Novel endoscopic and MRI-based methods for evaluating exocrine pancreatic function in pancreatitis and monogenic forms of diabetes

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Abstract

Background: Monogenic diabetes (Maturity onset diabetes in the young, MODY) are autosomal dominantly inherited diabetes syndromes characterized by diabetes due to beta cell dysfunction, with typical onset of diabetes before 25 years of age. Two MODY subtypes, HNF1B-MODY and CEL-MODY, have been associated with exocrine pancreatic dysfunction. Assessment of exocrine pancreatic function is a challenge due to few available tests with acceptable feasibility and diagnostic value.

Aims: In the present study we wanted to evaluate two novel methods for measuring pancreatic exocrine function; a rapid endoscopic secretin test and a dynamic magnetic resonance imaging (MRI) based protocol. We further wanted to use these two methods to assess the degree and nature of exocrine pancreatic dysfunction in CEL-MODY and HNF1B-MODY.

Materials and methods: We recruited 52 consecutive patients with suspected chronic pancreatitis, 23 patients with CEL-MODY and 8 patients with HNF1B-MODY. Suspected chronic pancreatitis patients underwent the endoscopic procedure only, while MODY patients were offered endoscopy and MRI procedure. In addition, 25 healthy controls underwent endoscopy and 20 healthy controls underwent the MRI protocol. In patients with suspected chronic pancreatitis, a renowned multimodal clinical score was used as reference standard. In MODY patients results from the examinations were compared to nutritional status as achieved from patient records before any treatment with pancreatic enzyme supplements. The endoscopic procedure started 30 minutes after secretin stimulation, with 15 minutes collection of duodenal juice. Duodenal juice was analyzed for bicarbonate in chronic pancreatitis patients, and for bicarbonate and digestive enzyme activities in MODY-patients and healthy controls. The MRI protocol consisted of anatomical imaging, followed by dynamic imaging before and after secretin stimulation. The dynamic series consisted of repeated duodenal fluid volume quantification with magnetic resonance

cholangiopancreaticography (MRCP) and measuring apparent diffusion coefficient (ADC) in pancreatic tissue using diffusion-weighted imaging.

Results: Using bicarbonate as diagnostic marker, the rapid endoscopic secretin test had acceptable diagnostic value in detecting chronic pancreatitis, and performed significantly better than fecal elastase 1. In healthy controls, increase in secretin stimulated duodenal fluid volumes correlated well with changes in ADC after secretin stimulation. In CEL-MODY patients we found moderately low bicarbonate levels and reduced pancreatic fluid output, and severely reduced digestive enzyme activity levels compared to controls. With exception from low levels of vitamin E, CEL-MODY patients revealed no signs of malnutrition. In HNF1B-MODY patients we found moderately reduced bicarbonate levels, reduced pancreatic fluid output and moderately reduced levels of digestive enzyme activities compared to controls. Pancreatic gland volumes were small in all but one HNF1B-MODY patients, but pancreatic volume output per gland size was increased.

Conclusions: Both rapid endoscopic secretin test and the MRI protocol are feasible and well tolerated examination modalities reflecting pancreatic exocrine function. CEL-MODY patients have moderately reduced ductal and severely reduced acinar pancreatic function, but compensated nutritional status. HNF1B-MODY patients have moderately reduced ductal and acinar pancreatic function, partly compensated by hypersecretion from a hypoplastic pancreas.

List of publications

Paper I: Erchinger F, Engjom T, Tjora E, Hoem D, Hausken T, Gilja OH, Dimcevski G. Quantification of pancreatic function using a clinically feasible short endoscopic secretin test. Accepted for publication in Pancreas, December 2012

Paper II: Wathle G, Tjora E, Ersland L, Dimcevski G, Salvesen ØO, Molven A, Njølstad PR, Haldorsen IS. Assessment of exocrine pancreatic function by secretin-stimulated MRCP and DWI in healthy controls. Journal of Magnetic Resonance Imaging, May 2013. Electronically published ahead of print

Paper III: Tjora E, Wathle G, Engjom T, Erchinger F, Molven A, Aksnes L, Haldorsen IS, Dimcevski G, Njølstad PR, Ræder H. Severe pancreatic dysfunction but compensated nutritional status in monogenic pancreatic disease caused by CEL-mutations. Pancreas, June 2013. Electronically published ahead of print

Paper IV: Tjora E, Wathle G, Erchinger F, Engjom T, Molven A, Aksnes L, Haldorsen IS, Dimcevski G, Ræder H, Njølstad PR. Exocrine pancreatic function in HNF1B-MODY is only moderately reduced: Compensatory hypersecretion from a hypoplastic pancreas. Diabetic Medicine, March 2013. Electronically published ahead of print

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1. Introduction

1.1 The normal pancreas

1.1.1 Structure and function

The pancreas is located retroperitoneally, deep into the upper abdomen. The location of the pancreas, its shared blood supply with other organs and its poor tolerance to manipulation make it one of the most unavailable abdominal organs for diagnostic and interventional procedures (figure 1).

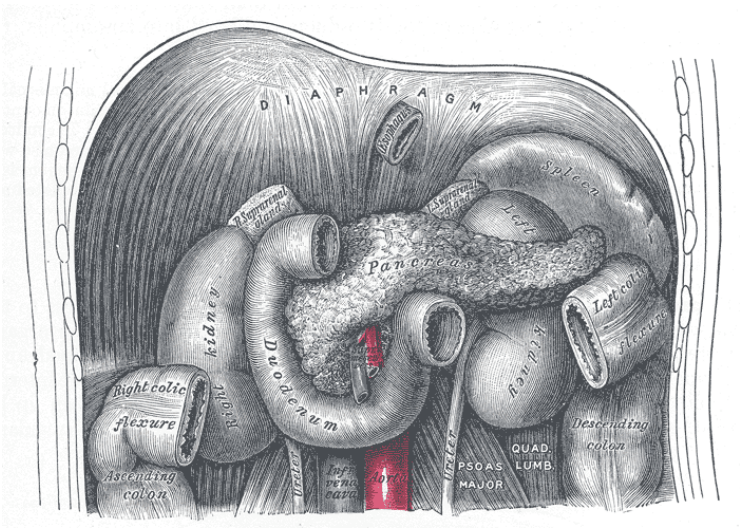

Figure 1. Classic illustration of the pancreas, showing its localisation and relation to other organs. The deep localisation of the pancreas in the abdomen is one of the reasons for its unavailability. Reprinted from (1) with permission from bartleby.com.

The pancreas is a mixed exocrine and endocrine organ. The exocrine compartment comprises >95 % of the gland, and consists of acinar cells, secreting enzymes, and ductal cells secreting a bicarbonate rich fluid. These are constitutes of pancreatic

juice, that is secreted to the duodenum and takes part in digestion of nutrients. The endocrine compartment consists of the islets of Langerhans, dispersed through the gland and produces hormones that primarily regulate glucose uptake in cells. This compartmentalising of the organ into two very different functional units is puzzling (2), but in some way both compartments take part in the same process, namely achievement and utilisation of nutrients.

The exocrine pancreas

The functional unit in the exocrine pancreas is the acinus with its draining ductule. The acini form berry-like structures connected to the ductuli, which comprise a branching drainage system to the main pancreatic duct, leading to the duodenum. The acini constitute 80 % of the pancreas (3), while ducts constitute 5 % (4).

Acinar compartment

The acinar cell has the highest rate of protein synthesis and excretion in the human body (3, 5), which is reflected by a highly developed endoplasmatic reticulum system (5). The main role of the acinar cells is to synthesize and excrete digestive enzymes to the pancreatic juice; enzymes that play a significant role in the intraintestinal digestion of nutrients. The main classes of digestive enzymes from the pancreas are proteases, lipases, amylase and nucleases (summarized in table 1) (3, 6). Proteases are excreted as proenzymes (3). Trypsinogen is activated to trypsin through proteolysis by enteropeptidase (or enterokinase) in the duodenal brush border (7). The other proteases are in turn activated by trypsin (6). This cascade of protease activation from proenzymes ensures that there is no uncontrolled proteolysis inside the pancreas, and failures in this system result in premature activation of proteases which can cause autodigestion and pancreatitis (8).

Enzymes	Cleavage site / Action	Product
Proteases		
Endopeptidases		
Trypsin	Lysine and arginine. Activates other proteases	Oligopeptides
Chymotrypsin	Aliphatic amino acids	Oligopeptides
Elastase	Small amino acid residues	Oligopeptides
Exopeptidases		
Carboxypeptidase A	Aromatic amino acids from C-terminal end	Aromatic amino acids
Carboxypeptidase B	Arginine and lysine from C-terminal end	Arginine and lysine
Lipases		
Pancreatic triglyceride lipase	Fatty acids at sn1 and sn3 of glycerides	Fatty acids
Colipase	Facilitates action of pancreatic triglyceride lipase	
Carboxyl-ester lipase	Glycerides, galactolipids, phospholipids, vitamin esters, cholesterol esters, ceramide	Fatty acids
Phospholipase A2	Phospholipids in sn2 position	Fatty acids
Amylases		
α-amylase	Starch	Glucose
Nucleases		
Deoxyribonuclease	DNA	Nucleic acids
Ribonuclease	RNA	Nucleic acids

Table 1. Some of the major pancreatic acinar cell products and their action. Adapted from (3, 6).

Ductal compartment

Ductal cells deliver the gross of fluid, bicarbonate and electrolytes to the pancreatic juice (3). The ductal cells are unique in their ability to produce large amounts of fluid

with bicarbonate concentration >140 mmol/L, which is way above bicarbonate concentrations in fluids from other cells in the gastrointestinal tract (4). This is exploited in pancreatic function tests, measuring peak bicarbonate concentrations in duodenal juice after pancreas stimulation.

Bicarbonate has an important role in neutralizing acidic chyme from the ventricle bringing it to near pH optimum for digestive enzymes and bile acids (9, 10).

Regulation of pancreatic secretion

Secretin

Secretin was discovered by Bayliss and Starling in 1902 (11), being the first demonstration of a hormone effect (12). Secretin is a peptide hormone, secreted from enteroendocrine cells as a response to acidic chyme entering the duodenum. Other stimulants to secretin secretion are bile acids and fatty acids (13). Secretin stimulates fluid secretion from pancreatic duct cells by stimulating the secretin-receptor. This in turn increases cytosolic cyclic AMP, activating protein kinase A (14). Protein kinase A opens the cystic fibrosis transmembrane regulator channel (CFTR) (15). The ion fluxes through CFTR and other ion channels as response to secretin stimulation are complex, and not fully understood (3, 4, 16). Exogenous bolus secretin stimulation causes a rapid increase in fluid secretion from the pancreas to the duodenum (17). Bicarbonate concentration in the duodenal fluid after secretin stimulation increases more gradually, and reaches a peak plateau 20-30 minutes after a bolus of secretin (figure 2) (17, 18).

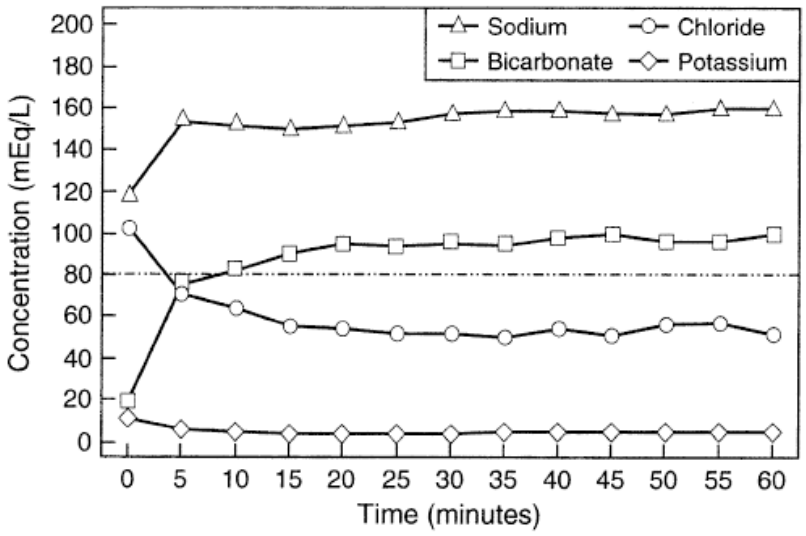

Figure 2: Changes in endoscopically collected duodenal juice after secretin bolus stimulation. Bicarbonate concentration reaches a peak plateau 20-30 minutes after secretin bolus. Chloride concentration decreases reciprocally. Reprinted from (18) with permission from Elsevier Inc.

In addition to its effects on duct cells, secretin also has effects on other cell types (19). Acinar cells are stimulated by secretin to increase enzyme secretion (20), which can be demonstrated by increased enzyme output in pancreatic juice after secretin stimulation in man (21, 22). Secretin also has choleretic effects by stimulating cholangiocytes to secrete bicarbonate rich fluid into the bile (23), but secretin does probably not stimulate the gallbladder to contract (24, 25).

Cholecystokinin

Cholecystokinin is also a peptide hormone released from enteroendocrine cells (26). It is secreted as a response to food elements, particularly fat and amino acids (27). The effects of cholecystokinin on human acinar cells are thought to be mainly via vagal neurons, stimulating through acetylcholine (28), but cholecystokinin receptors have also been demonstrated on human acinar cells, suggesting direct stimulatory effects as well (29). Cholecystokinin induces release of pancreatic enzymes to the pancreatic juice through calcium-mediated signalling (28). As its name indicates,

cholecystokinin causes gallbladder contractions, but also has choleretic effects, and potentiates secretin induced pancreatic ductal secretion (26).

Other hormones involved in pancreatic secretion

Receptors activated by vasoactive intestinal peptide (VIP) and gastrin releasing peptide (GRP) are located on human acinar cells and stimulate exocytosis (3). Similar receptors are also found on ductal cells (13).

The endocrine pancreas

Islets of Langerhans

The islets of Langerhans were described by Paul Langerhans, still a medical student, in 1869 (30). The cells in the islets secrete hormones regulating glucose homeostasis in the body. The islets of Langerhans are 100 to 200 μm in diameter (2), and constitute 2 % of total pancreas weight (31). They are spread throughout the gland, but are most abundant in the tail of the pancreas. The islets consist of four different types of hormone-producing cells, of which the insulin-producing beta cell is the most abundant, constituting about 50-60 % of the islet cells. In addition, there are alpha cells producing glucagon, pancreatic polypeptide (PP) producing cells and delta cells producing somatostatin (32). At least in mice, beta cells are evenly spread in all islets of Langerhans, but alpha cells are most abundant in the portions of the pancreas from the dorsal *anlage* (tail, body and superior part of the head), while PP-producing cells are most abundant in the part from the ventral *anlage* (inferior portion of the head, or duodenal pancreas) (33).

The secretion of insulin from beta cells is primarily regulated by the glucose concentration in the blood, keeping the blood glucose levels within tight limits. There are, however, many other mechanisms modulating insulin secretion from the beta cell. Insulin causes uptake of glucose into liver, muscle and fat, and inhibits glucose production in the liver (31). Glucagon is secreted primarily as a response to hypoglycaemia, activating glycogenolysis and gluconeogenesis which in turn increases the blood glucose.

Almost all arterial blood supply to the pancreas is led via the islets of Langerhans, indicating an insulo-acinar port vein system, supported by many observations indicating that insular hormones regulate growth and function of the exocrine pancreatic tissue (34).

The entero-insular axis

There are many modulators of insulin secretion. The islets are innervated by both vagal and enteropancreatic fibres. In addition, hormones also modulate insulin secretion (34). There is an old observation that orally ingested glucose is associated with a much greater increase in insulin response than equimolar amounts of glucose given intravenously. This has been named the incretin effect. Two incretin hormones have been identified, namely glucose-dependent insulotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1). Both of these hormones are secreted as a response to ingestion of food, especially food rich in carbohydrates and fat. Secretion of GIP seems more specifically to be induced by absorption of nutrients; hence secretion of this hormone may be reduced in malabsorption. GIP and GLP-1 both have effects on the beta cell by enhancing glucose-dependent insulin secretion. Both hormones induce beta cell proliferation, and inhibit beta cell apoptosis, in addition to many extra-pancreatic effects (35).

1.2 Clinical assessment of the exocrine pancreas

1.2.1 Imaging

Overview

Imaging is one of the cornerstones in diagnosis of pancreas disease, especially in the evaluation of chronic pancreatitis, and the differentiation between focal pancreatitis and malignant tumour (36). Several imaging modalities have been used, reflecting the difficulty of reaching an exact diagnosis in some cases. Traditionally transabdominal ultrasound (US), computer tomography (CT) and endoscopic retrograde

cholangiography (ERCP) have been the modalities of choice. However, many new modalities have emerged, increasing the possibilities but also the complexity in imaging of the pancreas. The different imaging techniques, their indications, advantages and disadvantages are summarized in table 2.

	Indications	**Advantages**	**Disadvantages**
US	First evaluation of suspected pancreatic disease Often very useful in children Useful in evaluating biliary tree	No radiation Cheap Widely available	Operator-dependent Visualization may be impaired due to abdominal fat or bowel gas Limited accuracy in detection of parenchymal pathology
CT	Most used modality in pancreas imaging in adults Diagnosis and staging of pancreatic cancer (may detect small masses early)	Widely available Increasing resolution Detects calcifications Contrast-enhanced imaging reveals vascularity and perfusion properties of lesions	Radiation Poor identification of ductal anomalies Limited sensitivity to early changes in chronic pancreatitis
ERCP	Allows cytology, stone extraction and procedures on the papilla	Gold standard for detecting ductal anomalies	Invasive. Significant risk for iatrogenic acute pancreatitis
EUS	Detection and staging of neoplasms Lymph node staging Detection and staging of chronic pancreatitis	Sensitive to parenchymal changes Allows fine needle aspirations and core biopsies Allows contrast enhancement and elastography	Invasive. Operator-dependent Limited availability Need for further evaluation of specificity of subtle changes
MRI	Evaluation of ducts	No radiation	Cost
MRCP	Acute and chronic pancreatitis Diagnosis of neoplasms	Visualization of ducts Early detection of chronic pancreatitis	Time consuming

	Indications	Advantages	Disadvantages
S-MRCP	Evaluation of ductal system	No radiation	Cost
		No contrast	Time consuming
	Quantification of exocrine pancreatic function	Excellent visualization of ducts	Need for further validation and optimalisation
DWI	Evaluation of lesions and parenchymal properties	Discriminates well between benign and malignant lesions	Promising, but further evaluation needed
		Promising in evaluation of cystic lesions	
Intraductal pancreatic endoscopy, US and OCT	Evaluation of intraductal and periductal lesions	Promising with regard to diagnosis and staging	Invasive. Not widely available
		Possibility to perform procedures and take biopsies	Complications
FDG-PET	Early detection of pancreatic cancer	Most sensitive modality in early pancreatic cancer	Not very widely available
			Less sensitive to more advanced cancers

Table 2. Imaging modalities. Abbreviations: US: Ultrasound; CT: Computerized tomography; ERCP: Endoscopic retrograde cholangiography; EUS: Endoscopic ultrasound; MRI: Magnetic resonance imaging; MRCP: Magnetic resonance cholangiopancreaticography; DWI: Diffusion weighted imaging; OCT: Optical coherence tomography; FDG-PET: ^{18}Fluorodeoxyglucose positron emission tomography. Based on (37-40)

Endoscopic ultrasound

Endoscopic ultrasound (EUS) is particularly suitable for evaluating the pancreas, due to the organ's proximity to the ventricle and duodenum, and the high image resolution of this method (40, 41). EUS also gives the opportunity of taking fine needle aspiration samples and core biopsies, doing elastography of the gland and to perform contrast enhanced imaging (41, 42). Many of the examinations needing EUS will probably be done by transabdominal US in the future, due to emerging advances in this modality (43).

Magnetic resonance imaging

Magnetic resonance imaging (MRI) of the pancreas has an increasing role in pancreas diagnostics. Evaluation of parenchyma is best done with T1-weighted sequences due to high protein contents in the tissue (44). These sequences are also used to determine pancreatic volumes by MRI (45). Dixon techniques, determining the relative differences between resonance frequencies of fat and water (46, 47), can be used to determine fat to water ratios of the parenchyma, and are used to detect and quantify pancreatic lipomatosis (45, 48).

In magnetic resonance cholangiopancreaticography (MRCP), T2 weighting and fat suppression causes water signal to brighten up. The water then becomes a contrast agent in water-containing compartments, causing the water filled pancreatobiliary tree to brighten up (44). This technique has made the invasive and potentially acute pancreatitis-provoking endoscopic retrograde cholangiopancreaticography (ERCP) almost obsolete as an imaging procedure, reserving ERCP to interventions in pancreatic disease (49-51). By administering secretin, increase of fluid pressure in the pancreatic duct system makes ectasias and stenoses become more evident, and changes in the ductal tissue compliance can be detected as altered calibre change after stimulation (52). In addition, secretin stimulation during MRCP gives the opportunity to evaluate pancreatic function, which will be dealt with in the next chapter of this thesis.

In diffusion-weighted imaging (DWI), the MR signal is sensitized to movement of water molecules. This mode detects whether movement of water molecules are random and unrestricted (Brownian movements), or if they are restricted by cell membranes or matrix. Signals can be detected at different water movement sensitivities, b-values, where low b-values detect large motions or long diffusion distances for water molecules and high b-values detect small motions or short diffusion distances. By obtaining two or more uptakes of the same area at different b-values, the apparent diffusion coefficient (ADC) can be calculated for a voxel through logarithmic regression analysis. The ADC is an estimate for the net water movement

within a voxel. For unvascularized tissues, the ADC would represent the diffusivity or true diffusion coefficient in the voxel, but as perfusion also contributes to water movement, the ADC is a combination of the perfusion and diffusion in the tissue. The higher b-values used, the lesser the perfusion affects the ADC (and the closer this value reflects the true diffusion coefficient). However, the noise to signal ratio increases with increasing b-values (53). The diffusion-weighted images are presented as ADC-value heat maps. As these images reflect the tissue properties, it has the potential to differentiate between pathological processes for example fibrosis restricting water movement with lower ADC values, whereas acute inflammation gives higher ADC values due to oedema (53). Diffusion-weighted imaging discriminates well between pancreatic cancer and benign lesions of the pancreas (54, 55), and also between different cystic lesions of the pancreas (56, 57). Secretin stimulated DWI is discussed in the exocrine pancreatic function section.

1.2.2 Exocrine pancreatic function testing

Clinical assessment of exocrine function of the pancreas has been performed for several decades (17). Tests can be divided into non-invasive and invasive based on the test procedure. The terms “direct” and “indirect” are often used, but definitions of these terms are diverse (58-60). I will discuss imaging function tests in the end of this chapter, as these new non-invasive procedures give results otherwise achieved through invasive function testing only.

Non-invasive exocrine pancreatic function testing

Non-invasive exocrine pancreatic function tests are appealing, as they are easy to perform, less time consuming and do not cause much physical discomfort for the patient. Many non-invasive tests have been developed, probably reflecting the lack of the “perfect” non-invasive test, that is cheap, easy to perform, and has an acceptable diagnostic accuracy. Generally, non- invasive tests have a rather good ability to detect severe pancreatic dysfunction, leading to pancreatic insufficiency (61, 62), but do

perform poorly on mild to moderate pancreatic dysfunction with limited or no detectable pancreatic insufficiency.

Faecal fat determination

Quantification of fat in faeces is the oldest of the indirect exocrine pancreatic function tests still in use. Modified versions of the van de Kamer method (63, 64) seem to be the most common to determine fat content, but near infrared (65, 66) and MRI (67) spectroscopy methods are also used. In a healthy individual eating normal western diet, faecal fat excretion is not altered by fat intake, and is about 4 g/day, which probably results from the digestive process, rather than the diet (68). In the case of fat malabsorption, ingested fat passes to the faeces and will, hence, be correlated to the intake (69). As faecal fat excretion is a result of increased faecal volume, rather than increased faecal fat concentration (64, 70), collection of faeces over a time period is necessary to determine faecal fat output. There are also day-to-day variations in gut motility causing variations in output of faeces, which necessitates three days collection of faeces and homogenisation of the samples to get a reliable estimate of faecal fat output (64). These practical and aesthetical obstacles to this test make the use of it limited. The van de Kamer test is considered the gold standard to detect steatorrhoea in patients (64), but as 90 to 95 % of the gland function needs to be diminished before steatorrhoea occurs (71, 72), determination of faecal fat excretion only detects severe pancreatic dysfunction leading to pancreatic insufficiency. Tests using spot samples to detect steatorrhoea have been studied. Microscopy of a Sudan dyed faecal sample (73) and acid steatocrit (74) are two examples. These tests will usually detect overt steatorrhoea, but do not have the accuracy of the three day collection method in milder forms (61, 75-77).

Breath tests

Breath tests using substrates marked with uncommon carbon isotopes represents another indirect approach to studying exocrine pancreatic function. The principle of these tests is that the subject investigated ingests a meal with a defined amount of a substrate marked with an uncommon carbon isotope, usually the non-radioactive

isotope ^{13}C. The substrate then needs to be digested and absorbed, before it is metabolized (78). By quantifying the amount of isotope in the carbondioxide of the expired air from the subject investigated, the ratio of metabolized substrate can be determined, reflecting the ratio of digestion and absorption. The most commonly used of these tests, is the ^{13}C mixed triglyceride breath test (79). In this test, ^{13}C marked octanoic acid has been placed in the second glycerol position in a triglyceride between two long chain fatty acids. The triglyceride has to be hydrolyzed by lipase in duodenum at the 1 and 3 position before the ^{13}C octanyl monoglyceride can be absorbed. Being a medium chain fatty acid, the substrate then undergoes rapid beta-oxidation in the liver, and the concentration of expired $^{13}CO_2$ reaches a peak after about 3.5 hours. To determine the ratio of digested substrate, a cumulative amount of $^{13}CO_2$ is determined by collecting expired air every 30 minutes for 6 hours after ingestion of the substrate. The ^{13}C mixed triglyceride breath test has performed well when compared with the faecal fat excretion test (80), and has been suggested to be a less cumbersome alternative to the latter. More interestingly, Keller et al. (81) recently found indications that by increasing the amount of ^{13}C-marked fat ingested, and keeping the subjects at rest while collecting samples, the ^{13}C mixed triglyceride test performed well in detecting moderate pancreatic dysfunction when compared to a classic secretin test. However, the patient material was small, and the specificity of the test has not been tested in patients with non-pancreatic causes of fat-malabsorbtion.

Faecal elastase 1

Human pancreatic elastase 1 is one of the proteolytic enzymes from the exocrine pancreas. The enzyme undergoes no significant intestinal digestion, hence, faecal elastase 1 levels are five to six times higher than concentrations in pancreatic juice (82). Immunological measuring of elastase 1 in faeces is a diagnostic test for pancreatic function (83). Elastase 1 concentrations can be measured in a small spot sample of faeces, samples are stable for several days in room temperature, and the results are not disturbed by use of oral pancreatic enzyme substitution therapy (83).

Hence, the test is feasible, easy to perform and relatively cheap, making it a popular test in a clinical setting (84). The test exhibits excellent sensitivity (82-100 %) in the case of severe pancreatic dysfunction, but has lower sensitivity in the case of moderate (33-100 %) and mild (0-65 %) pancreatic dysfunction (85). Specificity of the test has been difficult to assess, and values ranging between 29 and 96 % have been published (62, 85). Poor specificity in some studies has been attributed to two possible explanations. Firstly, watery diarrhoea causes dilution of the faecal samples, resulting in lower values of elastase 1 in the faecal samples. Lyophilisation of faecal samples has been demonstrated to prevent these false positives (86). Secondly, the faecal elastase 1 test seems to differ poorly between pancreatic and intestinal malabsorption, possibly due to secondary pancreatic dysfunction as result of disturbances in the entero-acinar axis (87-89). Lüth et al demonstrated that faecal elastase 1 test had a positive predictive value for primary pancreatic dysfunction of 50 % in their material of patients under investigation for possible pancreatic malabsorption, when compared to a secretin-caerulin test (87), concluding that the test is too inaccurate to be suitable for screening in such a patient material. In a recent pediatric material, the positive predictive value of lyophilized faecal elastase 1 was only 14 % in a retrospective material when compared to a short endoscopic secretin test evaluating digestive enzymes (90). This probably reflects that exocrine pancreatic dysfunction is a rare cause of malabsorption in children when compared to enteropathy and other causes of false positive faecal elastase 1.

Faecal chymotrypsin

Faecal chymotrypsin activity is also used as a tubeless test to assess exocrine pancreatic function. The principles and properties of the test are similar to the faecal elastase 1 test, but enzyme activity is measured instead of protein concentration detected immunologically. The sensitivity and specificity of the test is considered lower or in the same range as the faecal elastase 1 test (59, 62). Besides, the assay is not specific to human pancreatic chymotrypsin, making it mandatory to quit

pancreatic enzyme therapy several days before the sample is collected (if the test is not performed to monitor compliance) (62).

Invasive exocrine pancreatic function testing

Overview

Tests to evaluate pancreatic output to the duodenum have been developed over many decades (17). The principle for these tests is intubation of the duodenum to collect duodenal juice after stimulation of the pancreatic gland, either with hormones or nutrients (91). There are several tests available (61), and there has been no established standardization of the tests, with possible exception of that suggested by the Japanese Pancreatic Society (59). Despite this, the secretin test with or without the use of caerulein or another CCK analogue, has for a long time (and almost undisputedly (92)) been considered the gold standard in exocrine pancreatic function testing (93). The classic invasive tests are performed by intubating the duodenum with a two or three channels' tube. The most proximal channel opening keeps the ventricle empty by suctioning. The most distal channel opening is placed near the ligament of Treitz, and continuously collects duodenal juice by suctioning for analysis. Analysis is performed on peak concentrations or total output of bicarbonate and/or digestive enzymes in the collection period. In some modifications of the test, a channel proximal to the collection channel adds a solution of a carrier substance (most often polyethyleneglycol) at a constant rate to estimate recovery rate of duodenal juice (94). Collection time for these tests is one to two hours (59, 61).

Under these conditions, the classic, invasive pancreatic function tests are technically difficult to perform. Moreover they are expensive, time consuming and considered to be very unpleasant for patients. Hence, the use of them is very limited today (60).

Methods for stimulation of the exocrine pancreas

Several strategies for stimulation of the exocrine pancreas have been used. Use of nutrients, most commonly modifications of the Lundh test (95), seems to be less reliable in testing exocrine pancreatic function than intravenous secretagogues (59),

and is not common today (59, 96). To evaluate both ductal (volume and bicarbonate) and acinar (digestive enzymes) function, various combinations of secretin and CCK analogues have been used (59), however stimulation by secretin alone also produces an increase in secretion of digestive enzymes (81, 97, 98).

Peak concentrations or output?

Both total output and peak concentrations of bicarbonate and enzymes have been used to evaluate pancreatic function (60, 61). Schibli and co-workers (99) performed a retrospective evalutation of their tube based method, using both secretin and pancreozymin as stimulants, and estimating recovery rate by using polyethyleneglycol. They found that by measuring concentrations instead of total output of bicarbonate and digestive enzymes, there would be an increased variability, and also misclassifications of patients with cystic fibrosis, as these patients may secrete very small volumes of hyperconcentrated pancreatic fluid (100). The report did, however, not describe at what time points the concentration measures were made, and peak concentration was not used. The same authors argue that to determine output, a carrier substance is necessary to correct for intestinal losses, to avoid misclassifications of patients (99), increasing the complexity of their test. After all, peak concentrations of at least bicarbonate has proven to be useful and reliable, and makes the performance of the invasive tests easier and less prone to technical errors (59). Normal peak bicarbonate levels after secretin stimulation has by many been established to be 80-130 mEq/L (59, 61), though also this range lacks standardization (93).

Modifications of the invasive tests

Endoscopic tests

Several modifications have been tried to make these tests easier to perform. Use of an endoscope instead of the classic Dreiling tube eases duodenal intubation, and ensures correct placement for sample collection (93, 101, 102). This modification resulted in shorter examination time, and similar results as the Dreiling tube test with respect to

bicarbonate peak concentrations and output in a crossover study of healthy subjects (102) and in patients evaluated for chronic pancreatitis (103).

Intraductal collection

Intraductal collection of pure secretin stimulated pancreatic juice have been used (104), but the diagnostic value of this test has been demonstrated to be limited (105). In addition, the test carries a risk for complications associated with cannulation of the pancreatic duct.

Shortening collection time ("rapid collection")

There have been several attempts to shorten collection time. In paediatrics, a test analysing digestive enzymes in duodenal juice collected for 10-15 minutes after secretin and/or CCK-analogues has been used to some extent for more than 20 years (22, 90, 97, 106). However, several studies have found unacceptably low diagnostic accuracy when shortening collection time to 10-20 minutes (99, 107-109). In all these studies, collection of duodenal juice has been performed immediately after stimulation.

Optimizing time of collection after stimulation

There have also been attempts to determine the optimal timing for collection of duodenal juice after secretin stimulation. One study demonstrated peak concentrations of bicarbonate occurring in different individuals with abdominal pain and suspected chronic pancreatitis at 15 minutes, 30-40 minutes and 45-55 minutes during one hour collection of duodenal juice after secretin stimulation, concluding that extended collection is necessary for acceptable diagnostic accuracy (109). The diagnostic accuracy of collecting at one of these time points was, however, not determined. Stevens and co-workers (110) demonstrated retrospectively that collection of duodenal juice at 30 and 45 minutes after secretin stimulation gave a sensitivity of 94 % and a specificity of 85 % when compared to one hour collection time. This was based on the "rediscovery" that bicarbonate concentration increases for 20-30 minutes after secretin stimulation and before it reaches a peak plateau (17, 18). Identical

timing in increases of bicarbonate concentration to a peak plateau was found in healthy subjects, patients at risk for chronic pancreatitis, chronic pancreatitis patients and patients with manifest steatorrhoea (110). Hence, the poor reliability of the other short endoscopy tests described above can be attributed to the fact that duodenal juice was collected while bicarbonate concentration was still increasing. As a result of this observation, Jensen and co-workers (111) performed a rapid endoscopic secretin test in patients with suspect and manifest chronic pancreatitis. Duodenal juice was collected through an endoscope in one, single portion from 30 to 40 minutes after secretin stimulation. The samples were analyzed for bicarbonate, lipase, elastase and zinc. The results from this test were compared to results from the Lundh test as a reference standard. Bicarbonate concentration was the biomarker with best diagnostic value. There was a positive predictive value of 88 % and negative predictive value of 83 % when bicarbonate concentration of 60 mEq/L was chosen as a cut off. Duodenal lipase concentration from the endoscopic test did not have much diagnostic value in itself, but correlated acceptably with duodenal lipase from the Lundh test, and performed well in detecting patients with pancreatic exocrine insufficiency compared with patients having normal or reduced (but sufficient) pancreatic function. Even though the latter study has some limitations in methods and reference standard that will be dealt with later in this thesis, it represents a promising approach to the rapid endoscopic stimulation test of the pancreas.

Image-based exocrine pancreatic function testing

Secretin-stimulated MRCP

Pancreatic fluid output to the duodenum can also be demonstrated by MRI based techniques. By imaging with a fluid sensitive MRI protocol at defined time points before and after secretin stimulation, increase of fluid content in the duodenum can be measured semi-quantitatively (112-114) or quantitatively (115-117). The semi-quantitative method is based on grading duodenal filling after which anatomical landmarks that obtain fluid signals (e.g. Grade 0: no fluid filling in duodenum, Grade 1: fluid signal in duodenal bulb, Grade 2: fluid filling up to genu inferius duodeni,

Grade 3: Fluid filling beyond genu inferius) (112). Quantification of output can be performed by simply measuring the increase in the duodenal width after secretin stimulation (117). However, increase in volume can also be determined by imaging coronal slices, defining a region of interest (ROI) containing duodenum and proximal jejunum, and relating increase in water intensity per voxel to water intensity in a voxel from a compartment containing pure water (115, 116). Volumes determined by this method have demonstrated excellent linearity with volumes instilled through a duodenal tube in healthy volunteers (115), as has determination of ingested water (118). As the main volume of the pancreatic excretion originates from the ductal cells (3), determination of volume increase in the duodenum most probably reflects ductal function (119), which has been confirmed indirectly by a significant correlation between MRI-estimated volume output and bicarbonate concentration in pure pancreatic juice (114).

Several groups have tried to evaluate the diagnostic value of MRI-estimated pancreatic fluid output. Two small materials have compared the semi-quantitative MRI method to invasive secretin-stimulated function tests. In one of them, a considerable overlap was found between normal and reduced pancreatic function using the intraductal secretin test as the reference standard (114), giving a sensitivity and specificity of 72 % and 87 % respectively. A more recent study demonstrated no overlap between normal and reduced pancreatic function in patients with suspected chronic pancreatitis, using a full time endoscopic secretin test as reference standard (113). There are also few studies comparing quantitative MRI tests to invasive tests. One small study compared duodenal width 10 minutes after secretin to results from the Lundh test, finding excellent discrimination between normal and reduced pancreatic function, but poor discrimination between mild and severe exocrine dysfunction (117). A recent study with 65 patients demonstrated a highly significant correlation between peak bicarbonate concentration from a 60 minutes endoscopic secretin test and increase in duodenal volumes 10 minutes after secretin stimulation (119). The discriminating properties of the test were, however, not shown in this study.

Some studies have demonstrated that function testing with secretin stimulated MRCP performs well when compared to non-invasive tests (120-123). As indirect tests perform poorly in detecting mild and moderate exocrine pancreatic dysfunction, the value of these studies is limited in evaluating diagnostic accuracy.

Several studies have evaluated the secretin-stimulated MRCP function test's ability to detect chronic pancreatitis when this diagnosis is determined by a multimodal approach (119, 124, 125). In two of these studies, patients with established and severe chronic pancreatitis were well discriminated from subjects with no detected pancreatic disease, but mild to moderate or early chronic pancreatitis did overlap considerably with subjects with no known pancreatic disease (119, 124).

Diffusion-weighted imaging

Function testing with DWI before and after secretin stimulation has also been evaluated. In a small study, Erturk and co-workers (126) demonstrated increase in ADC after secretin stimulation to a peak, followed by a decrease in healthy subjects. This peak in ADC was observed between 90 seconds and four minutes after secretin stimulation, while in patients with severe chronic pancreatitis, there was no such peak during 10 minutes observation time. Interestingly, in patients at risk for chronic pancreatitis because of chronic alcohol abuse, a delayed peak (appearing more than four minutes after secretin stimulation) was observed, discriminating healthy controls from subjects at risk and chronic pancreatitis patients (126). This delayed peak in subjects at risk may reflect early changes in the pancreas found on EUS in patients with chronic alcohol abuse (127). In another study, the discriminatory properties of time to peak after secretin stimulation was not reproduced between healthy controls and patients with mild and moderate chronic pancreatitis, even though quite similar protocols were used (128). Both studies suffer from the lack of other functional evaluation of the pancreas. This secretin induced peak in ADC does, however, deserve further investigation, as diffusion weighted imaging gives an opportunity to evaluate the direct pancreatic tissue response to stimulation.

1.2.3 Analysis of duodenal juice

Bicarbonate analysis

Bicarbonate concentration in duodenal juice may reach 140 mM or even higher (3). Bicarbonate is the one of the two salts of carbonic acid:

$$CO_2\uparrow + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

According to the Henderson-Hasselbalch equation most of the carbonic acid is in the bicarbonate form at pH 8-9, as in duodenal juice (this is also the case at pH 7.3 as in serum) (129). Measuring bicarbonate concentration has traditionally been done by back titration (130), modified from a method originally described by van Slyke almost 100 years ago (131). This method exploits the volatility of carbonic acid as shown in the carbonic acid equation.

The pH in the sample is measured, before the sample is acidified with a given amount of hydrochloric acid, and shaken vigorously. This almost completely left-skews the carbonic acid equation, and allows the carbon dioxide to evaporate, for practical purposes eliminating all the carbonic acid from the solution. The solution is then titrated back to the original pH, using sodium hydroxide. The difference between amount of hydrochloric acid used to acidify and sodium hydroxide used to titrate back to original pH equals the amount of carbonic acid lost in the acidification process (130). Back-titration is time consuming, and demands a large amount of duodenal juice, which is not always readily available. Recently, two papers have been published presenting promising results on automation of bicarbonate determination, one of them using a “blood-gas” machine, measuring partial pressure of carbon dioxide and pH in appropriately diluted samples of duodenal juice (132), the other measuring bicarbonate concentration using the phosphoenol pyruvate carboxylase enzyme reaction (133, 134).

Digestive enzyme analysis

Action of enzymes is linked to lowering the activation energy threshold in a chemical reaction. Enzymes may speed up a chemical reaction already going on, or initiate a reaction when this activation energy threshold is too high for the reaction to occur spontaneously (135). Several digestive enzymes have been used as indicators of exocrine pancreatic function. Which of these enzymes that are best for determining exocrine function is unclear (59). Enzymes may either be determined by quantification of amount or catalytic activity analyses. In exocrine function testing, enzyme catalytic activity is the most used (faecal elastase 1 is an important exception) (59).

The principle for an enzyme activity assay is incubating the enzyme with substrate(s) for the enzymatic reaction, and measure decrease of substrate or increase of end product resulting from the enzymatic reaction. This is preferably done in a first order reaction system, because of the close to linear reaction rate when substrate is in excess (136).

There is a large number of test principles available, exemplified by a review on lipase assays (137). No standard method exists for assessment of digestive enzymes in duodenal juice (138). In enzyme catalytic activity assays, factors like temperature, pH, substrate saturation, presence of other substances and salt concentrations need to be controlled, as they may all alter enzyme activity(136).

Enzyme catalytic activity can be measured continuously, measuring reaction products at different time points, or by measuring reaction products after termination of the reaction (136). Enzyme catalytic activity is measured in units (U) or katal (kat). One U is defined as the amount of enzyme that catalytically transforms one micromole of a substrate per minute under standard conditions. To comply with SI units, the katal unit was made, in where one katal equals the catalytic activity that will raise the rate of reaction by one mole per second in a specified assay system (139).

Stability considerations and conservation of duodenal juice

Special measures are needed in the handling of duodenal juice for analysis. Bicarbonate is unstable, and will evaporate when the samples are acidified. Activated proteases are abundant; ready to proteolyse other enzymes and indeed themselves.

Stability of bicarbonate

According to the Henderson-Hasselbalch equation, at pH 8-9, most of the carbonic acid is in the bicarbonate form, and negligible amounts are prone to evaporate (129). This is confirmed by the observation that bicarbonate samples are stable for at least 6 hours on ice and in room temperature (140). Further escape of carbon dioxide is avoided by storing samples in gas tight containers. Contaminations that lower the pH of the duodenal juice will cause a left-skew of the carbonic acid equation, leading to more instability of the sample. Samples with pH < 6-7 will, hence, be unstable, and results from analysis will not be reliable. To my knowledge, little data exist on freezing samples for analysis on bicarbonate.

Stability of digestive enzymes

Several factors must be taken into consideration when storing samples of duodenal juice for analysis of digestive enzymes, as wrong storing conditions will irreversibly destroy the samples. Among the most commonly measured digestive enzymes, pancreatic lipase is the most unstable one, as demonstrated both in vitro (17, 141) and in vivo (142).

Effect of proteolysis

Pancreatic lipase is subject to rapid proteolysis catalyzed by proteases, especially chymotrypsin, effectively inactivating catalytic activity and binding to colipase (143, 144). Also trypsin is inactivated trough chymotrypsin catalyzed proteolysis (145). Amylase (146) and chymotrypsin (141) activity are relatively stable to degradation.

Also the amounts of elastase measured quantitatively by immunological methods are stable to degradation in faeces and duodenal juice (82).

Effect of temperature

Storage of duodenal juice at room temperature rapidly inactivates lipase and trypsin, but not amylase, as is also the case with storage at 4°C (146). Decline in lipolytic activity in untreated duodenal juice has been demonstrated at - 20°C (141), and even at -80°C when stored for more than four months (147).

Effect of additions

Lipase activity was protected by addition of a chymotrypsin inhibitor and was further correlated to amount of lipase measured by quantitative methods, suggesting that loss of lipase activity during storage at -20°C is due to proteolysis (141). Addition of glycerol (17) and food substrates (148) seem to stabilize activity of digestive enzymes in duodenal juice during storage.

Effect of thawing and refreezing

Freezing at -80°C, thawing and refreezing did reduce activity of lipase, chymotrypsin and trypsin, but not amylase in pure pancreatic juice. However, in this material, the effect on activity of the first freezing compared to fresh, never frozen pancreatic juice was not determined (138).

1.2.4 Exocrine pancreatic insufficiency

The exocrine pancreas has a large functional reserve capacity. Excess losses of lipids in the faeces, steatorrhoea, is the clinically most important symptom, and also occurs as the primary symptom of malabsorbtion caused by exocrine pancreatic insufficiency (149). DiMagno showed in the early seventies that >90 % of the lipase output had to be abolished before steatorrhoea occurred (71, 150). However, the lipase output threshold for developing steatorrhoea seems to be somewhat variable between individuals (72).

Fat malabsorption

The development of fat malabsorption has been suggested to be caused by several interacting mechanisms (151). In alcoholic chronic pancreatitis, lipase seems to be more vulnerable to the disease process than at least trypsin, as shown by decreased lipase/trypsin ratio in chronic pancreatitis compared to healthy controls (150). Destruction of ductal compartments in pancreatic disease cause bicarbonate output to decline, causing lower pH levels in the duodenum (9). Pancreatic lipase is very pH sensitive, and may be irreversibly destroyed if pH gets to low (152). The ability of bile acids to form micelles is also less in the acidic duodenal environment found in patients with exocrine pancreatic insufficiency (10). Pancreatic lipase is also subject to earlier inactivation in the intestinal lumen than proteases and amylase (142), partly due to high susceptibility to proteolysis (143, 153). Human gastric lipase may play a modifying role in development of fat malabsorption. There are observations indicating that amount and efficacy of gastric lipase may be up-regulated in severe chronic pancreatitis, compensating for some of the loss of pancreatic lipase (154, 155). The quantitative contribution of gastric lipase in severe pancreatic insufficiency does, however, still remain controversial (156).

Malabsorption of other nutrients

Loss of nitrogen in the faeces, creatorrhea, does usually not occur until late in the development of pancreatic insufficiency (71). This has been attributed to gastric protease activity (149). However, recent studies using a breath test based on ^{15}N labelled casein in pancreatic insufficient patients indicate that creatorrhea underestimates loss of protein due to maldigestion, as colonic fermentation of proteins causes production of ammonia which is subsequently exhaled. Hence, the role of protein malabsorption may be larger than assumed earlier (157). Starch malabsorption due to low levels of pancreatic amylase has also been demonstrated (158). The role of salivary amylase is questionable in pancreatic insufficiency (159). However, there are findings indicative of substantial colonic salvage of short-chain fatty acids produced

by fermentation of carbohydrates in patients with exocrine pancreatic insufficiency, resulting in less energy loss (160).

Contributing drivers to malabsorption

Intestinal bacterial overgrowth seems to be a common complication in exocrine pancreatic insufficiency, aggravating diarrhea and malabsorption (161). Also postprandial motility responses are altered in chronic pancreatitis, possibly aggravating postprandial pain and intestinal malabsorption (162). Figure 3 gives an overview of the different drivers to malabsorption in exocrine pancreatic insufficiency.

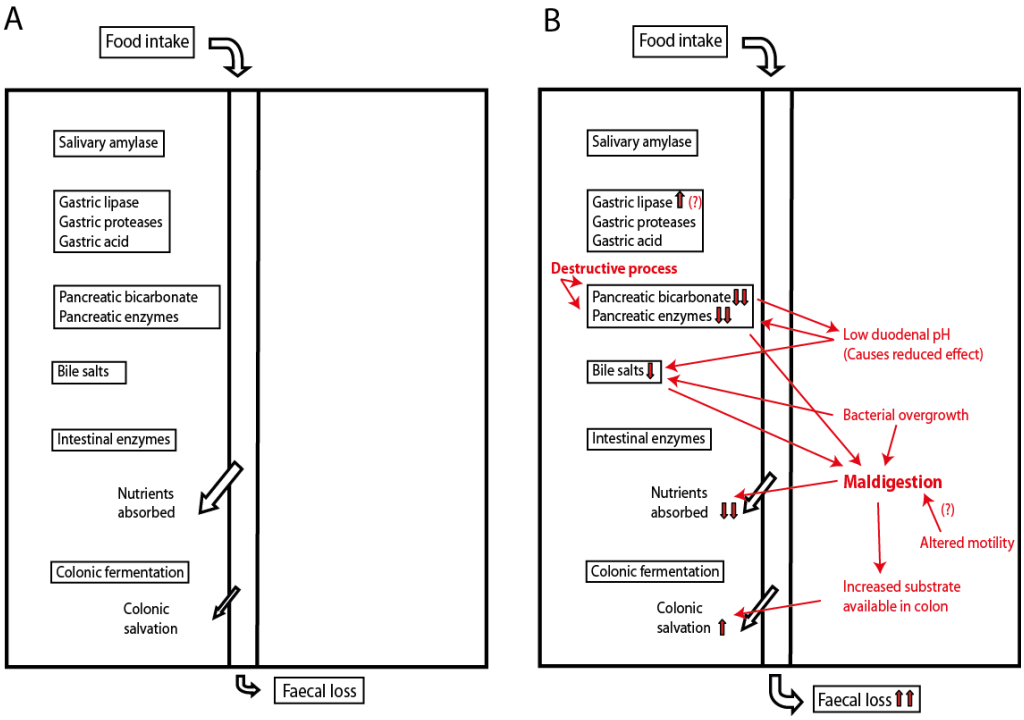

Figure 3: Mechanisms of faecal nutrient loss in exocrine pancreatic dysfunction. Panel A. The situation in the healthy individual, with addition of salivary, gastric and pancreatic enzyme systems and bile as the chyme passes. Pancreatic bicarbonate is a substantial contributor to optimal pH for digestive processes in duodenum. In addition, intestinal enzymes cause some digestion, leading to absorption of most of the nutrients. Colonic salvation is probably negligible. Panel B. The situation in pancreatic exocrine insufficiency. The destructive process in the pancreas leads to low output of digestive enzymes and bicarbonate. Lack of digestive enzymes causes maldigestion, which is further aggravated by lower duodenal pH, causing digestive enzymes and bile salts to function sub-optimally. Increased amount of nutrients not absorbed promotes bacterial overgrowth which causes diarrhoea and binding of bile salts. In addition motility disorders may play a role. The net result is less substrate available for absorption, and increased faecal loss of nutrients. To compensate, gastric lipase activity increases, and there is a substantial increase in colonic salvation of short fatty acids from fermented carbohydrates. The figure is based on (151).

1.3 Diseases affecting exocrine pancreatic function

1.3.1 Disorders of pancreas morphogenesis

Pancreas hypoplasia and agensis

The pancreas is of endodermal origin, starting as a dorsal and a ventral budding evaginating from the endoderm. The dorsal budding subsequently develops into the dorsal *anlage*, eventually becoming the tail, body and superior part of the head of the pancreas. The ventral *anlage* becomes the inferior or duodenal part of the head of the pancreas. Normally, the ducts from the two *anlagen* fuse into the common duct of Wirschung (163, 164). Most of the knowledge about pancreas developmental biology originates from studies on mice (164), the frog *Xenopus laevis* (165) and Zebrafish (166). The finding that a mutation in the genes PDX1, EIF2AK3, PTF1A, HNF1B, RFX6 and GATA6 can cause agenesis or hypoplasia of the pancreas has demonstrated their crucial role also in human pancreatic development (table 3) (163, 167). Functional studies of endocrine and exocrine function in the remaining pancreas in patients with these mutations may increase our understanding of how these genes affect pancreas morphogenesis.

Mutated gene	Phenotype related to pancreas	Reference
PDX1	Neonatal diabetes. Pancreas: agenesis/ hypoplasia of body and tail/ normal	(165, 168, 169)
EIF2AK3	Neonatal diabetes. Pancreas: hypoplasia/normal	(170)
PTF1A	Pancreas: agenesis	(171)
HNF1B	Neonatal diabetes/MODY. Pancreas: hypoplasia (?)/normal	(172, 173)
RFX6	Neonatal diabetes. Islet agenesis. Pancreas: hypoplasia/small normal	(174, 175)
GATA6	Neonatal diabetes/MODY. Pancreas: agenesis/hypoplasia	(176, 177)

Table 3: Genes which, when mutated, cause agenesis or hypoplasia of pancreas. Phenotype is caused by haploinsufficiency in the case of HNF1B and GATA6, and homozygous mutations in the other genes.

Pancreas divisum

Pancreas divisum is a very common anomaly of the pancreas, affecting 1.5 – 6.0 % of healthy individuals (178). A cause for this incomplete fusion of the dorsal and ventral *anlagen* has not been identified, but it is interesting to note that mice with haploinsufficiency in hedgehog signalling develops a similar phenotype (163). Pancreas divisum has been regarded as a risk factor for pancreatitis, but this is controversial, and recent studies may indicate that this common variant probably must be regarded as an incidental finding rather than a cause in pancreatitis (178).

Annular pancreas

Annular pancreas is a rather rare anomaly, characterized by encirclement of the duodenum by pancreatic tissue from the ventral *anlage*. The anomaly may be a result of defective hedgehog signalling, reducing the integrity of the duodenal wall. This has, however, not been demonstrated in man. Duodenal obstruction symptoms are the most common presentation of annular pancreas (179).

1.3.2 Inborn primary exocrine pancreatic diseases

Cystic fibrosis

Cystic fibrosis is the most common lethal genetic disease in the white population. Cystic fibrosis is an autosomal recessive disease, caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) encoding gene (*CFTR*), causing impaired or lost function of the CFTR. There are >1500 mutations in the CFTR, but only some of them are known to be associated with disease development. Disease causing mutations are classified after how they affect function of CFTR, a classification that also may predict disease severity (180). By not fully elucidated mechanisms, impaired or lost function of CFTR results in thick, poorly soluble mucus, blocking lumina of hollow organs (180, 181). This in turn eventually leads to reduced or destroyed organ

function, inflammation and opportunistic infections. Organs commonly involved include, lungs, sinuses, gastrointestinal tract, liver, pancreas, sweat glands and reproductive organs (180). There are great differences in vulnerability of the different organs to *CFTR* mutations. While male cystic fibrosis patients are almost inevitably infertile due to destroyed vas deference, genetic and environmental modifiers may cause very different phenotypes of liver or intestinal disease in patients with the same mutations, even among siblings (182). Lung insufficiency is the main cause of death in cystic fibrosis patients, and as environmental factors modify lung function to a great extent, lung drainage and aggressive treatment of opportunistic infections is one of the cornerstones in cystic fibrosis treatment (180).

In the pancreas, thick mucus may block the ducts, eventually leading to destruction of the gland. Both ductal and acinar compartments are then affected (100), and many patients develop diabetes in early adult age, indicating that also islets are affected by the destructive process (183).

To what extent the exocrine pancreatic function is affected is more predictable from genotype than most other manifestations, and homozygote loss-of-function mutations in *CFTR* will almost inevitably cause intrauterine destruction of pancreas, and pancreatic insufficiency from birth or early childhood (182, 184, 185). Patients with milder mutations are most often pancreatic sufficient, but some of these patients develop exocrine pancreatic insufficiency later in life (184, 186, 187). Pancreatic sufficient, but not pancreatic insufficient patients with cystic fibrosis are prone to pancreatitis (185). Pancreatic insufficiency causes maldigestion, which is often aggravated by other gastrointestinal manifestations associated with cystic fibrosis (182, 188).

Poor nutritional status caused by maldigestion, but also by increased energy demands, is common in cystic fibrosis patients (189). Before modern cystic fibrosis treatment, malnutrition was the major cause of death, illustrating the importance of treating maldigestion and providing nutritional support (180).

Treatment based on understanding of the genetic and pathogenetic factors in cystic fibrosis has lead to an incredible improvement of life expectancy in these patients the last decades (180, 190).

Other inborn diseases of the exocrine pancreas

Shwachman-Diamond syndrome

The Shwachman-Diamond syndrome (191) is characterized by low number of one or more haematological cell lines, increased susceptibility to severe infections, pancreatic dysfunction, skeletal abnormalities and poor growth. The phenotype is variable, and manifestations tend to improve during childhood (192). However, there is an increased risk for leukaemia and myelodysplastic syndrome in these patients (193). Pancreatic dysfunction in Shwachman-Diamond syndrome is an isolated acinar defect, with low levels of digestive enzyme output, but normal bicarbonate secretion (159, 194), and probably not increased diabetes risk (195). Shwachman-Diamond syndrome is a recessive trait, and most patients have mutations in the *SBDS* gene (159, 196). These mutations cause impaired assembly of ribosomes, which is probably the pathogenetic basis for the disease (197).

Johanson-Blizzard syndrome

The Johanson-Blizzard syndrome is a rare syndrome characterized by absent alae nasi, teeth anomalies, pancreatic dysfunction, deafness, hypothyroidism, mental retardation and several other malformations (198). Also in Johanson-Blizzard syndrome, the pancreatic dysfunction is an isolated acinar defect, with seemingly normal function of ducts and islets (199, 200).The syndrome is autosomal recessive, caused by mutations in the *UBR1* gene which codes for one of the ubiquitin ligases (201).

Pearson syndrome

Pearson marrow-pancreas syndrome is a disorder caused by mutations in mitochondrial DNA. The syndrome is characterized by bone-marrow disease in addition to multi-organ failure, among them, the pancreas. The prognosis is generally poor (202).

Histiocytosis-lymphadenopathy plus syndrome

The histiocytosis-lymphadenopathy plus syndrome (earlier named H-syndrome or pigmented hypertrichosis with insulin-dependent diabetes mellitus, PHID) is caused by homozygous mutations in the *SLC29A3* gene, which codes for a nucleoside transporter (203). The syndrome phenotype is very heterogeneous, and is associated with histiocyte infiltration and multi-organ failure, among them diabetes and pancreatic exocrine dysfunction (demonstrated by low faecal elastase), as reviewed in (204).

Isolated enzyme deficiencies

There are some reports on isolated, inborn pancreatic enzyme deficiencies (163). Insufficient enteropeptidase activity in the intestinal mucosa causes impaired activation of trypsinogen, with resulting lack of active digestive proteases. The condition is associated with severe malabsorbtion in infancy (205), and is linked to mutations in the proenteropeptidase gene (206).

1.3.3 Chronic pancreatitis

Chronic pancreatitis is a progressive inflammatory disease of the pancreas leading to fibrosis and loss of pancreatic exocrine and eventually endocrine function (207). The progressive destruction of the gland has been regarded as the hallmark of chronic pancreatitis versus acute pancreatitis. However, severe acute pancreatitis and recurrent acute pancreatitis may induce chronic pancreatitis, and novel insights in causative factors and different disease mechanisms has altered the understanding of acute and chronic pancreatitis to being a disease continuum rather than two distinct conditions (207-209).

Clinical features of chronic pancreatitis

Pain is the main symptom in patients with chronic pancreatitis (207). The pain may come in attacks, or be more chronic. The pain is often excruciating, hence, opiate demanding, and seems to be a combination of nociceptive and neuropathic pain (210).

With progressive destruction of the gland, exocrine dysfunction develops, and eventually exocrine and endocrine insufficiency (211). Often the pain then recedes. This is thought to be a burnout phenomenon, indicating that the destruction of pancreatic tissue is a part of the pain causing process, though this hypothesis is controversial (210, 212).

Pathophysiology

The pathophysiology of chronic pancreatitis is linked to pancreastasis, misdirection of zymogens, and intracellular and interstitial activation of zymogens (207, 213, 214), leading to activation of multiple signalling pathways causing further pancreastasis with activation of zymogens, inflammation and cell death (215, 216). This again leads to fibrosis, eventually causing destruction of the architecture of the gland (chirrosis) (217).

Etiology

Alcohol abuse is the most common cause of chronic pancreatitis (207, 218). The mechanism of alcohol in inducing chronic pancreatitis is, however, not fully understood (219), and as <10 % of alcohol abusers develop chronic pancreatitis (220), other genetic and/or environmental factors must have triggering effects in addition. Also cigarette smoking is an independent factor associated with chronic pancreatitis (221, 222). Drugs may also induce pancreatitis, though most often mild acute pancreatitis (223). Taken together, the toxic effects of these substances may point towards a role of the pancreas as a vulnerable xenobiotic-metabolizing organ (207, 224).

Autoimmune pancreatitis is a rather novel disease entity, characterized by focal or global inflammation in the pancreas and increase in immunoglobulin G4 and other serological markers (225). Autoimmune pancreatitis has been estimated to cause 2-4 % of cases with chronic pancreatitis, but these prevalence numbers will probably increase with the increasing awareness of this condition among clinicians (226). Several genetic risk factors for chronic pancreatitis have been identified. Severe

mutations in the cationic trypsinogen gene (PRSS1) are the only known causes of hereditary pancreatitis (due to high penetrance), while the other mutations are regarded as genetic risk factors for pancreatitis, depending on other genetic factors or environmental factors to cause chronic pancreatitis (216, 227). With increasing knowledge about risk factors for pancreatitis, the idiopathic chronic pancreatitis group is declining (207). Tropical calcific pancreatitis has been regarded as an idiopathic entity observed in patients in low income countries in the tropical parts of the world. The condition has been attributed to cassava intoxication and malnutrition (228). However, recent studies weaken these associations (229), and findings of genetic risk factors in a substantial part of these patients, question whether tropical calcific pancreatitis actually is a disease entity on its own (230).

Diagnostic approach

The diagnosis of chronic pancreatitis may be challenging. In moderate to severe large duct disease (most common manifestation of alcoholic chronic pancreatitis), the development of pancreatic calcifications and ductal abnormalities visible on imaging often make the diagnosis evident with commonly available modalities. On the other hand small duct disease may have remarkably normal findings on imaging, and the diagnosis is often challenging (231). Early chronic pancreatitis findings are also often non-specific (207).

Demonstrating histological changes in representative biopsies form the pancreas has been defined as the reference standard in diagnosing chronic pancreatitis. However, the difficult accessibility of the pancreas, the high risk of complications and the risk of not getting a representative specimen justifies this approach only in cases of suspected pancreatic cancer (231). Hence, imaging and function testing are the cornerstones in the diagnosis of chronic pancreatitis.

Imaging

Cambridge criteria

The Cambridge criteria came in 1983, and were based on parenchymal changes found on CT and US examinations, and ductal changes found on ERCP (232). These criteria have later been revised and are still in use (table 4) (233). As MRCP is the modality of choice in evaluating pancreatic duct anatomy, the ERCP criteria have been adapted for MRCP and secretin stimulated MRCP (44). MRI has also been shown to be very sensitive in revealing parenchymal changes in chronic pancreatitis (44).

Terminology	CT or US	ERCP		Additional features
		Main duct	*Abnormal side branches*	
Normal	Main pancreatic duct < 2 mm Normal gland size and shape Homogeneous parenchyma	Normal	None	
Equivocal	Only one of: • Main pancreatic duct between 2 to 4 mm • Gland enlargement < 2x normal • Heterogeneous parenchyma	Normal	<3	
Mild	Two or more above	Normal	>3	
Moderate	Cavities <10 mm Duct irregularity Focal acute pancreatitis Increased echogenity of duct wall Contour irregularity	Abnormal	>3	None
Marked	As above + one or more of: • Cavities > 10 mm • Gland enlargement >2x normal • Intraductal filling defects or calculi • Duct obstruction, stricture, gross irregularity • Contiguous organ involvement	Abnormal	>3	One or more of: • Large cavity • Obstruction • Filling defects • Severe dilatation • Irregularity

Table 4. Cambridge imaging criteria for chronic pancreatitis (233).

Endoscopic ultrasound

Endoscopic ultrasound is very sensitive for detecting parenchymal changes of the pancreas. This may actually be a problem, as small changes in the pancreatic tissue can be seen in subjects with no other signs of pancreatic disease, in whom changes may be due to old age (234) or risk factors for chronic pancreatitis, such as alcohol abuse (127). Hence, standard criteria for chronic pancreatitis have been riddled with too many false positives (41, 235). To increase specificity, the more restrictive (and complicated) Rosemont criteria have been proposed (table 5) (236), but further evaluation is needed to see if these criteria actually are more specific (237).

	EUS finding	Description	Correlation	Strength
Parenchymal criteria	Hyperechoic foci with shadowing	Length or width >2 mm	Calcifications	Major A
	Lobularity with honeycombing (>3 contiguous lobules)	Structures >5 mm with enhancing rim and echo-poor centre	None	Major B
	Lobularity without honeycombing	As above	None	Minor
	Hyperechoic foci without shadowing	As above	None	Minor
	Hyperechoic strands	Lines > 3 mm in at least 2 different directions	None	Minor
	Intraparenchymal cysts	None	None	Minor
Ductal criteria	Main pancreatic duct calculi with shadowing	None	None	Major A
	Irregular main pancreatic duct/ectatic contour	None	None	Minor
	>3 dilated side branches	None	Side branch ectasias	Minor
	Main pancreatic duct dilatation	>4 mm head, >3.5 mm body, >1.5 mm tail	None	Minor
	Hyperechoic main pancreatic duct margin	Echogenic structure covering >50 % of entire main duct	Ductal fibrosis	Minor
	Strictures	None	None	Minor

Table 5. Rosemont criteria for chronic pancreatitis (236).

Consistent: 1 Major A + ≥ 3 Minor; 1 Major A + 1 Major B; 2 Major A.

Suggestive: 1 Major A + < 3 Minor; 1 Major B + ≥ 3 Minor; ≥5 Minor.

Indeterminate: 3-4 Minor; 1 Major B + 0-3 Minor.

Normal: ≤ 2 Minor

Exocrine pancreatic function testing

Reduced bicarbonate output as measured through the classic secretin stimulation test has been regarded the most sensitive marker for chronic pancreatitis (61). The addition of cholecystokinin or one of its analogues and measuring digestive enzymes has been done in many protocols (59). Lankisch and co-workers used reduced output of one or more digestive enzymes as the first sign of diminishing exocrine pancreatic function in chronic pancreatitis, followed by reduction in bicarbonate output (238). However, at least in the 60-minute secretin endoscopic test, addition of cholecystokinin and measuring digestive enzymes did not enhance the test in comparison to secretin alone (239). These conflicting results probably reflect the lack of standardization of tests and reference standards, but may also partly be due to different courses of early chronic pancreatitis with respect to acinar or ductal involvement (240). As non-invasive tests detect only moderate to severe pancreatic dysfunction (62), these tests detect late changes in chronic pancreatitis, leading to substantial loss of exocrine tissue (61).

Validation of diagnostic modalities

There have been some attempts to validate the different diagnostic modalities against histopathological changes of the pancreas. There are fairly good correlations between histopathological changes and chronic pancreatitis found on ERCP (241), EUS (242-244) and invasive pancreatic function tests (244-246). However sensitivity for minimal change pancreatitis and early chronic pancreatitis is limited for all modalities. There also seem to be differences between patients whether structural or functional changes are most evident in the early phases of chronic pancreatitis (240, 244, 247). Furthermore, patients may have calcifications on pancreatic imaging with normal exocrine pancreatic function (248, 249).

Multimodal approach

As chronic pancreatitis can be challenging to diagnose, a multimodal approach is often needed. Layer and co-workers (250) published a clinical scoring system, weighing the different clinical findings according to their specificity for chronic

pancreatitis (table 6). As pancreatic calcification (visible on imaging available in the late 1970s and early -80s) and typical histological changes are regarded as pathognomonic for chronic pancreatitis, these findings alone were considered enough for establishing the diagnosis. This scoring system is quite simple, and has been found to be useful in modified forms in later studies (251, 252). Other clinical scoring systems have also been proposed (217, 233). To my knowledge, none of these scoring systems have been validated.

Clinical findings	**Score**
Pancreatic calcification	4
Typical histological changes	
Characteristic findings on ERCP	3
Steatorrhoea (> 7 g/day) *or*	2
Abnormal cholecystokinin test	
Recurrent acute pancreatitis attacks or chronic abdominal pain	
Diabetes mellitus	1

Table 6. The Mayo clinic scoring system for chronic pancreatitis (or Layer score) (250). A sum of scores ≥ 4 suggests chronic pancreatitis.

1.3.4 Diabetes mellitus

Diabetes mellitus comprises a heterogeneous group of metabolic disesases characterized by hyperglycaemia. The hyperglycaemia results from defects in insulin secretion, insulin action on target organs or both. The disease process causes abnormal metabolism of carbohydrates, fat and protein. The diagnostic criteria for diabetes mellitus are related to presence of hyperglycaemia or increased levels of glycosylated haemoglobin (HbA1C) (253):

1. HbA1C ≥ 6.5 %
2. OR: Fasting (>8 hours) plasma glucose ≥ 7.0 mmol/L
3. OR: Plasma glucose ≥ 11.1 mmol/L two hours after oral glucose tolerance test
4. OR: Plasma glucose ≥ 11.1 mmol/L in random sample and diabetes suspect symptoms

Inappropriate hyperglycaemia or elevated HbA1C not fulfilling the above criteria classifies as impaired glucose tolerance or prediabetes. Short-term complications to diabetes are related to hyperglycaemia, ketoacidosis and intracellular starvation, in addition to iatrogenic hypoglycaemia. Long-term diabetes-associated complications are caused by microvascular injury, namely nephropathy, retinopathy and neuropathy, and by macrovascular injury; cardiovascular disease. Good metabolic control is the key to prevent complications (253). Diabetes is one of the most common chronic diseases in the world, and there is a global increasing incidence of diabetes and its complications, especially in developing countries (254, 255). By far the most cases of diabetes are type 1 or type 2 (253).

Type 1 and type 2 diabetes mellitus

Type 1 diabetes accounts for 5-10 % of cases with diabetes. It is caused by autoimmune destruction of beta cells in the islets of Langerhans (type 1A), or is idiopathic (type 1B). Several genetic risk factors have been identified, the strongest among them related to the HLA system, but these risk factors only represent a vulnerability to develop diabetes. Hence, environmental factors must also exist, but the hunt for the "smoking gun" has not identified any yet. As the insulin producing beta cells are destroyed by the disease process, and insulin sensitivity normally is unaffected, insulin supplementation is the only medical treatment option (256).

Type 2 diabetes is estimated to account for 80-90 % of diabetes cases. However, the real number may be lower, as non-type 1 and non-type 2 diabetes may mimic type 2 diabetes. Type 2 diabetes represents an imbalance between insulin production and peripheral insulin sensitivity, principally caused by an imbalance between intake and expenditure of nutrients. Insulin resistance in liver, adipose tissue and muscle is the primary manifestation, and diabetes occurs when beta cells cannot compensate with

insulin. With progression of disease, beta cell function also declines. Overnutrition and obesity is a risk factor. Also, many genetic risk factors for type 2 diabetes have been identified, telling us that type 2 diabetes is a very heterogeneous disease, and that genetic risk is difficult to predict from other sources than family history. Treatment is primarily by reducing overnutrition. Drugs increasing insulin effect and reducing insulin resistance may be used. Eventually insulin supplementation may be needed to achieve metabolic control (257).

Diabetes and the exocrine pancreas

Pancreatic diabetes

Pancreatic diabetes, or type 3C diabetes, is diabetes secondary to destructive processes in the exocrine pancreas. Chronic pancreatitis is the most common cause, but pancreatic diabetes may also be caused by other pancreatic diseases, pancreatic malformations, pancreatic trauma and pancreatectomy (258). The prevalence of pancreatic diabetes has been estimated to be 0.5-1.2 % of diabetes patients, but recent reports suggest the number to be higher (259). In a recent, retrospective study diabetes patients were reclassified, and 9 % of the patients were classified as pancreatic diabetes patients (260). However, because of the retrospective design of this study, with seemingly no possibility to find out whether changes in the exocrine pancreas were primary or secondary to endocrine disease, this number may be spuriously high. There have been several studies estimating that the risk for developing diabetes in patients with chronic pancreatitis ranges from 41-86 % (261). The risk estimates will necessarily depend on diagnostic criteria for chronic pancreatitis, time of follow up and therapeutic traditions in the cohort studied. Diabetes is also a common complication in cystic fibrosis. One study showed that in cystic fibrosis patients ≥ 30 years of age, 43 % had developed diabetes, and 27 % were glucose intolerant (262).

The primary cause of pancreatic diabetes is insulin deficiency. In chronic pancreatitis, the beta cell mass is reduced due to chronic inflammation and development of fibrosis of the islets. Beta cells seem to be the most vulnerable cell type in islets of

Langerhans to these noxious processes (263). Secondarily, hepatic insulin resistance often develops in pancreatic diabetes, possibly as a result of reduced pancreatic polypeptide from the islets (264). In addition, insulin stimulation may be impaired by reduced secretion of the incretin hormones, GIP and GLP-1, as a result of maldigestion in patients with pancreatic exocrine insufficiency, as has been demonstrated by increased incretin and insulin response to pancreatic enzyme replacement therapy (265-267).

Exocrine pancreatic function in diabetes

It has been known for a very long time that diabetes patients may develop pancreatic dysfunction (268). Mild exocrine pancreatic dysfunction was found in 43 % of type 1 diabetes patients undergoing a secretin pancreozymin test (269), and there was no progression of exocrine pancreatic dysfunction at follow-up 20 years later. The authors concluded that mild, non-progressive exocrine pancreatic dysfunction is common among diabetes patients, but that most of them do not need therapy (270).

Hardt and co-workers demonstrated low levels of faecal elastase and steatorrhoea in type 1 and type 2 diabetes patients (271, 272), but demonstrated no clinical effect of pancreatic enzyme therapy (273). However, low faecal elastase and steatorrhoea has been disputed as a marker of exocrine pancreatic dysfunction in diabetes patients (274).

The cause of impaired exocrine pancreatic function in diabetes is not known. Pancreas volume is reduced in diabetes patients, and has been demonstrated to be correlated to faecal chymotrypsin levels (275). Loss of trophic effects of insulin and other hormones have been suggested as a mechanism, as has diabetic neuropathy and microangiopathy (276). Misclassification of pancreatic diabetes may also be one reason (258).

Maturity onset diabetes in the young (MODY)

Overview

Maturity onset diabetes in the young (MODY) is a group diseases with autosomal dominant inheritance characterized by onset of non-ketotic diabetes in at least one family member before age of 25 years and evidence of primary beta cell dysfunction (277). Molecular studies have identified genetic background for MODY in at least 13 genes (table 7) (278, 279). Prevalence of known MODY mutations is difficult to assess, as clinical suspicion and availability of genetic testing may vary in a population (280). From the large Norwegian health survey for Nord-trøndelag County (HUNT2), clinical criteria for MODY was present in 2.2 % of participants with diabetes. The most common form, HNF1A-MODY was confirmed genetically in 0.4 % of diabetic subjects (281). In studies from the Norwegian childhood diabetes registry, the minimum prevalence of monogenic diabetes was recently estimated to be 1.1 % of the diabetic child population, HNF1A-MODY being most abundant also among the childhood MODYs (282). GCK-MODY is also rather frequent in a diabetes population, while HNF4A-MODY and HNF1B-MODY occur sporadically. The other MODY forms are reported in only one or few families, and for some genes, the link between genotype and phenotype may even be unconvincing (278).

MODY is often due to mutation in a transcription factor (283, 284). Study of disease mechanisms in MODY may therefore increase our knowledge both on how factors of the transcriptional network are essential for development and function of beta cells, and also on other intracellular mechanisms crucial for normal beta cell function.

Identifying MODY patients may also have clinical implications. For example, HNF1A-MODY patients often respond excellently to treatment with sulfonylurea, due to the mutation induced dysfunction upstream to the sulfonylurea receptor (285, 286). Another example is GCK-MODY patients who develop mild fasting hyperglycaemia, but usually no need for treatment or diabetes associated complications (287, 288).

Common name	Alternative name	Gene mutated
MODY1	HNF4A-MODY	*HNF4A*
MODY2	GCK-MODY	*GCK*
MODY3	HNF1A-MODY	*HNF1A*
MODY4	PDX1-MODY	*PDX1*
MODY5	HNF1B-MODY	*HNF1B*
MODY6	NEUROD1-MODY	*NEUROD1*
MODY7	KLF11-MODY	*KLF11*
MODY8	CEL-MODY	*CEL*
MODY9	PAX4-MODY	*PAX4*
MODY10	INS-MODY	*INS*
MODY11	BLK-MODY	*BLK*
MODY12	ABCC8-MODY	*ABCC8*
MODY13	KCNJ11-MODY	*KCNJ11*

Table 7. The different known MODY forms, adapted from (278, 279). A clinical MODY phenotype with unknown genetic background is designated MODY X.

MODY and the exocrine pancreas

There are few studies on exocrine pancreatic function in subjects with MODY mutations. Two of the MODY forms, HNF1B-MODY and CEL-MODY, have been attributed to exocrine insufficiency.

HNF1A-MODY

In mice, the *hnf1a* gene is expressed in both exocrine and the endocrine pancreas during development to adult stage, indicating roles in both exocrine and endocrine pancreas development (289). Despite this, our group demonstrated only moderately high prevalence of faecal elastase deficiency and steatorrhoea, as well as moderately reduced pancreas volume in HNF1A-MODY patients compared to type 1 diabetes patients. These findings, and the observation that faecal elastase deficiency was more abundant in patients with long lasting diabetes, indicate that exocrine pancreatic dysfunction in HNF1A-MODY is secondary to diabetes, and not a primary result of the mutation in *HNF1A* (290, 291).

HNF1B-MODY

Reduced pancreas volume is a well-known finding in most HNF1B-MODY patients, but not all, and has been attributed to atrophy (292, 293). Haldorsen and co-workers demonstrated lack of pancreatic tissue corresponding to the body and tail of the pancreas in mutation carriers, suggesting that volume reduction is due to agenesis of the dorsal pancreas, and not diffuse atrophy (173). This is supported by demonstration of hypoplastic pancreata in two fetuses with mutations in *HNF1B* (172), and that *hnf1b* deficiency in mice leads to pancreas agenesis (294).

Low levels of faecal elastase have been demonstrated in many, but not all HNF1B-MODY patients, compatible with exocrine pancreatic dysfunction (173, 292, 293). Low levels of vitamin D and vitamin E have also been demonstrated in HNF1B-MODY patients (173). However, the few reported levels of faecal fat excretion have been normal or nearly normal, indicating that severe exocrine pancreatic insufficiency is probably not common (292, 295, 296).

Whether pancreatic exocrine dysfunction precedes diabetes in HNF1B-MODY has to my knowledge not been addressed before the works of the present thesis.

Diabetes development in HNF1B-MODY seems to be a combination of reduced beta cell mass and increased peripheral insulin resistance (297-299).

CEL-MODY

Carboxyl-ester lipase (CEL) is a digestive enzyme secreted by acinar cells of the pancreas. In the pancreas, the *CEL*-gene is expressed in acinar cells only, but not in ductal or islet cells (300). In 2006 Ræder and co-workers described a syndrome of exocrine pancreatic dysfunction and development of diabetes from young adult age caused by heterozygous mutations in the variable number of tandem repeats (VNTR) region of the *CEL*-gene. The syndrome was named CEL-MODY or MODY8, and was demonstrated in two families (301). In CEL-MODY, low faecal elastase and lipomatosis of the pancreas develops from childhood, preceding diabetes (45). There is a severe pancreatic insufficiency in CEL-MODY patients, demonstrated by low faecal elastase, high levels of faecal fat, and low levels of vitamin E (301, 302). CEL-MODY is a rare condition (303), and to my knowledge, no patients from other families than the two described in (301) have been published.

How mutations in *CEL* cause pancreatic disease is unknown. Recently, Johansson and co-workers published functional studies, demonstrating altered physico-chemical properties of the mutant protein, and high propensity to form aggregates, suggesting that misfolded proteins may be the noxious factor (304). The CEL-MODY phenotype has not been reproduced in *cel* knockout- or transgenic mutated *cel* mouse models (305, 306). The expression of the *CEL*-gene only in the acinar cells of the pancreas, and the exocrine pancreatic dysfunction preceding diabetes, suggests that diabetes in CEL-MODY may be secondary to exocrine pancreatic disease, type 3C.

2. Aims of the present study

Since the first molecular backgrounds for MODY were discovered in the 1990's, this group of diseases has served as an invaluable tool for understanding biology and function of the beta cell. The MODYs are well suited as disease models, showing different mechanisms in diabetes development, as the phenotype is caused by a single mutation only. This contrasts the very complex genotype-phenotype relationship in type 1 and type 2 diabetes. In addition, research on MODY phenotypes has had direct implications for treatment of these subtypes of diabetes. There are few similar models for exocrine pancreatic function. Cystic fibrosis, Shwachman-Diamond and Johanson Blizzard syndromes are all majorly confounded by other contributions to the phenotype than exocrine pancreatic dysfunction. As the exocrine pancreas is affected in HNF1B-MODY and CEL-MODY, we wanted to investigate these two conditions as models of disease in the exocrine pancreas. To do this, we needed develop a feasible test battery able to reveal the different aspects of exocrine pancreatic disease.

Hence, the aims of this study were to:

1. Develop a rapid endoscopic secretin test protocol, and evaluate its feasibility and diagnostic accuracy in detecting exocrine pancreatic dysfunction in patients with chronic pancreatitis
2. Develop an MRI-based protocol, involving anatomical uptakes, secretin-stimulated MRCP and secretin-stimulated DWI, and to evaluate this protocol in healthy controls
3. Use the rapid endoscopic secretin test and the MRI protocol to evaluate the degree and nature of exocrine pancreatic dysfunction in patients with CEL-MODY

4. Use the rapid endoscopic secretin test and the MRI protocol to evaluate the degree and nature of exocrine pancreatic dysfunction in patients with HNF1B-MODY

5. Relate the volume of the small pancreases in patients with HNF1B-MODY to their exocrine and endocrine pancreatic function, and to study whether the small pancreas in patients with HNF1B-MODY is attributable to hypoplasia or atrophy.

6. Relate exocrine pancreatic dysfunction to signs of malnutrition in patients with CEL-MODY and HNF1B-MODY

3. Main results

Paper I describes the use of a rapid endoscopic secretin test protocol in diagnosing chronic pancreatitis. Consecutive patients with suspected chronic pancreatitis and healthy controls underwent the rapid endoscopic secretin test. Three samples of duodenal juice were collected through the endoscope, starting 30 minutes after secretin stimulation. Bicarbonate concentration was measured in all samples, and the highest bicarbonate concentration was designated the peak, and used as a marker for exocrine pancreatic function. The results from the endoscopic test were compared to faecal elastase 1 concentration in the same patients and controls. A well recognized multimodal clinical scoring system for chronic pancreatitis was used as reference standard. The rapid endoscopic secretin test performed well in diagnosing chronic pancreatitis, and performed significantly better than faecal elastase.

Paper II describes the use of secretin stimulated MRCP and secretin stimulated DWI for testing pancreatic function in healthy controls, relating the results to pancreatic morphology and to peak bicarbonate concentrations from the rapid endoscopic test described in paper I. On secretin stimulated MRCP, post secretin volume increase in duodenal fluid filling, reflecting pancreatic secretion rate, was highest 1-5 minutes after secretin stimulation, before declining through the 13 minutes observation time. On secretin stimulated DWI, the ADC values increased after secretin stimulation, reaching a peak after one minute, before declining to pre-stimulation level at the next observation points. The increases in duodenal fluid volumes were positively correlated with ADC values at all time points. There was also a weak, but significant positive correlation between peak bicarbonate concentrations from the endoscopic tests and duodenal fluid volume increase one minute after secretin stimulation.

Paper III describes evaluation of exocrine pancreatic function in patients with CEL-MODY and healthy controls with rapid endoscopic secretin test, secretin stimulated MRCP and secretin stimulated DWI. In the duodenal juice samples, bicarbonate concentration and activity of digestive enzymes were measured. The results were related to body mass index and clinical chemical markers of nutrition status in the patients, obtained before commence of pancreatic enzyme therapy. A severe acinar and moderate ductal pancreatic dysfunction was demonstrated in CEL-MODY patients compared to healthy controls. This is compatible with severe steatorrhoea in CEL-MODY patients. Despite this, the patients were seemingly well nourished, and low vitamin E levels were the only clinical chemical marker indicating malnutrition. Vitamin E levels correlated significantly with duodenal lipase levels.

Paper IV describes evaluation of exocrine pancreatic function in patients with HNF1B-MODY and healthy controls with rapid endoscopic secretin test, secretin stimulated MRCP and secretin stimulated DWI. Results were related to pancreas gland volume of the patients and their nutritional status. Small pancreases, lacking body and tail, were found in all but one of the patients. Acinar and ductal function was moderately reduced in HNF1B-MODY. Ductal function, but not acinar function correlated positively with pancreatic gland volume. There was a non-significant trend towards earlier onset of diabetes in patients with smaller pancreas volume adjusted for body surface area. Total duodenal fluid volume increase to pancreatic gland volume ratios were higher in patients than in controls, suggesting compensatory hypersecretion in the remaining gland.

4. General discussion

4.1 Rapid endoscopic secretin test in the evaluation of exocrine pancreatic function

4.1.1 Methodological aspects

Endoscopic procedure

There has been a lack of feasible direct methods for determining exocrine pancreatic function. Indirect tests may have good enough diagnostic accuracy in the case of high pre-test probability of exocrine dysfunction (for example in cystic fibrosis (307, 308)), but the sensitivity and specificity of these tests are poor in general screening (62, 90). Despite being "gold standards" the traditional invasive tests have mostly been abandoned from daily clinical practice, due to the challenges in using them (93).

In our test, we aimed to collect secretin stimulated duodenal juice when the bicarbonate concentration reaches a peak plateau (18, 110). This gradual increase in bicarbonate concentration was first described in early works on secretin response (17). Stevens and co-workers (110) retrospectively calculated the optimal collection period for duodenal juice after secretin stimulation, while Jensen and co-workers (111) actually were the first to publish the use of delayed, rapid endoscopy test. In this study, one sample was collected between 30 and 40 minutes after secretin stimulation, and analyzed for levels of bicarbonate, elastase and lipase. The test was validated against the Lundh test (111).

Secretin stimulation alone has also been demonstrated to induce secretion of digestive enzymes (20-22), but an optimal period for collection of duodenal juice after a single dose of secretin has not been determined. However, Jensen and co-workers (111) achieved acceptable agreement between lipase levels achieved from the Lundh test and the rapid endoscopic secretin test. This finding suggests that digestive enzyme

activity determined in samples from a rapid endoscopic secretin test may be a representative estimate of pancreatic acinar status.

One important improvement in our endoscopic secretin test protocol was collecting three samples of duodenal juice. Bicarbonate is difficult to assess, as acidic contamination of the sample will left skew the equilibrium of the carbonic acid equation, causing carbon dioxide to evaporate:

$$CO_2\uparrow + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

Contamination of the duodenal juice will come from the ventricle, the duodenal mucosa and the biliary system, but contamination from the ventricle constitutes the largest error source, as both volume and acid effects will take part. We took measures to avoid contaminations from the ventricle by emptying it thoroughly by suctioning. However, we collected duodenal juice through the same suctioning channel. To avoid contamination from the endoscope, we saturated the suctioning channel with duodenal juice by discarding the juice collected the first 15 seconds. Still, there may have been remnants of gastric fluid in the endoscope. Furthermore, the gastric fluid which had entered the duodenum before the procedure commenced had been neutralized by pancreatic juice, with resulting loss of bicarbonate. In Steven's retrospective material, the ventricle had been emptied before secretin stimulation. Furthermore, the duodenum had been emptied two times in connection with collection of samples before 30 minutes had passed (110). These measures may have caused the duodenal juice collected at 30 minutes in Stevens' material to be purer samples of pancreatic juice than the first sample collected in a rapid endoscopy test. This suggestion is supported by our finding, that the first sample collected had significantly lower bicarbonate levels than the next two samples in 29 healthy controls (figure 4A,

unpublished results). It may also explain the lower reference level in the material from Jensen et al. (111), where only one sample was collected. In the healthy controls, we found peak bicarbonate concentrations in sample one, two and three (figure 4B).

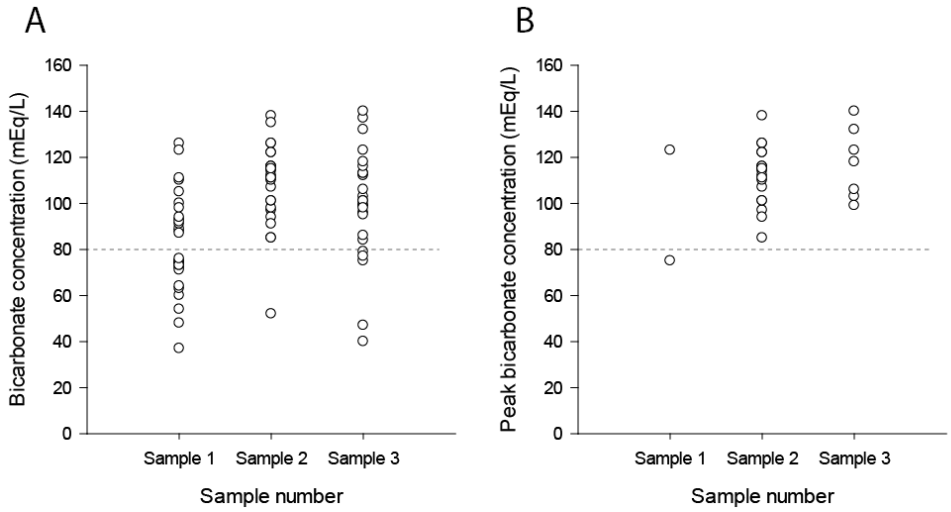

Figure 4. A. All bicarbonate results in 29 healthy control subjects who underwent rapid endoscopic secretin test. B. Peak bicarbonate results in the same control subjects. Peak bicarbonate concentrations were most common in sample 2 and 3. Using peak values of bicarbonate from three samples collected over 15 minutes gave acceptable specificity, with only one peak value under the cut-off value among the healthy controls. The dashed line represents the cut-off value of 80 mEq/L.

By using only the first sample for analysis, 11 out of 29 healthy controls (38 %) would have been misdiagnosed as having pancreatic exocrine dysfunction by using the cut-off of 80 mEq/L. By using only the two first samples, one of the healthy controls (3 %) would have been misdiagnosed. As the peak bicarbonate level is used, the accuracy of the test will increase with increasing number of samples, and hence, increased time used for sampling. Our fifteen minutes of sampling resulted in acceptable specificity of the test among the healthy controls (1 healthy control (3 %) was misdiagnosed), within slightly more than normal endoscopy duration. The bias caused by contamination of the working channel of the endoscope could be overcome by inserting a tube through the working channel to collect duodenal juice, as was done by Raimondo et al. (107). This, however, would complicate the procedure, as the

diameter of the collection tube would be smaller than the working channel, and extra external suction would have to be applied.

Conservation of duodenal juice

Samples collected through the endoscopic procedure were immediately sealed with an air tight screw cap and placed on ice. If possible, bicarbonate was determined the same day. Alternatively, the samples were stored in air tight containers, snap frozen on liquid nitrogen and stored till day of analysis. Pilot studies from our laboratory have not revealed any systematic changes in bicarbonate concentration between fresh samples and samples analyzed after conservation procedures described above (unpublished results).

Digestive enzymes were not possible to determine on the same day, and needed to be conserved. Deterioration of lipase activity attributed to proteolysis has been demonstrated to occur even at -20°C (141). To avoid destruction of samples, we added a commercially available cocktail of protease inhibitors (cOmplete, Roche diagnostics). This effectively inhibited chymotrypsin activity, as shown in figure 5.

In addition, samples were snap-frozen and stored on liquid nitrogen till day of analysis, to avoid any proteolytic inactivation of enzyme activity during storage. Samples were thawed on melting ice before analysis to keep pre-analytic proteolysis on a minimum. We have not determined the effect of snap-freezing, storage on liquid nitrogen and thawing procedures, but all samples were treated equally. Once thawed and then re-freezed duodenal juice samples were not used for enzyme activity analyses (138).

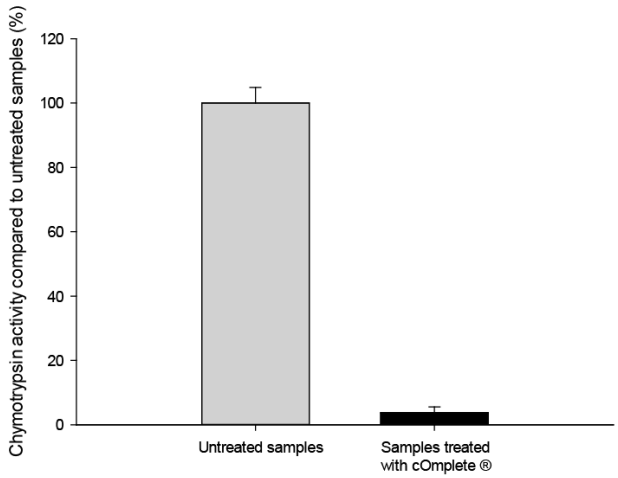

Figure 5. Relative chymotrypsin activity in 10 triplets of samples treated with cOmplete protease inhibitor cocktail (Roche) compared to the same untreated samples. The protease inhibitor cocktail reduced chymotrypsin activity to 3.8 % (SD: 1.7 %) of activity measured in untreated samples.

Determination of bicarbonate concentrations in duodenal juice

We used back-titration of samples to determine bicarbonate concentrations by a method modified from Van Slyke (130, 131). This method is considered as a reference standard in bicarbonate determination (132, 133). However, back-titration is time consuming as one sample takes 30 minutes to analyze. Back-titration is also technically challenging and demands a substantial sample volume, as at least 0.5 mL (preferably 1 mL) of duodenal juice is needed (130).

Determination of digestive enzyme activities in duodenal juice

We analyzed activities of several digestive enzymes to evaluate whether there was a global enzyme deficiency or if only one or few enzymes were affected. In addition, this approach gave us the opportunity to cross-validate our enzyme activity results. We chose to analyze enzymes with major roles in digestion of triglycerides, starch and

protein. Duodenal elastase activities were also analyzed, because faecal elastase 1 is a marker used to detect exocrine pancreatic dysfunction in CEL-MODY (301) and HNF1B-MODY (173, 292, 293).

To analyze enzyme activities, we wanted to use swift assays with possibility to measure several samples at the same time. In addition we wanted to use small sample volumes, as duodenal juice was not always abundant when sampling. To achieve this we fitted enzyme assays to 96 wells microplate. Commercially available enzymes were used as standards on each microplate to reduce inter-assay variation caused by incidental differences in assay conditions. However, the assays were not validated against other external standards, making comparison of our results to results from other laboratories difficult. To determine intra-assay variation, we measured each sample in triplicates.

As described in paper III and paper IV, all four enzyme assays had excellent linearity determined from dilution series of standard enzymes within measuring range. The intra-assay variance was also acceptable for all enzyme assays with possible exception from the pancreatic lipase assay (coefficient of variance: 14.7 %). The large intra-assay variance in the lipase kit may have been caused by incomplete emulsification of the substrate. Lipid droplets in a well of the microplate may then have caused altered fluorescence properties in the sample. Incomplete emulsification of substrate is a well-known cause of inaccuracy in lipase assays (137). To improve emulsification, we sonicated the pancreatic lipase substrate for 30 seconds, but it may still have been incompletely emulsified. Using larger volumes of the pancreatic lipase substrate and duodenal juice samples could possibly have improved intra-assay variation, as single lipid droplets then would constitute a smaller part of the total volume. However, then the assay would not fit into a microplate setting. Inter-assay variations have not been determined.

4.1.2 Diagnostic aspects

Diagnostic value of the rapid endoscopic secretin test

Peak bicarbonate concentration as diagnostic marker in chronic pancreatitis

In paper I, we used a rapid and easy to perform endoscopic secretin test in the diagnostic work-up of patients with suspected chronic pancreatitis. In lack of a feasible gold standard for the test, we determined the diagnostic value of the test in comparison to a much used multimodal diagnostic score (250-252). We found a specificity of 100 % for the test in our material when using a peak bicarbonate concentration cut-off value of 80 mEq/L. The peak bicarbonate value is a result of secretory capacity of the pancreatic ducts (3). Shortening collection time increases the risk of not sampling when the bicarbonate concentration has reached the peak value, hence reducing the specificity of the test. Poor specificity has in fact been one of the main problems with earlier rapid endoscopic tests of exocrine pancreatic function (99, 107, 108). Hence a high specificity of our test is very promising. Using the same cut-off, the sensitivity of the test was found to be 73 %. Comparing sensitivity of our test to other tests is difficult, as no gold standard for exocrine function has been used in our study, and there are observations indicating that 10-20 % of unselected patients with chronic pancreatitis do not have exocrine pancreatic dysfunction (252).

Concerns regarding the multimodal approach

We evaluated whether the patients had chronic pancreatitis or not by using a modified version of the Mayo score (or Layer score) (250). The patients were classified as having chronic pancreatitis if they were scored ≥4. Our modification of the score is quite similar to the Lüneburg score proposed by Lankisch et al (251).

The scoring system deserves some concerns. We used both faecal elastase < 200 µg/g and duodenal bicarbonate < 80 mEq/L as signs of pancreatic exocrine dysfunction in the evaluation of diagnostic value of duodenal bicarbonate. This is a circular reasoning that spuriously may elevate the sensitivity and the specificity of the test, as a positive test will elevate the score by 2 points. This could be solved by using only

faecal elastase as a measure of reduced exocrine function. However, as faecal elastase has poor sensitivity and specificity, and would hence reduce the validity of the reference standard (62). In addition, the diagnostic value of faecal elastase is also tested in our material for comparison. Hence, the diagnostic value for faecal elastase could be spuriously elevated if this was the only marker for exocrine dysfunction. Another solution could have been using and external marker for exocrine dysfunction, for example three day faeces collection for determination of faecal fat. However, the sensitivity and specificity of faecal fat is also poor in chronic pancreatitis (71, 72, 252). In addition, most of the participants in the study declined to collect faeces for three days (Erchinger F. et al. unpublished results). We were aware of this circle reasoning problem when we chose to use both duodenal bicarbonate and faecal elastase in the scoring system. To avoid misclassification, we offered examination with EUS and classification with Rosemont score as an external classification system to patients where different results from faecal elastase and duodenal bicarbonate caused discrepancy in the classification of whether or not they had chronic pancreatitis.

The Layer score is quite conservative due to its multimodal approach (251), and is much based on changes in the pancreas visible on imaging. This may have resulted in misclassifying small duct disease and early chronic pancreatitis as normal in our material (231), because imaging classification may be equivocal or normal despite present disease. As MRI is considered a more sensitive method in detecting parenchymal changes (44), inclusion of MRI results as a criterion in our modified score may have counteracted this tendency to misclassification.

The rapid endoscopic test beyond chronic pancreatitis

In paper I, we demonstrated that the rapid endoscopic secretin test had very good diagnostic value in the diagnosis of chronic pancreatitis. By measuring the peak bicarbonate values, we only evaluate the ability of the pancreatic duct to make bicarbonate rich fluid. This has been shown to be a reliable and sensitive parameter of exocrine pancreatic function in chronic pancreatitis (61), and evaluation of acinar

function does not seem to enhance the test (239). This is not necessarily the case in the evaluation of other conditions. In Shwachman-Diamond and Johansson-Blizzard syndromes, the acinar function is impaired, but not the ductal function, leading to normal bicarbonate values (199). In cystic fibrosis, small output of highly concentrated pancreatic juice may cause high bicarbonate concentrations in some patients, even though the total ductal function is severely impaired (99, 100). Hence, in evaluating the nature of the exocrine pancreatic dysfunction in different conditions, we need estimates for output of digestive enzymes and fluid volume output from the pancreas.

4.2 Imaging evaluation of exocrine pancreatic function

4.2.1 Methodological aspects

Secretin stimulated increase in duodenal fluid volume

We used a magnetic resonance imaging (MRI) protocol to examine pancreas morphology and function. In paper II we used this protocol on 20 healthy subjects with assumed normal exocrine pancreas.

Pancreatic fluid volume output was estimated by calculating intraintestinal fluid volume increase in the duodenum and proximal jejunum at defined time points after secretin stimulation. By drawing regions of interests (ROI) around duodenum and proximal jejunum in six 10 mm thick slices in the coronal plane, the whole duodenum and most of the proximal jejunum were included for calculations of volume increase, allowing a non-invasive estimation of pancreatic volume output. We found a mean secretion rate in healthy subjects of 6.4 mL/min during the first 13 minutes after secretin stimulation, which is comparable with maximal flow of approximately 4 mL/min after secretin stimulation demonstrated earlier by fluid collection (309). Our results are also comparable with other secretin stimulated MRCP materials (116, 118, 121).

As ductal cells are by far the main contributor of water in pancreatic juice (3), increase in duodenal fluid volume after secretin stimulation probably mainly reflects ductal function, which is also to some degree supported by the correlation between duodenal fluid filling after one minute and peak bicarbonate concentrations from rapid endoscopic secretin test.

The increase in fluid volume in the duodenum and proximal jejunum is an estimate of pancreatic fluid output. Any secretin-induced changes of fluid volume in the ROI not caused by pancreatic output will confound this estimate. Extra-pancreatic fluid may come from proximal to the ROI (e.g. the ventricle), or may be lost distal to the ROI (e.g. more distal parts of jejunum). In addition, increased secretion from the duodenal and jejunal mucosa and the biliary system may confound our results. Secretin has been shown to slow gastric emptying in animal models (310, 311); hence, gastric fluid from above the region of interest does probably not constitute a major confounder to this estimate. Some fluid may have spilled out distal to the region of interest, leading to underestimation of fluid output. However, the region of interest was drawn to include all intestinal segments with changes in water signal through the imaging series, probably making at least systematic effects of this possible confounder negligible. Fluid filling beyond the duodenal genu inferius at 15 minutes after secretin stimulation has been used as a landmark for normal pancreatic function (312). It is therefore unlikely that undetected fluid filling in intestinal segments distal to the proximal jejunum cause significant underestimation of total fluid increase after secretin stimulation.

Secretion of bicarbonate rich mucoid fluid from the duodenal mucosa is one of the main mechanisms protecting from acid mediated injury in the duodenum (313), but no effect of secretin has been demonstrated on this epithelial bicarbonate secretion (314). Bile is another possible confounder to the estimated pancreatic output. Secretin has choleretic properties (23, 315, 316), but the choleretic effect of secretin is probably equal in patients and controls, and therefore has probably no influence on differences of total volume increase between the groups. However, increased biliary output may

confound the results of the secretin stimulated duodenal volume increase per pancreatic gland volume ratio presented in paper IV. In this ratio, the effect of increased biliary flow will increase both with lower pancreatic output and with smaller pancreas gland size. From studies performed on patients with surgical biliary drainage, the maximum increase in biliary flow after secretin stimulation has been estimated to be approximately 10 mL/15 minutes (315, 316), which is a relatively small amount compared to the total increase in duodenal volume. When correcting for bile flow by subtracting 10 mL from increase in duodenal fluid volumes 13 minutes after secretin stimulation in *HNF1B* mutation carriers and healthy controls, the increase in duodenal fluid volume to pancreatic volume ratios are (SD) 3.5 mL/cm^3 (2.7) mL/cm^3 and 1.1 (0.7) mL/cm^3 respectively, the difference still being significant (p=0.04).

Diffusion weighted imaging and apparent diffusion coefficient – what do we measure?

We also evaluated pancreatic tissue properties by diffusion weighted imaging (DWI) before and after secretin stimulation in healthy controls. The apparent diffusion coefficient (ADC) was calculated from two uptakes with b-values of 50 and 800 sec/mm^2 respectively. The ADC value is a result of the net sum of water movement in a voxel (53). We detected an increase in ADC after secretin stimulation in healthy controls. This increase is probably a combination of increased tissue perfusion and increased tissue diffusivity as a response to secretin. The latter may reflect the movement of water from the bloodstream to the ducts occurring instantly after secretin stimulation (4). In DWI, higher b-values detect smaller water movements (53). As perfusion causes larger movements of water than diffusion, our ADC values are probably more reflecting changes in diffusion properties of the tissue than the results from Erturk and co-workers (126) who used b-values of 0 and 400 sec/mm^2 respectively when calculating ADC.

We demonstrated significantly positive correlations between increase in duodenal fluid volume and ADC values in the pancreatic tissue. As suggested in paper II, this

may indicate that diffusion and perfusion properties of the pancreas may predict the pancreatic tissue secretory capacity. This notion is supported by the results from Akisik and co-workers (128) who demonstrated lower pre- and post-secretin ADC values in chronic pancreatitis patients compared to patients with normal pancreases. However in paper III, we demonstrated a non-significant trend towards higher pre-secretin ADC values in CEL-MODY patients, even though their secretory capacity is reduced. This indicates that pre-secretin ADC values are also resulting from other tissue properties than secretory capacity alone.

4.3 Pancreatic disease in CEL-MODY and HNF1B-MODY

4.3.1 Evaluation of exocrine pancreatic function

Acinar and ductal function

In paper III and IV, we demonstrated significantly lower peak bicarbonate concentrations in duodenal juice in both CEL-MODY and HNF1B-MODY patients than in controls, indicating reduced ductal function. The mean bicarbonate levels seem to be moderately reduced in both CEL-MODY and HNF1B-MODY patients. This is supported by significantly lower, but still present duodenal fluid volume increase after secretin stimulation observed on MRCP.

We used duodenal juice collected in the rapid endoscopy test for determination of digestive enzyme activity levels. In paper III, we describe a large, global digestive enzyme deficiency in CEL-MODY patients compared to healthy controls, indicating severe acinar failure in this condition. In paper IV, HNF1B patients had significantly lower activity levels of pancreatic lipase and elastase, and there was also a non-significant trend towards lower levels of amylase and chymotrypsin, indicating that digestive enzyme levels are reduced in this condition, but not as much as in CEL-

MODY. As we did not quantify the amount of digestive enzymes in duodenal juice in patients with CEL-MODY and HNF1B-MODY, lower levels could theoretically be due to reduced activity in normal amounts of enzymes. However, this is unlikely, as low faecal elastase levels have been demonstrated in both CEL-MODY (301) and HNF1B-MODY (173, 292).

Diagnostic value of endoscopic test and imaging studies in detecting CEL-MODY and HNF1B-MODY

Peak bicarbonate concentrations and duodenal fluid volume increase

We demonstrated significant differences in secretin stimulated pancreatic fluid volume output between healthy controls and CEL-MODY patients, and between healthy controls and HNF1B-MODY patients, confirming reduced ductal function as demonstrated by reduced peak bicarbonate concentration levels in the endoscopic test. In the CEL-MODY group, there was considerable overlap between patients and healthy controls, in contrast to the bicarbonate results. This is demonstrated by the receiver operating characteristic curves (Figure 6A). There was a similar, non-significant trend in the HNF1B-MODY group (Figure 6B). This may represent poorer diagnostic accuracy of secretin stimulated pancreatic output volume compared to peak bicarbonate concentration, as has also been suggested in a recent work by Lieb and co-workers (317). In this study, peak bicarbonate concentration had better diagnostic value than total volume of duodenal juice collected for one hour after secretin stimulation in patients with chronic pancreatitis classified after multimodal clinical criteria. However, there are several weaknesses in this study worth mentioning: No duodenal carrier substance was used, making quantification of fluid lost from collection impossible, hence underestimating the volume secreted (99). In addition collection time was one hour, even though the peak flow of pancreatic juice after secretin stimulation occurs almost immediately, followed by a slow decline in flow (17). If reduced peak flow is the main indicator of reduced exocrine function, the signal to noise ratio will be lowered by one hour collection time.

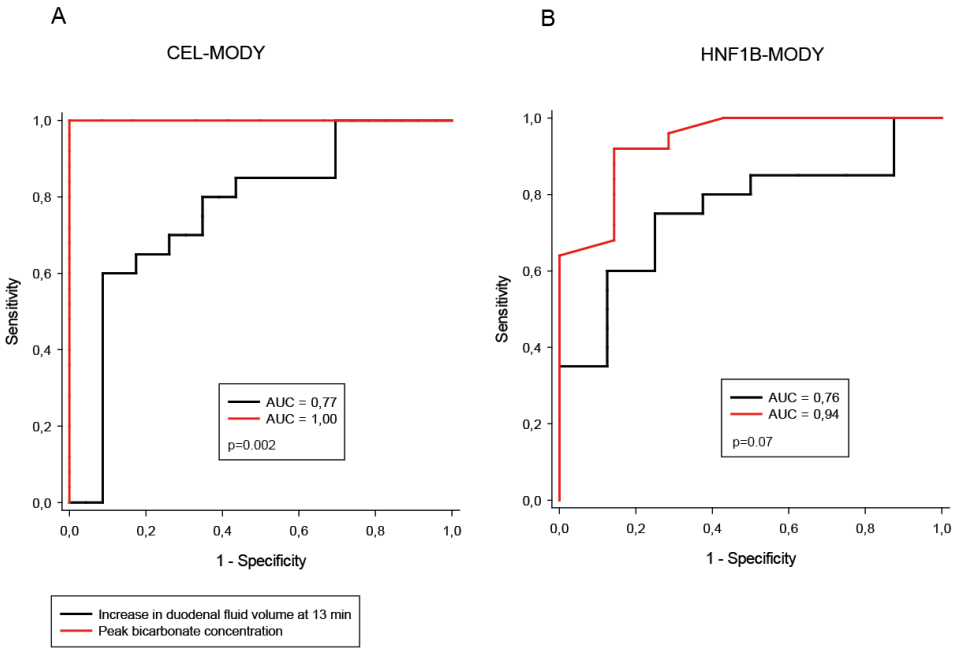

Figure 6: Receiver operating characteristic curves demonstrating differences in ability to discriminate patients from controls between peak bicarbonate concentrations achieved from rapid endoscopic secretin test, and increases in duodenal fluid volume 13 minutes after secretin stimulation achieved from magnetic resonance imaging. Panel A demonstrates that using peak bicarbonate concentrations detect patients with CEL-MODY significantly better than duodenal fluid volume increase. There is a non-significant trend towards a similar difference in HNF1B-MODY as demonstrated in panel B.

On the other hand, use of peak bicarbonate concentration may have overestimated the pancreatic exocrine dysfunction in CEL-MODY and HNF1B-MODY. As MRI based secretin stimulated exocrine pancreatic function testing is appealing because of its non-invasiveness, further studies are warranted to evaluate diagnostic accuracy, and also to optimize timing for imaging after secretin stimulation.

Digestive enzyme activity levels

As is the case with faecal elastase concentrations (301), digestive enzyme activity levels in duodenal juice were excellent diagnostic markers for CEL-MODY as there was virtually no overlap between the CEL-MODY patients and the controls. As with

bicarbonate, there was considerable overlap between the HNF1B-MODY patients and controls with respect to digestive enzymes, fitting well with these patients' clinical phenotype and our conclusion that pancreatic function is only moderately reduced.

4.3.2 Pathophysiological aspects in CEL-MODY and HNF1B-MODY

Pancreatic function in CEL-MODY

Acinar and ductal dysfunction

In paper III, we demonstrate that peak bicarbonate concentration is reduced in CEL-MODY patients to a median of 38 mEq/L, which is 33% of the median in healthy controls. This reduction is less than the median lipase level in CEL-MODY, which was only 2.4 % of the median in healthy controls. This apparently indicates that ductal function is reduced to a less degree than acinar function in CEL-MODY. There are, however, other bicarbonate-containing contributors to duodenal juice that could bias the peak bicarbonate concentration. I have discussed bile as a contaminant before in this thesis. Results from biliary drainage studies indicate that after secretin stimulation, bile bicarbonate concentration increases to a mean peak level of approximately 30-40 mEq/L (315, 316). This could cause a relatively higher median for bicarbonate concentration. However, there are observations indicating that the increase in biliary flow and bicarbonate concentration reaches a peak within 10 minutes after a single dose secretin stimulation, before it decreases, and reaches basal state before 30 minutes, when we start collecting duodenal juice (318). Another possible contaminator is bicarbonate rich mucoid secretions from the duodenal mucosa, but due to the pH gradient effect of this layer (313), the contribution to total bicarbonate concentration in duodenal juice is difficult to estimate. We do, however, demonstrate a mean increase in duodenal fluid volume in CEL-MODY patients that is 70% of the mean volume increase in healthy controls. Regardless of contaminants biasing this result, this observation supports the notion that ductal function is more preserved than acinar function in CEL-MODY.

CEL-MODY may be a primary acinar disease

From our clinical studies of CEL-MODY, we observe that there is a severe reduction of pancreatic acinar function and relatively moderate reduction of ductal function, both of which have been observed to precede diabetes. This may indicate that the disease process is primarly in the acinar compartment with secondary destruction of the ductal and beta-cell compartment, fitting well with the observation that the *CEL* gene is expressed in acinar cells of the pancreas, but not in ductal cells or in the beta-cells (300). This contrasts the situation in Shwachman-Diamond syndrome (194) and Johansson-Blizzard syndrome (199). These are also conditions causing primary disease in the acinar compartment of the pancreas, but ductal cell function is intact, and diabetes risk is probably not increased (195, 200). How this primarily acinar defect causes a global pancreatic dysfunction is not known. We have demonstrated that the mutated *CEL* in CEL-MODY has amyloid properties and easily form aggregates, and that the mutant protein is excreted, and found in pancreatic and duodenal juice (304). The secondary effect on ductal cells and beta-cells could be directly cytotoxic, by similar effects as the hypothesized mechanisms of islet amyloid polypeptide in type 2 diabetes (319), but misfolded self-protein may also resemble pathogen-associated molecular patterns, initiating chronic inflammation and cell senescence, as is one of the models for explaining Alzheimer's disease (320). Development of diabetes mellitus in cystic fibrosis and chronic pancreatitis are both conditions associated with beta-cell failure secondary to exocrine pancreatic dysfunction. The pathophysiological mechanisms of secondary diabetes development in these conditions are complex, involving beta cell dysfunction secondary to chronic inflammation and destruction of beta- cells secondary to fibrosis (263), but possibly also loss of beta-cell maintenance signalling from the exocrine tissue (321).

Pancreatic function in HNF1B-MODY

The size matters

In paper IV we demonstrate a strong correlation between pancreatic gland volumes and both secretin stimulated peak bicarbonate concentrations and duodenal fluid

volume increase 13 minutes after secretin stimulation. Both correlations indicate that ductal function is dependent on pancreatic gland size. In our material, we did not find correlations between digestive enzymes and pancreatic gland volumes. It is, however, not unlikely that such a correlation applies to acinar function as well. The amount of digestive enzymes excreted undergo changes as a result of diet composition (322), probably causing more variability in excreted digestive enzymes in a population. This increased variability may have obscured a correlation between duodenal digestive enzyme activity and pancreatic gland volume. Relation between pancreatic size and faecal chymotrypsin levels have been demonstrated in diabetes patients in a recent paper from Philippe and co-workers (275). It is also well known that a substantial part of patients undergoing pancreatic resections develop exocrine pancreatic insufficiency (323). However, in addition to the volume of remaining pancreatic tissue, the degree of exocrine pancreatic insufficiency is dependent on type of surgery e.g. preservation of ventricle and duodenum (323), and status of the remaining pancreas after surgery (324). In one study examining six children who had undergone 85-95 % pancreatectomy due to hyperinsulinism, all children had reduced pancreatic exocrine function, and four of them had pancreatic function test compatible with pancreatic exocrine insufficiency (325).

The observed trend towards earlier age of diabetes onset in subjects with reduced pancreatic volumes suggests a possible relation between reduced pancreatic volume not only to pancreatic exocrine function but also to endocrine function. Such a relationship could be explained by lower beta cell mass in a smaller pancreas, but may also be due to similar defects in the transcriptional network causing pancreatic hypoplasia, peripheral insulin resistance and beta cell dysfunction (295, 297-299, 326).

Pancreatic hypersecretion in HNF1B-MODY

The pancreatic hypersecretion observed in HNF1B-MODY patients may be a compensatory mechanism to reduced pancreatic mass, at least in the ductal compartment. This is supported demonstration of pancreatic hypersecretion after

pancreatic resection in dogs (327), but also in man there are observations supporting pancreatic tissue plasticity, leading to recovery of functional parameters in both ductal and acinar compartments after pancreatic resection (328).

Hypersecretion from the pancreas has been demonstrated in patients with liver cirrhosis and other severe liver disease (329, 330), and also in hemochromatosis (329, 331), but even though mild liver affection is a common finding in *HNF1B* mutation carriers (292, 293, 332), liver disease do not explain the relative hypersecretion we observe in these patients.

The demonstration of hypersecretion from small pancreases of HNF1B-MODY patients indicates normal or nearly normal function of the exocrine pancreatic tissue, compensating for reduced pancreatic mass. This observation supports the earlier suggestion from our group (173), that pancreas volume is reduced due to hypoplasia and not atrophy in HNF1B-MODY.

Normal function of exocrine pancreatic tissue is further confirmed by the demonstration of the same post-secretin increase in ADC to a post-secretin peak in HNF1B-MODY patients, in contrast to no increase in ADC in CEL-MODY patients, which has also been demonstrated in chronic pancreatitis patients (126).

4.3.3 Exocrine pancreatic function and sufficiency in CEL-MODY and HNF1B-MODY

In CEL-MODY patients, we demonstrate a negative rank correlation between faecal fat loss and peak duodenal juice pancreatic lipase activity. This finding directly demonstrates that the exocrine pancreatic dysfunction found in CEL-MODY patients causes pancreatic insufficiency. This is further supported by subnormal vitamin E levels in untreated CEL-MODY patients (301, 302), and the positive linear correlation between lipid-adjusted α-tocopherol and peak duodenal pancreatic lipase activity levels we demonstrate in paper III.

Several of the HNF1B-MODY patients participating in our study were, understandably, reluctant to collect faeces for three days, making estimation of faecal fat loss impossible in this small group. However, other materials have demonstrated that faecal fat loss is within normal or nearly normal limits in HNF1B-MODY patients (292, 295, 296), indicating that pancreatic exocrine function is probably sufficient in these patients. This is also supported by the absence of abdominal symptoms in the HNF1B-MODY patients described in paper IV. The demonstration of subnormal levels of vitamin D and E found in HNF1B-MODY patients (173), and our demonstration of a positive linear correlation between 25-hydroxyvitamin D and peak duodenal pancreatic lipase activity do, however, oppose this notion.

As we have not quantified the fat intake in patients when determining faecal fat loss, we are not able to quantify the fat absorption rate in CEL-MODY patients (64), hence, we are not able to estimate a threshold value for how much duodenal peak pancreatic lipase activity must be impaired for fat malabsorption to occur. A reduction of pancreatic lipase output to 5-10 % of normal value has long been established as a threshold for impaired absorption of fat, hence, pancreatic insufficiency (71, 72). Our peak values from spot samples of duodenal digestive enzyme activities in patients compared to healthy may be good enough estimates for digestive enzyme output. This is supported by the finding of pancreatic insufficiency in CEL-MODY patients with a median peak duodenal pancreatic lipase acitivity level of 2.4 % of the median in healthy controls, and apparent exocrine pancreatic sufficiency in HNF1B-MODY patients, of whom all subjects had duodenal pancreatic lipase activity levels above 10 % of the median in healthy controls. However, in CEL-MODY and HNF1B-MODY, these peak values probably overestimate the enzyme output, as we also demonstrate less fluid volume output in patients than in healthy controls.

4.4 Clinical consequences of exocrine insufficiency

It is well established that exocrine pancreatic dysfunction leads to pancreatic insufficiency, causing increased faecal losses of nutrients, which may subsequently lead to malnutrition. This is the case in both chronic pancreatitis (207, 333-336) and cystic fibrosis (189). Malnutrition may be divided into negative energy balance leading to weight loss and poor physical performance, and micronutrient deficiency (337).

4.4.1 Macronutrient status

In paper III we discuss the macronutrient status in CEL-MODY. It is somewhat puzzling that despite severe exocrine insufficiency in CEL-MODY patients, with substantial steatorrhoea (301) and severely reduced digestive enzyme activity levels and bicarbonate concentration in the duodenum, they seem to be well nourished. Before any pancreas enzyme replacement treatment, most of the patients did have body mass indexes within normal limits, some were even overweight and obese, and as a group, they did not differ from the healthy controls (301). Weight gain after treatment with pancreas enzymes was also rather modest, and occurred only in some patients (302). In paper I, we found that the mean body mass index in chronic pancreatitis patients was not significantly different from the healthy controls. However, we did not determine how many of these patients actually had severe exocrine pancreatic dysfunction leading to insufficiency. It is also worth noting that three of the chronic pancreatitis patients were underweight, with body mass indexes between 15 and 18.

In contrast to CEL-MODY, weight loss is a common complication in pancreatic insufficient patients with chronic pancreatitis (207, 333) and cystic fibrosis (189), leading to considerably increased morbidity (338).

To further discuss the different clinical outcomes in chronic pancreatitis, cystic fibrosis and CEL-MODY, three conditions causing severe exocrine pancreatic

insufficiency, we need to look into the factors affecting energy balance. Energy balance, or macronutrient status, may, according to the first law of thermodynamics, be decomposed into the following equation:

Food intake [Energy in]

=

(Faecal loss + Energy expenditure) [Energy out] + Weight gain [Energy stored]

In the question of macronutrient nutritional status over time, the status of energy storages e.g. whether there is weight gain or weight loss, is one indicator of this balance, as shown in figure 7.

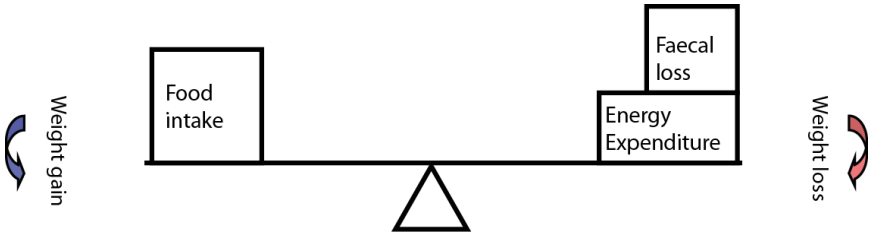

Figure 7: Model demonstrating energy balance in the healthy, adult individual. Food intake is balanced to loss of nutrients and energy expenditure. There is no net gain or loss of energy storages, hence no weight gain or weight loss. In the child, growth is also on the expenditure side of the balance, necessitating increased food intake.

Food intake

Food intake over time is strictly regulated, leaving the body weight in adults remarkably stable (339). This homeostasis of appetite regulation over time is regulated through complex central nervous mechanisms reviewed in (340), and seems to be more protective against weight loss than weight gain (341, 342). Cytokines from chronic inflammation may counteract this homeostasis, causing reduced appetite, leading to anorexia (343).

Faecal loss of nutrients

In exocrine pancreatic insufficiency, substantial amounts of nutrients may be lost in faeces. Mechanisms leading to faecal loss of nutrients in exocrine pancreatic insufficiency have been discussed in chapter 1.2.4. If additional gastrointestinal disorders are present, they may add on to the poor uptake of nutrients further increasing faecal loss (182).

Energy expenditure

Total energy expenditure comprises three elements; resting energy expenditure (or resting metabolic rate), energy expended on physical activity and thermogenesis (344). In inflammatory conditions, resting energy expenditure is increased, probably as a result of increased metabolic needs from activated leukocytes, as reviewed in (345).

Energy balance in exocrine pancreatic insufficiency

Energy balance in chronic pancreatitis

Cytokines from the chronic inflammatory process in chronic pancreatitis may counteract the homeostatic increase in food intake (343, 346). Severe postprandial pain is also common in chronic pancreatitis, often leading to anorexia (347). In many patients with chronic pancreatitis , nutritional status is further deteriorated by chronic alcoholism (333). Exocrine insufficiency leads to increased faecal loss of nutrients. Resting energy expenditure has been demonstrated to be increased in chronic pancreatitis, probably as a result of chronic inflammation, though alcoholism may play a role also in this matter (348). Hence, there are several drivers to negative energy balance which may exceed the homeostatic mechanisms, causing decompensation and weight loss as illustrated in figure 8.

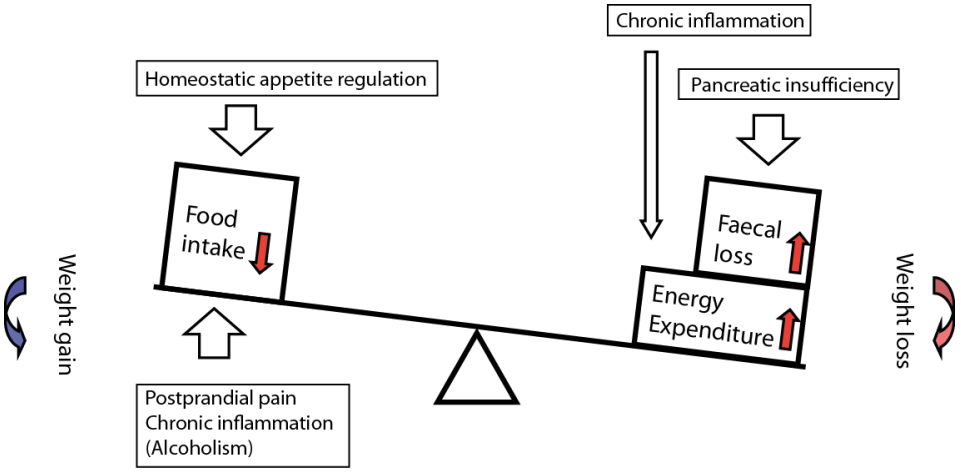

Figure 8: Drivers to negative energy balance in chronic pancreatitis. Increased faecal loss of nutrients and increased energy expenditure lead to increased demand for food. However, postprandial pain, chronic inflammation and alcoholism counteract the homeostatic appetite regulation which may lead to decompensation and weight loss in chronic pancreatitis.

Energy balance in cystic fibrosis

Also in cystic fibrosis, cytokines from chronic inflammation as well as from acute exacerbations probably counteracts homeostatic increase in food intake (349). Gastrointestinal disorders causing pain in association with food intake, as esophagitis and distal intestinal obstruction syndrome, are quite common in cystic fibrosis patients, often restricting food intake (189). Exocrine pancreatic insufficiency cause faecal loss of nutrients, which may be further increased by viscous mucus impairing nutrient absorption, and also by cystic fibrosis associated liver disease (189). It is well known that resting energy expenditure is increased in cystic fibrosis patients (344). The reason for this is complex, and has been associated with inflammation (350) and deteriorating lung function (351). The energy imbalance that occurs when this situation decompensates in cystic fibrosis is illustrated in figure 9.

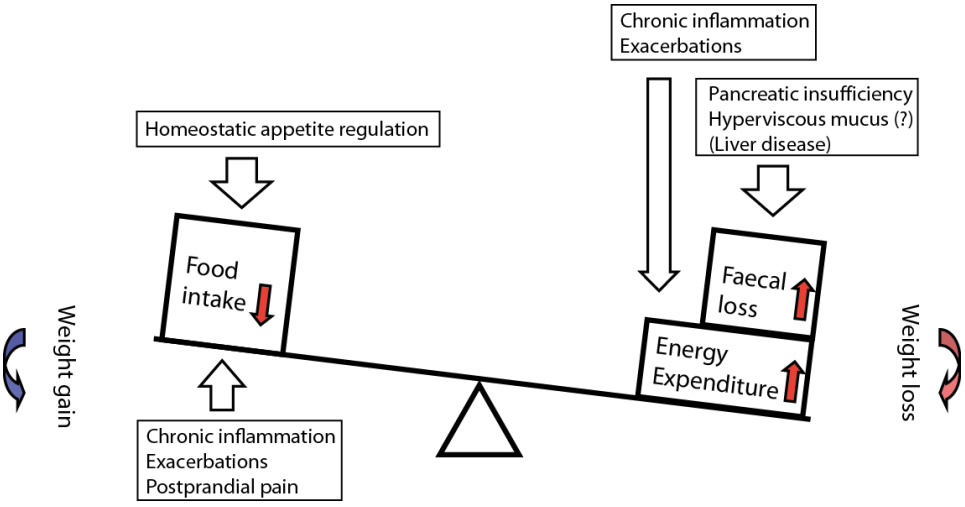

Figure 9. Drivers to negative energy balance in cystic fibrosis. Also in cystic fibrosis, need for food intake is increased due to increased loss of nutrients and increased energy expenditure. Chronic inflammation and inflammation caused by exacerbations counteract homeostatic appetite regulation. In addition, postprandial pain is common in cystic fibrosis, also leading to less food intake and energy balance decompensation.

Energy balance in CEL-MODY

The process causing destruction of the pancreas in CEL-MODY is not fully characterized, but may involve chronic inflammation. However severe pain and other inflammatory signs are not present, and food intake is probably not much negatively affected in CEL-MODY. Results from paper III confirms that faecal fat loss is caused by pancreatic insufficiency. There are no signs of other mechanisms leading to increased faecal loss of nutrients. Many of the patients develop diabetes, but low values of HbA1C (301) indicate good glycemic control, and energy loss through glycosuria must therefore be negligible if present. We have not measured the resting energy expenditure in CEL-MODY patients, but as inflammation, if present, is probably not severe, resting energy expenditure is probably not increased very much in comparison to healthy subjects. A proposed model of the energy balance in CEL-MODY is illustrated in figure 10.

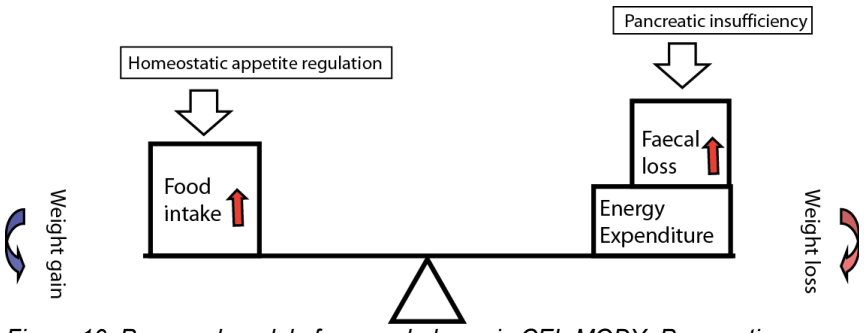

Figure 10. Proposed model of energy balance in CEL-MODY. Pancreatic insufficiency causes increased faecal loss of nutrients. This is compensated by homeostatic appetite regulation leading to increased food intake. CEL-MODY may prove to be a purer model disease for pancreatic exocrine insufficiency than chronic pancreatitis and cystic fibrosis.

CEL-MODY as a model disease for pancreatic insufficiency

According to this model, there are several additional drivers besides exocrine pancreatic insufficiency to decompensation, causing malnutrition in chronic pancreatitis and cystic fibrosis. In CEL-MODY, we have not found other drivers for malnutrition than exocrine pancreatic insufficiency, which may explain why CEL-MODY patients compensated and did not lose weight, despite no treatment with pancreatic enzyme supplements. A similar compensated situation can be demonstrated indirectly by the need for additional strict diet, and still a rather modest effect of the lipase inhibitor orlistat in treatment of obesity (352-354), despite the strong inhibition of gastric and pancreatic lipases by orlistat in vivo (355, 356).

Due to no known extrapancreatic manifestations, CEL-MODY may prove to be an excellent model disease for isolated severe exocrine pancreatic insufficiency. These beneficial conditions for studying consequences of exocrine pancreatic insufficiency are further strengthened by the fact that the diagnostic work-up of the patients was performed before knowledge about the patients' exocrine pancreatic status was established, and therefore not biased by treatment with pancreatic enzyme replacement therapy.

Our observations that untreated CEL-MODY patients compensated their energy balance despite severe, untreated exocrine pancreatic insufficiency emphasize the importance of addressing all contributors to a decompensated nutritional status in patients with chronic pancreatitis and cystic fibrosis in addition to providing pancreatic enzyme replacement therapy.

4.4.2 Micronutrient status

Vitamin E

Our group has earlier demonstrated vitamin E deficiency in CEL-MODY patients (301, 302). A significant increase in vitamin E levels in CEL-MODY patients resulting from treatment with pancreatic enzymes (302), suggests that vitamin E status in CEL-MODY is dependent on exocrine pancreatic function. In paper III, we demonstrated a strong correlation between pre-pancreatic enzyme treatment lipid-adjusted α-tocopherol levels and duodenal lipase levels, further supporting that the low vitamin E levels are due to pancreatogenic fat malabsorption. We used α-tocopherol as a marker for vitamin E status, because this is the most biologically active of the tocopherols (357). Lipid adjusted levels were used, as α-tocopherol is transported via lipoproteins, hence, the concentration is dependent on lipid status (358). This correction is probably important in the CEL-MODY patients studied, as they had a trend towards lower cholesterol levels than healthy controls (301). We did not find any significant or suggestive correlation between duodenal lipase levels and total cholesterol, LDL cholesterol or HDL cholesterol (not shown). Low levels of vitamin E were earlier demonstrated in two HNF1B-MODY patients (173), but was not found in the HNF1B patients described in paper IV. One possible reason for this may be that earlier investigations in HNF1B-MODY patients have made them aware of their small pancreases, causing them to take supplements, such as cod liver oil. However, as vitamin E levels in (173) were not corrected for lipid status, they may have been erroneously low if any of the patients had altered lipid profiles.

The dependency of the pancreas in the uptake of vitamin E has been known for several decades (359, 360), and negative correlations between vitamin E status and faecal fat loss have been demonstrated in chronic pancreatitis earlier (335, 360, 361). However, increased oxidative stress has been suggested to be an additional contributor to low levels of vitamin E in patients with chronic pancreatitis (362-365) and cystic fibrosis (366, 367).

Lack of vitamin E may be associated with neuropathy. In CEL-MODY patients, demyelinising neuropathy was a common finding (302), but no significant correlation between neurophysiological status and vitamin E status was found (unpublished results). It is, however, worth noticing that peripheral neuropathy (though axonal) also seems to be common in cystic fibrosis (368). As an antioxidant, vitamin E may also be a disease modifier in chronic pancreatitis and cystic fibrosis (364, 367). It therefore seems prudent to diagnose and treat vitamin E deficiency in conditions associated with pancreatic exocrine insufficiency.

Vitamin D

There was no difference in 25-hydroxyvitamin D levels between CEL-MODY patients and healthy controls (301). One would expect malabsorption of vitamin D in severe exocrine pancreas insufficiency, as vitamin D deficiency is common in cystic fibrosis (369) and chronic pancreatitis (370, 371). However, the precursor of 25-hydroxyvitamin D, previtamin D, is synthesized in the skin from UVB exposure from the sun (372), and samples from CEL-MODY patients were drawn in September, after months of sun exposure. There are great seasonal changes in 25-hydroxyvitamin D levels (373), and during the six darkest months of the year, the UVB exposure is not adequate to provide a sufficient vitamin D status at our latitude (372). Hence, an adequate intake and absorption of vitamin D is necessary to sustain normal vitamin D levels in the winter months, and probably would 25-hydroxyvitamin D deficiency in untreated CEL-MODY patients be more common in this period of the year.

In paper IV, we describe three HNF1B-MODY patients with 25-hydroxy vitamin D deficiency, as has also been found before (173). In this material, all samples were drawn in the period November to April, when vitamin D levels are dependent on ingested and absorbed nutrients. We demonstrated a positive correlation between duodenal lipase levels and serum levels of 25-hydroxyvitamin D, suggesting the dependence on normal exocrine pancreatic function. However, in HNF1B-MODY patients, levels of duodenal lipase were only moderately reduced compared to healthy controls, and none of the patients had duodenal lipase levels below 10 % of the median from the controls. This may indicate that vitamin D absorption is more sensitive to reduced exocrine pancreatic function than fat absorption in general. One possible cause of this is linked to the sterol binding capacity of elastase 1, possibly facilitating uptake of 25-hydroxyvitamin D (374). However, our finding may also reflect that peak lipase activity concentration underestimates the level of pancreatic dysfunction in HNF1B-MODY as discussed earlier. Finally, the finding may be coincidental, as vitamin D deficiency is quite common at our latitude during winter months (375, 376).

The most obvious consequence of vitamin D deficiency is osteoporosis, which is common in both chronic pancreatitis (336) and cystic fibrosis (369). In one study, low faecal elastase and low levels of 25-hydroxy vitamin D was more abundant in patients with osteoporotic fractures compared to controls, but occult celiac disease, causing both low faecal elastase and osteoporosis, may have been a confounding factor (377). Osteoporosis was diagnosed in three and osteopenia in two of the nine CEL-MODY patients participating in the study by Vesterhus and co-workers (302) (not published). Vitamin D is also important in many extra-skeletal processes, like cell cycle regulation and immune regulation (372), and there is increased risk for cancer and autoimmune diseases in case of vitamin D deficiency (378). Interestingly, recent results indicate that improving vitamin D status may have beneficial effects on beta cell function and insulin resistance in type 2 diabetes patients (379-381). As consequences of vitamin D deficiency may be detrimental, and this is a common finding in patients with exocrine pancreatic dysfunction (maybe even in only

moderate exocrine pancreatic dysfunction), screening for and treating vitamin D deficiency seems reasonable in patients with exocrine pancreatic disease.

Vitamin A

The vitamin A family comprises carotenoids and retinoids. Only one of the untreated CEL-MODY patients had vitamin A (retinol) deficiency, but the mean vitamin A level was significantly lower in patients than in controls (301). Vitamin A deficiency is common in chronic pancreatitis (335, 382) and cystic fibrosis (383, 384). At least in alcohol induced chronic pancreatitis, vitamin A levels seem to be less affected than vitamin E levels (335), as was found in CEL-MODY patients. This may partly be explained by brush border retinyl hydrolases in the small intestine (385). However, it is unclear how much this enzyme system compensates for lacking pancreatic lipase activity.

Night blindness is the most commonly detected effect of vitamin A deficiency (386), but vitamin A is also involved in cell differentiation, immune function, pulmonary function and bone health (387). None of the CEL-MODY patients had physical findings compatible with clinical vitamin A deficiency. As antioxidants, carotenoids may also have disease modifying properties in chronic pancreatitis and cystic fibrosis (364, 367).

Vitamin K

Vitamin K is an important cofactor in posttranslational modification of coagulation factors. Gut flora is the main provider of vitamin K. No parameters on vitamin K status have been measured in CEL-MODY patients. On the other hand, no bleeding disorders have been reported by the patients.

Vitamin K deficiency is a concern in patients with cystic fibrosis, probably due to the combination of pancreatogenic malabsorption and frequent use of antibiotics affecting gut flora (387).

Cobalamins

Cobalamin deficiency was detected and treated parenterally in one of the CEL-MODY patients in (302). Cobalamin status has not been investigated systematically in the other patients, but casuistically, we know about three CEL-MODY patients receiving regularly injections of cobalamin supplements (not published). Pancreatic proteases take part in the complex mechanism binding cobalamin to intrinsic factor (388), and cobalamin deficiency has been attributed to pancreatic exocrine insufficiency (388, 389), though this is probably not a common complication (347).

The observation is interesting, as neuropathy in CEL-MODY patients could also be explained by cobalamin deficiency.

4.4.3 Should we treat with pancreatic enzyme supplements?

In paper III, we detect pancreatic insufficiency with severe lipase deficiency, negatively correlated with faecal fat, and positively correlated with vitamin E status in CEL-MODY patients. Probably also vitamin D status is affected by this condition, as discussed above. Intuitively, recommending pancreatic enzyme supplement therapy to all CEL-MODY patients seems reasonable.

However, we need to define an indication for treatment and a treatment goal. The steatorrhoea does not cause weight loss despite being severe. We have detected micronutrient deficiency in CEL-MODY patients, and at least vitamin E status seems to be caused by pancreatic insufficiency. Some, but not all the patients present subjective symptoms of diffuse abdominal pain or discomfort and diarrhoea.

Three systematic reviews, have evaluated the effect of pancreatic enzyme supplements on faecal fat loss, two of them in chronic pancreatitis and one of them in chronic pancreatitis and cystic fibrosis. All three conclude that enzyme supplements reduce faecal fat loss (390-392). One of them also evaluates the effect on weight loss and quality of life in chronic pancreatitis, finding no significant effect of treatment (391). The results are, however, based on few studies. Effect of pancreatic enzyme

supplements on pain in chronic pancreatitis has also been evaluated in a systematic review (393), concluding with no significant effect. To my knowledge, no studies have evaluated the effect of pancreatic enzyme treatment on fat-soluble vitamin status or other micronutrients; on the contrary, there are observations that fat soluble vitamins may be deficient despite enzyme supplements (366, 382).

It seems reasonable to treat pancreatic insufficient patients with cystic fibrosis and chronic pancreatitis with pancreatic enzyme supplements, as energy balance is threatened in these conditions, and faecal losses are reduced by therapy. It is probably also reasonable to try pancreatic enzyme supplements in CEL-MODY patients with abdominal symptoms as well, as there is a reasonable linkage between the symptoms they present and the deficiency of pancreatic enzymes demonstrated in paper III. In addition, abdominal symptoms were improved on enzyme therapy in the majority of CEL-MODY patients participating in the treatment study (302). The correlation between lipid adjusted vitamin E status and duodenal lipase levels in CEL-MODY patients demonstrated in paper III indicate a lipase dependent uptake of vitamin E, which was also demonstrated by improvement of vitamin E levels on pancreatic enzyme supplements (302). The positive correlation between 25-hydroxyvitamin D and duodenal lipase in HNF1B-MODY patients demonstrated in paper IV may indicate a similar lipase dependency for uptake of this vitamin, necessary for adequate vitamin D status at least in the dark months of the year. Hence, poor fat-soluble vitamin status despite adequate vitamin supplementation may be an indication for trying pancreatic enzyme supplements in the otherwise asymptomatic CEL-MODY and HNF1B-MODY patient. Our findings also emphasize the importance of clinical follow-up of these patients despite stable weight, as they are at high risk to develop micronutrient deficiencies.

In children and adolescents with CEL-MODY, our group has detected low faecal elastase and pancreatic lipomatosis (45). The children and adolescents with CEL-MODY participating in the study referred to in paper III, also had reduced fluid output from the pancreas compared to age matched controls (data for subgroup not

shown). It is probably reasonable to treat children and adolescents with CEL-MODY with pancreatic enzyme supplements, as they have higher energy requirement per kg than adults (394), possibly making them more prone to imbalance in the equation above. As protein-energy malnutrition has detrimental effects on growth (395), and lack of fat soluble vitamins may cause osteopenia with later osteoporosis (396), clinical follow-up with anthropometry and determination of fat soluble vitamins on regular basis is crucial in children and adolescents with CEL-MODY.

Another subgroup of CEL-MODY patients that might have additional benefit from treatment with pancreatic enzyme supplements is patients with prediabetes and mild diabetes. Treatment with pancreatic enzyme supplements may cause increased meal induced levels of GIP and GLP-1, subsequently increasing insulin secretion. Increased incretin and insulin secretion following a meal has been demonstrated in patients with pancreatic exocrine insufficiency due to chronic pancreatitis (265, 266) and cystic fibrosis (267). However, no effect on long-term glycaemic regulation has been demonstrated as a result of pancreatic enzyme treatment.

In paper IV we demonstrate only moderately reduced levels of digestive enzymes in patients with *HNF1B* mutations, and no gastrointestinal symptoms were reported. These patients do probably not need supplements with pancreatic enzymes, but will also need clinical follow-up of nutritional status, especially in childhood and adolescence, as fat-soluble vitamins are low in some of them. From our findings, this especially applies to patients with small pancreas volumes adjusted for body surface area.

5. Conclusions

Through the work presented in the present thesis, we demonstrate that our modified rapid endoscopic secretin test using peak bicarbonate as diagnostic marker, is feasible, well tolerated by the patients and has acceptable diagnostic accuracy in diagnosing chronic pancreatitis. We further demonstrate that measuring duodenal fluid volume increase by secretin stimulated MRCP and ADC values by secretin stimulated DWI give correlated results, both probably reflecting exocrine pancreatic function. By using these two methods in evaluating exocrine pancreatic function in CEL-MODY patients we demonstrate severely reduced acinar function and moderately reduced ductal function, while function of both these compartments are only moderately reduced in HNF1B-MODY. In HNF1B-MODY, ductal function is dependent on pancreas volume, and there is compensatory hypersecretion. We find support that the pancreas in HNF1B-MODY is small due to hypoplasia and not atrophy. Despite severe pancreatic insufficiency in CEL-MODY patients, they show remarkably few signs of malnutrition, probably due to increased food intake. Fat-soluble vitamin levels are vulnerable to exocrine pancreatic insufficiency, and should be monitored closely in patients with CEL-MODY and HNF1B-MODY.

6. Future perspectives

Improving the diagnostic methods

Despite its potential role in diagnosing exocrine pancreatic disease, invasive pancreatic function testing is almost never used in clinical settings. This is probably due to lack of feasibility of most invasive pancreatic function tests (93). Further development of our feasible, rapid endoscopic secretin test therefore seems reasonable. We are planning to perform measurements of enzyme activities in duodenal juice from patients with suspected chronic pancreatitis to evaluate whether this improves diagnostic value of the test.

To do this, we need to evaluate and improve pre-analytic measures and methods for analysis of bicarbonate and digestive enzymes. By treating duodenal juice samples with protease inhibitor before snap-freezing and storing on liquid nitrogen, we were able to conserve them well enough to discriminate well between healthy controls and patients with pancreatic disease. However, the effect of these measures needs further evaluation. Also the precision and accuracy of the digestive enzyme activity assays need to be evaluated further. Both bicarbonate analysis and digestive enzyme activity assays have been done by rather work intensive methods, and automation of these analyses would be a major improvement.

To evaluate the nature of exocrine pancreatic dysfunction in MODY patients, we needed an estimate of pancreatic fluid output. We used a functional MRI protocol to achieve this. By diffusion-weighted imaging we were also able to directly study tissue response to stimulation. Despite some concerns regarding diagnostic value of the secretin stimulated MRCP protocol, our MRI protocol represents a promising approach to exocrine pancreatic function testing. The MRI protocol is appealing as it is non-invasive with minimal discomfort for the patient, as well as it combines function testing with state of the art imaging modalities in one procedure. We plan to further optimize the secretin stimulated MRCP protocol, and to evaluate it in other

patient groups with exocrine disease, such as patients with cystic fibrosis and chronic pancreatitis.

As a paediatrician, I also see the need to evaluate exocrine pancreatic function in children with more precise tools than what is available today. Further evaluating the rapid endoscopic secretin test and the MRI protocol in children with suspected pancreatic disease would therefore be of interest.

Duodenal juice beyond function testing

As secretin stimulated duodenal juice is not only expressing the result of exocrine pancreatic function, but also represents fluid proximal to the processes going on in the pancreas, we have stored samples of duodenal juice in a biobank for further investigation. There are a few studies using these properties by measuring cytokines in duodenal juice in patients with chronic pancreatitis (397-399). We plan to investigate the disease causing process in CEL-MODY by measuring the cytokine profile in these patients compared to healthy controls. Similar profiles may be measured in duodenal juice from patients with chronic pancreatitis and cystic fibrosis.

We have also searched for protein fingerprints from the disease process with discovery proteomics of the duodenal juice from a few of the CEL-MODY patients, and are currently validating the results in a larger CEL-MODY patient material.

Clinical investigations in MODY patients

The phenotype of HNF1B-MODY is heterogeneous. Patients recruited to the present study have been identified through our MODY registry, thus with diabetes as important part of the phenotype. Hence, the HNF1B-MODY patients described in this thesis may represent a group with more affected pancreases than the *HNF1B* mutation carrier population in general. A supplementary estimate of the prevalence of pancreas hypoplasia in these patients could be made by imaging studies in *HNF1B* mutation carriers recruited through for example kidney disease registers. In HNF1B-MODY patients we demonstrated a non-significant trend towards earlier debut age of diabetes

with smaller body surface area adjusted pancreas volume. A similar correlation could be done in a larger patient material to increase power of the analysis; as demonstrating such a relationship would be of prognostic value for the patients.

The compensated nutritional status in patients with CEL-MODY is intriguing. CEL-MODY may be a purer model disease for global pancreatic exocrine dysfunction than chronic pancreatitis and cystic fibrosis, and energy balance studies in nearly asymptomatic patients with CEL-MODY with and without pancreatic enzyme supplement treatment would be informative with respect to contribution of pancreatic insufficiency to negative energy balance, and clinical effect of enzyme supplements.

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