Chronic intestinal ischemia: Measurement of the total splanchnic blood flow

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1. HD Zacho, NB Kristensen, JH Henriksen and J Abrahamsen. Validation of 99mTechnetium labelled mebrofenin hepatic extraction method to quantify meal induced splanchnic blood flow responses using a porcine model. Journal of Applied Physiology. 2012;**112**:877-82.
2. HD Zacho, JH Henriksen, and J Abrahamsen. Measurement of the total splanchnic blood flow for the diagnosis chronic intestinal ischemia: Reference values and correlation with body composition. World J of Gastroenterology. 2013;**19**:882-88

1. Background

1.1. Introduction

Chronic intestinal ischemia (CII) is a relatively rare condition and according to the Danish Vascular Registry (http//www.karbase.dk), approximately 45 patients underwent a revascularization procedure due to CII in 2011 in Denmark. This clinical entity is characterized by common, but unspecific symptoms such as abdominal pain and weight loss [1], and is caused by insufficient blood flow to the gastrointestinal tract at times of peak demand - during digestion of a meal. During fasting conditions the intestinal blood supply is adequate thus no symptoms are experienced at rest. In the majority of cases CII develops due to atherosclerosis in the intestinal arteries. The complex of symptoms is also known as intestinal angina.

CII may in some individuals proceed within weeks, months or years to acute intestinal ischemia which is associated with high mortality and morbidity [2;3]. The prevalence of acute intestinal ischemia is reported to be as a high as 14.1% amongst patients older than 50 years admitted to hospital for acute abdomen [4]. More than 50 % of patients dying from acute intestinal ischemia have experienced symptoms of CII before the fatal event [3;5], underlining the importance for correct diagnosis and treatment of patients with CII beforehand.

Traditionally, the CII-diagnosis is exclusively based upon morphology of the intestinal arteries; however, a large inconsistency exists between CII-symptoms and the presence of atherosclerosis/stenosis in the intestinal arteries impeding the diagnosis. The noticeable lack of exact diagnostic methods may hamper optimal selection of patients for revascularisation [6].

1.2. A historic view

The presence of occlusion of the mesenteric arteries was described already in the last part of the 15th century by Antonio Beniviene from Florence, Italy [7]. Only little attention was paid to this clinical entity until Councilman [8] in 1894 described three cases of (acute) mesenteric ischemia due to occlusion of the superior mesenteric artery (SMA). In 1901, Schnitzler [9] explored the possibility that CII was associated with occlusion of the mesenteric arteries. This first account was followed in 1918 by the presentation of a case story in a paper entitled “Angina Abdominis” by Goodman [10]. The case described a history of CII in an 82-year-old female in whom symptoms of CII were partly relieved by minor food restriction and administration of nitro-glycerine several times a day. Goodman [10] and Conner [11] were the first to describe the analogy between the symptoms of CII and those experienced in patients suffering from angina pectoris, and a few years later Dunphy [12] described the clinical symptoms and signs, abdominal pain and weight loss, of a vascular disease that may, for a period of months or years, precede a complete occlusion of the mesenteric arteries and cause acute intestinal ischemia.

Twenty years later, in 1957, Mikkelsen [13] defined the complex of symptoms as ‘intestinal angina’ and proposed a potential surgical procedure to circumvent the narrowing of the origin of the SMA. A successful thrombendarterectomy was reported by Shaw and Maynard [14] the following year, and soon after the availability of durable grafts for arterial bypass surgery cleared the way for retrograde and antegrade arterial bypass from the aorta to the visceral arteries [15;16]. The method currently preferred for revascularisation remains percutaneous treatment thereby avoiding open surgery. This procedure was introduced in 1980 by Uflacker [17].

1.3. Measuring the total splanchnic blood flow (SBF)

The first method to measure the total hepatic or splanchnic blood flow (SBF) was based on the “Fick principle” as described by Bradley et al [18] in 1945. They performed their investigation in 23 sub-

jects who were being treated for primary or secondary syphilis. This method requires catheterisation of a liver vein and a peripheral artery, as well as continuous infusion of an indicator extracted by the liver. The method is based on the following four assumptions [18]: 1. The indicator is removed from the blood exclusively by the liver; 2. The concentration of indicator in the peripheral blood corresponds to its concentration in the blood entering the liver; 3. The level of indicator in the hepatic vein is representative of the level in total pooled hepatic output; and 4. The hepatic blood flow altered neither by the infusion of the indicator nor by the presence of the catheter in the liver vein. The first indicator used was Bromsulfalein. Unfortunately, it undergoes enterohepatic circulation and fatal allergic reactions have been reported, and Indocyanine green was therefore introduced [19;20] owing to its more suitable properties and this indicator is still in use though Technetium-99 labelled agents have been launched [21]. These commercially available hepatobiliary agents are Technetium-99 labelled equivalents of iminodiacetic acid e.g. 99mTc labelled mebrofenin (99mTc-MBF) which has the advantage of requiring smaller plasma samples and permitting determination of SBF in presence of hyperlipidaemia [21].

1.4. Anatomy

The gastrointestinal tract receives blood from three arteries directly from the abdominal aorta. The coeliac artery (CA) divides into the common hepatic artery which supplies the liver with arterial blood, and the left gastric and the splenic arteries which supply the ventricle, the spleen, and the cranial part of duodenum to the major duodenal papilla of Vater. The SMA also originates from aorta and supply the pancreas, the jejunum from the duodenal papilla, the ileum and colon to splenic flexure. The remaining part of the colon and rectum receive their blood supply from the inferior mesenteric artery (IMA). Anatomical variations in the gastrointestinal blood supply are numerous. Under normal conditions, the gastrointestinal blood flow comprises 20 – 25 % of the cardiac output.

All the venous blood from the gastrointestinal tract drains via the splenic vein, the superior mesenteric vein and the inferior mesenteric vein into the portal vein and enters the liver at the porta hepatis. The liver thus has a dual blood supply from the hepatic artery and the portal vein; during fasting approximately 25 % of the blood is received from the common hepatic artery and 75 % from the portal vein.

A redundant collateral network between the three intestinal arteries is present at all times. The collateral path between CA and SMA is present through the arterial gastroduodenal and pancreaticoduodenal arcades along the greater curvature of the ventricle. Between the SMA and IMA the collateral blood supply is located in the arterial arcades in ileum and colon, the most developed is known as the Riolan artery and the Marginal artery of Drummond. In case of ischemia in the gastrointestinal tract, the collateral blood supply can develop further, thus accommodating the demand for oxygen even in the presence of significant stenosis or occlusion of the intestinal arteries without clinical symptoms of intestinal ischemia. Atherosclerosis is the most common cause of stenosis in the intestinal arteries.

1.5. Atherosclerosis in the intestinal arteries

Atherosclerosis of the intestinal arteries is often an affirmation of generalized progressive atherosclerotic disease. Atherosclerosis is strongly associated with age. Thus, in an autopsy study of an unselected Finnish population, 67% of subjects aged 80 years or more presented with mesenteric artery stenosis, whereas the rate was 6% among those aged less than 40 years [22]. Others report incidences of atherosclerosis in the intestinal arteries of around 20 % [23;24]. The incidence of significant stenosis of at least one of the intestinal arteries is 40% in patients with abdominal aortic aneurysm, 29% in patients with occlusive disease of the iliac segment, and 25% in patients with peripheral arterial disease [25]. However, only few of these patients will eventually develop CII. The above-mentioned autopsy study found that 15 % (18 patients) had atherosclerotic stenosis in at least two mesenteric arteries, but found only one case of bowel necrosis. Similar discrepancies have been described repeatedly in the literature [24;26;27], which has given rise to the question: "Does intestinal angina exist?" As early as in 1869 Cheine described a case of complete obliteration of the three visceral arteries with no signs or symptoms of intestinal ischemia and explained in minute details the collateral system of the vessels supplying the viscera with blood [28].

1.6. Physiology

After a meal, the blood flow through the intestinal arteries increases; its augmentation depends on the size of the meal and its content of protein, fat, and carbohydrates. A combination of all three nutrients and the presence of bile acid is the most potent inducer of the postprandial blood flow [29-31]. The postprandial increase in SBF is accommodated partly by an increase in cardiac output and partly by a decrease in flow to other organs, e.g. skin and skeletal muscles [32;33]. Anticipation and ingestion of food enhance the organ-specific blood flow in the mouth, oesophagus, and stomach [34]. The increased volume of blood flow in the intestines precedes the arrival of chyme [35], and segment-specific, increased blood flow persists as long as the chyme remains in the specific intestinal segment [34;36]. In healthy volunteers, it seems that the postprandial increase in SBF is relatively larger than the increase in splanchnic oxygen uptake (SO2U); thus, the saturation in the hepatic vein will increase slightly postprandially (study I, study III) [37].

1.7. Clinical features of chronic intestinal ischemia

Symptoms of ischemia develop when the genuine and collateral blood supply no longer accommodate the need for oxygen. In CII, the baseline SBF is sufficient, but the postprandial increase in SBF is inadequate and sudden onset of abdominal pain will therefore develop in relation to food intake (study I). Pain is the principal symptom and is often described as colicky, crampy, or dull. Due to the intensity of the abdominal pain, patients soon refrain from eating large meals with a consequent weight loss. A weight loss which arises because of the reduced caloric intake rather than because of malabsorption.

The pain is thought to be caused by hypoxia in the intestinal villi, but a steal syndrome may also be involved [38]. The pain can last up to several hours after the meal and may worsen over time. If it becomes continuous, acute intestinal ischemia should be considered. The presence of abdominal pain and severe weight loss often mislead the physician into considering a malignant disease. The patients may suffer from diarrhoea, obstipation [39], nausea, and in case of involvement of the CA gastroparesis and intractable ulcers may accompany the ischemic entity [40]. Thus, a high index of suspicion is required in order to make the correct diagnosis; and even when the diagnosis has come to mind, it remains difficult to decide which patients would benefit from revascularization. A successful revascularisation of one or more of the intesti-

nal arteries will restore a sufficient blood flow allowing the patients to eat without experiencing abdominal pain. The quantitative effect of revascularisation has only been investigated in six patients showing a normalisation of the meal-induced SBF increase [41].

1.8. Diagnosis of CII

According to international guidelines [42;43], the diagnosis of CII is based upon the presence of the two essential symptoms: postprandial abdominal pain and weight loss in combination with an angiography showing that at least two of the three intestinal arteries are significantly stenotic (> 70 % lumen reduction) or occluded. The angiography can be performed using MR, CT, or conventional digital subtraction angiography; the latter is considered the gold standard.

Only a few places in the world take the physiologic consequences of the intestinal arterial stenosis into account when making the diagnosis of CII [44-46]. However, it is widely recognized that stenosis in other vascular territories (e.g. the coronary arteries, the carotid arteries, and peripheral arterial disease) does not automatically indicate ischemia; and physiology is consequently evaluated along with investigation of the morphology in these vascular territories [47;48].

This is particularly important in the intestinal vascular territory due to the redundancy of the collateral vessels; thus, symptoms need not develop even when all three intestinal arteries are affected. This is known from autopsy studies [22] and follow-up studies performed in patients with known stenosis or occlusion of one or more of the intestinal arteries [24]. On the other hand, symptoms of intestinal ischemia may very well develop with only one artery involved (single-vessel CII) and even in patients with no signs of morphologic changes in the intestinal arteries; this is known as non-occlusive-mesenteric-ischemia (NOMI). NOMI is a completely different clinical entity, which can be seen in low-flow states like septic shock, cardiac insufficiency, or haemorrhage, where blood is redistributed from the gastrointestinal tract to more vital organs. Furthermore, NOMI can be observed in healthy people during strenuous exercise; the symptoms are related to mucosal ischemia [49] and are of shorter duration.

The existence of single-vessel CII has only been addressed in a few studies and the diagnosis has been doubtful with the consequence that clinicians have generally been reluctant to perform revascularization. A paper has recently been published by the Enschede group who used functional tests for the diagnosis of single-vessel CII. The outcome of revascularization in patients with an isolated > 70 % stenosis and an abnormal tonometry-test was 71 -75 % of the patients experienced relief of symptoms with more than one year of follow-up [50;51].

1.8.1. Digital subtraction angiography (DSA)

Digital subtraction angiography (DSA) is the gold standard for detection of arterial stenosis and determination of stenosis degree in the arterial system. The method has the possibility of performing both angiography and an endovascular procedures in one session [44]. The use of iodine-containing contrast agents - with its possible side effects - is limited in patients with diabetes or renal impairment

The potential complications which can develop are due to the invasive nature of DSA, the most common complications reported are bleeding, external iliac artery dissection, pseudoaneurysms, cerebral air embolus, and deep venous thrombosis. The reported complication rate is 2.1 %. [52].

DSA is increasingly being replaced by non-invasive investigations such as CT- and MR-angiography. However, the lack of comparative studies using these modalities in the splanchnic arterial system is remarkable.

1.8.2. CT-angiography

Owing to its non-invasive properties CT-angiography is increasingly replacing conventional DSA in the evaluation of stenosis in the arterial system. The use of multi-detector row computed tomography (MD-CT) permits a detailed assessment of the intestinal arteries and the short acquisition time minimises respiratory and motion artefacts [53].

The key advantage – apart from that the investigation is non-invasive – is the ability to visualise not only the vessels but other possible causes of abdominal symptoms (e.g. tumours, thrombus-formation, bowel wall thickening or external compression) [44].

The major drawback of CT-angiography is the frequent inability to visualise the IMA, the use of a potential nephrotoxic contrast agent and the radiation dose [44], which approximates 10.8 mSv for one abdominal examination [54].

1.8.3. MR-angiography

The immense development within magnetic resonance imaging (MRI) during the past decade has rendered the use of MRI in patients with CII increasingly interesting. It is a non-invasive investigation with no radiation exposure, and in contrast to DSA and CT-angiography, which solely address the morphology of the intestinal arteries MRI may also provide information regarding tissue perfusion, arterial and venous blood flow and oxygenation. It is possible to perform fast-scan MR-angiography (acquisition time 17 s) with a sensitivity of 89 % and specificity of 99 % for detecting stenotic vessels when compared to conventional MR using MD-CT [55].

MRI has previously shown promising results for quantitating the blood flow in the portal- and superior mesenteric vein [56;57]. Using MR oximetry it is possible to assess the oxygenation of the venous blood before and after a meal. In patients with CII the meal induces a decrease in venous saturation unlike healthy individuals that show an equal or increased saturation (study I, study III) [37;58].

Another promising study reports the possibility to quantify the small-bowel perfusion to differentiate normal individuals from those with CII [59]. Despite the promise of such reports, MRI's capability of functional assessment of CII has hitherto not been applied to the clinical setting.

MRI is fighting the disadvantage of incompability with claustrophobia and implanted devices (e.g. pacemakers, cochlear implants and metal devices) [44], and a high rate of patient discomfort [60].

1.8.4. Duplex ultrasonography (DUS)

Duplex ultrasound (DUS) is the preferred method when screening patients for stenosis/occlusion of the intestinal arteries [44]. It is inexpensive and non-invasive. DUS employs the fact that blood velocities can be converted to a degree of arterial stenosis [61-63]. Moreover, DUS has been evaluated as a functional test; Assessment of the portal blood flow before and after a meal is possible using DUS. However, the inter observer variation of the investigation is only slightly lower than the meal-induced increase in portal blood flow [64].

In healthy young volunteers the meal induced differences in blood flow velocity-response and resistivity-index in the coeliac and superior mesenteric arteries can be assessed using DUS [35] as

a surrogate marker for the blood flow. However, calculation of the volume blood flow is dependent upon an accurate cross-sectional area of the vessel, which is rather difficult by DUS especially in the intestinal arteries and almost impossible in patients with disseminated atherosclerosis.
The shortcomings of the DUS-modality is the necessity of an experienced user [44].

1.8.5. Tonometry
Tonometry is a functional test to detect ischemia; the investigation is based on the knowledge that if blood flow does not accommodate the metabolic demand, ischemia develops. During ischemia tissue depends on anaerobic metabolism to take place and CO2 will accumulate in the tissue. In the intestines CO2 will easily diffuse from the mucosal layer to the gut lumen, thereby increasing the luminal PCO2 and consequently enhancing the lumen to blood PCO2-gradient [65;66]. A tonometer balloon is placed in the patient's stomach or bowel (at the ligament of Treitz), the balloon is inflated and deflated at regular intervals and the PCO2 in the aspirated gas is measured.
The use of tonometry after meal-stimulation has been proposed; unfortunately many nutrients interfere with the PCO2 in the lumen. Hence, the effect of various meals on the PCO2 in ventricle has been assessed, and using food with no or low impact on the PCO2 is of great importance [67].
Gastric exercise tonometry has been suggested by Kolkman et al as an alternative to meal stimulation. The idea was that heavy exercise would provoke ischemia by redirecting blood from the intestines to the muscles [49;68-70;70]. However, a large proportion (24 %) of patients with two- or three-vessel disease are not capable of performing the relevant amount of exercise [45] due to cachexia.
This challenge can be overcome by using 24 hour tonometry with high doses of proton pump inhibitors and food with low or no impact on PCO2 [71].
Tonometry has the great advantage of enabling diagnosis of single-vessel CII and celiac artery compression syndrome [46;69;72].
As a diagnostic tool in patients with CII, tonometry is - like other functional tests – only applied to daily clinical practice in few centres worldwide. This might be due to the test being rather time consuming and clinicians being unaccustomed to this type of test. Nevertheless, selecting patients for revascularisation using tonometry enhances the clinical outcome [46].

1.9. Conclusion leading to present thesis
The existence of the clinical entity CII has hitherto been questioned due to the discrepancy between its morphology and symptoms. Selecting patients for revascularisation is customarily based solely upon the morphology of the intestinal arteries. This implies that some patients forego treatment while treatment is offered to those patients whose stenosis of the intestinal arteries is found coincidentally when investigating for the origin of their abdominal pain. A diagnostic method for detection of CII that takes the physiologic consequences of arterial stenosis into account is therefore much needed.

2. Aim of the thesis
The total SBF can be measured using the Fick principle with continuous infusion of a tracer and catheterization of a hepatic vein and an artery. By doing so it is possible to determine the SBF and SO2U during fasting and after a standard meal in order to quantify the meal induced increases in these variables.

2.1 Aim
The overall aim of this thesis was to investigate and validate a method to determine the SBF and to employ the method as a diagnostic tool in patients suspected of CII.
The specific aims were:
To give the historic background for the clinical entity CII, its diagnosis and treatment.
To measure the SBF before and after a test-meal in a group of patients suspected of CII and to correlate the results with angiography, and to analyse the diagnostic outcome in this group of patients (study I).
To compare the SBF measured by the Fick principle with a liver extraction-independent paraaminohippuric acid (pAH) dilution method in a porcine model. Moreover, as a change in the hepatic extraction of 99mTechnetium labelled Mebrofenin (99mTc-MBF) could impact the calculation of SBF, thus the arteriovenous 99mTc-MBF difference was monitored and its impact upon SBF measures assessed. Finally, the potential metabolism of 99mTc-MBF in the intestines was assessed (study II).
To determine the SBF and SO2U before and after a standard meal in a group of middle-aged healthy volunteers with angiography-proven normal intestinal arteries. In addition the aim was to relate the size of SBF and SO2U to the anthropometric measures of the body, for this purpose a cohort of patients suspected of chronic intestinal ischemia due to weight loss and abdominal pain but with angiography-proven normal intestinal arteries was included (study III).

3. Materials and methods

3.1. Study populations.

3.1.1. Study I
This study consisted of 50 consecutive patients referred to our Department for routine investigations; determination of the SBF before and after a test meal and simultaneously angiography due to clinical suspicion of CII. Prior to referral other causes of abdominal pain and weight loss were excluded by appropriate diagnostic methods. The investigations were performed during a 6-year period, from June 2002 to October 2008. Only patients investigated with SBF and angiography during the same session were included, the only exclusion criterion was reinvestigation. A retrospective review was then performed of 50 patients who met these criteria: Twenty-one women with a mean age of 67 years range (44-85), body mass index (BMI) 18 kg/m2 (12-25) and 29 men at a mean age of 66 years (45-82), BMI 20 kg/m2 (12-26). Hospital records were examined in order to obtain information about patient characteristics as atherosclerotic stigmata and risk factors (Table I). Four patients were excluded from analysis, one patient was given atropine due a vasovagal syncope, one patient had delayed gastric emptying verified by lack of postprandial increase in plasma glucose concentration, one patient did not obtain steady state condition of the infused tracer thus SBF could not be calculated, and finally one patient was excluded due to partial ileus with inconclusive angiography.

3.1.2. Study II
Sixteen female Duroc × (Danish Landrace × Yorkshire) pigs were obtained from the Faculty of Agricultural Sciences of Aarhus University. The pigs were transferred to an intensive care facility; each pig weighed approximately 50 kg on the day of operation. On the day of the study the mean weight was 60 kg (range 56 to

68 kg). One pig was excluded due to incorrect placement of the catheters.

3.1.3. Study III

Twenty healthy volunteers in the age 40 - 70 years (ten women) participated in the present study. None of the volunteers had any signs of cardiovascular disease, no abdominal complaints or weight loss for a year before the investigation. Apart from appendectomy n = 2, no former abdominal surgery was performed in any of the volunteers.

During a period from June 2002 to October 2011 180 patients suspected of CII were routinely referred to measurement of the SBF due to weight loss and abdominal pain. For comparison only patients with a digital subtraction angiography showing three normal intestinal arteries were included in the present study. Thus 32 patients participated, 31/32 (97 %) patients suffered from postprandial abdominal pain and 25/32 (78 %) had experienced an unintentional weight loss (mean 10.4 kg). Three patients were excluded, two due to ischemic colitis verified by biopsy and one patient due to pulmonary cancer metastasizing to the ventricle and colon. Table II presents the anthropometric data of the healthy volunteers and the patients.

Further details of the study populations are given in study I, II, and III articles.

Table I: *Demographic data of the study population (Study I) entering analysis, n = 46. Data is shown as mean, ranges and numbers.*

	Female n = 21	Male n = 25
Age, years	67 (44-85)	65 (45-82)
Weight, kg	52 (34-76)	69 (38-92)*
Height, cm	160 (150-176)	174 (161-187)*
Weight loss	n = 18	n = 19
Postprandial pain	n = 21	n = 23
Smoking incl. ex-smoking	n = 16	n = 17
Hypertension	n = 9	n = 10
Ischaemic heart disease	n = 4	n = 9 *
Diabetes	n = 1	n = 2

** Indicates a significant ($p < 0.05$) difference between females and males.*

3.2. Methods

3.2.1. Catheterization in patients

After an overnight fast and at least one hour of resting in a supine position the subjects underwent catheterization of the femoral artery and vein ad modum Seldinger using local analgesia. For the artery a 5F sheath was used and 7F for the vein. The venous catheter (Swan-Ganz, Edwards, CA, USA) was placed in a central hepatic vein using fluoroscopy, the 4F arterial catheter (pigtail catheter, Cordis, NJ, USA) in the abdominal aorta.

3.2.2. Blood sampling

Blood samples were taken simultaneously from a central hepatic vein and the abdominal aorta every ten minutes during the first hour. After one hour, the participants ingested a 4000 kJ/400 mL standard liquid meal based on dairy products consisting of 33% protein, 33% carbohydrates and 33% fat. Blood samples were then collected every ten minutes for another hour, and blood glucose concentrations were measured for each sampling time as a control of gastric emptying and intestinal absorption.

3.2.3. Splanchnic blood flow, splanchnic oxygen uptake and extraction fraction

The SBF, which in normal subjects equals the hepatic blood flow, was measured using a standardized protocol by indirect Fick principle with 99mTechnetium labelled Bridatec (Mebrofenin®, GE Healthcare, Suluggia, Italy) as the indicator. The method was originally introduced by Bradley et al [18] and used with corrections for unsteady state, and for a small urinary excretion of 99mTc-MBF as recommend by Henriksen & Winkler [21]. The radiation equivalent dose was below 2 mSv.

In brief, a bolus injection of 99mTc-MBF was given followed by a constant infusion with 2.0 mL/min. In total less than 80 MBq was used in patients (study I and III) and 250 MBq in pigs (study II). To obtain steady state an equilibration period of 20 minutes was interposed before blood samples were taken. Splanchnic plasma flow (SPF) was calculated as: SPF = E/ (Ca - Chv), where E is the hepato-biliary excretion rate of 99mTc-MBF which equals the corrected infusion rate [21]. Ca and Chv are the concentrations of 99mTc-MBF in the abdominal aorta and the hepatic vein, respectively. The level of 99mTc-MBF in plasma samples was determined by Cobra II Auto-Gamma, gamma counter (Packard Bioscience Company, Frankfurt, Germany). At least 10,000 counts were obtained corrected for decay, background and dead-time.

SBF was calculated as SPF/ (1 – haematocrit fraction). The splanchnic SO2U was then calculated as: Haemoglobin [g/mL] x (arterial oxygen saturation – hepatic venous oxygen saturation) x SBF [mL/min] x 1.34 mL O2/g of haemoglobin. The blood samples were analysed using ABL 700 series (Radiometer Medical A/S, Brønshøj, Denmark) and operated according to the instructions from the manufacturer.

The Extraction Fraction (EF) of 99mTc-MBF was calculated as (Ca - Chv)/ Ca.

At the end of the session the wedged hepatic vein pressure and free hepatic vein pressure were measured by a capacitance transducer in order to exclude the presence of any portal hypertension and resulting porto-systemic shunting.

Table II. *Demographic data from 20 healthy volunteers and 29 patients suspected of chronic intestinal ischemia but with normal intestinal arteries (study III). All results are given as mean and range.*

	Healthy volunteers		Patients suspected of CII, but with normal angiography		
	Female N = 10	Male N = 10	Female N = 15	Male N = 14	p-value* difference between healthy volunteers and patients
Age, years	53.8 (40 - 64)	54.5 (43 – 69)	63.0 (44 - 85)	60.4 (45 - 76)	0.01
Weight, kg	72.0 (55.0 - 92.0)	83.0 (65.0 - 94.8)	57.9 (34.0 - 114)	74.9 (52.0 - 98.0)	0.02
Height, cm	165 (160 - 170)	177 (170 - 188)	165 (153 - 172)	176 (170 - 186)	0.88
BMI, kg/m^2	26.5 (21.2 - 34.5)	26.6 (21.7 - 30.6)	21.4 (12.5 – 41.9)	24.0 (15.0 - 31.6)	0.02
Body fat, %	34.4 (28.0 - 48.2)	21.9 (13.1 - 29.4)	Not available	Not available	
Lean body mass, kg	47.4 (39.6 - 54.6)	65.5 (55.8 - 70.6)	Not available	Not available	
Body surface area, m^2	1.81 (1.56 - 2.07)	2.01 (1.77 - 2.16)	1.61 (1.25 – 2.29)	1.89 (1.64 - 2.19)	0.02

**The p-value is the difference between healthy volunteers (n = 20) and patients (n = 29).*

3.2.4. Angiography

In patients an angiography was performed during the same session via a pigtail catheter in the abdominal aorta as a control of the morphology of the intestinal arterial blood supply. The angiography consisted of an antero-posterior and lateral horizontal abdominal projection to visualize the individual origin of the mesenteric artery branches. Iomeprol (Iomeron® 200 mg iodine/mL, Bracco, Milan, Italy) 15 mL for each image was used as contrast agent, a total of 45 mL in average.

Each of the three mesenteric arteries were classified as either normal (0 – 9 % lumen reduction), having a slight stenosis (10 – 49 % lumen reduction), moderate stenosis (50 – 69 % lumen reduction), significant stenosis (70 – 99 % lumen reduction) or occlusion. The investigator evaluation of the angiographies was blinded to the results of the SBF; both positive and negative controls were included.

3.2.5. Study I

The result of the angiography was categorized as either group 1) No need of intervention 2) Intervention needed (at least two arteries with significant stenosis or occlusion). Group 1 was further subdivided in 1a: normal (no affection of any degree of any abdominal artery) or 1b: slightly abnormal (three or less arteries insignificantly affected and/or one artery with more than a 70% stenosis).

3.2.6. Study II

Operation: Permanent indwelling catheters made of Tygon (S-54-HL, 1.02 mm i.d. × 1.78 mm o.d.; Buch & Holm A/S, Herlev, Denmark) were implanted into the abdominal aorta, inferior vena cava, portal vein, hepatic vein, and mesenteric vein of each pig 14 days (range 13 to 15 days) before sampling, as illustrated in figure 1.

Surgery was performed under general anaesthesia. The caudal branch of the saphenous artery was located by palpation. Both

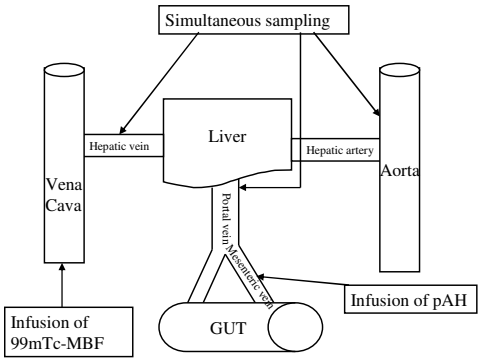

Figure 1: *Placement of catheters in the pigs during infusion and sampling. In the inferior vena cava 99mTechnetium labelled Mebrofenin® (99mTc-MBF) is infused continuously. Para-aminohippuric acid (pAH) is infused continuously in the mesenteric vein. Blood samples are collected simultaneously from the hepatic vein, portal vein and abdominal aorta.*

the arterial and venous catheters were implanted using a guidewire (THSF-25-145; Cook Denmark, Bjaeverskov, Denmark). From the point of insertion, each catheter was 35 cm long. The arterial and vena cava catheters were exteriorized in the lumbar region and tunnelled subcutaneously to the exteriorization point using long needles. The portal and hepatic vein catheters were implanted through an incision in the left medial lobe of the liver [73] as shown in figure 2. A branch of the portal vein was identified by passing a guidewire from the incision to the portal vein to verify the position of the tip in the portal vein by palpation. The location of the hepatic vein branch was verified by cardiac arrhythmia induced by passing the guidewire into the right atrium. Sutures that passed through the liver parenchyma anchored the catheters. The portal vein catheters were placed with the tip at the porta hepatis. A purse-string suture was placed on the vena mesenterica superior, and the catheter was introduced through a small incision with a tip length of 10 cm. The hepatic, portal, and

mesenteric vein catheters were exteriorized in the paralumbar groove. After surgery, the catheters were filled with saline containing heparin (100 IU/mL; Heparin LEO, LEO Pharma A/S, Ballerup, Denmark), benzyl alcohol (0.1%; benzyl alcohol + 99%, Sigma-Aldrich, St. Louis, MO), and benzyl penicillin (0.2%; Benzylpenicillin, Panpharma, NordMedica A/S, Copenhagen, Denmark). The pigs were treated with antibiotics for five days and analgesics for three days after surgery.
All the exteriorization points of the catheters (five for each pig) were cleaned daily and treated with antibiotics by drip (Streptocillin Vet.; Boehringer Ingelheim Denmark A/S, Copenhagen, Denmark).

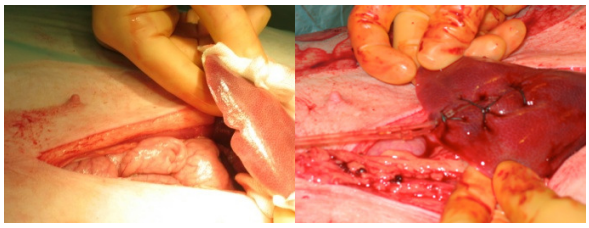

Figure 2: *Placement of the portal and the hepatic vein catheter was performed using a guidewire through an incision in the left medial liver-lobe. A branch of the portal vein was identified by passing a guidewire from the incision to the portal vein to verify the position of the tip in the portal vein by palpation. The location of the hepatic vein branch was verified by cardiac arrhythmia induced by passing the guidewire into the right atrium. The catheters were anchored to the liver parenchyma.*

The pigs were trained to stay in balance cages before sampling, and they were trained to accept human contact by daily scrubbing with a brush. All the pigs recovered quickly after surgery. The placement of all catheters was verified by autopsy and photo documentation ten days after sampling. All catheters were placed correctly in 15 of the 16 pigs. Two hepatic venous catheters were observed in one pig, and the pig was excluded from analysis. The average distance between the tip of the mesenteric vein catheter and the portal catheter was 21 cm, and the average distance between the tip of the hepatic catheter and that of the inferior vena cava was 5.9 cm.
Sampling: In pigs blood samples were collected simultaneously from the central hepatic vein, the portal vein and the abdominal aorta as a set at the same frequency as in patients. The pigs were fed a 15000 kJ/1500 mL standard liquid meal based on dairy products and consisting of 33% protein, 33% carbohydrates and 33% fat; once this meal was ingested, they were fed their regular meal, which was primarily based on barley, wheat and soybeans. Each plasma sample was divided into two vacutainers; one for gamma counting and one for analyses of pAH. The plasma samples were analysed for glucose and lactate using D-glucose oxidase and L-lactate oxidase, respectively (YSI 7100; YSI Inc., Yellow Springs, OH, USA).
Calculations: The SBF was determined by an indicator dilution method using paraaminohippuric acid (4-paraaminohippuric acid 99%, Acros, Geel, Belgium) as the indicator. The pAH was infused at a constant rate of 2.0 mL/min (0.057 mmol/min), and an equilibration period of 60 minutes was interposed to obtain a steady state before blood sampling was initiated.
At concentrations below 0.3 mmol/L, pAH has been shown to be solely and almost completely cleared by the kidneys [74], and the hepatic acetylation of pAH was corrected for by deacetylating the samples [75]. Given that the infused volume is much smaller than the SBF, the SPF can be determined as SPF = I / (Chv – Ca), where I

Figure 3: *Day of sampling (study II), the pigs stay in balance cages during the day of sampling*

is the pAH infusion rate, and Chv and Ca are the concentrations of pAH in the hepatic vein and abdominal aorta, respectively. Determination of portal blood flow was applied to calculate the intestinal flux of oxygen, lactate and glucose. Portal plasma flow (PPF) was determined as PPF = I/ (Cportal – Ca), Cportal is the concentration of pAH in the portal vein.
The plasma pAH was deacetylated before the total pAH concentration was determined by the method described by Harvey and Brothers [76] using a continuous flow analyser (Autoanalyzer 3, method US-216-72 Rev.1; Seal Analytical Ltd, Burgess Hill, England). To deacetylate pAH, the plasma was deproteinized by the addition of an equal volume of 20% trichloroacetic acid (w/v), and the supernatant was incubated at 100°C for 1 h. The SBF and oxygen consumption were calculated as previously described [73].

3.2.7. Study III
Healthy volunteers were subjected to a dual-energy X-ray absorptiometry (DEXA) scan. X-rays with two different energy levels are used. The variation in tissue density attenuates the X-rays penetrating the body. The specific density for each kind of tissue is used to calculate the relative mass of e.g. fat, bone or lean mass. Body composition with percentage body fat and fat free mass were measured by whole-body scanning with a Delfi Discovery A S/N 70879 using a standard software version 12.6 (Hologic, MA, USA) for quantification. During evaluation, each participant wore only their underwear and a t-shirt, and was immobilized in supine position, keeping the arms and legs away from the body. The duration of one examination was around 10-15 minutes. The equipment was calibrated daily, according to the manufacturer's instructions.

3.3. Statistics
Statistical analysis was performed using STATA®11 (StataCorp LP, College Station, Texas). All results are presented as mean values, standard deviation (s.d.), standard error of the mean (SEM) and ranges. Paired and unpaired Student's t test was used for intergroup comparisons and to compare the baseline and postprandial periods. Relations between variables were analysed using linear or multiple linear regression analyses and as repeated measurements when appropriate. A p-value < 0.05 was considered statistical significant.

Table III: *Results (study I) of SBF and splanchnic oxygen uptake (SO2U). "Normal SBF" is the group of patients with a normal postprandial increase in SBF (increase in SBF > 250 mL/min).*
"Abnormal SBF" is the group of patients with an abnormally low postprandial increase in SBF (increase in SBF < 250mL/min). Results are shown as mean and range.

	Normal SBF, n = 36	Abnormal SBF, n = 10
Baseline SBF, mL/min	1008 (525 - 1932)	897 (530 - 1422)
Postprandial increase in SBF, mL/min	591 (254 - 1353)	81 (-130 - 248) †
Postprandial increase in SBF, %	63 % (13 – 152)	9,6 % (-21 - 45) *
Baseline SO_2U , mL O2/min	46 (27 – 74)	41 (28 - 50)
Postprandial increase in SO_2U , %	53 (4 – 135)	21 (-2 - 40) *
Rise in blood glucose level, mM	2,3 (0,5 - 6,3)	1,1 (0,5 - 1,1)*

** Indicates significant difference (p < 0.05) between the two groups.*
† Selection criterion

4. Results

4.1. Functional versus radiological assessment of Chronic Intestinal Ischemia (study I)

A concordant categorization by SBF and angiography was observed in 44 of 46 patients. The mean
baseline SBF for all patients was 985 mL/min range (525 to 1932). The median coefficient of variation was 9 % range (1 to 35). The measured postprandial increase in SBF was 480 mL/min (-130 to 1353) in the entire group of patients (Table III). SBF was categorized as normal in 36 of 46 patients resultantly no intervention should be performed in this group. The corresponding angiographies in these 36 patients were all in group 1: No need of intervention. The remaining ten patients showed abnormal postprandial increase in SBF. Of these patients eight had the angiographic classification; intervention needed, and two patients had the angiographic classification normal (1a). The diagnosis of CII based on the postprandial SBF was independent of body weight, body surface, and age.
Given that angiography is considered "Gold Standard" the nosologic sensitivity is 8/(8+0) = 1.0, the nosologic specificity is: 36/(36+2) = 0.95.
Follow-up: None of the patients with normal postprandial increase in SBF underwent any form of revascularization, and every patient with angiography and SBF indicating need of revascularization were referred to the Department of Vascular Surgery. In two patients the SBF and angiography were not concordant and the patients were subjected to medical treatment.

4.2. Validation of 99mTechnetium labelled mebrofenin hepatic extraction method to quantify meal induced splanchnic blood flow responses using a porcine model (study II)

The mean SBF was measured at each sample time for both methods; no differences were detected between the methods at any time (shown in Fig. 4). The mean baseline SBF was 2,961 mL/min

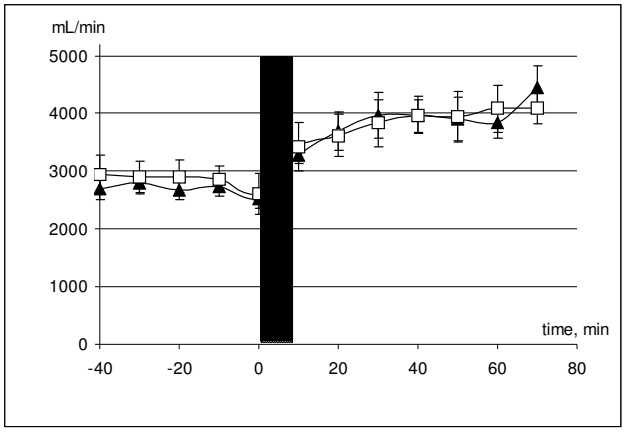

Figure 4: *The mean SBF measured in mL/min with 95% confidence interval (+/- 1.96*SEM) is shown as a function of time (minutes) for fifteen pigs (study II). Zero denotes the time of feeding. ▲ Mean SBF measured by the Fick principle and 99mTc-MBF. □ Mean SBF measured by the indicator-dilution method using pAH*

(c.i. 2,678 to 3,244) measured by pAH and 2,762 mL/min (c.i. 2,586 to 2,937) measured by 99mTc-MBF, this value increased to 3,977 and 3,981 mL/min, respectively (both p < 0.001), after ingestion of the meal.
During baseline, SBF showed no change with time (p = 0.73) by either flow method analysed as a linear regression model with repeated measurements. After the meal, the SBF showed a significant correlation with time (p < 0.001).
The data sets for baseline and postprandial SO2U and L-lactate release, as well as blood pH, are presented in Table IV. The SO2U (figure 6a) calculated as the net oxygen flux for the total splanchnic territory reached a plateau within 30 minutes after the meal. The total SO2U was divided equally between the liver parenchyma and the intestines (0.13 < p < 0.95) for each sample during the baseline period and after the meal.
The intestinal glucose uptake (figure 6b) presented a significant (p < 0.001) increase of 0.16 (c.i. 0.06 to 0.26) mmol/min from the baseline level for all postprandial blood samples, maximum was reached 30 minutes after feeding, and baseline level was not reached within the observation period. The plasma glucose in the arterial samples returned to baseline within 60 minutes after the meal.
The plasma concentration of lactate (figure 6c) in the arteries and the portal and hepatic veins increased immediately (p < 0.001) after feeding, and the highest value was obtained after 50 minutes. This result was consistent with the observed significant (p < 0.001) postprandial decrease in blood pH (figure 6d) in the The intestinal lactate flux was close to zero at baseline, reflecting arterial and portal and hepatic vein samples, although the numerical change was relatively small, as shown in Table IV.no net absorption or production of lactate in the intestines during the fasting state. After ingestion of the standard meal, the intestinal lactate flux increased significantly (p < 0.001), and a plateau was reached within 30 minutes after the meal. In contrast, the hepatic lactate flux did not change despite a lactate-rich meal. During the entire investigation, the net production/release of L-lactate from the liver was 0.42 mmol/min prior to the meal versus 0.45 mmol/min after the meal (p = 0.95).
No net absorption, metabolism or enterohepatic cycling of 99mTc-MBF was encountered. The mean difference between the arterial and portal concentrations of 99mTc-MBF, expressed as a percentage of the arterial concentration, was 0.21% (c.i.: -0.12%

Table IV: *Oxygen uptake, lactate flux and pH in pigs (study II) before and after a standard meal (n=15).*

Item	Baseline[1]	Postprandial[2]	p-value[3]
Mean oxygen uptake, mmol/min			
Total splanchnic	7.19 ± 0.20	9.81 ± 0.30	< 0.001
Intestinal	3.75 ± 0.16	4.92 ± 0.13	< 0.001
Hepatic	3.49 ± 0.15	4.82 ± 0.22	< 0.001
Mean net plasma L-lactate-flux, mmol/min			
Total splanchnic L-lactate-flux	0.49 ± 0.04	1.83 ± 0.08	< 0.001
Intestinal L-lactate-flux	0.07 ± 0.02	1.38 ± 0.06	< 0.001
Hepatic L-lactate flux	0.42 ± 0.04	0.45 ± 0.07	ns
Mean pH			
Arterial	7.47 ± 0.002	7.43 ± 0.007	< 0.001
V. Portae	7.40 ± 0.003	7.35 ± 0.004	< 0.001
V. Hepatica	7.39 ± 0.002	7.34 ± 0.004	< 0.001

*1. Baseline values are based on the five samples before the meal and given as mean+/- SEM (Standard Error of the Mean, n=15*5) based on flow determined by the indicator dilution method (SBF-pAH)*
*2. Postprandial values are given as mean value +/- SEM (n=15*5) of the five samples 30 to 70 minutes after the meal. Based on flow determined by the indicator dilution method (SBF-pAH)*
3. Significant difference before and after the meal is tested by the paired t-test

to 0.54%), which is not significantly different from zero, and no significant changes with time (p = 0.48) occurred.
A decrease in the EF of 99mTc-MBF was seen throughout the investigation, as shown in Fig 7. The EF showed an almost linear decrease (p < 0.001) from 40% at the beginning (40 minutes prior to feeding) to 20% 20 minutes after feeding; From 20 minutes after the meal to the end of the investigation the EF remained constant (p = 0.16).
Average values and ranges of SBF and SO2U during fasting and after the meal are shown in Table V.
Healthy volunteers: Visceral arteriography revealed no significantly stenotic vessels or occlusions in the group of healthy volunteers. The baseline SBF and SO2U values did not change over time and the variation in SBF was significantly larger between individuals than within individuals (p = 0.002).

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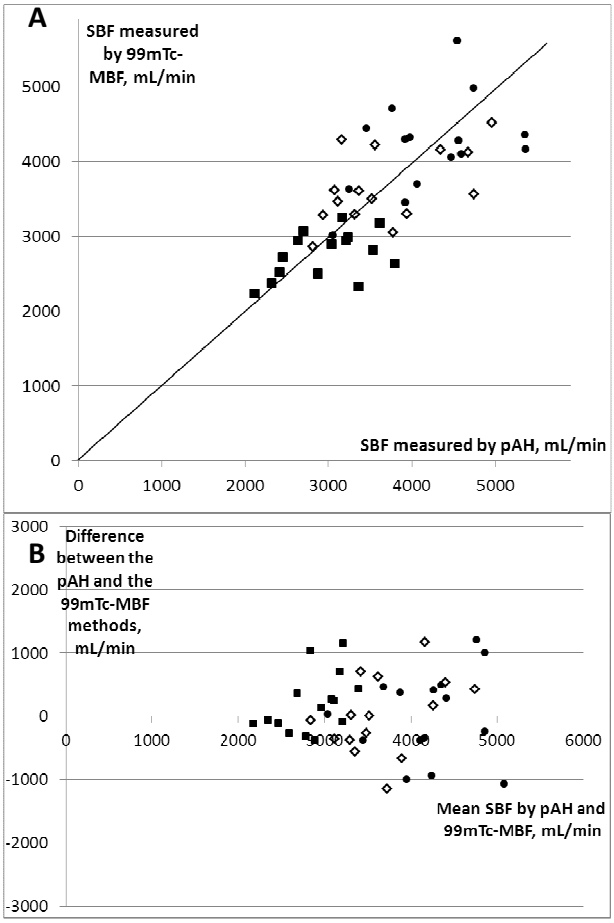

Figure 5 (study II): *Measurements were performed every ten minutes twelve times in each pig. For each pig measurements were merged into three periods indicated by the following symbols: ■ Baseline period ◊Measurements 10 to 30 minutes after the meal ●Measurements 40-70 minutes after the meal.*
A: SBF measured by the 99mTc-MBF-method is shown as a function of the SBF measured by the pAH-method
Line of identity is shown. B: Bland Altman-plot of the raw SBF measurements.

4.3. Measurement of the total splanchnic blood flow for the diagnosis of chronic intestinal ischemia: Reference values and correlation with body composition (study III)

The mean body weight among patients (65.4 kg) were significantly (p = 0.016) smaller than in the group of healthy volunteers (77.5 kg). Nevertheless no difference was found between the mean baseline SBF in patients (1080 mL/min) and healthy volunteers (1087 mL/min) p = 0.95 or the mean postprandial SBF which was 1718 mL/min in patients and 1787 mL/min in healthy volunteers. SBF is shown as a function of time in figure 8.

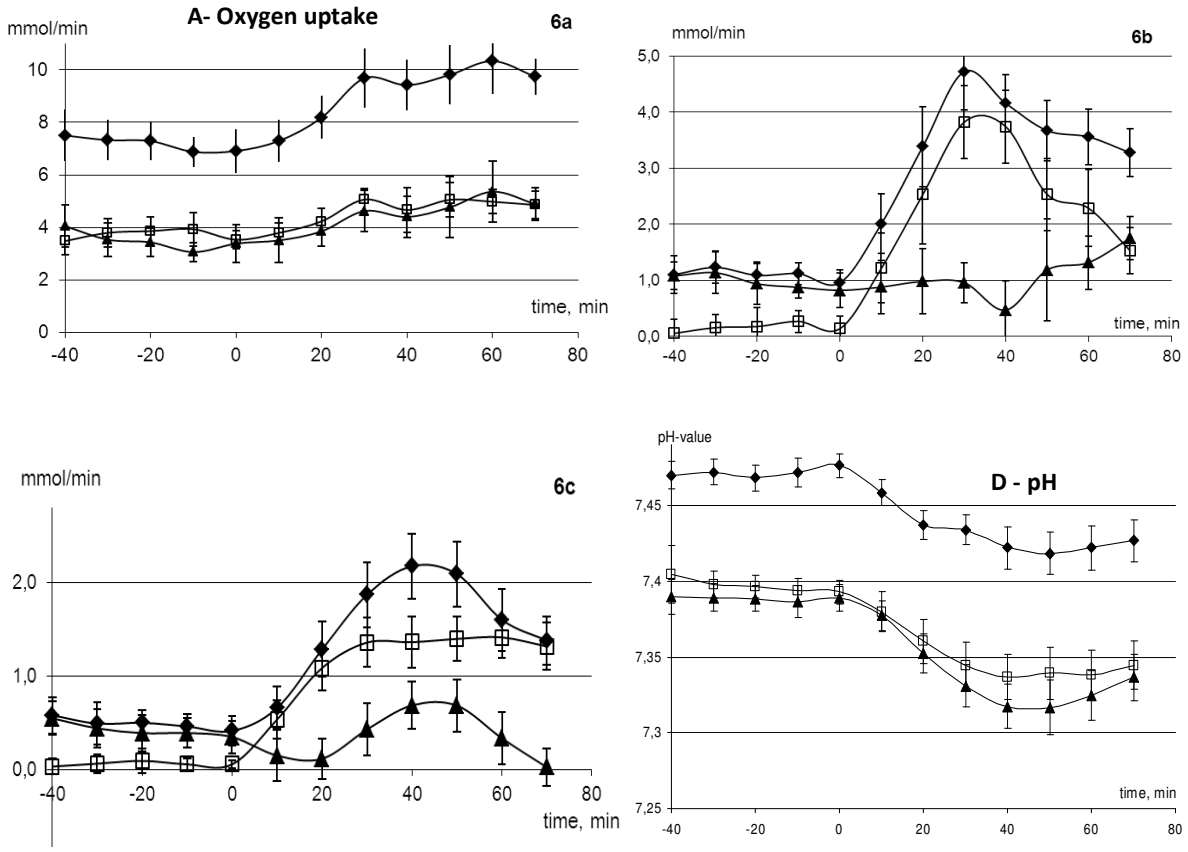

Figure 6, (study II): *Zero is the time of feeding.*
*A: shows the oxygen uptake with 95% confidence interval (+/- 1.96*SEM) at baseline and after the meal ingestion as a function of time (minutes). ▯ Denotes the total splanchnic oxygen uptake □ Denotes the intestinal oxygen uptake ▲Denotes the hepatic oxygen uptake calculated as the difference between the total splanchnic and intestinal oxygen uptake. B: Mean net glucose flux in mmol/min with 95% confidence interval (+/- 1.96*SEM) shown as a function of time (minutes). ▯ Denotes the total splanchnic glucose flux □ Denotes the intestinal glucose flux ▲ Denotes the hepatic glucose flux calculated as the difference between the total and intestinal glucose flux. C: The mean lactate flux measured in mmol/min with 95% confidence interval (+/- 1.96*SEM) shown as a function of time (minutes). ▯ Denotes the total splanchnic lactate flux □ Denotes the intestinal lactate flux ▲Denotes the hepatic lactate flux calculated as the difference between the total and intestinal lactate flux. D: The mean pH value with 95% confidence interval (+/- 1.96*SEM) shown as a function of time (minutes). ▯ Arterial □ portal vein ▲Hepatic vein*

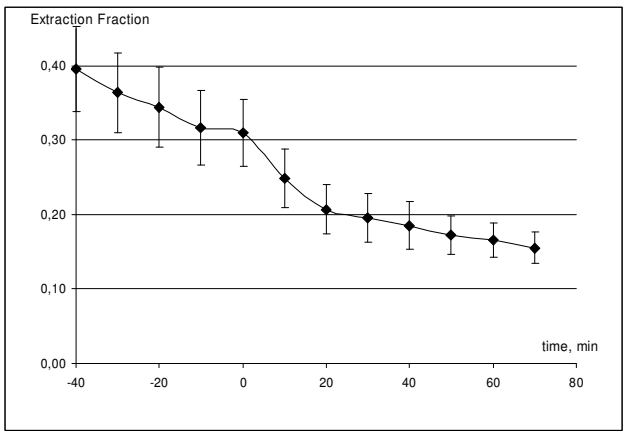

Figure 7: *Extraction Fraction of 99mTc-MBF in the liver shown as a function of time (minutes). Zero is the time of feeding (study II).*

The baseline value and the increase in SBF after the meal were independent of age, sex, body weight, body surface area, and lean body mass in the group of healthy volunteers. Contrary to this SO2U differed significantly between the two genders (p = 0.001); but when gender was corrected for lean body mass, this difference disappeared (p = 0.99). Thus, the gender-related difference in baseline SO2U and postprandial increase were solely attributed to the gender-related difference in lean body mass (p = 0.001).

A significant decrease in EF was seen during the baseline period and until 20 minutes after the meal.

Patients: In the patient group both baseline SBF and the postprandial increase did not depend on weight (p = 0.47 and p = 0.12, respectively). But when combining all individuals (n = 49) independently of health-status weight did have a significant (p = 0.025) impact on the postprandial increase but not on the baseline SBF. Thus an average person weighing 60 kg will demonstrate a 606
mL/min (95 % confidence interval: 510 to 701 mL/min) postprandial increase in SBF. Each additional kg bodyweight will augment the postprandial increase by 5.4 mL/min.

No significant difference between genders or any relation to age was found. Figure 9a shows the mean baseline SBF for each individual – patients and healthy volunteers - as a function of weight. Figure 9b shows the postprandial increase in SBF for each individual as a function of weight. Baseline and postprandial SO2U were

	Healthy volunteers		Patients suspected of CII, but with normal angiography		
	Female N = 10	Male N = 10	Female N = 15	Male N = 14	† p-value difference between healthy volunteers and patients
Fasting plasma glucose, mM	5.7 (5.1 - 6.9)	5.8 (5.2 - 6.9)	5.7 (4.5 – 11.6)	5.6 (4.7 – 7.5)	0.59
Postprandial plasma glucose, mM	6.8* (5.9 – 7.8)	7.2* (6.3 – 8.8)	7.0* (5.4 – 12.1)	7.2* (5.9 – 9.9)	0.27
Mean fasting SBF, mL/min	1044 (731 - 1319)	1129 (800 - 1390)	1040 (619 - 2781)	1123 (429 - 2740)	0.95
Mean postprandial SBF, mL/min	1731* (1386 - 1987)	1844* (1485 - 2343)	1581* (906 - 2340)	1863* (1332 - 3163)	0.54
Postprandial rise in SBF, mL/min	686 (515 - 915)	714 (314 - 1145)	542 (-441 - 1014)	707 (293 - 1325)	0.47
Mean fasting oxygen uptake, mL/min	43.4 (32.1 – 53.8)	58.1 (40.5 - 84.5)	44.5 (34.6 - 76.1)	51.8 (15.2 – 78.2)	0.48
Mean postprandial oxygen uptake, mL/min	65.5* (49.1 - 84.0)	89.6* (60.1 - 118.9)	68.3* (46.2 - 104.7)	82.2* (45.0 - 118.2)	0.63

Table V: *Results of glucose, splanchnic blood flow (SBF) and oxygen uptake from 20 healthy volunteers and 29 patients suspected of chronic intestinal ischemia but normal intestinal arteries (study III). All results are given as mean and range.*
†The p-value is the difference between healthy volunteers (n = 20) and patients (n = 29).
** Indicates a significant (p < 0.001) increase compared to fasting values.*

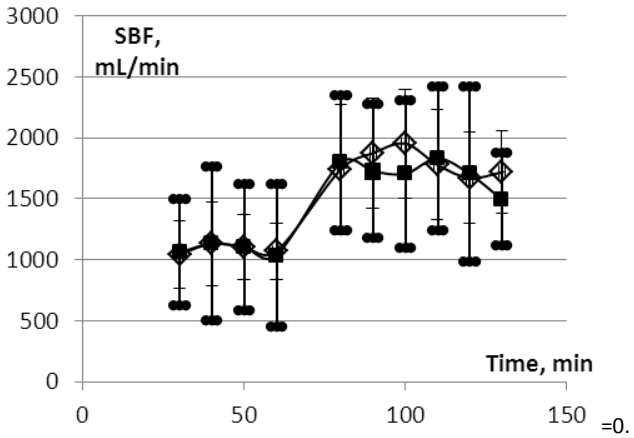

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Figure 8 (study III): *The mean splanchnic blood flow (SBF) +/- s.d. is shown as a function of sampling time (study III).*
■ (and fat error bars) denotes patients suspected of CII but with normal angiography of the intestinal arteries (n = 29).
◊ (and slim error bars) denotes the healthy volunteers (n = 20). The first 60 minutes are fasting values. The standard meal of 4000 kJ was served after 60 minutes.

48.0 mL O2/min and 75.0 mL O2/min, respectively and did not differ significantly from the healthy volunteers (p = 0.48 and p 68) as shown in table V. Patients also showed a significant difference between genders regarding postprandial SO2U (p = 0.03).

5. Discussion

International guidelines [42;43] on CII suggest exclusively use of investigations describing the morphology of the intestinal arteries to diagnose CII. The diagnostic criteria are a combination of relevant symptoms – postprandial abdominal pain and weight loss - and at least two significant stenosis/occlusions in the intestinal arteries. This approach is not taking the well documented knowledge regarding the frequent presence of inconsequential arterial stenosis into account [22;25;26]. Furthermore, by doing so patients with single vessel disease or NOMI are neglected.
The main advantage of using the measurement of SBF before and after a standard meal as a diagnostic test in patients suspected of CII is that the investigation measures the physiologic response rather than the morphology of the intestinal arteries and thereby assessing whether ischemia is present. Many studies have been conducted on patients suspected of CII but only a few have addressed the physiology when diagnosing ischemia in the gastrointestinal tract.

5.1. Study I
A simultaneous measurement of the SBF and angiography was performed for each patient and data from both modalities were analysed independently to ensure objective data.
The findings in this study showed a very good agreement between the postprandial increase in SBF and angiography. Given that angiography is considered the Gold standard SBF makes the diagnosis CII with a nosologic sensitivity of 1.0 and a nosologic specificity of 0.95. Only two patients had diverging outcome with an abnormal SBF but a normal angiography. Ischemic lesions in the colon were verified by biopsy in one of these patients. This patient might have suffered from microvascular or non-occlusive disease not identified by angiography.
The measured baseline SBF is concordant with previously published results (Hansen et al. 1977; Madsen et al. 2006). A study in healthy humans (Madsen et al. 2006) suggests that SBF might be

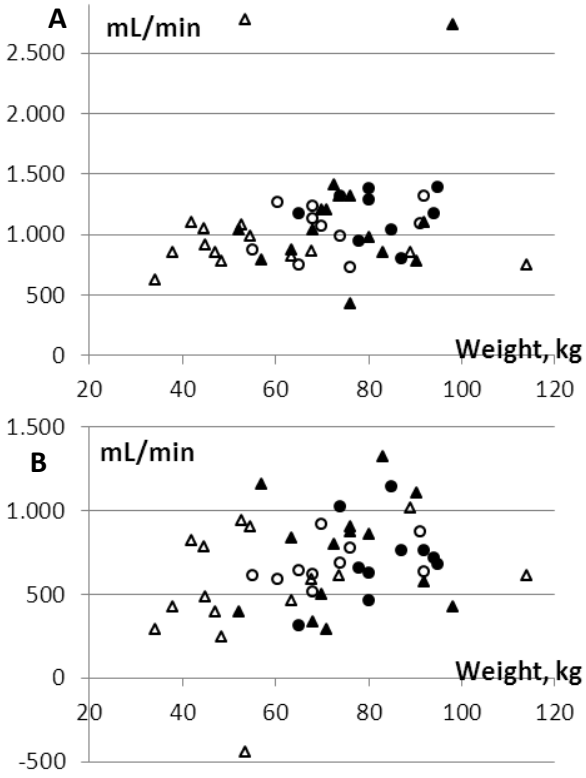

Figure 9 (study III): *Δ Denotes patients with normal angiography and ○denotes healthy volunteers. Hollow symbols are women and full symbols are men. A: Mean baseline SBF as a function of bodyweight for each individual. The linear correlation between bodyweight and baseline SBF is given by the equation. SBF = 858 mL/min + 3.2 mL/min*bodyweight, the correlation is however not significant (p = 0.35). When excluding the two individuals with hyperperfusion, the correlation between bodyweight and SBF only changes little and remains non-significant. B: Mean postprandial increase in SBF for each individual as a function of bodyweight. The linear correlation between bodyweight and the postprandial increase in SBF can be described by the equation: SBF (increase) = 282 mL/min + bodyweight * 5.4 mL/min (p = 0.025). Excluding the two individuals with hyperperfusion at baseline only changes the correlation between bodyweight and the postprandial increase in SBF little, it remains statistically significant*.

related to age and body surface area. It was, however, not the case in our study.
In the present cohort, the meal-induced increase in SBF was abnormal in 10/46 patients. Eight of the patients had an angiography with significant stenoses or occlusion of at least two of the intestinal arteries. They were thus referred to revascularization. A study of a similar group of patients has shown that an abnormal response in SBF only led to revascularization in 10 % of patients (Moller & Madsen 2002). This is in contrast to our results.

5.2. Study II

Study II demonstrated an excellent agreement between 99mTc-MBF and pAH in the measurement of the SBF for each individual sampling time. When plotting SBF measured by the two methods against each other, as presented in figure 5a, departure from the line of identity is revealed. There is a tendency towards larger discrepancy between the two methods in the high flow area. The departure from line of identity is random, though the 99mTc-MBF-method tended to underestimate the SBF during baseline the difference being non-significant. When applying the method to patients, baseline SBF is based on the mean of 5 individual measurements in order to overcome the known variation within individuals.
The corrections applied to the 99mTc-MBF-method for urinary excretion of 99mTc-MBF will lower the estimated value of SBF. When using the correction we assume a steady production of urine. Urine was collected from the start to the end of tracer infusion and pooled; one representative sample was used for analysis. The pigs were kept fasting and without water overnight before sampling, it thus seems credible that urine production would be much larger after the feeding than during fasting. By using the correction based on average urine production an over-correction may be applied to the estimated baseline SBF values, thus underestimating the true baseline SBF, and an insufficient correction could have been applied to the postprandial SBF values. The present study would have benefitted from repeatedly measures of urine production and sampling of urine. The mean postprandial increase in SBF of 1,016 and 1,219 mL/min measured by pAH and 99mTc-MBF, respectively, was greater than that generally observed in pigs fed conventional finishing diets [75].
During the observation/sampling period, a steady state was not obtained for 99mTc-MBF, as evaluated from the arterial blood curve; when calculating the SBF, a correction for the non-steady state was built into the algorithm using the extracellular volume as the volume of distribution for 99mTc-MBF. The corrections for non-steady state and for urinary excretion of 99mTc-MBF both tended to decrease the estimated value of SBF. Prior to the meal, there was a tendency for 99mTc-MBF to underestimate the SBF by 199 mL/min (c.i. -62 to 461) when compared to pAH, although this was not significant (p = 0.12). After feeding, this tendency was not seen, which may potentially be because the EF did not decrease significantly after the meal, due to a non-steady state in the excretion system. The difference could also be caused by not obtaining representative blood samples for the analysis of pAH, which was infused in the mesenteric vein. This problem ought to be overcome by the fact that the tip of the mesenteric vein catheter was placed 21 cm from the porta hepatis and the hepatic catheter used to calculate the SBF was placed downstream of the liver.
The present study showed that meal-induced SO2U reached a plateau within 30 minutes after the meal; moreover, the total SO2U was equally divided between the intestines and liver as seen before [77]. The meal-induced increase in oxygen uptake thus consists of two components: the intestinal thermogenesis related to the energy expenditure for digestion and absorption of nutrients, as well as the hepatic element for the conversion and storage of nutrients. The pigs were followed for 70 minutes after feeding, which was not sufficient to cover the total postprandial metabolism The length of the investigation was based on the objective to apply a clinical diagnostic method to a porcine model. The clinical diagnostic method aims at quantifying the meal induced SBF response rather than pursue the return to baseline. This is, off course, a limitation of the study. Although the net glucose uptake after the meal did not reach the baseline level within the observation time, the arterial glucose concentration returned to baseline within 60 minutes after the meal, reflecting both peripheral insulin-dependent and insulin-independent glucose uptake.
A net production of L-lactate from the liver was observed during the fasting state and after feeding, which can be explained by the production of lactate by the perivenous hepatocytes. The lactate enters the systemic circulation and is taken up by the periportal hepatocytes and degraded. Some of the circulating lactate is metabolized in the brain and skeletal muscles during rest [78;79].

Concomitantly with the increase in circulating lactate, a decrease in both arterial and venous blood pH was seen in this study.
Four of the pigs in the present study were examined six days later, with blood samples collected hourly six times after a standard lactate-free meal. The samples were analysed as described above, and the postprandial arterial pH was 7.4 and did not differ from the baseline level [75]. This result indicates that the postprandial decrease in pH was associated with the rapid absorption and accumulation of lactic acid in the blood
It was hypothesized that 99mTc-MBF is not metabolized in the intestines; and thus, the arterial content of 99mTc-MBF could be used instead of the portal content. This was confirmed by the present study that found no difference between the content of 99mTc-MBF in the two vascular territories at any time.

5.3. Study III

Study III aimed at establishing a reference value for SBF before and after a standard meal. As patients with CII are often severely underweight the relation between weight and SBF was investigated too; hence the aim of this study was to recruit healthy volunteers with a weight span comparable to patients with CII. However none of the healthy volunteers with stable weight without any signs of chronic disease had a BMI < 20. Confronting this challenge a group of patients with measurements of SBF performed due to suspicion of CII and a weight span from 34 kg to 114 kg but with angiography proven normal intestinal arteries served as an additional study group.
A mean baseline SBF of 1087 mL/min increasing to 1787 mL/min after ingestion of a standard meal as found in this study are supported by Madsen et al [37], who performed a study in a comparable population of healthy volunteers but without knowledge of arterial morphology.
Previous investigations of SBF were performed in volunteers between 20 and 30 years of age which are not representative of the population of interest when focusing on CII. Such studies have reported a total splanchnic plasma-flow during fasting around 900 mL/min [80-82] corresponding to a SBF of 1500 mL/min, given a normal haematocrit. These flow values are 35 % higher than the results presented in this study. Even though we found no age-related effect in a span from 40 to 85 years, this may, however, be the case when comparing young adults to middle-aged and elderly individuals.
The frequent presence of atherosclerotic changes in asymptomatic 60 – 70 years old adults renders knowledge about the arterial morphology in a study population essential. This has to the best of our knowledge been neglected in all previous studies. The present study demonstrates no atherosclerosis in any mesenteric arteries of the healthy volunteers, only mild impressions in the coeliac trunk was visualized in a few subjects, and solely patients with normal intestinal arteries were included in the present study.
Healthy volunteers showed no correlation between SBF and body composition described as body weight, body surface area and lean body mass. This is in contrast to a previous study [37] which showed that both baseline SBF and SO2U were directly related to the body surface area. A reason for this discrepancy might be that the ranges of body surface area or lean body mass amongst the healthy volunteers in the present study were too small to detect a possible correlation. It is a shortcoming of the present and similar earlier studies that underweight healthy individuals were not represented in the study-population as the majority of patients suspected of CII are underweight.
In the present study this limitation was encountered by using a group of patients representing weights ranging from 34 kg to 114 kg. When included in the analysis, weight did augment the postprandial increase further by 5.4 mL/min for each kg bodyweight. Calculated from this even patients weighing 34 kg (representing the lightest patient at our clinic) should experience a mean postprandial SBF increase of 465 mL/min (95 % c.i.: 275 to 656). Excluding the two patients with hyper perfusion at baseline only changes these values little (mean postprandial SBF will increase 4.9 mL/min for each kg). These findings support previous studies [37;41] stating that all individuals should demonstrate a postprandial response larger than 250 mL/min independent of bodyweight. However, the present study recommends that body weight is taken into account when using the postprandial SBF to diagnose CII.
Interestingly baseline SBF was not influenced by weight. A minor tendency towards an increased baseline SBF by 2.2 mL/min (95 % c.i. -1.6 to 6.1, p = 0.35) for each kg increase in body weight was seen, however, a significant correlation between weight and baseline SBF was not demonstrated.
In both groups a significant difference in the SO2U between genders was found, in the healthy volunteers who had a DEXA scan performed, the difference between genders was uniquely correlated to the differences in lean body mass. Whether this explanation applies to the patient-group as well, remains unknown as this group was not subjected to a DEXA-scan.

5.4. General discussion

5.4.1. Extraction Fraction

In the three studies a similar pattern in EF has been observed though not described in study I. Following the ingestion of the meal, a change in the gastrointestinal and hepatic hemodynamics was observed, along with a consequent decrease in EF during the first twenty minutes after the meal. The decrease in the EF of 99mTc-MBF was seen in patients (study I, study III), healthy volunteers (study III), and pigs (study II). This is an inherent consequence of the Fick Principle that EF will decrease when the flow increases owing to the formula: SPF = E/ (Ca - Chv). However, it remains unexplained why the EF decreased throughout the baseline period which was characterized by a constant SBF. From twenty minutes after the meal and during the rest of the investigation the EF shows a decreasing tendency, but it remains insignificant.
A decrease in EF from 46 % to 26 % during continuous infusion has been previously observed in a similar set-up in healthy humans and in patients with fatty liver and cirrhosis [21]. Other studies have previously shown that the first pass hepatic EF after a single bolus injection of 1.7 mg 99mTc-MBF into the portal vein is 91% [83]. This is in contrast to the present findings when using continuous infusion. This observation may be due to a limited capacity of the hepatocytes to process 99mTc-MBF from the blood to the bile. The exact kinetics of 99mTc-MBF transport in the liver requires further studies.

5.4.2. Advantages and limitations of the SBF method

Assessing the physiological consequences of intestinal arterial stenosis is of great importance as revascularization is aimed at relieving symptoms based on ischemia and not reliving the patient of a stenosis. However, the method has some inherent limitations. Most importantly the investigation is invasive with catheters inserted in both the arterial system as well as the ve-

nous system. The possible complications related to the invasive nature of the investigation are the same as can be seen when performing a DSA; external iliac artery dissection, pseudo aneurysms, cerebral air embolus, and deep venous thrombosis [52]. None of these complications were seen in any of the patients in our study-populations.
The meal volume of 400 mL may seem large to a cachectic patient, yet all the volunteers (study III) and 87 % of the patients (in study I) were able to ingest the entire meal, only one patient left more than 125 mL of the meal. Gastric emptying and intestinal absorption of glucose was controlled by measuring the blood-glucose concentration every ten minutes.
The method for measuring the SBF for diagnosing CII is based upon the difference between the mean baseline SBF (based on five blood samples) and the mean postprandial SBF (based on seven blood samples). This is a rather rough measure. We know that baseline SBF has fluctuations and this is more evident after the meal. It could be suggested to take into account the maximum value of postprandial SBF or the time from the meal to the max SBF the latter being much dependent on gastric emptying and intestinal absorption. This relationship has not been investigated in the present thesis.
When applying the SBF method for the diagnosis CII only the global perfusion of the gastrointestinal tract is assessed. By this method it is not possible to determine whether small parts of the intestine e.g. in the waterbed areas may suffer from local ischemia. This is a well-known weakness of the method.
In the study populations used, an increased gradient between the wedged and free hepatic pressure was encountered in none of the individuals. If such an increased gradient was present it would influence the calculation of the SBF as some of the blood may be shunted past the liver. The difference between the 99mTc-MBF-concentration in arterial and hepatic vein blood would be falsely lowered resultantly the SBF would be falsely elevated. This potential bias was avoided by measuring the wedged to free hepatic pressure gradient in all individuals (study I, study III).

5.4.3. Reference value and relation with body weight
The importance of proper reference values is immense. Hitherto, reference values has been based upon only four healthy individuals with normal angiography of the intestinal arteries [41] and 18 healthy volunteers without knowledge about the intestinal arterial morphology [37]. In the first mentioned study the relation between SBF and body weight was not investigated, in the latter a direct relation between SBF and body surface area was found in baseline, but no association after the meal. Nevertheless the possible effect of body composition when measuring the SBF has not yet made its way to the clinical setting. When applying the SBF-method to patients suspected of having CII a postprandial increase above 250 mL/min has been considered normal independently of body weight. When including a greater number of individuals with a large weight span (from 34 to 114 kg) the effect of bodyweight on the postprandial increase cannot be disregarded (study III).
The present study only supports a cut-off level of 250 mL/min in patients weighing 34 kg. When taking the effect of body weight into account 250 mL/min is too low for diagnosing CII amongst patients weighing more than 34 kg. Consequently, it would be expected that more patients would be diagnosed with CII using a cut-off value based on body weight.

6. Conclusions

This PhD thesis explores a method to determine the SBF and its potential use as a diagnostic tool in patients suspected to suffer from CII.
When the method is applied to patients it may be able to diagnose CII in patients with weight loss and postprandial pain in spite of normal morphology at angiography (study I). Measurements of the SBF thus have a place in the diagnostic armamentarium for the detection of chronic intestinal ischemia. SBF provides knowledge about the circulatory physiology in intestines in patients with weight loss and abdominal pain with or without intestinal arterial stenosis.
The method has been validated against a method independent of the hepatic extraction of tracer using pAH (study II). An excellent agreement was found between the 99mTc-MBF method based on hepatic extraction and the pAH dilution methods for the measurement of splanchnic blood flow in a conscious porcine model. The decreasing EF of 99mTc-MBF did not influence the measurement of SBF. In the same set-up metabolism and recirculation of 99mTc-MBF in the intestines was rejected based on the consistency between the portal and arterial contents of 99mTc-MBF during the fasting state and postprandial. This study leads us to conclude that an arterial blood sample can be used instead of a portal blood sample, making the method applicable to patients.
Finally the method was applied to healthy volunteers and patients with weight loss and abdominal pain but without intestinal arterial stenosis (study III). Study III revealed a small variation in baseline-SBF within subjects but a large variation between subjects; this underlines the importance of using the meal induced change in SBF for the diagnosis of CII. Secondly this study - in a population covering a wide weight-range - recommends that body weight is taken into account when diagnosing CII by the postprandial increase in SBF.

7. Perspectives

The present PhD thesis answers the questions posed at the beginning of the research project but several new questions have been raised since then, providing opportunity for new studies to be launched.
Study III recommends that body weight is taken into account when diagnosing CII by the postprandial increase in SBF. Using the weight factor, one could expect the number of patients diagnosed with CII to increase. The consequences of this approach should – of course - be evaluated in a prospective setting.
It is postulated that a successful revascularization will normalise the postprandial SBF, such a study has already been initiated at our department but only few patients have been recruited at present, the study will consequently continue.
In order to make the investigation less invasive and more comfortable to the patients it would be of great interest to study whether is it possible to avoid arterial catheterisation, by using peripheral venous blood instead of arterial to calculate the SBF. Or to shorten the duration of the investigation e.g. use a baseline SBF based on two blood samples and the postprandial increase based on three blood samples 30 to 50 minutes after the meal.
Another potential study is to measure the SBF in underweight healthy volunteers or to investigate the exact kinetics of 99mTc-MBF during continuous infusion.

8. Summary

A redundant collateral network between the intestinal arteries is present at all times. In case of ischemia in the gastrointestinal tract, the collateral blood supply can develop further, thus accommodating the demand for oxygen even in the presence of significant stenosis or occlusion of the intestinal arteries without clinical symptoms of intestinal ischemia. Symptoms of ischemia develop when the genuine and collateral blood supply no longer can accommodate the need for oxygen. Atherosclerosis is the most common cause of obliteration in the intestinal arteries.
In Chronic intestinal ischemia (CII), the fasting splanchnic blood flow (SBF) is sufficient, but the postprandial increase in SBF is inadequate and abdominal pain will therefore develop in relation to food intake causing the patient to eat smaller meals at larger intervals with a resulting weight loss.
Traditionally, the CII-diagnosis has exclusively been based upon morphology (angiography) of the intestinal arteries; however, substantial discrepancies between CII-symptoms and the presence of atherosclerosis/stenosis in the intestinal arteries have been described repeatedly in the literature impeding the diagnosis of CII.
This PhD thesis explores a method to determine the total splanchnic blood flow (SBF) and its potential use as a diagnostic tool in patients suspected to suffer from CII. The SBF can be measured using a continuous infusion of a tracer and catheterisation of a hepatic vein and an artery. By measuring the SBF before and after a standard meal it is possible to assess the ability or inability to enhance the SBF and thereby diagnosing CII.
In Study I, measurement of SBF was tested against angiography in a group of patient suspected to suffer from CII due to pain and weight loss. A very good agreement between the postprandial increase in SBF and angiography was found. The method was validated against a well-established method independent of the hepatic extraction of tracer using pAH in a porcine model (study II). An excellent agreement was found between the two methods for the measurement of SBF. In the same set-up metabolism and recirculation in the intestines of the 99mTechnetium labelled tracer was rejected based on the consistency between the portal and arterial contents of tracer. Based on this study we concluded that an arterial blood sample can be used instead of a portal blood sample, making the method applicable to patients. In study III, 20 healthy volunteers and 29 patients with weight loss and abdominal pain but normal morphology of the intestinal arteries were investigated. A reference value for the meal induced SBF-increase and the relation to bodyweight was established designating that bodyweight should be taken into account when diagnosing CII based on measurement of SBF.
The clinical method for measuring the SBF based on hepatic 99mTc-MBF extraction is a robust method. It allows determination of the postprandial increase in SBF providing knowledge about the circulatory physiology in intestines in patients with weight loss and abdominal pain with or without intestinal arterial stenosis. Future studies within this field could include measurement of the SBF before and after revascularisation in order to quantify the effect of revascularisation or investigate whether arterial blood sampling could be avoided or the amount of blood samples (and thus the time spend) could be reduced.
The three studies were presented at eleven national and international congresses and Helle Damgaard Zacho has been awarded three prizes for the presentations.

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