Validation of $^{99m}$Technetium labelled mebrofenin hepatic extraction method to quantify meal induced splanchnic blood flow responses using a porcine model

Helle Damgaard Zacho$^1$, Niels Bastian Kristensen$^2$ Jens Henrik Henriksen$^3$ and Jan Abrahamsen$^1$

$^1$Department of Clinical Physiology, Viborg Hospital, Viborg, Denmark.
$^2$Department of Animal Health and Bioscience, Aarhus University, Denmark.
$^3$Department of Clinical Physiology, Hvidovre Hospital, University of Copenhagen, Denmark

Running head: Measurements of the total splanchnic blood flow

Please address all correspondence to:
Helle Damgaard Zacho
Department of Clinical Physiology
Viborg Regional Hospital
Heibergs Allé 4
8800 Viborg
Denmark

Tel(+45) 78 44 33 06
Fax(+45) 78 44 33 99
E-mail: helle.damgaard@viborg.rm.dk

Manuscript category: Original article
ABSTRACT:

Background: The aim of this study was to evaluate the measurement of the total splanchnic blood flow (SBF) using a clinical diagnostic method based on Fick’s principle and hepatic extraction of 99mTc-mebrofenin (99mTc-MBF) in comparison with a paraaminohippuric acid (pAH) dilution method in a porcine model. Another aim was to investigate whether enterohepatic cycling of 99mTc-MBF affected the SBF measurement.

Materials and methods: Five indwelling catheters were placed in each pig (n=15) in the portal, mesenteric, and hepatic veins, as well as in the aorta and the vena cava. The SBF was measured using both methods. The portal blood flow, the intestinal and hepatic oxygen uptake, the net fluxes of oxygen, lactate, and glucose, and the extraction fraction (EF) of 99mTc-MBF were measured before and for 70 minutes after feeding.

Results: The mean baseline SBF was 2,961 mL/min vs. 2,762 mL/min measured by pAH and 99mTc-MBF, respectively and increased significantly to 3,977 mL/min and 3,981 mL/min postprandially. The hepatic EF of 99mTc-MBF decreased from 40% at the start of the investigation to 16% 70 minutes after feeding.

The arterial-portal difference in 99mTc-MBF concentration was 0.21% (p=0.48), indicating no intestinal extraction or metabolism.

Discussion: The clinical method for measuring the SBF based on hepatic 99mTc-MBF extraction is robust when compared to the indicator dilution method, despite the decrease seen in hepatic extraction of 99mTc-MBF. Because there was no difference in the content of 99mTc-MBF between the arterial and portal vein plasma, the SBF can be calculated from an arterial and a hepatic vein sample.

KEYWORDS: Chronic intestinal ischemia, glucose absorption, paraaminohippuric acid, oxygen consumption, meal stimulation,
**INTRODUCTION:** According to international guidelines (1), chronic intestinal ischemia is diagnosed by the presence of two main symptoms: a) postprandial abdominal pain, also known as intestinal angina (14), and b) weight loss, as well as stenosis or occlusion of at least two of the three intestinal arteries. The physiologic consequences of these stenoses are rarely taken into account when deciding which patients should be offered revascularization. One way to address this challenge is to measure the total splanchnic blood flow (SBF). One clinical method that has been thoroughly described in the literature is based on the Fick principle; this method requires the catheterization of a liver vein and a peripheral artery, as well as the continuous infusion of an indicator extracted by the liver (2; 3; 8). The indicators used include ⁹⁹ᵐ⁻Tc-Mebrofenin (⁹⁹ᵐ⁻Tc-MBF) or HIDA (Hepato-Iminodiacetic Acid) and indocyanine green (8). Using this technique, the SBF can be measured before and after a standard meal to determine the baseline blood flow in the fasting state and the enhancement of blood flow to the gastrointestinal tract after a meal. The outcome of SBF measurements has been evaluated against clinical symptoms and angiographic findings (6; 21), but the actual flow measurements have not been compared to a method that did not include liver extraction.

Using a porcine model with permanent indwelling catheters in the superior mesenteric vein, portal vein, and hepatic vein, the SBF can be measured by the downstream dilution of a continuously infused indicator or, more specifically, paraaminohippuric acid (pAH) infused into the superior mesenteric vein. Subsequent sampling from the portal and hepatic veins and an artery enable the measurement of the portal vein and hepatic vein (total SBF) blood flow. This method is known as the indicator dilution method or Stewart’s principle (9; 11; 12; 19).
The aim of this study was to compare the SBF measured by the Fick principle with a liver extraction-independent pAH dilution method in a porcine model before and after a test meal. Moreover, a change in the hepatic extraction of 99mTc-MBF could impact the calculation of SBF, thus the arteriovenous 99mTc-MBF difference was monitored and its impact upon SBF measures assessed.

MATERIALS AND METHODS: The present research complied with the Danish Ministry of Justice Law no. 382 (June 10, 1987), Act no. 726 (September 9, 1993), concerning experiments with animals and the care of experimental animals, and the protocol was approved by the Danish Animal Experiments Inspectorate.

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, and each pig weighed approximately 50 kg. 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 anesthesia. The caudal branch of the saphenous artery was located by palpation, and a 5- to 7-cm incision was made medial to the artery. Both 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 tunneled 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 (17). 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).
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.
The placement of all catheters was verified by autopsy and photo documentation ten days
after sampling.
All the pigs recovered quickly after surgery and weighed an average of 60 kg (range 56 to 68
kg) on the day of the study. Autopsies were performed 10 days (range 9 to 11 days) after
sampling, and there were no signs of infections of the subcutaneous catheter tunnels or at the
points where the catheters penetrated the peritoneum. 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:** All blood samples were collected simultaneously from the central hepatic vein, the portal vein and the abdominal aorta as a set. For each pig, a set of blood samples were obtained every 10 minutes from 40 minutes prior to feeding (-40 min) to the time of feeding (0 min), and a mean baseline SBF was calculated based on the five sampling times. At the time of feeding (0 min), the animals 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. Blood sample sets were then collected every 10 minutes from 10 minutes (+10 min) to 70 minutes (+70 min) after feeding (seven sampling times). In 521 of 540 attempts, sufficient blood samples were obtained simultaneously from the three sites at the scheduled time. Each plasma sample was divided into two vacutainers; one for gamma counting and one for analyses of pAH.

The blood plasma was analyzed for glucose and lactate as a control for gastric emptying and intestinal absorption.

**Splanchnic blood flow (SBF) and oxygen uptake measured by the 99mTc-MBF hepatic extraction method:** The SBF was determined using 99mTechnetium-labeled mebrofenin (Bridatec®, GE Healthcare, Suluggia, Italy) (99mTc-MBF) as an indicator following the method based on the indirect Fick principle introduced by Bradley *et al.* (2; 3). In brief, a bolus injection of 99mTc-MBF (2.4 mg in 24 mL) was administered,
followed by a constant infusion of 99mTc-MBF at 1.95 mL/min (0.36 MBq/min, 0.2 mg/min). In total, less than 250 MBq was used. To reach a steady state, an equilibration period of 20 minutes was interposed before blood sampling was initiated. Splanchnic plasma flow (SPF) was determined by SPF = E / (C_a – C_{hv}), where E is the turnover rate of 99mTc-MBF, which equals the infusion rate corrected for the non-steady state and for a small urinary excretion of the indicator (8), and C_a and C_{hv} are the concentrations of 99mTc-MBF in the abdominal aorta and the hepatic vein, respectively. The levels of 99mTc-MBF in the plasma samples were determined using a Cobra II Auto-Gamma gamma counter (Packard Bioscience Company, Frankfurt, Germany). The counts were corrected for radioactive decay, counted for at least 10,000 counts (v.c. ≤ 1%), and the dead time of the counter did not pose a problem. The SBF was determined as SPF / (1 – hematocrit fraction). The splanchnic oxygen uptake was then calculated as Hb x (arterial oxygen saturation – hepatic venous oxygen saturation) x SBF, where Hb is the hemoglobin concentration. The blood samples were analyzed for blood pH, blood gases, and oximetry variables (ABL 700 series; Radiometer Medical A/S, Brønshøj, Denmark; used according to the manufacturer’s instructions). The plasma samples were analyzed for glucose and lactate using D-glucose oxidase and L-lactate oxidase, respectively (YSI 7100; YSI Inc., Yellow Springs, OH, USA).

**SBF by the paraaminohippuric acid indicator dilution method:** The SBF was determined by an indicator dilution method using paraaminohippuric acid (4-paraaminohippuric acid 99%, Acros, Geel, Belgium) (pAH) 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 (18), and the hepatic acetylation of pAH was corrected for by deacetylating the samples (14). Given that the infused volume is much smaller than the SBF, the splanchnic plasma flow (SPF) can be determined as SPF = I / (Chv – Ca), where I is the pAH infusion rate, and Chv and Ca are the concentrations of pAH in the hepatic vein and abdominal aorta, respectively. The plasma pAH was deacetylated before the total pAH concentration was determined by the method described by Harvey and Brothers (7) using a continuous flow analyzer (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 (17).

Determination of portal blood flow was applied to calculate the intestinal flux of oxygen, lactate and glucose. In a small number of blood samples (24 out of 180) estimated portal blood flow exceeded hepatic blood flow due to displacement of the portal sampling catheter. In this case portal blood flow was set equal to the hepatic blood flow in the flux calculation. This has no influence on the comparison of the SBF measurement by 99mTc-MBF or pAH as a complete and uniform mixing of the blood can be expected after passing the liver.

Statistical analyses: Statistical analysis was performed using STATA® 11 (StataCorp LP, College Station, Texas). The five baseline SBF measurements were not dependent on time and were analyzed using a linear regression model with repeated
measurements. The flow data for each sampling time were analyzed as paired samples (n=15) using the Student’s $t$-test to compare the different methods and to compare the baseline and postprandial periods. Estimates of the mean are given with 95% confidence intervals. The assumptions were verified by plotting the individual differences between SBF-pAH and SBF-MBF against the average SBF and by a Q-Q plot for each sampling time.

**Sample size calculation:** The calculation of sample size was based on a paired sample from a normal distribution testing no difference between the two methods. The intention was to detect a difference of 200 mL/min between the two methods with a significance level of 5 % and a risk of type two error of 10%. The standard deviation of the difference was considered to be 230 mL/min. The required number of pigs was 14.

**RESULTS:**

The mean SBF with the 95% confidence interval (c.i.) for each sample time is shown in Fig. 2 as a function of sampling time for both 99mTc-MBF and pAH. No differences were detected between the two methods at any time. The mean baseline SBF was 2,961 mL/min (c.i. 2,678 to 3,244) and 2,762 mL/min (c.i. 2,586 to 2,937) measured by pAH and 99mTc-MBF, respectively, and this value increased to 3,977 and 3,981 mL/min, respectively (both p < 0.001), after ingestion of the meal. A non significant difference of 199 mL/min (c.i. -62 to 461) was observed (p = 0.12) between the mean baseline SBF, as measured by the two methods. An analysis of the baseline SBF using a linear regression model with repeated measurements showed no change with time (p = 0.73) by either flow method. After the meal, the mean flows were observed to be similar (p = 0.98) between the two methods (mean
difference 4.2 mL/min; c.i. -309 to 300 mL/min). After the meal, the SBF showed a
significant correlation with time (p < 0.001).

Fig 3a presents the measurements of SBF by the two methods plotted against each other, and
Fig. 3b shows a Bland Altman plot of the differences between the two methods. As the
individual data are not independent but repeated measurements (12 measurements) on the
same individual (15 individuals in all) Fig. 3a and 3b are only a visualization of the raw
measurements and are not used for statistical analysis.

The data sets for baseline and postprandial oxygen consumption and L-lactate release, as
well as blood pH, are presented in Table 1. The postprandial values were calculated based on
the five sets of blood samples from each pig from 30 minutes after the meal to 70 minutes
after the meal.

The oxygen consumption calculated as the net oxygen flux for the total splanchnic territory,
the intestines and the liver reached a plateau within 30 minutes after the meal. The total
splanchnic oxygen consumption 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 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. A maximum of
3.86 (c.i. 3.13 to 4.60) mmol/min was reached 30 minutes after feeding, 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, while the net glucose flux remained
significantly higher than at the baseline (p < 0.001).

The plasma concentration of lactate 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 in the arterial and portal and hepatic vein samples, although the numerical change was relatively small, as shown in Table 1.

The intestinal lactate flux was close to zero at baseline, reflecting 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).

The mean difference between the arterial and portal concentrations of 99mTc-MBF, expressed as a percent of the arterial concentration, was 0.21% (c.i.: -0.12% to 0.54%), which is not significantly different from zero, and no significant changes with time (p = 0.48) occurred.

A decrease in the hepatic extraction fraction (EF) of 99mTc-MBF was seen throughout the investigation, as shown in Fig. 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).

**DISCUSSION:**

The method based on the liver extraction of 99mTc-MBF is used to investigate the total SBF in humans. The main purpose of this study was to validate this diagnostic method by comparing it to the pAH method that is independent of liver extraction. This study 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 Fig 3a, 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 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 overcorrection was applied to the baseline SBF values, thus underestimating the true baseline SBF, and an insufficient correction was 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 (14).

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. 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 (8). 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% (5). This is in contrast to the present findings when using continuous infusion. The observed decrease might be due to a limited capacity of the hepatocytes to process 99mTc-MBF from the blood,
and the exact kinetics of 99mTc-MBF transport in the liver warrants further studies. Previous studies on the kinetics of 99mTc-MBF have concentrated on the excretion of 99mTc-MBF in the kidneys (13), and the properties of 99mTc-MBF as a cholestatic agent (16). The demonstrated decrease in the EF of 99mTc-MBF was most pronounced during the start of the observation period and the first 20 minutes after the meal. The SBF was, however constant throughout the baseline period and thus not affected by the decreasing EF.

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 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 splanchnic oxygen consumption reached a plateau within 30 minutes after the meal; moreover, the total splanchnic oxygen consumption was equally divided between the intestines and liver as seen before (15). The meal-induced increase in oxygen consumption 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. In a human study by Jensen et al. (10), normal subjects were
investigated for 6 hours after a mixed meal, and the splanchnic oxygen uptake did not return
to baseline by the end of the study. 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, of 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 (4; 20). 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 after six days, with blood samples
collected hourly six times after a standard lactate-free meal. The samples were analyzed as
described above, and the postprandial arterial pH was 7.4 and did not differ from the baseline
level (14). 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 is confirmed
by the present study that found no difference between the content of 99mTc-MBF in the two
vascular territories at any time.
CONCLUSION:

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.

No difference was observed between the portal and arterial contents of 99mTc-MBF during the fasting state or postprandial. Therefore, an arterial sample can be used instead of the portal blood sample, making the method applicable to patients.

The 99mTc-MBF method may be suitable to detect splanchnic ischemia and other perfusion disturbances like those seen in cirrhosis and portal vein thrombosis in man.
Acknowledgements: Birgit H. Løth, Anne Krustrup, and Ole H. Olsen for skilful technical
assistance. Students attending the course ‘Experimental Animal Nutrition and Physiology 2008
and 2009 (KU Life, Copenhagen, Denmark)
This study was supported by grants received by the Rosa and Asta Jensen Foundation, the
Danielsen Foundation. The Danish Ministry of Food, Agriculture and Fisheries. Helle Zacho
was a recipient of grants from Region Midts Research Foundation.
No conflicts of interest.


Table 1. Oxygen consumption, lactate flux and pH in pigs before and after standard meal (n=15).

<table>
<thead>
<tr>
<th>Item</th>
<th>Baseline</th>
<th>Postprandial</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean oxygen consumption, mmol/min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total splanchnic</td>
<td>7.19 ± 0.20</td>
<td>9.81 ± 0.30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intestinal</td>
<td>3.75 ± 0.16</td>
<td>4.92 ± 0.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hepatic</td>
<td>3.49 ± 0.15</td>
<td>4.82 ± 0.22</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

| **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      |                |

| **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.
Figure 1. Placement of catheters in the pigs during infusion and sampling. In the inferior vena cava, 99mTc-Technetium labelled Methenamine (99mTc-MB) is infused continuously. Para-aminohippuric acid (pAH) is infused continuously in the mesenteric vein. Blood samples are collected simultaneously from the hepatic, portal vein and abdominal aorta.
Figure 2. The mean SBF measured in mL/min with 95% confidence interval (+/- 1.96*SEM) is shown as a function of sampling time for fifteen pigs. 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
Figur 3a. SBF measured by the 99mTc-MBF-method is shown as a function of the SBF measured by the pAH-method.

The raw flow data are not independent as they have been measured repeatedly (12 times) in each pig. Line of identity is shown.

- Baseline period.
- Measurements 10 to 30 minutes after the meal.
- Measurements 40-70 minutes after the meal.
Figure 3b. Bland Altman-plot of the raw SBF measurements.

The individual flow data are not independent as they have been measured repeatedly (12 times) in each pig.

■ Baseline period.

◊ Measurements 10 to 30 minutes after the meal.

● Measurements 40-70 minutes after the meal.
Figure 4: Extraction Fraction of 99mTc-MBF in the liver shown as a function of time. Zero is the time of feeding.