in muscle as oxidation substrates, or in the epithelium of the cecum and colon as a major (60-70%) source of energy. While the energy produced from fermentation products in the colon may not be of great significance for people in affluent societies, it has been hypothesized to be able to serve as an important source of energy for people afflicted by poverty in third world countries [16]. The SCFAs are also able to trigger secretion of the glucose-regulating hormone GLP-1, thus
possibly enabling regulation of energy metabolism via fatty acids in the colon [26].

Dietary fatty acids were previously thought to only be taken up by the small intestine through passive diffusion. Research has however uncovered that there are distinct transporters that partake in long-chain fatty acid (LCFA) absorption in the GIT [133, 134], in addition to the absorption of colonic SCFAs carried out by the aforementioned MCTs. These proteins belong to a set of proteins collectively denoted as lipid-binding proteins (LBPs) [135]. The LBPs include plasma membrane fatty acid binding proteins (FABP_m), FAT/CD36\(^4\) and the transporter FATP4, which is the fourth member of the SLC27 FA transporter family (SCL27A4) [135, 136]. The expression of these genes seems to be regulated by the peroxisome proliferator-activated receptors (PPAR), which are nuclear receptors that are activated by LCFA\(s\) [137] and LCFA\(s\) are thereby able to increase LBP gene expression [135]. With regards to the role of SLC27a4 or FATP4, recent findings however indicate that FATP4 (SCL27A4) may not be necessary or responsible for intestinal FA absorption [130]. It is not located at the brush-border membrane, and deleting FATP4 expression in the intestine did not appear to affect the absorption of lipids, nor did it protect from HFD-induced obesity [133]. This implies that FATP4 may be dispensable for intestinal FA transport, but not necessarily that it is without function in this context [130].

The intestinal mucosa has a very high turnover rate and this is regulated by the nutrients in the diet. Lipids have been found to be the strongest inducer of cell-renewal of the intestinal mucosa, which in the rat can undergo hypoplasia after just a couple of days of fasting [135, 138]. Comparing mice fed either a high-fat (HFD; 40% fat by weight) or a control diet (3% fat by weight) for just three weeks, it has been found that the HFD drastically alters intestinal lipid handling. The HFD-fed animals showed an increased uptake of linoleic acid compared with control-fed animals, also having enhanced but reversible intestinal cell proliferation and an upregulation of genes involved in FA absorption and lipoprotein synthesis, e.g. Fatp4, Fabps, Apoa-Iv, and Fat/Cd36 [135]. Gene expression levels were also found to return to baseline values, except for Fapt4, once the control diet replaced the HFD. These findings show that the intestine can quickly adapt to a HFD by increasing its absorption and handling capacity of FAs.

\(^4\) CD36 is also responsible for “tasting” fat in the tongue, contributing to fat taste preference and perception, and partaking in the initiation of the early cephalic phase of digestion (Abumrad and Davidson [133] Down-regulation of this protein occurs after high-fat feeding, and has been proposed as a mechanism for increased fat consumption – if proven correct a sort of diet-induced “tolerance” for fat.
Once absorbed through the apical enterocyte membrane, further handling of FAs depends on their chain length [126, 129]. Medium and short-chain (under 10 carbon atoms in chain length) FAs are able to pass directly through the enterocytes to the portal vein for uptake into the circulation. FAs with a carbon atom chain length equal to or exceeding 12 are instead together with 2-MAG passed to the endoplasmic reticulum (ER), with the help of a fatty acid-binding protein, for resynthesis into TAGs [12, 133]. These are then incorporated into lipoproteins known as chylomicrons (CM), which also contain phospholipids and proteins, notably among them the key apolipoprotein Apo B-48 (a truncated form of another lipoprotein, Apo B-100). Apolipoproteins are present on the surface of lipoproteins and both solubilize and regulate the transport of the lipoproteins and their lipids.

Lipoproteins can transport lipids in the form of TAGs, phospholipids and cholesterol esters (CEs) [139]. CMs are only produced by the intestine, and once synthesis is complete, the CMs are secreted by exocytosis at the basolateral side of the enterocyte, entering the bloodstream after initial passage through the lymphatic system [12]. The TAGs in CMs are then released throughout the body through hydrolysis by the enzyme lipoprotein lipase (LPL), located at capillaries [133]. The released free fatty acids (FFAs) are then absorbed by peripheral tissues, for continued metabolism, e.g. energy utilization in muscle and storage in adipose tissue, whereas glycerol is returned to the liver [126]. The chylomicrons themselves are through the action of LPL converted into chylomicron remnants, which under physiological conditions are removed by the liver after having bound to the LDL receptor and other proteins (e.g. hepatic triglyceride lipase, HTGL) [139]. The liver is then able to secrete other lipoproteins, such as very-low density lipoproteins (VLDLs). These are instead characterized by the lipoprotein Apo B100. VLDLs incorporate triglycerides stemming from liver uptake, newly synthesized FAs, as well as cholesterol, and carry these to peripheral tissues. The TAGs in VLDLs are hydrolyzed when the particles enter plasma, once more utilizing LPL for this breakdown, and VLDLs also transfer some TAGs to HDLs in exchange for CE. Through these actions, the VLDL particles successively turn into IDLs (intermediate-density lipoproteins). Not all IDLs are removed by the liver, and these particles can be further broken down into low-density lipoproteins (LDLs) – rich in cholesterol and CE. These are mainly (70-80%) catabolized through the LDL receptor (LDL-R) pathway. Once in the adipose tissue, fatty acids in TAGs constitute by far the largest energy reserve in the body (often in the hundreds of thousands of

\[LPL\] is also expressed on macrophages that reside on the wall of blood vessels and which have been implicated in the atherogenic potential of lipoproteins, perhaps due to their high LPL expression [139, 140].
calories), each gram being convertible into 9 kcals of energy, more than twice the energy content of each gram of protein or carbohydrates (4 kcals) [126, 141].

**Obesity**

Obesity⁶ is a serious health concern as it increases the risk of pathological conditions and negative outcomes such as type-2 diabetes (T2DM), dyslipidemia, hypertension, coronary heart disease (CHD), stroke, metabolic syndrome, liver and gallbladder disease, cancer, osteoarthritis, cognitive decline, sleep disturbances, and overall mortality [143-152]. Even simply being overweight (BMI 25-28.9 kg/m²) has been associated with a 72% increase in the risk of cardiovascular disease (CVD) [153]. The risk also seems to increase as BMI increases, as the same study found an almost two-fold increase for individuals with a BMI of 29-32 kg/m². Another study found no increase in CHD for just overweight subjects, but the number of comorbidities (hypertension, gallbladder disease, high blood cholesterol, osteoarthritis) increased with increasing weight class [151]. Being either overweight or obese was especially associated with increased risk of hypertension and T2DM – for the latter, the risk increased for overweight men and women between 3 and 4 times, respectively, and up to about 18 and 13 times for the most obese group. The link between excess body weight and the metabolic syndrome has also been clearly established, as 59.6% of obese and 22.4% of overweight men could be diagnosed with the condition in an American population [152]. In contrast, it was only present in 4.6% of normal-weight men.

However, with regards to overall mortality, large meta-analyses – the most recent looking at 2.88 million individuals with over 270 thousand deaths, have found little or even reduced mortality in overweight subjects [154]. Obese individuals [149] or at least those severely obese (BMI ≥ 35 kg/m²), instead appeared to have an increased mortality [154]. Proposed mechanisms of a protective effect of overweight or grade I obesity (BMI 29-32 kg/m²) as found in the study by Flegal et al., were a metabolic reserve, cardio-protective effects of fat tissue or higher likelihood of receiving better medi-

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⁶ When referring to the “Obesity epidemic”, the term obesity is typically (and thus also here-in) used as an umbrella term for all those classified as having a body mass index (BMI)>25.0 kg/m². Using the common health indicator BMI, a healthy weight is defined as a value between 18.5 and 24.9 kg/m² [142] Per definition, overweight is instead defined as a BMI spanning 25 to 29.9 kg/m², whereas actual obesity is defined as a BMI equal to or exceeding 30 kg/m². BMI is calculated by dividing weight in kilograms (kg) by the squared height in meters (m²).
cal care. Previous studies have however indicated higher mortality due to overweight and obesity. Calle et al. looked at over 1 million Americans followed for 14 years, registering a total of 201,622 deaths, and found that the within-normal-weight BMIs of 23.6-24.9 for men and 22.0-23.4 for women were associated with the lowest death rates [155]. A smaller study indicated the optimal BMI as being 23-25 for Caucasians and 23-30 for Afro-Americans and furthermore that obesity depending on its severity could reduce longevity by 5-20 years [156].

Notwithstanding the debatable increase or decreases in mortality due to obesity or optimal BMI, it may produce a vicious circle with regards to weight gain. The very brain circuits tasked with regulating the feeding and feeding-related reward behaviors, such as the orbitofrontal cortex (OFC) and amygdala, may be damaged by obesity, possibly by producing increased inflammation [157]. A study from our lab has also found reductions in obese vs. normal-weight subjects in regional brain gray-matter volume (GMV), particularly in the left dorsolateral-prefrontal cortex (DLFC), which is associated with successful appetite regulation [150], and which our more recent meta-analysis also finds to be less active in obese vs. normal-weight subjects when viewing images of food [158]. Exacerbating the situation even more, fat cells can be gained during the vulnerable period of childhood, but once adulthood has been reached, research suggests that our fat cell numbers stay constant [160]. At an adult age, there is instead a constant annual fat cell turnover of about 10% – implying a precise regulation of this vast cell population. Spalding et al. further found that even following substantial weight loss, only the size of adipocytes was further subject to change in adults. Furthermore, once obesity has set, losing weight may develop into a constant battle with persistent hunger sensations (strong enough throughout millions of years to ensure survival until this day). In an elegant study from 2011, Sumithran et al. studied subjects enrolled in a 10-week weight loss trial and who were followed for one year while maintaining a weight-neutral diet. The authors found that one year after weight loss, levels of the satiety-promoting hormones leptin, insulin, peptide YY and cholecystokinin remained low, and the hunger hormone ghrelin was increased, all thereby significantly shifted towards a state favoring weight regain [161]. Not surprisingly then, so were also rated hunger sensations, which were also increased at the one-year follow up.

7 The path to obesity may indeed be set in childhood. Of overweight children, most (77%) continue to stay obese as adults, a figure that can be compared with the ten times lower risk (7%) for children that were classified as lean [159]. Notably, in the study by Freedman et al., the risk for being obesity in adulthood was even higher for overweight children of even younger age (<8 years old).
Causes of obesity

It is generally accepted that the cause for the current obesity epidemic, as it is often called, is multifactorial [7]. It is believed to involve metabolic, cognitive, genetic, environmental and societal factors [7, 162-165]. The exact etiology of the development of obesity in today’s society is however unknown. Stripped of all actual complexity, whatever develops into obesity does so by producing an imbalance of input and expenditure of energy, with the latter literally outweighing the former [166]. The body has elaborate neural and peripheral systems that actively aim to regulate food intake and energy expenditure in order to maintain energy homeostasis, whereby under most long term conditions (with the exception of growth and child-bearing) the equation “energy intake – energy expenditure” cancels out or approximates zero. Maintaining the current obesity epidemic in the U.S. has e.g. been calculated to require a daily energy surplus of 220 kcal (0.9 MJ) per person [166]. The energy imbalance that has led to the current state of U.S. body weight surplus might however be more than a magnitude lower, weighing in at a merely 7.16 kilocalories (30 kJ) per day, or close to the amount of calories contained in one gram of alcohol. To lose weight, one rule has been frequently touted: that by reducing average daily energy intake by about 500 kcal (2 MJ), this would result in weight loss of about 0.5 kg per week (based on the rule that a pound of fat (454 grams) contains about 3500 kcals of energy) [166]. This assumption, which has even been promoted by government agencies such as the U.S. National Institutes of Health (NIH), has however been contested by recent estimates by Hall and colleagues. As the body adapts dynamically to weight loss, changing the energy requirements for physical activity, its resting metabolic rate and also the amount of metabolically more expensive lean tissue, the relationship between energy intake reduction and weight loss turns out to be more complex. Using the model by Hall et al., a 100 kg man adhering to a daily 500-kcal reduction in his diet would not lose 22 kg of weight in one year, but rather (and alas) only half as much.

During attempts of weight loss the body’s energy homeostasis will be disrupted in a manner that elicits compensatory mechanisms designed to strongly defend a set body weight by reducing energy expenditure and increasing appetite [167]. As recently demonstrated these changes can persist under long-term weight reduction maintenance, even when previously lost weight has been partially regained [161]. These compensatory mechanisms are believed to have been under strong long-term evolutionary pressure to ensure an adequate level of adiposity and supply of nutrients for survival in an environment where procurement of food might be dangerous and difficult [168]. The currently most important known hormones that partake in this energy homeostasis and convey the status of current energy stores include insulin,
leptin and ghrelin [167]. Circulating levels of the two former hormones correlate positively with adipose tissue mass, and both leptin and insulin are considered as satiety-suppressing or anorexigenic hormones. In contrast, ghrelin instead shows a negative correlation with adipose tissue mass and constitutes a satiety-enhancing or orexigenic hormone. Whereas insulin is secreted by beta cells of the pancreas in response to nutrients, especially glucose [169], leptin is secreted by the adipose tissue [170]. As such, most obese patients have higher levels of this hormone compared with lean subjects, but this does not successfully suppress appetite to the point of inducing weight reduction. The cause for this may be that obese subjects display resistance to the anorexigenic effects of higher circulating leptin concentrations [171], just like central obesity often leads to insulin resistance [148]. Finally, ghrelin is primarily produced by cells in the stomach, rises before meals, and is able to increase food intake in both rodents and humans [172-174].

A large body of research has argued for the main drivers of the global rise in obesity prevalence as being the simultaneous changes to activity patterns and our food system, which today promote passive obesity by increasing the accessibility to energy-rich food, high in both fat and sugar. Together, the aforementioned two factors have been able to combine forces especially in the last 40 years, a period during which the greatest increase in obesity prevalence has taken place [7]. The availability of fats and refined carbohydrates, e.g. sugar, did indeed start to increase by the 1970s, concurrent with the beginning of the increase in obesity prevalence.

High-fat and high-sugar foods, while rich in energy but unfortunately often lacking in nutrients, are today readily purchasable at more-than affordable prices for many of those afflicted by obesity. These foods are generally considered as highly palatable, in fact so palatable that they may, at least in vulnerable individuals, be able to act in an addictive manner [175]. The sight of or intake of food does indeed activate the same brain reward-associated regions that e.g. cocaine activates, and even more so in obese individuals, and this has been hypothesized to contribute to the obesity epidemic. Food addiction, in which food acts similar to the action of illicit drugs of abuse, is however not an uncontested model [158, 175, 176]. Whatever the case may be, palatable foods are nevertheless at least hard to resist and tend to be overconsumed, especially under situations of physical [177] and social stress [178], against what would seem to be the purely rational choice.

Some researchers have recently gained increasing traction for an old but long-neglected hypothesis: that it is sugar and the marked increase in added sugar consumption that has been at the heart of driving the contemporary increase in the prevalence of obesity [179]. Sugar consumption has indeed
increased dramatically during the last century, having tripled worldwide during the 1899-1957 period, and it has increased in many countries now afflicted by the obesity epidemic [179-181]. Daily kilocalories from sugar now almost amount to about 500 in many countries, about a quarter of the daily energy requirements of an adult person [179, 182]. Added sugar contains equal amounts (if it is not high-fructose corn syrup, HFCS) of glucose and fructose. It is the latter type of monosaccharide that has come under attention for being especially obesogenic.

Not only is fructose inherently sweeter than glucose, making fructose-containing sugars like syrup and sucrose popular additives to foods for increasing palatability [184], but fructose has also been found to act like alcohol in the liver, e.g. by promoting the storage of fat as well as inflammation [185]. As hinted at in the previous paragraph, that fructose might be obesogenic and be metabolized like alcohol is an old observation [186], but it is only more recently that the mechanisms have begun to be unraveled. In comparison to glucose, fructose and alcohol are able to overload the liver with calories, when comparing equal caloric ingestion of glucose, sucrose and alcohol [185]. Fructose also only weakly stimulates insulin [185, 187], a hormone that among its vital roles also confers signals of satiation [169, 188, 189]. Fructose is however avidly taken up by the liver, where its different metabolism as compared with glucose is able to induce hepatic fat accumulation (activating de novo lipogenesis, DNL), dyslipidemia (with small, dense LDL and hypertriglyceridemia), impaired hepatic energy utilization, hepatic and peripheral insulin resistance with elevated glucose levels, and intra-myocellular lipid accumulation [185, 187]. Indeed, a recent RCT found that fructose may induce features of the metabolic syndrome, simultaneously increasing blood pressure [190]. The latter may have occurred through an increase in uric acid, known to be fructose-inducible, as lowering uric acid levels reduced blood pressure. Finally, fructose may, just as alcohol, elicit addictive brain responses while promoting a hormonal appetite balance favoring CNS hunger signaling [185]. This is supported by a 2013 study where the effect of fructose ingestion compared with glucose ingestion was compared in terms of the resulting regional brain blood flow changes. Fructose appeared to activate the brain differently than glucose, producing a lower deactivation in food-regulating brain regions such as the hypothalamus [191]. A high-fructose diet given to rats can even worsen memory impair-

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*While fruits also contains fructose, and are “banned” in some diets such as the popular low-carbohydrate high-fat diets (LCHF), fruit intake has been associated with a decrease in body weight [183], likely by providing a high fiber content and being more slowly digested. Fructose intake from natural sources such as fruits, vegetables and grain products has furthermore not increased but decreased in the years trailing the obesity epidemic [182].*
ments induced by a diet deficient in n-3 fatty acids [192] - a dietary situation that someone binging on sugary foods conceivably may experience.

Regarding its relevance for the obesity epidemic, sugar consumption has indeed increased dramatically throughout the last 150 years, and this has also been the case for fructose. For U.S. adolescents, daily fructose consumption is currently at 73 g (12% of caloric intake), up from a daily global sugar consumption of only 15 g before the beginning of the 1900’s [180, 193]. The most notable threefold increase occurred in the first part of the 20th century, but importantly, overall for all ages, average fructose intake continued to increase in the 1977-2004 period, by a more modest 32% [182]. At the same time, calorie intake from sugar-sweetened beverages (SSBs) almost doubled for children aged 2-18 and more than doubled for adults [4]. Today SSBs provide the greatest proportion of added fructose and amount of calories from beverages in the U.S. diet [4, 182] – interestingly with alcohol in second place (for adults). However, while the obesity epidemic has taken place in the last couple of decades and fructose intake has increased in parallel, the proportional increases in daily caloric intake (18%) and overall carbohydrates (41%) have nevertheless been even greater [182]. Promisingly, added sugar intake in the U.S. seems to have decreased by 25% during the latest decade, promisingly alongside a status quo for the daily calorie intake and a flattening out of the obesity prevalence [194, 195].

Animal studies where 60% of calories are provided by fructose have shown that this monosaccharide can also induce obesity as well as features of the metabolic syndrome (hyperinsulinemia, hypertriglyceridemia, and hyperuricemia) [196]. Other studies have failed to see fructose-induced weight gain, but intriguingly instead found that a high-fructose diet exacerbated subsequent weight gain when animals were switched to a high-fat diet, possibly by inducing leptin resistance [197]. Intake of SSBs, including fruit juice, is associated with an increased risk of obesity in children, but this effect may be accounted for by excessive energy intake9 [204]. This is also supported by a meta-analysis of controlled feeding trials, which found that overfeeding with fructose compared with non-fructose carbohydrates leads to significant weight gain in humans. The effects were however not seen under isocaloric conditions and most analyzed studies were of short (all <12 weeks) duration [204]. An older meta-analysis looking at 88 studies also

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9 Soda may not be good for several reasons. Not only does it break down the enamel of shiny teeth, but this effect seems to happen irrespective of whether the soda contains actual sugar or is artificially sweetened (diet soda) [198]. Furthermore, while there are studies refuting the link between intake of SSBs and obesity [199-201], the findings of these studies have been questioned as the authors have been found to have ties to e.g. the beverage industry [202, 203].
supported a link between SSB intake and increases in energy intake as well as body weight [205]. A meta-analysis of over 300,000 participants by Malik et al. from 2010 has nevertheless found that intake of SSBs was associated with an increased risk of developing the metabolic syndrome (20% increase) and type 2 diabetes (26% increase) [181], when comparing the subjects with the highest and lowest intakes of SSBs.

While diet and sedentarism constitute the classical culprits for causing obesity, other research suggests that chemicals in our environment, such as endocrine-disrupting chemicals (EDCs), may play a significant role [165, 206], potentially especially through early-life exposure [207]. EDCs that may lead to obesity and insulin resistance in humans include hexachlorobenzene [208], the DDT metabolite dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs) [209], as well as phthalates [210], and bisphenol A (BPA). For example, urinary concentrations of the BPA, a chemical found in plastic containers and in the lining of metal containers (e.g. those very same ones for drinks that are high in sugar and also blamed for causing obesity), have been found to be higher in urine of overweight and obese children, as well as adults [211, 212]. Notably, almost all U.S. citizens have been shown to be exposed to BPA. This exposure may almost exclusively (99%) occur through ingested foods [212]. Furthermore, research suggests that BPA may accumulate in fat tissue [213], which could possibly exacerbate any obesity-promoting effect of such an EDC, but also complicate the possibility of teasing out cause and effects with regards to higher levels in obese subjects. The 2012 U.N. report on EDCs and their consequences for human health highlighted the link between EDCs and obesity, among other serious conditions such as diabetes, prostate and breast cancer, and Parkinson’s and Alzheimer disease [214]. In the report, the authors declared that the still ongoing increase in metabolic disorders could not be fully explained by other already established risk factors.

Sleep may also be a contributor to the current problem of global body overweight. The quality and quantity of sleep can influence glucose metabolism, energy expenditure, appetite, food intake and appetite-regulating hormones, as well as how the brain responds to rewarding food stimuli [177, 215-220]. Sleep deprivation has also recently been shown to produce changes in the expression pattern of genes involved in e.g. metabolism, and to decrease the sensitivity of adipose tissue to insulin [217, 221], in sum providing many potential mechanisms through which sleep can influence the risk of becoming obese. Such an association has also been shown: for each hour of increase in average sleep time, the risk of being or becoming obese may be decreased by 24-61% [222, 223]. In another study, every hour of shortened sleep at age 5-11 was instead associated with an increase of 0.93 in BMI at age 32 [224]. As many of the described possible culprits for the obesity epi-
demic, the average sleep time has also changed in parallel with the rise of the obesity epidemic, so much indeed that this by itself has been described as an epidemic on a global scale [225]. Average sleep time has by some estimations declined by more than an hour per night, with almost a third of working age Americans (30-64 years old) reporting that they get less than 6 hours of sleep each night [225].

Meanwhile, whereas several early studies had indicated (and more recent studies continue to indicate) a high heritability of obesity, implying a strong genetic component, this has so far not been born out in the large-scale studies [226] into the genetics of obesity that followed the completion of the human genome project [227]. So far, the genetic variants found responsible for explaining why throughout the general population some people are susceptible to obesity only account for slightly more than one percent of the variation in BMI, with variants of the gene FTO having the most pronounced effect [226, 228, 229]. This is however far from what studies had suggested should be the heritability of obesity, namely around 40-70%, the discrepancy possibly attributable to the fact that the common form of obesity is a complex polygenetic condition. In contrast, there are some monogenic forms of obesity, albeit rare but in some cases extreme such as those caused by mutations targeting leptin, and genetic studies of these conditions have provided important insights into the complex pathways that regulate appetite and energy metabolism [226, 230].

Our genetic contribution comes from our parents, who also to a great extent determine the environment predating birth (prenatal) and moreover generally the early decades of our lives. This environment can clearly be decisive for the development of obesity [159, 160]. The prenatal and early-life environment may be crucial for obesity susceptibility by permanently setting obesity-contributing factors in an unfavorable direction [231], encompassing factors such as CNS appetite regulation, food preference, resting metabolism through differential skeletal muscle (fiber) composition and metabolism, and the tendency for non-exercise activity thermogenesis (NEAT; a kind of energy expenditure through small, often non-overt movements, e.g. fidgeting-like activities) [231]. Together with early-formed food and activity habits shaped by an environment with high-energy food intake, and which promotes sedentarism, this may explain why, when set early in life, it can be so difficult to deviate from the path to obesity.

The effects of obesity on the body
Pathophysiologically, obesity is associated with elevated fasting insulin levels [169] and insulin resistance, which is also at the core of the metabolic syndrome. Insulin resistance and the metabolic syndrome with elevated glu-
Cose levels have foremost been associated with obesity of the upper body or abdominal region – termed central obesity [143, 148, 232]. In a complex interplay, insulin resistance by itself further increases the risk of obesity and of the metabolic syndrome, the latter being diagnosed based on the presence of some out of a constellation of risk factors (abdominal obesity, dyslipidemia, hypertension, insulin resistance) [190]. The metabolic syndrome is a condition already afflicting more than a third of the adult U.S. population and has been associated with a 78% increase of cardiovascular events or death [233].

Oxidative stress [234], as well as pro-inflammatory molecules, such as tumor necrosis factor-α (TNF-α), C-reactive protein and interleukin 6 [235, 236], are also increased in obese subjects, and inflammation may already be increased in early adulthood obesity [237]. A pro-inflammatory state is especially associated with central obesity – typical of the so-called metabolic syndrome – in which there is an excess of body fat at the abdominal subcutaneous and visceral fat depots [232, 238]. Abdominal obesity also leads to dyslipidemia, marked by increasingly higher levels of so-called “small, dense” low-density lipoprotein (LDL) particles and lower levels of high-density lipoproteins (HDLs) [148]. It is especially this type of obesity and its inflammatory state that may be detrimental to metabolic and cognitive health [238, 239], whereas gluteo-femoral or lower body obesity may instead be protective [240, 241].

Obesity in men is often associated with higher degrees of abdominal obesity (“an apple shape”) than in women, who instead tend to have higher fat accretion around the gluteo-femoral region (“pear-shaped obesity”), although following menopause women tend to increase their amount of abdominal fat due to decreasing estrogen [240]. Men may therefore, at least at a lower age, have a higher risk of the negative metabolic outcomes of obesity as observed in epidemiological studies. Indeed, the risk of the metabolic syndrome rises as much as 60% in women after menopause [240]. Recent evidence also supports the existence of underlying metabolic gender differences in the different adipose tissue depots, with men having a higher expression of fatty acid transporters in their abdominal subcutaneous adipose tissue (SAT), as compared with females [242]. While women had a higher free fatty acid (FFA) uptake in their SAT than men, only men showed a higher intake in their SAT compared with their femoral fat depot. Such regional differences have not been observed with regards to FFA release from different adipose tissue depots, and therefore lend support to the observation that men more easily can accrue fat in their upper body fat depots.

The release of non-esterified fatty acids (NEFAs) from the adipose tissue has in many studies found to be enhanced in people who are obese [232, 238],
although the evidence for this has recently been disputed [243]. NEFAs are released during normal fasting by means of lipolysis of the stored pool of triacylglycerols in adipocytes, and serve as the main source of energy during fasting. In spite of higher insulin levels that accompany obesity and which would normally inhibit release of excess NEFAs by suppressing lipolysis, as there is a simultaneous resistance to insulin NEFA release has been indicated to occur at higher-than-normal levels in obesity. The excess of NEFAs has several negative effects, e.g. producing muscle insulin resistance and promoting excess fat storage in the liver [232], a condition that is on the rise and is associated with obesity [244]. The liver also becomes more insulin resistant due to excess fat accumulation and therefore does not respond adequately to the action of insulin, thereby contributing to an increase in blood glucose levels in subjects without an adequate insulin secretion. By promoting a fatty liver, an increase in NEFAs may also exacerbate the inflammatory state produced by the visceral adipose tissue, by promoting hepatic production of inflammatory mediators such as cytokines and PAI-1 [232, 238, 245]. Finally, the insulin resistance that NEFAs contribute to in the liver leads to an increase in VLDLs and LDLs, producing an atherogenic lipid profile associated with an increased risk of CVD. Obesity itself is further associated with a prothrombotic state with abnormalities in fibrinolytic and coagulation factors, decreased levels of adiponectin (which could serve as an anti-inflammatory molecule), increased plasma glucose and elevated blood pressure [232]. The increased body weight seen in obesity does not only increase the mass of adipose but also of supporting lean tissue, in combination increasing oxygen demand to non-physiological levels, thus putting an excessive burden on the cardiovascular system and ultimately restructuring the heart to produce left ventricular hypertrophy – a condition present in up to 70% of obese women with hypertension [143, 246, 247].

More recent theories however contest the idea of increased NEFA release as the major causative factor for insulin resistance. Rather, they suggest that what may be detrimental in obesity is when fat deposition is dysregulated or fat oxidation is impaired [243, 248]. In this scenario, when the subcutaneous adipose tissue does not function properly, excess fatty acids are stored in sites such as the visceral adipose tissue, skeletal muscle, the heart and liver [248]. This is described as ectopic fat deposition and may induce insulin resistance through a mechanism called lipotoxicity. E.g., increased deposition of fat in skeletal muscle\(^\text{10}\), intramyocellular lipid accumulation, is closely associated with impaired insulin action [243], and impaired fat oxidation may also contribute to insulin resistance [243, 251].

\(^{10}\) That skeletal muscle is important for glucose metabolism becomes evident when considering that this tissue is responsible for 70-90% of the insulin-mediated glucose uptake [249, 250].
Regarding potential negative effects of obesity on the central nervous system (CNS), obesity has been associated with an increased risk of e.g. Alzheimer disease (AD) and depression [252, 253]. As obesity is also related to an increased risk of stroke and cardiovascular disease, and as several cardiovascular risk factors confer a higher risk of developing mild cognitive impairment (MCI) – a condition that by itself confers risk for later developing AD – there appears to be a link between these conditions [252].

Both MCI and AD are characterized by brain atrophy that can even precede the clinical onset of these conditions, and studies have also found that certain brain regions show signs of injury and atrophy in obesity [150, 157, 252, 254]. Compared with normal-weight elderly subjects, those who were obese were found to have signs of atrophy in several brain regions [252]. The study was based on 94 cognitively healthy adults (mean age 77.3 years) with similar morbidity at 10-year post-brain scan follow up, and found that even being just overweight at old age was associated with atrophy in e.g. the basal ganglia and corona radiata. In a subsequent study, authors from the same group looked at 700 patients with MCI or AD, and found that the relationship between brain structure and obesity also held true for this population. The brain tissue reduction was more generalized throughout the brain, each one-unit increase in BMI being associated with between 0.5 and 1.5% lower brain volume. Furthermore, higher BMI was associated with ventricular expansion in AD but not MCI patients. The authors concluded that the findings provide further evidence that obesity also affects the risk of developing cognitive impairment [255]. A study from researchers at our lab looking at 292 subjects also found a decrease in gray matter volume in obese compared with normal-weight subjects between the ages of 70 and 75 [150].

Alarmingly, there is also evidence that neuronal injury already occurs in overweight and obese young adults. Mueller et al. found a negative correlation between serum levels of the neurodegenerative marker neuron-specific enolase (NSE), and gray-matter density in hippocampus and cerebellum in overweight and adult subjects aged 20-41 [237]. Such associations between NSE and brain regions involved in memory and cognition were not however seen in the normal-weight controls. Furthermore, another recent study suggests that visceral obesity may be especially responsible for these brain volume reductions, and intriguingly only found associations for younger and not older obese subjects [256]. Possible mediators of cerebral injury in obesity may be hypertension, type 2 diabetes and hyperinsulinemia, resulting in neurotoxicity from decreased amyloid clearance and glycation end-products that produce neurofibrillary tangles [252, 255]. Since the associations however remained even after having adjusted for fasting plasma glucose levels and
T2DM, this suggests that other mechanisms may also be involved in the possible detrimental effects of obesity on the brain.

Prevalence and cost of obesity

Given the negative metabolic, clinical and societal, as well as economical [144] outcomes of obesity – essentially an energy surplus that in forlorn days was something that the brain of every animal strived for – the global trend of increasing obesity is all the more a cause for great concern. Diseases can largely be categorized into two categories, the first being non-communicable diseases (NCD), mainly constituted by cardiovascular disease, diabetes, respiratory disease and cancer, and are set apart from the second category of communicable diseases, such as HIV, malaria and TBC. In a 2008 WHO report, it was stated that deaths from NCDs outnumbered those from communicable diseases in 2005, as they amounted to an estimated total of 35 million. Since these diseases are considered to mainly be caused by the four factors unhealthy diet, smoking, physical inactivity and harmful use of alcohol, an estimated 80% of these deaths should in fact be preventable [257].

With a current overweight world population of almost 1.5 billion people and a third of these people being classified as obese [8], the state of affairs is even worse in the U.S., with over two thirds (68.8%) of the adult American population now being classified as either overweight or obese, and more than one third (35.7%) as being obese [194]. While the obesity prevalence for adult men and women in the U.S. has doubled over the last three decades, the percentage of obese children has in fact tripled during the same time period [258, 259]. Fortunately, the trend of an increasing prevalence of obesity at least seems to be leveling off during the most recent decade [194], promisingly so also in younger populations [260] for which recent data even suggest a slight decline at least in the U.S. [261].

Notwithstanding some positive signs of an abating increase in obesity prevalence, based on current trends, it has recently been predicted that the global prevalence of overweight and obesity will continue to rise for several decades to come [262]. In 2030, an estimated extra 65 and 11 million more obese adults in, respectively, the U.S. and United Kingdom alone, has been calculated to result in an extra 5.7-7.3 million cases of stroke and heart disease, 6-8.5 million cases of diabetes and 492-669 thousand additional cases of cancer [262]. Before the year 2050, by taking obesity prevalence and associated life expectancy into account, the average life expectancy in the U.S. has even been suggested to possibly start declining from its so far continuous increase [263].
The cost of this is projected to be 26-55 million quality-adjusted life years (QALYs), and will carry with it roughly 50-70 billion USD higher medical costs. The current dismal trends in obesity prevalence have even come so far as to make a number of governments impose taxes on unhealthy food, as was recently the case in Denmark and is currently the case in Hungary [264, 265], measures that recent science says should be able to have an impact on the consumption of unhealthy types of food.\textsuperscript{11}

\textsuperscript{11} According to the recent study by Mytton et al., as food consumption is generally quite insensitive to variation in prices, a tax of at least 20 % would be required to have a substantial impact on cardiovascular events and obesity [265]. For example, a 20 % tax on sugary drinks in the U.S. would lower the prevalence of obesity by about 3.5 %.
Diet-induced obesity in animal models

To gain an understanding of the mechanisms and consequences of obesity caused by the modern diet, characterized by high fat and sugar content, animal models can serve as an essential tool [266-268]. Not all humans [269], nor all animals, gain weight in an obesity-promoting environment with easy access to highly palatable food, and understanding this phenomenon and its underlying causes is central to nutritional and obesity research. Certain diets, especially those high in fat [267], can induce obesity in a range of different animal species, ranging from rats to monkeys as reviewed by West and York [270]. Through diet, it is also possible to induce obesity in animals using diets with lower fat content, but this does not appear to be as effective [271, 272].

Alongside the obesity epidemic in humans, there has been an increase in the average weight of at least 24 examined populations of animals, including rodents and primates in laboratories [273]. This intriguing phenomenon begs the question whether some environmental factor may also be driving obesity in these animals, or whether epigenetic factors, able to act across generations [274], may have responded to our direct or indirect obesogenic treatment of these animals.

High-fat diets for modeling diet-induced obesity

That diets high in fat can induce obesity in animals as well as humans has been known for a long time [275, 276], as extensively reviewed by e.g. Hariri and Thibault [6, 267, 268, 277]. The rationale for using laboratory animals to investigate the obesity-inducing effects of such diets is therefore scientifically justified [267]. It was in the middle of the 20th century that the diet-induced obesity (DIO) model first was established, where Ingle gave a palatable ad libitum diet to Sprague-Dawley rats, and as the title of the article succinctly states, apparently found “A simple means of producing obesity in the rat” [276]. The area progressed a couple of years later with the use of high-fat diets (HFD) containing 5-50% fat to induce obesity in mice [278, 279], in what later became known as “dietary obesity” [267].

In 1970, Schemmel et al. studied the tendency of seven different rat strains to gain obesity on a HFD [280]. Of the seven studied, it turned out that only one strain did not become obese in response to this dietary treatment. The HFD was found to be able to increase body weight from 44-55 grams at weaning to 346-693 grams 20 weeks later (the two heaviest strains at that point being the Osborne-Mendel and the Sprague-Dawley), compared with an increase to 304-445 grams for the grain-fed rats of the same strains. West et al. found similar results in mice when they studied nine inbred strains fed
either a HFD (32.6% energy from fat) or a chow diet (11.6% energy from fat) [266]. Six strains (e.g. C57BL/6J) increased in body weight and adiposity, whereas three strains were unresponsive. The observed body weight increase in response to a HFD was interestingly not due to hyperphagia (increased food intake), as in terms of food energy at the end of the 7-week treatment, the mice consuming the HFD (6/7 strains) actually ate less compared with the mice of the same strains that were fed the chow diet.

To achieve even greater weight gain than in response to a HFD in rats, in 1976 Anthony Sclafani and Deleri Springer produced the first “cafeteria diet” (CAF diet). This diet was meant to simulate our western diets, with an assorted (and regularly changing) selection of palatable foods such as “chocolate chip cookies, salami, cheese, banana, marshmallows, milk chocolate, and peanut butter.” (in addition to the chow, high-fat diet and sweetened milk that was always available to the rats) [275]. Both HFDs and cafeteria diets are used today for the study of the mechanisms underlying obesity susceptibility and the consequences thereof [135, 281-286]. For HFDs, the percentage fat content varies widely (20-60%) but is typically between 40 and 60% by energy contribution – lower than the initial studies done half a century ago, where the percentage reached up to 85% – as extensively reviewed by Buettner et al., and by Hariri and Thibault [267, 268]. Buettner et al. concluded that HFDs with over 40% of energy from fat and not too extreme amounts of n-3 omega acids are suitable for inducing obesity in animal models [268].

As access to HFD can result in DIO, the reverse, that lowering fat intake can decrease body weight, should also be true. Research both using animal models and in humans does indeed support this notion [286-288]. In their recent meta-analysis looking at cohort studies and randomized controlled trials in human subjects, Hooper et al. found that simply reducing total fat intake could produce an average weight loss on the order of 1.6 kg [288], without having a negative effect on metabolic parameters. Notably, simply replacing fat by simple\textsuperscript{12} (i.e. not complex) sugars may not however produce significant weight loss or improve metabolic health in obese subjects with the metabolic syndrome [289]. A comparable weight loss to that seen in the analysis by Hooper et al. may be hard to achieve in humans just by increasing energy expenditure [290], as the average energy surplus, suggested by the average increase in body weight over time, is too great (300-600 kcal/day) to be overcome by reasonable daily increases in physical activity, according to Katan et al. Even though low-fat and other diets (such as low-carbohydrate diets) may lead to weight loss, this typically seems to plateau after six

\textsuperscript{12} Simple sugars or monosaccharides include glucose, galactose and fructose, whereas complex sugars or oligo-/polysaccharides include starches and cellulose [126].
months in humans [291], a phenomenon also observed in rats [286]. Even though the weight loss achieved might be modest, it still contributes to considerable health benefits, such as improved insulin resistance, dyslipidemia and hypertension [292].

The obesity-prone Sprague-Dawley model
In 1983 Levin et al. presented data for a model of Sprague-Dawley (SD) rats, which when switched to an ad libitum HFD high in energy, gained weight in a differential manner [293]. Whereas about half gained significant amounts of weight and developed obesity, the other half was apparently resistant to such diet-induced effects [294]. These categories have since been respectively referred to as being diet-induced obese (DIO) or obesity-prone (OP), and diet-resistant (DR) or obesity-resistant (OR) rats. The OP/OR model using SD or Wistar rats has since its inception been extensively used to model genetic, metabolic and physiological mechanisms underlying DIO development [294-300]. Compared with OR rats, rats with the OP phenotype have been found to have a lower sympathetic tone [293, 294] with lower expression of uncoupling proteins that are important for oxidizing excess fat [301]. In addition, studies have found that OP rats have lower total energy expenditure, coupled with lower expression of fat oxidation enzymes and lower relative fat oxidation [295, 296, 302-304]. At the endocrine level, they show higher insulin and leptin levels and defective leptin signaling in the brain [285, 294, 305], whereas at the transcriptomic level they display a proadipogenic expression profile [297, 306, 307]. Recent studies also support a defective dopaminergic reward system in OP rats that could lead to reduced reward from food intake and thereby promote the hyperphagia that is typical of OP vs. OR animals [308, 309]. Previous research has furthermore also indicated that OP and OR animals have significant differences in their FA profile. A study by Li et al. looked at transcriptomic differences between OP and OR Wistar rats and found that there were significant differences between the two phenotypes after the 16-week HFD treatment, e.g. with increased oleic (18:1n-9) and stearic acid (18:0) in plasma, and higher palmitic (16:0) and palmitoleic acid (16:1n-7) in the liver.

Mechanisms underlying HFD-induced obesity in humans
The mechanisms for why diets high in fat may produce obesity may be several [267]. For one, it may be difficult for the body and the food intake regulating brain regions to accurately assess variation in the amount of fat consumed, as it is, in relative terms, generally much lower than the amount of fat stored in the body [141, 231]. In addition, humans may typically consume the same volume of food, possibly making energy density – more than twice as high for each gram of fat compared with carbohydrates or protein – a de-
fining determinant in daily energy intake variation [6]. Other theories for obesity development due to HFDs include a less flexible oxidation adjustment in response to varying amounts of fat, altered meal patterns and poor satiation or sensation of fat [267].

As an example, on a daily basis the human body has to deal with approximately 60-90 grams of fat intake. This is lower than the daily intake of carbohydrates at 150-300 grams, and roughly equal to daily protein intake of 60-100 grams [141]. However, the body only stores about 400-800 grams of carbohydrates in mostly muscle glycogen stores, and the daily protein turnover is on the order of 300-400 grams. Assuming an energy density of 7700 kcals for one kilogram of fat tissue and a man weighing 80 kg with a body fat percentage of 20 (a rather slim individual by modern standards13), this would mean that the fat stores of this individual would equal almost 125 000 kcals. In relative terms, fat ingested on a typical day for such an individual would be 150-230 times lower than the energy content of that person’s fat depots, compared with a factor of only 2-4 for the ratio of carbohydrate intake to carbohydrate stores. This larger discrepancy between stored and ingested energy for fat has thus been hypothesized to underlie a greater ability for dietary fat to lead to obesity [141, 231].

Metabolic and cognitive effects of HFDs

With regards to their implications in the genesis and pathogenesis of obesity, there is a wealth of scientific evidence to prove that high-fat diets may be detrimental both in terms of the weight trajectory [277] as well as to metabolic and cognitive health [254, 268].

Metabolic effects

In skeletal muscle, HFDs may induce insulin resistance by altering the expression of genes involved in the oxidative phosphorylation (OXPHOS) of mitochondria, the essential “power plants” of our cells. By taking muscle biopsies from metabolically healthy males before and after a 3-day HFD treatment, it was observed that PGC1α and PGC1β, genes involved in mitochondrial biogenesis, decreased by 20 and 25%, respectively. OXPHOS-related genes (e.g. SDHB of complex II and SLC25A12) were also downregulated [282]. The changes were mirrored by those seen in a parallel experi-

13 Measuring adiposity or obesity in humans is not routinely done using measures that calculate percent body fat as these are more expensive and not as readily available as those required to simply calculate BMI. However, the cut-off for obesity for men is at 25% body fat; for women at 35% [232].
ment conducted in C57BL/6J mice that were put on a 3-week HFD, although
to an even greater extent, perhaps owing to the longer treatment. Similar
expressional changes have also been reported in skeletal muscle of predia-
betic and type-2 diabetic subjects [310, 311]. HFDs may however not induce
peripheral insulin resistance through decreased mitochondria, as a later study
found that the number of mitochondria actually increased while insulin re-
sistance developed in response to a HFD, thus allowing for greater fat oxida-
tion [312]. In line with this evidence, a contemporary study found that HFDs
might cause insulin resistance by contributing to excessive beta oxidation of
fatty acids, ultimately causing mitochondria to fail [251].

Both HFDs and CAF diets also appear to alter gene expression in adipose
tissue and the liver, including genes involved in energy metabolism (e.g.
UCP3, DGAT, SCD), adipose tissue differentiation, transcription factors
(e.g. Pparγ) and signal transduction [283, 307, 313, 314]. HFD treatment
may also result in activation of anti-apoptotic genes while downregulating
apoptotic-inducing genes [313] and cellular stress-related genes in the adi-
pose tissue [283]. Mice that were subjected to an 8-week HFD also showed
higher leptin, insulin and corticosterone levels in combination with central
resistance to peripheral leptin administration [314]. Additionally, exposure
to a HFD may decrease the adipose and plasma levels of the fatty acid pal-
mitoleic acid (16:1n-7), thus decreasing the possibly metabolically beneficial
effect of this FA (see later section) [121].

The cafeteria versus high-fat diet
Recent research also supports a more weight-inducing effect of CAF diets,
coupled with an even more adverse metabolic and inflammatory response. In
a study from 2011, a high-fat and CAF diet were compared with a chow diet
regarding their ability to induce metabolic and inflammatory changes in
Wistar rats [281]. Both the CAF diet and the HFD produced hyperglycemia,
insulin resistance, increased non-esterified FAs (NEFAs) plasma levels,
macrophage infiltration into adipose tissue (typically seen in obesity) and
signs of liver steatosis (fatty liver) and pancreatic islet dysfunction. These
changes were however for most parameters more pronounced in the CAF
diet group. The CAF diet group continued to gain weight throughout the 15-
week dietary intervention, indicating homeostatic feeding dysregulation,
whereas the HFD group initially decreased food intake when the treatment
was initiated and had a weight trajectory that appeared to level off after 7
weeks, even though this group also gained significantly more weight than
the control group.
CNS and cognitive effects of HFDs

Several recent studies have found that diets high in fat, some also high in sugar, can produce inflammatory or neurodegenerative effects in the brain of both humans and rodents [254, 315]. In the study by Posey et al., the inflammation was associated with local accumulation of stearic and palmitic acid in the hypothalamus, but the link to specific types of FAs may be complex [316]. The inflammatory effects due to a HFD can already be seen within 24 hours, much quicker than the typical timeframe for HFD-induced inflammation in peripheral tissues [254]. By activating local inflammation, HFDs are therefore hypothesized to be able to produce an insulin- and leptin-resistant state in the hypothalamus and increase the risk of DIO caused by HFD access [316]. Blocking this hypothalamic inflammation has in animal studies been shown to prevent DIO from occurring. Looking at the epidemiological level, a study from 2011 looking at 1233 older people found an association between high-calorie diets and the development of mild cognitive impairment, a condition that can precede Alzheimer disease (AD) [317]. A previous study had however only found associations between calorie and fat intake, and the risk of developing AD, in those patients carrying the allele APOE ε4 [318].
Desaturases – fat metabolizers and health indicators

Individual fatty acids (FAs), dietary FA intake or FA composition in e.g. plasma, adipose tissue, muscle and liver have been linked with metabolic regulation – encompassing everything from oxidation to energy storage – and pathological states such as obesity, insulin resistance and cognitive health [121, 192, 319-327]. Important for regulating the FA profile are the enzymes involved in de novo lipogenesis, desaturation and elongation of FAs [127, 328].

FA composition can be measured in various tissues and lipid fractions, e.g. in plasma or serum, liver, muscle and adipose tissue. Within hours of a meal the proportion of FAs in serum triglycerides can be affected [127]. Changes in cholesterol esters and red blood cell phospholipids can instead be seen over a couple of days. Finally, long term changes in FA composition, occurring over the span of months to years, are seen in the adipose tissue [329].

Some FAs can be newly synthesized using a pathway called de novo lipogenesis (DNL), which uses the fatty acid synthase (FAS) enzymatic pathway [330]. DNL primarily yields palmitic acid (16:0) and mainly occurs in the liver, but has also been found to occur in the adipose tissue [328]. FAs produced by DNL, along with other FAs provided by the diet, can be used for fat storage and energy by the body [126]. These FAs can however also serve as substrates for producing FAs of longer carbon chains and more double bonds. As the properties of FAs are modulated by their chain length, isomeric form and how unsaturated they are, these longer FAs can therefore also have important differences in their physical, chemical and biochemical properties [126, 331]. The enzymes carrying out FA elongation and desaturation are appropriately called, respectively, elongases and desaturases [331]. These enzymes are located in the endoplasmic reticulum (ER) and the FAs produced by these enzymes can either be long-chain (LCFA; 16 or 18 carbon atom chains) or very-long chain (VLCFAs; 20 or more carbon atom chains) [120]. These enzymes are furthermore regulated by a variety of factors such as diet, hormones, and circadian rhythms, as extensively reviewed by e.g.
Elongases

Elongation is carried out in the ER by four individual enzymes, of which the rate-limiting are the initial condensing enzymes, the elongases, also known as ELOVLs (Elongation of very long chain fatty acids) [120]. The elongases function in a manner similar to FAS, as their enzymatic action is to add mal-
onyl-CoA, thereby elongating the FA chain by two carbon units in each elongation step. Seven mammalian elongases have been identified so far (ELOVL1-7) and like desaturases, the different ELOVL proteins appear to have different FA substrate preferences [120]. Saturated FAs and monounsaturated FAs are the preferred substrates for ELOVL1, ELOVL3, ELOVL6 and ELOVL7, whereas polyunsaturated FAs are preferred by ELOVL2, ELOVL4 and ELOVL5. The different elongases also display different expression patterns, with the genes Elovl1, Elovl5 and Elovl6 being ubiquitously expressed. In contrast, Elovl2, Elovl3, Elovl4 and Elovl7 appear to have more tissue-specific expression patterns, which could be due to tissue-dependent demands of VLCFAs [120]. Mutations in ELOVL genes can furthermore lead to specific alterations in the FA profile, and these enzymes seem to have important functions for lipid homeostasis in e.g. liver and adipose tissue, and more specifically also for producing VLFCA for membrane integrity, during cold exposure and for normal skin-barrier function [120, 330].

Desaturases

The mammalian body has three desaturases, the delta-5 (D5D, \( \Delta 5 \)), delta-6 (D6D, \( \Delta 6 \)) and delta-9 (D9D, \( \Delta 9 \)) desaturase. The last one is however more commonly referred to as stearoyl-CoA desaturase (SCD) and is arguably the most studied of the elongases and desaturases. The desaturases derive their prefix delta number from the position at which they introduce double bonds in the FA chain [120]. Since mammals do not have delta-12 and delta-15 desaturases, the two PUFAs linoleic acid (LA) and \( \alpha \)-linolenic acid (ALA) need to be provided by the diet for synthesizing longer PUFAs (e.g. arachidonic and eicosapentaenoic acid) in the mammalian body [120].

Stearoyl-CoA desaturase 1

SCD is responsible for producing MUFAs from SFAs, introducing a single double bond at the delta 9 position of FAs [120]. The preferred substrates for these enzymes are the SFAs oleic acid, and to a somewhat lesser extent, palmitic and [332]. The SCD enzymes exist in several isoforms, with e.g. four known isoforms in mice, all with specific expression patterns. Scd1 is expressed in brown and white adipose tissue, liver and sebaceous glands. Scd2 is almost ubiquitously expressed with the exception being the liver. Scd3 is expressed in sebaceous, Harderian and preputial glands and Scd4 is expressed in the heart [332]. In rats, there are two known isoforms, of which both are present in brain, liver, kidney and testis, whereas Scd1 is exclusively expressed in adipose tissue.

The effects of SCD-1 deficiency have been extensively studied in mice, in which those made deficient in SCD-1 exhibit several interesting phenotypes.
These mice are e.g. resistant to diet-induced obesity (DIO) [320, 333], have lower adiposity [320], display greater insulin sensitivity [334], but may have either decreased or increased inflammation [335, 336]. SCD-1 seems to be intimately linked with the adipose-derived hormone leptin and may function as a switch between lipogenesis and oxidation of FAs [333, 337]. Recent evidence furthermore seems to implicate a skin-specific function for SCD-1 in regulating DIO, as targeting the loss of SCD-1 to the skin was able to protect mice from DIO on a high-fat diet [338], whereas this was not the case in mice with an isolated or combined adipose- and liver-specific deletion of SCD-1 [339].

**Palmitoleic acid – a possible “lipokine”**

Interestingly, although loss of SCD-1 seemingly can protect from DIO in mice, the product of this enzyme, palmitoleic acid (16:1n-7), has been found to function as a “lipokine”, able to stimulate the action of insulin in liver and muscle [121]. This FA was furthermore found to be able to suppress hepatic SCD-1 activity, thereby decreasing hepatic synthesis of triacylglycerols and preventing FA accumulation in the liver. The finding by Cao et al. from 2008 is also interesting as dietary intake of 16:1n-7 is low and rarely estimated, yet the 16:1n-7 is the second most abundant MUFA in human adipose tissue [332]. Moreover, recent findings suggest that the release of 16:1n-7 may be greater from gluteo-femoral fat, which could be part of the explanation for why lower body fat may be more beneficial than that in the upper and abdominal region, as the level of 16:1n-7 also was inversely correlated with insulin resistance [241]. There are nevertheless conflicting reports regarding whether 16:1n-7 is associated with improved or worsened insulin sensitivity (for a review see Hodson and Fielding [332]). For example, a prospective study of 50-year old men found that higher serum proportions of palmitoleic acid, as well as other saturated FAs, was associated with an increased risk of both cardiovascular and overall mortality [324].

**Desaturase and elongation indices**

By using the ratio of the product to the precursor of the different desaturases and elongases, indices of these enzymes can be calculated. The desaturase indices have been employed to obtain approximate values of desaturase enzyme activities in other tissues and have been shown to reflect the mRNA expression of the desaturase enzymes in e.g. subcutaneous adipose tissue and certain lipid fractions [241, 340, 341].

There are two SCD-1 indices that are commonly employed: SCD-16 (calculated from the ratio 16:n-7/16:0) and SCD-18 (18:n-9/18:0 ratio) [331]. The delta-6 desaturase index can instead be estimated from several different ratios (e.g. 18:3n-6/18:2n-6 or 20:3n-6/18:2n-6) of which the 18:3n-6/18:2n-6
ratio appears to be the more common one [342-344]. Finally, to calculate the delta-5 desaturase index, the ratio 20:4n-6/20:3n-6 is employed. Indices for FA elongation can also be calculated, e.g. using the ratio 18:1n-7/16:1n-7 or 18:0/16:0 [241].

Fatty acids and desaturases: Links to metabolic and cognitive health

The desaturases have been found to correlate with measures of adiposity in rats, where, although not commonly used for laboratory animals, BMI was correlated with SCD-1 indices in genetically obese WNIN/Ob and WNIN/GR-Ob – mutant inbred Wistar rats that within a year can achieve a weight of more than 1 kg before (on average) dying six months later [345]. The more than two times heavier (736 vs. 314 grams) WNIN/Ob rats had higher SCD-16 indices in plasma, liver and adipose tissue depots compared with lean Wistar rats [346]. This was also the case for the WNIN/GR-Ob strain and in both of these extreme rat strains the SCD-16 and SCD-18 ratios were highly correlated with measures of adiposity – BMI and an adiposity index (AI). Similar observations have also been made in human subjects, where SCD-16 and D6D have been positively correlated with BMI and HOMA-IR (a measure of insulin resistance), whereas the association for D5D with these parameters was the opposite [344].

In both prospective and cross-sectional studies, associations have been found between high intake of saturated fatty acids (SFAs) and low levels of LA in serum, and the metabolic syndrome (often referred to as MetS) [323, 347]. In the study from 2005, Warensjö et al. found that a high SCD-1 and D6D desaturase index, but a low D5D index, significantly predicted development of the metabolic syndrome during the 20-year follow up. In both of the studies, looking at the same cohort of men, low serum proportions of LA was also predictive of later developing this condition. Looking at the possible outcome of such conditions, Laaksonen et al. found that having a higher intake of both LA and PUFAs was associated with a lower risk (61% and 62%, respectively) of dying of cardiovascular disease [348]. Similar risk reductions, although more pronounced with regards to PUFAs, were also seen for LA and PUFAs when analyzing the serum proportions of esterified fatty acids. The risk reduction also carried over for overall mortality. These findings resonate with those of Warensjö et al., who found an increased risk of overall and cardiovascular mortality in men with low serum LA and PUFA proportions. The risk was instead increased in men with high proportions of SFAs. Furthermore, a high SCD and D6D index, but a low D5D index, also predicted both overall and cardiovascular mortality [324]. High SCD activity, as estimated by the SCD-16 ratio, was the strongest predictor of mortality.
(30% risk increase per 1-SD increase). This was hypothesized to be attributable to a high SCD-1 activity being indicative of an increased lipogenesis, ectopic fat deposition and insulin resistance, or alternatively to be secondary to increased SFA intake [324].

In skeletal muscle, an organ essential for regulation of glucose metabolism, FA accumulation has been linked with the severity of insulin resistance. A study in rats found that SFAs as opposed to PUFAs were able to induce insulin resistance both in vitro and in vivo [349]. Additionally, the SFAs increased the SCD-16 index and SCD-1 protein level in the skeletal muscle, whereas there was no difference in these variables between the control- and PUFA-fed animals. A study in humans found that regular aerobic exercise decreased n-6 PUFAs in skeletal muscle [350]. Simultaneously, the proportion of oleic acid (OA; 18:1n-9) to palmitic acid (16:0) increased, perhaps indicating greater membrane fluidity, and these changes may have been associated with the improvement in insulin sensitivity seen after the exercise intervention. Additionally, baseline FA composition in skeletal muscle has been associated with insulin resistance in humans, both in Caucasians and Pima Indians [351]. Insulin resistance has been found to correlate positively with a high proportion of SFA, such as palmitic acid, and palmitoleic acid, but a low proportion of PUFAs such as LA in skeletal muscle [351].

Regarding FAs and cognitive health, a recent study from our lab looking at 252 elderly people found that the self-reported dietary intake of the n-3 PUFAs eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) was associated with cognitive performance and global gray matter volume [325]. The serum concentration of DHA has also been associated with cognitive functioning in mid-life adults (34-54 years old) [352]. At older ages, a plasma FA composition low in n-3 PUFAs has also been found to increase the risk of developing Alzheimer disease (AD) and plasma levels of n-3 PUFAs have been found to be lower in AD patients [353, 354].
Aims

- The aims of Paper I and II of this thesis were to characterize the mRNA expression patterns of all receptors of the *Adhesion* GPCR family as well as a large number of transporter genes belonging to the solute carrier (SLC) family, in a segment-detailed model of the gastrointestinal tract of the rat, thereby providing anatomical roadmaps for the distribution of these gene families in the GIT. As both *Adhesion* GPCRs and SLCs have important functions for normal physiology and states of disease [114, 355, 356], and as they are potentially interesting as future drug targets, these studies would provide important information for further physiological, pharmacological as well as pathological analyses of these genes and their respective proteins, and hopefully highlight interesting genes in the studied gene families where such further characterization could be of even greater importance.

- Given the role for fatty acid (FA) composition and desaturases in metabolism and pathology [323, 324, 334], in Paper III and IV we aimed to investigate how these factors respond under different dietary conditions including diet-induced obesity (DIO). Levels of biochemically important FAs and FA enzyme indices, among them indices of desaturase activities, were investigated in lipid fractions involved in FA handling and metabolism: subcutaneous adipose tissue triacylglycerols (SAT-TG), liver phospholipids (liver PL), liver triacylglycerols (liver TG), plasma cholesterol esters (PL-CE) and plasma phospholipids (PL-PL). Three dietary groups were investigated: an *ad libitum* HFD group of rats, a calorically restricted HFD group and a group with *ad libitum* access to chow. In Paper III, in order to study intraspecies responses to a HFD, modeling DIO, we further divided the *ad libitum* HFD group into the DIO-modeling phenotypes obesity-prone (OP) and obesity-resistant (OR). Differences between the OP and OR phenotypes in desaturase indices and biochemically important FAs were investigated in the five abovementioned lipid fractions. To study how desaturase indices and FAs are co-regulated across lipid fractions and how this is affected by diet as well as weight gain, we studied all three dietary groups in Paper IV, looking at how the associations of desaturase indices between tissues are affected by diet and weight gain.
Materials and methods

In the following section, a brief overview is presented of the main methods employed in the papers of this thesis (Paper I and II: tissue selection, data mining and qRT-PCR; Paper III and IV: gas chromatography). For more details, please refer to the separate papers.

Paper I and II

Tissue isolation for quantitative real-time qPCR of Dark Agouti rat GIT subsections

For Paper I and II, the tissue analyzed using qRT-PCR was obtained from Dark Agouti rats. The tissue consisted of the entire GI wall from the following twelve sections (about 5 to 10 mm in length) of the gastrointestinal tract (GIT): distal esophagus (a few mm from the stomach), corpus and antrum of the stomach, proximal duodenum (1 mm from the pylorus), distal duodenum (4 cm from pylorus), proximal jejunum (9 cm from pylorus), distal jejunum (19 cm from pylorus), proximal ileum (29 cm from pylorus), distal ileum (2.5 cm from the ileocecal valve), cecum, proximal colon (5 cm from ileocecal valve) and distal colon (12 cm from the ileocecal valve).

The polymerase chain reaction

qRT-PCR is based on the polymerase chain reaction (PCR), a method developed in the 1980s and that earned the inventor Kary Mullis the Nobel Prize in Chemistry in 1993 [357]. PCR relies on a repeated three-step reaction at cycling temperatures – specific for the three steps denaturation, annealing and elongation – resulting in amplification of any nucleic acid sequence, e.g. DNA or RNA. Whereas the original PCR method did not allow quantitative measurements of the amount of nucleic acid present, the refined PCR method qRT-PCR, which was developed in 1992 by Higuchi et al., provided this essential functionality [358]. As the name implies, in qRT-PCR the quantity of nucleic acid amplified is detected in real-time. By assuming a certain efficiency of the PCR amplification, this continuous detection enables calculation of the amount of product present not only at the end of the reaction – traditional PCR – but also at the beginning. To monitor the amount of prod-
uct, fluorescent probes or dyes are used. qRT-PCR has wide uses, e.g. for expression analysis, detection of pathogens and single nucleotide polymorphism (SNP) analysis. In our papers, we used qRT-PCR to characterize the anatomical distribution of SLC gene mRNA (Paper I) and to analyze the mRNA expression patterns Adhesion GPCRs (Paper II) in the twelve abovementioned segments of the GIT from healthy Dark Agouti rats.

Data mining and primer design for qRT-PCR

Unigene is an NCBI resource where all nucleotide sequences for genes are collected with information of the collection origin. This resource was utilized for Paper II to screen for intestinal Slc genes in the rat. An initial screening procedure examined the expression annotation in this database and we subsequently downloaded the Unigene data for rat from NCBI’s FTP-page and queried it for Slc genes – all genes having a symbol starting with “Slc”, expressed in intestinal tissues using local scripts. The representative mRNA sequences of the retrieved hits, together with the mRNA sequences for rat housekeeping genes and all known Adhesion GPCR genes, were downloaded from NCBI’s GenBank (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide) and used for primer design. The Beacon Primer Design 7.0 software (Premier Biosoft, USA) was used to design the primers, with primers designed to have optimal melting temperatures approaching 62°C to avoid the formation of primer dimers. The rat Adhesion GPCR primers were positioned within TM regions.

Quantitative Real-time PCR

qRT-PCR was done using a MyiQ thermal cycler (Bio-Rad Laboratories, Sweden). For Paper II and Paper III, all real-time PCR experiments were run in duplicates and triplicates, respectively. A negative control for each primer pair was included on each plate; in addition for Paper III a positive control consisting of 25 ng of rat genomic DNA was also included on each plate.

Data analysis and relative expression calculations

Bio-Rad iQ5 software v2.0 software (Bio-Rad Laboratories, Sweden) was used to process qRT-PCR data and obtain threshold cycle (Ct) values. All melt curves were analyzed to ensure that only one product with the expected melting point was amplified and that this was separate from the negative control. The software LinRegPCR was used to calculate the PCR efficiencies for each sample. Grubbs' test (GraphPad, USA) was then applied to exclude potential outliers (P < 0.05) when calculating the average PCR efficiency for each primer pair. To convert Ct values into relative quantities with the standard deviation, the delta Ct method was used [359]. The highest expression
was normalized to 1. The GeNorm method (Vandesompele, et al., 2002) was used with data from the most stable housekeeping genes to calculate normalization factors for each tissue to compensate for differences in cDNA quantity. Subsequently, the normalized quantities were calculated and maximum expression was set to one, with all relative expression values shown as fold decrease with respect to the detected maximum expression.

Paper III and IV

Gas chromatography

Gas chromatography (GC), which was used for analysis of fatty acids in Paper III and IV, was developed more than a century ago. Primitive forms of chromatography were apparently used as early as in ancient Rome, with the purpose of being able to distinguish a pigment mixture from a pigment dye and to establish their quality, but the chromatography process may be even more ancient than that [125]. Today GC offers excellent sensitivity and is a fast method for obtaining quantitative results from as little as pictograms for some of the GC detectors. GC involves two major steps, of which the first one is the preparation of methyl ester form of FAs (FAMEs), which is then followed by analyzing the FAMEs using GC [360]. The first step requires proper sample preparation, whereby either bound or free FAs (BFAs and FFAs, respectively) are extracted. The majority of FAs in natural samples occur as BFAs, and elevated levels of FFAs may be indicative of organ damage or ischemia where phospholipids may have been degraded [125]. Interestingly, the most common contaminants are introduced by laboratory personnel, as lipids are present in e.g. cosmetics. Once the samples have been prepared, lipids have to be separated from non-lipids, as the lipids are presumed to be contaminated by such substances. This process usually requires several steps prior to GC analysis, and can be achieved using several different techniques, e.g. microwaves or solid phase extraction [125].

To make the FAs volatile they are derivativized, e.g. by esterification to methyl groups using transesterification reagents. This process yields low molecular weight non-polar methyl esters, which is one of the most common FA ester preparation reactions [361]. These are more easily analyzed by GC compared with FFAs [360]. GC as used in GLC mentioned above consists of two phases, a liquid and a stationary phase. Injection of a sample into the gas phase is followed by volatilization of the sample [361]. This is then passed into the liquid phase. There the sample will be retained in a column, the retention time depending on how avidly the components are retained by the stationary phase. This will thereby affect how soon the components of the sample exit the column, where they can be detected. Detection is based on
the conversion of the sample into an electrical signal, the strength of which will depend on the quantity of the specific component. GC analysis using capillary columns identifies FAs only based on their retention time, successfully separating FAs between 2 and 24 in carbon chain length [360]. Quantification can however be especially difficult for PUFAs as these can be oxidized during sample handling.
Conclusions

Paper I and II

Solute carriers (SLCs) play a crucial role for the function of cells in the gastrointestinal tract (GIT) as well as for the ability of the GIT to obtain nutritive and non-nutritive substances, including drugs [77, 108]. Pharmaceuticals are furthermore mainly targeted at G-protein coupled receptors (GPCRs), of which the Adhesion family is a large but hitherto largely uncharacterized subfamily [86, 362]. In Paper I and II of this thesis we provided unique, comprehensive overviews of the localization of genes encoding Adhesion GPCRs and SLCs along the GI tract. We used a detailed division of the GI tract, which took into account subtle, otherwise undetectable differences in the localization of expression of each gene between proximal and distal gastrointestinal subsegments. Using this model, mRNA expression patterns of all 30 rat Adhesion GPCR and SLC genes from a total of 28 SLC gene families – for a total of 78 SLC genes – were analyzed using qRT-PCR.

Many of the genes studied in Paper I and II were found to be differentially expressed when comparing the proximal and distal parts of “classical” GI tract regions. These results indicate that rather than treating the GI segments as genetically homogenous entities, thorough proximodistal expression patterns should be considered and need to be kept in mind when comparing the expression of genes from neighboring segments. These may very well show similar expression patterns for a particular gene of interest, but due to differing expression patterns for other genes and transporters they may function differently. To therefore take such proximodistal differences into account may facilitate better understanding of the absorption processes, help in conceptualizing the function of pharmacological agents, as well as decipher pathological changes along the GI tract.

In sum, in this thesis we present the most comprehensive and detailed characterization of the expression of all 30 Adhesion GPCR and 78 solute carrier genes in the rat GI tract. The results show that the majority of genes from both membrane protein families are expressed in widespread to ubiquitous patterns in the GIT. Among the remaining genes, most are detected in more than five segments. We characterized the expression of eleven Slc orphans and similarly most of the analyzed Adhesion GPCRs have no putative func-
tion or ligand. Widespread distribution of SLC and Adhesion GPCR genes in the GIT highlight the importance of these genes and their encoded proteins for SLCs in gut physiology. Our twelve-segment model provides a unique illustration of the intestinal mRNA distribution pattern of membrane proteins, and as proximodistal differences in gene localization were found for many genes in our two papers, this suggests that a detailed anatomical division could be of added value when analyzing gene, and likely protein, expression in the gastrointestinal tract.

Paper III and IV

In Paper III of this thesis we used an established model of diet-induced obesity (DIO) to examine differences in fatty acid (FA) composition and FA enzyme indices between rats that when exposed to an energy-rich high-fat diet (HFD), can be divided into those that are either more prone (OP) or resistant (OR) to subsequent weight gain. We found that after a 5-week HFD, obesity-prone (OP) rats had a higher stearoyl-CoA desaturase 1 (SCD-1) activity, as measured by the SCD-16 index, when compared to their obesity-resistant (OR) counterpart. We furthermore found a significant increase in the proportion of palmitoleic acid, as well as a significant decrease in the proportion of linoleic acid, in the same obese phenotype. We did not however observe any significant differences between these two phenotypes in the studied liver or plasma lipid fractions, suggesting that such changes, which have been observed by others, may occur at a later timepoint, or may require a different diet in this specific animal model of DIO. The higher SCD-1 activity may be driven by insulin, which was higher in OP versus OR animals, and which is known to be able to increase SCD-1 activity. OP animals may also induce SCD-1 to prevent excess deleterious storage of saturated FAs. Alternatively OR animals may actively downregulate SCD-1 to increase oxidation of FAs. The combination of a high SCD-16 index, which may indicate a high SCD-1 activity, and a low proportion of linoleic acid, may indicate that these factors are either outcomes or mechanisms involved in producing DIO in OP versus OR animals.

In Paper IV of this thesis we studied the impact of three different dietary conditions – an ad libitum chow or HFD, and a restricted HFD – on the associations between desaturase indices. We found significant correlations between many of the plasma and liver lipid fractions. The fractions that were significantly correlated did not however appear to be the same throughout the three different groups. This suggests that diet and weight gain should be taken into account when desaturase indices of other tissues such as the liver are indirectly inferred from plasma measurements. Furthermore, the subcutaneous adipose tissue (SAT) fraction was not correlated with virtually any
other lipid fraction in any of the three dietary groups, and this may indicate that such correlations are primarily seen in longer DIO interventions, or when studying other plasma lipid fractions. It may also suggest a differential regulation of the desaturation process in the SAT compared with other lipid fractions and tissues, at least under the studied conditions. We found that only animals on an *ad libitum* HFD showed significant correlations between the SCD-16 index in adipose tissue and weight gain. This suggests that the use of desaturase indices as markers of adiposity may be less reliable unless dietary or weight parameters are taken into consideration.
Strengths and limitations

Paper I and II

In spite of their involvement in normal physiology and disease, the proximodistal mapping of many solute carriers (SLCs) in the gastrointestinal tract (GIT) had previously not been performed in a thorough manner, although there was evidence indicating that SLC transporter genes were differentially expressed along the gut axis, even when comparing proximal and distal subsegments [363-365]. In terms of its coverage, both Paper I and II employed a longitudinally highly detailed model of the GIT for mRNA expression analysis. This allowed us to virtually survey this complex organ system in its entirety, thereby examining inter-subsegmental differences in mRNA expression of large proportion of the SLC family of transporters and the entire rat Adhesion G-protein coupled receptor (GPCR) family.

In addition, many of the SLC and Adhesion GPCR genes had previously not been examined in subsections of the GI tract at all or using qRT-PCR, a method providing excellent sensitivity. Better knowledge of the overall Adhesion GPCRs’ and SLCs’ proximodistal distribution in the GI tract could provide fundamental physiological information and information relating to absorption of substrates as well drug design/delivery methods [94, 366-368]. QRT-PCR enables simultaneous analysis of a high number of mRNA expression profiles throughout complex systems, thereby increasing the probability of linking localization and physiological roles, which remain undetermined for a large number of SLCs and the majority of the Adhesion GPCRs. It should however be noted that mRNA does not always correspond to protein levels [369, 370]

Longitudinal GIT distribution profiles of the SLC genes reported by others tended to be in good agreement with our results. For example, the Slc15a1 gene encoding the di/tripeptide transporter PEPT1, has been bound to be expressed at high levels throughout the small intestine, while the cecum and stomach did not contain mRNA for this transporter [159, 371]. Dmt1 or Slc11a2, encoding the divalent metal ion transporter 1, is furthermore expressed in the stomach and throughout the small and large intestine [372, 373]. As we included the entire GI wall, the dissected tissue may furthermore not be representative of its finer constituents, such as the mucosa, mus-
cle layer as well as nerve and lymphoid tissue. As such, the mRNA expression presented in these two papers may rather predominately belong to any one of these tissues and this would require further studies in order to characterize the expression in each tissue component or cell type. In a study by Ito et al. looking at orphan GPCRs, many of them belonging to the Adhesion family, several genes were found to only be expressed in either the mucosa or the muscle layer [374].

The tissues analyzed in Paper I and II were furthermore collected in the morning under fasting conditions. Gene expression levels are known to oscillate in a circadian manner and may therefore be at different levels at other time points [375]. Feeding condition is also known to affect the expression of transporters in the GIT [356]. A study in mice by van den Bosch et al. however found that only 16 out of a total of 243 investigated SLC genes were differentially expressed after 24 hours of fasting [376].

Furthermore, the rat may not be ideal for making comparisons with humans, especially with regards to correlations between gene expression and physiology in the colon as rats are coprophagic and reingest their cecal fermentation contents, but as there also are other numerous differences in e.g. GIT physiology [10, 35, 377].

Paper III and IV

In Paper III and IV we used an established rat model, the Sprague-Dawley rat, to model diet-induced obesity (DIO). We specifically focused on the estimated desaturase activities and fatty acid (FA) composition in plasma, adipose tissue and liver lipid fractions to investigate potential differences between animals that are obesity-prone and obesity-resistant when put on a high-fat diet (HFD). Although FAs are a crucial part of the metabolism and are implicated in the development of DIO as well as many other biological mechanisms, these analyses had previously not been done in this DIO model. Furthermore, we present the first analysis of how the estimated desaturase activities are correlated across five lipid fractions under three dietary conditions: one modeling DIO with ad libitum HFD access, and two isocaloric conditions with either HFD or chow. Since we had two groups that were calorically matched, as well as two different diets, we could study the effect of diet as well as more pronounced weight gain (caused by ad libitum vs. restricted HFD access) in Paper IV.

Initial measurements of the FA composition would have been of value as this would have allowed us investigate whether individual FA or desaturase indices could have been used to predict which animals were prone or re-
sistant to DIO on a HFD. Initial measurements would also have allowed us to resolve whether the differences observed in Paper III were already present at the onset of the experiment, perhaps implying an already unfavorable metabolic state in the rats that gained the most in body weight or, if they were not, whether such changes only appeared when fed a HFD. The latter would instead have pointed to a diet-dependent vulnerability, perhaps insignificant when kept on a regular, less energy-dense chow diet. This diet dependency could also have been studied in a longer experiment; continuing to observe FA compositional changes after e.g. having switched back to a regular chow diet.

As there is a wealth of different diets that are employed to induce DIO in animal models – including a “true” cafeteria or supermarket diet that literally tries to mimic the overeating-promoting diets that we humans face in modern everyday life [275] – it would also have been of value to examine the effects of additional types of diets. As saturated (SFAs) and unsaturated fatty acids (MUFAs/PUFAs) have different effects on desaturase activities [331, 378], FA composition and overall metabolism [321], more refined diets in terms of FA composition would have been an interesting addition. However, just as cafeteria diets aim to do, typical commercially available HFDs are also put together to mimic the effects of western diets on humans [268], diets that seldom have just one type of FA, such as strictly SFAs, MUFAs or PUFAs. Just varying the degree of unsaturation can however yield significant insights, such as the study by Sampath et al. into the potential role of SCD-1 as a lipogenic switch [333].

There were quite few significant differences between OP and OR animals found in Paper III. Whereas SCD-18 may have been affected by a high dietary oleic acid content or a high degree of elongation of palmitic to stearic acid in the body [344, 378], previous studies have indicated SCD-18 in both humans and rats, as well as D6D and D5D in humans, to be significantly altered when comparing obese versus lean subjects and animals [343, 346, 379]. As these associations have also been found using other obesity measures such as adiposity, which in rats may more accurately reflect the degree of obesity in response to HFDs [267], having measured body fat would have given us additional parameters to evaluate in relation to desaturase indices and FA composition. The findings may also have been affected by the choice of adipose tissue depot, as we examined the subcutaneous inguinal fat depot, or by the duration of the HFD treatment.

In contrast to Paper I and II, where we looked at mRNA expression, we looked at the estimated outcome of protein activities in Paper III and IV. Similarly then, the estimated activities of the desaturase enzymes may not be reflective of the actual gene expression, although such associations have
been found in other studies, for example for the adipose and hepatic tissue [340, 341].
Perspectives

As outlined in the introduction and emphasized by the recent U.N. report on non-communicable disease, diet plays a large role for the death toll in modern society [257], preventing us from living even longer and especially healthier lives into old age. Diet affects our metabolism through countless ways but the mechanisms are still not fully accounted for or understood. It would therefore be of relevance to study how different diets are able to affect the expression of solute carriers (SLCs), which are essential for nutrient and non-nutrient absorption in the gastrointestinal tract (GIT), and whether such interactions play significant roles for drug absorption. As many diseases are increasingly being linked to inactivity, endocrine-disrupting chemicals and poor sleep habits, examining how alterations in such parameters, e.g. acute or chronic sleep deprivation, affect levels of SLCs or GPCRs in the GIT, would also provide further insight into the dynamics of these membrane proteins in health and disease. For such studies it would also be essential to examine the various tissues and cell layers of the GIT, to ascertain where any potential differences occur, thereby increasing the chance of establishing their physiological mechanisms and relevance for the question of study.

It would also be of great interest to see how the gut flora, essential for human and gastrointestinal health, can affect expression of genes and proteins of the GIT. This could be achieved by altering the gut flora, either by introducing other pathogenic or commensal bacterial species, or by disrupting the normal flora by means of antibiotics. The latter case would be of high relevance to the clinical setting, as antibiotic treatments are an everyday phenomenon, and yet have many unforeseen or unintended possible consequences that may involve disruption to the GI flora. As changes to the gut flora have already been linked to conditions such as insulin resistance, obesity, stress and memory alterations, one could try to establish the possible chain of events that might lead to such outcomes. Possible mechanisms of an altered gut flora may involve direct – through its own signaling molecules – or indirect ones, by e.g. interacting with the GIT and altering its gene expression profile, signaling molecules or vagus nerve signaling. Some studies have promisingly already been done in this area [380, 381]. Furthermore, we are currently at our lab planning studies for examining the effects of altering the microbiota in both humans and animals.
The gut flora likely also interacts with many GPCRs in the GIT and may turn out to contain substrates for activation of the *Adhesion* family, thereby perhaps partaking in producing overexpression of these receptors, as seen in e.g. some cancers of the GIT.

As for fatty acids (FAs) and desaturases, additional studies are needed to ascertain whether SCD-1 induction in the adipose tissue of rats can be protective in DIO. A recent study in mice has indeed indicated that an adipose tissue-specific deletion of SCD-1 does not protect from DIO [339]; whether this is the case in the DIO Sprague-Dawley model would be an interesting issue to investigate. It would also be interesting to study to what extent desaturases are co-regulated across different lipid fractions when only the degree of unsaturation, or the degree of e.g. fructose to total carbohydrates, are varied in the provided diet. Whether physiological parameters, such as acute or chronic exercise, stress or sleep deprivation, influence their co-regulation would also be interesting to examine.

Furthermore, it would also be of interest to study whether obesity-prone and obesity-resistant animals also show differences in SCD-1 mRNA or protein levels in adipose tissue, as well as for other genes involved in FA metabolism, when simultaneously examining other tissues and adipose tissue depots, even though we could only detect a difference for the SCD-16 index in the subcutaneous adipose tissue. There are many fat depots that can be studied both in rats and in humans, and these have been associated with different metabolic properties, harmful, neutral or protective [241, 242, 346, 382]. It would therefore be interesting to look for regional differences in FA handling and desaturase regulation in response to DIO, calorie restriction and other conditions.
Svensk sammanfattning


Vi använde tekniken kvantitativ realtids-PCR, en teknik som kan kvantifiera uttrycket av gener, för att mäta uttrycket av 78 stycken SLC-gener och alla Adhesion GPCR-gener, 30 stycken, i råttans magtarmkanal. Denna magtarmkanal var uppdelad i tolv olika segment, för att tillåta kartläggning av genuttrycket längs magtarmkanalens olika delar. De delar som vi undersökte var en mer närbelägen och en mer avlägsen belägen del i magsäcken, tolvfingartarmen, tunntarmens två delar (ileum och jejunum) respektive tjocktarmen, samt även matstrupen och blindtarmen.

Vi fann att de flesta (21 av 30) av Adhesion GPCR-generna var uttryckta i de flesta av de undersöka magtarmsegmenten. Mer begränsat uttryck hade de gener som tillhörde den sjunde gruppen av Adhesion GPCR-familjen. Av de SLC-gener som vi undersökte, var mer än hälften (56 procent) uttryckta i alla de magtarmsegment som genuttrycket analyserades i. Störst antal av de
undersökta SLC-generna uttrycktes i delar av magtarmkanalen som tar upp näringsämnen och andra substanser, det vill säga tunn- och tjocktarmen.

I och med att både Adhesion GPCR- och SLC-gener uttrycktes i stora delar av de segment av magtarmkanalen som vi undersökte i denna råttmodell, kan man dra slutsatsen att många av dessa proteiner med stor sannolikhet har betydelsefulla funktioner i magtarmkanalen. Detta stödjer även befintlig kunskap. Många SLC-gener och deras proteiner vet man idag ansvarar för livsavgörande upptag av olika näringsämnen, t.ex. socker, aminosyror och andra näringsämnen från innehållet i magtarmkanalen, så att de därefter kan användas som byggstenar i kroppen. Likaså är de viktiga för att reglera saltbalansen i magtarmkanalen och för att ta upp många läkemedel. I och med att många Adhesion GPCR-gener uppvisade uttrycksmönster som var mer begränsade till vissa specifika magtarmsegment, kan detta vidare tyda på att de kan utgöra framtida läkemedelsmål. GPCR:er, som Ahdesion-familjen som sagt tillhör, utgör redan idag den grupp av proteiner som flest läkemedel idag verkar mot. Vidare tyder de skillnader vi såg mellan närliggande segment i vår modell av magtarmkanalen, att man kan finna skillnader i genuttryck mellan dessa segment, och att man därefter bör tänka på detta då man analyserar genuttryck i magtarmkanalen, för att kanske använda mer detaljerade, det vill säga mer noggrant uppdelade, modeller.

Övervikt och fetma är idag ett stort problem i Sverige, USA och övriga världen. En av de centrala frågorna i denna utveckling, som ofta kallas för en epidemi, är varför inte alla individer går upp i vikt när de får tillgång till en energirik diet som innehåller mycket socker och fett. Fett utgörs av fettsyror som är viktiga som energikälla och lagras framförallt som sådan i fettväven. Fettsyror är emellertid även viktiga för att celler ska kunna fungera normalt då de utgör byggstenar i deras cellmembran där bland annat GPCR:er och SLC:er fäster. För kommunikation (signalering) mellan celler, för inflammation, reglering av födointag och många andra processer i kroppen har fettsyror också avgörande roller. Man har på senare år noggrant studerat funktionen av särskilda enzymer som deltar i regleringen av vilka fettsyror som kroppen bildar och reglerar om dessa ska användas för att generera energi till cellerna, användas som lagring för senare användning, eller användas för att skapa långa fettsyror med särskilda egenskaper. Dessa enzymer kallas för desaturaser och av dessa finns det tre välkaraktäriserade hos däggdjur: delta-5, delta-6 och delta-9 desaturaser. Det sistnämnda desaturas-enzyme kallas oftare för ”stearoyl-CoA desaturase” eller SCD-1 och utgör det mest välstuderade av de tre desaturaserarna. I försök där man har tagit bort SCD-1 från möss har man till exempel kunnat visa att de inte går upp i vikt då de får en fettrik diet. Funktionen för desaturaser-enzymerna är att göra fettsyror mer omättade och tillsammans med andra enzymer, elongaser, kan längre och mer omättade fettsyror därmed skapas. Med hjälp av kvoter mel-
lan de fettsyror som dessa enzym skapar och de fettsyror som användes till processen, kan man uppskatta aktiviteten för enskilda desaturas- och elongasenzym. Dessa kvoter har använts i studier på både människor och råttor, och man har, tillsammans med olika nivåer på fettsyror, sett att de har samband med bland annat mått på vikt och fetma, utveckling av ämnesomsättningssrelaterade sjukdomar och till och med dödlighet.

I andra delen av denna avhandling studerade vi den roll som framförallt desaturas-enzymerna och vissa fettsyror har för viktuppgång. Vi gjorde detta med hjälp av en råttmodell som ska försöka efterlikna situationen för människor, nämligen att alla individer inte går upp i vikt på en fettrik diet, så kallad högfettsdiet. Genom att ge råttor en fettrik diet kunde vi sedan dela upp dessa, utifrån viktuppgången efter fem veckor, i de som hade gått upp mer och de som hade gått upp mindre i vikt. Vi tog sedan och analyserade fettsyreproportionerna i fettraktioner från underhudsfett, levervävnad och plasma från blod. Dessa användes för att studera hur desaturas- och elongas kvoterna skiljde sig i dessa fettraktioner mellan de två olika typerna av råttor. Vi fann att de råttor som var mer benägna att gå upp i vikt hade en högre SCD-16-kvot, jämfört med råttor som inte var lika benägna att gå upp i vikt. Kvoten SCD-16 indikerar aktiviteten av desaturas-enzymet SCD-1. Vidare hade de råttor som var benägna att gå upp i vikt lägre proportioner av fettsyran linolsyra i fettväven. Denna fettsyra är en fleromättad fettsyra som tidigare studier har visat kan ha positiva egenskaper, bland annat för hur kroppen kan hantera blodsocker. Höga halter av linolsyra har vidare även kopplats till minskad risk för det så kallade metabola syndromet. Våra resultat kan tyda på att förändringarna i underhudsfettet hos råttor som går upp i vikt, i form av ökad SCD-16-kvot och minskade proportioner linolsyra, kan vara av betydelse för att avgöra huruvida råttor går upp mycket eller mindre i vikt på en fettrik diet. Det är även möjligt att de råttor som var benägna att gå upp i vikt ökade sin produktion av enzymet SCD-1 för att försöka minska på lagringen av så kallade mättade fettsyror, som kan vara farliga i för höga koncentrationer. De råttor som var benägna att gå upp i vikt hade nämligen också högre proportioner av en fettsyra som produceras av SCD-1, och som i vissa studier har visat sig vara kopplat till gynnsamma effekter på ämnesomsättningen.

I den sista delen av denna avhandling (fjärde arbetet) studerade vi hur ovan nämnda desaturas-enzyme i sig regleras mellan olika vävnader, med olika fettsyresammansättningar och olika funktioner för omsättningen av fettsyror. Vi undersökte detta genom att jämföra desaturas-kvoterna hos råttor som fått åta obegränsad mängd fettrik kost eller vanligt råttfoder, samt hos råttor som åt fettrik kost men som var begränsade i deras födointag så att de inte fick i sig mer kalorier än de råttor som åt det mer energisnåla råttfördet. Vi fann att desaturas-kvoterna speglade varandra mycket väl då man mätte dessa kvoter

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i plasma från blod och i levern. Däremot tycktes underhudsfettets desaturas-
kvoter inte spegla de i plasma eller levern, utan i stället var det här som en av
kvoterna, SCD-16 för SCD-1-enzymet, tycktes vara kopplat till hur mycket
djuren hade ökat i vikt under det fem veckor långa försöket. Denna koppling
syntes dock bara för de råttor som hade fått äta obeegränsad mängd av den
fettrika dieten. Sammantaget tycks denna kvot, åtminstone i denna rättmo-
dell, kunna användas som ett mått på viktuppgång hos råttor som fått en
fettrik diet. Att vi inte såg några kopplingar mellan desaturas-kvoterna i fett-
väven och de i plasma och levern, kan tyda på att enzymerna regleras an-
norlunda, men de funna starka kopplingarna mellan lever och plasma innebär
att kvoter beräknade från plasma i stor utsträckning tycks motsvara de som
ses i levern.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.