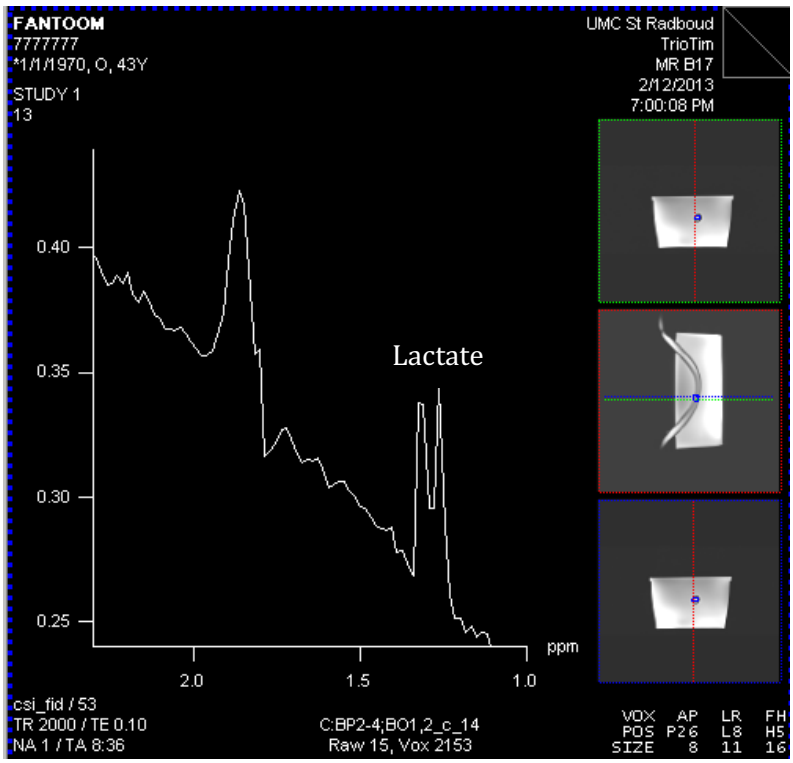
Magnetic Resonance Spectroscopy as a diagnostic tool for Chronic Splanchnic Syndrome



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Acknowledgement

This thesis is based on an internship followed at the Medisch Spectrum Twente (MST) in Enschede and the Universitair Medisch Centrum (UMC) St Radboud in Nijmegen. The thesis is part of a research line to create a noninvasive and less time consuming test for the diagnosis of chronic splanchnic syndrome.

The technological experiments were performed at the radiology department in Nijmegen, with the use of clinical MRI-scanners. The experiments were performed under supervision of A.J. Wright and Prof. dr. A. Heerschap from the radiology research department.

My medical competencies were trained and assessed at the department of vascular surgery, Medical Spectrum Twente under supervision of dr R.H. Geelkerken, vessel surgeon. Different medical tasks including participating in inhospital and outpatient diagnostic processes, with the focus on imaging and under direct supervision performing surgical skills.

The University of Twente in Enschede was the third party in this research, the process was under supervision of dr. ir. B. ten Haken and drs. P.A. van Katwijk.

Some basic knowledge of medical and technological background in the area of splanchnic ischemia is necessary to understand this research and the future perspectives.

Summary

Stenosis or occlusion of the celiac artery (CA), the superior mesenteric artery (SMA) and/or the inferior mesenteric artery (IMA), most commonly caused by atherosclerosis, can lead to ischemia of the intestines. In this master thesis a new diagnostic tool to diagnose chronic splanchnic disease (CSD) and chronic splanchnic syndrome (CSS) is investigated for further development. Nowadays the diagnostic process encompasses first the anamneses. The symptoms of the classical triad are questioned; these three characteristic ischemic symptoms are postprandial pain, loss of weight, and epigastric bruit. Secondly, non-invasive as well as invasive diagnostic tools, have to be taken into account. All results are discussed with several specialists, a vessel surgeon, gastrointestinal surgeon, and radiologist, to complete the diagnostic process. This study is aimed to ease the diagnosis with a diagnostic tool that is beneficial for the patients and the physicians. The 'new' diagnosis should be less invasive, time consuming and usable at multiple locations as the complicated current diagnosis of CSD or CSS.

Lactate is a potential marker for ischemia, because it is generated as the end product of anaerobic glycolysis. For the diagnosis of patient with CSD or CSS, the lactate concentration can be measured in the intestinal circulation, the portal vein. The main idea in this research line is to analyse portal flow Magnetic Resonance Spectroscopy (MRS) as a diagnostic tool to distinguish between CSD or CSS. MRS is a non-invasive and non-harmful technology to image the human body. In previous studies it seems that lactate was not detectable in a flowing solution with a velocity above 65 ml/min. This study will use another sequence with which it seems to be possible to measure lactate. Multiple experiments are performed to answer the following question: *'Is it possible to measure physiological and pathological concentrations of lactate in the portal vein with the use of Magnetic Resonance Spectroscopy, to diagnose patients with Chronic Splanchnic Syndrome?'*

This study contains seven different experiments. First, sequence determination, in which a suitable sequence is determined for the detection of lactate in volume flow velocities above 65 ml/min. This is possible with the preprogrammed [csi_fid_bir45_nodec_lowres] sequence, consisting of Chemical Shift Imaging (CSI) and B_1-insensitive rotation (BIR). The goal in the second experiment is to detect lactate in a velocity above human blood flow, around 1000 ml/min. The velocity is stepwise increased and it seems that at a velocity of 1350 ml/min lactate is still detectable with MRS. A surface coil is used in the previous two parts, which is not suitable to measure a human body. In the third experiment different coils are used, after which it is concluded that an extended version of the portal vein phantom is necessary to use a body with spine coil combination. This combination is closest to real clinical settings and therefore desirable.

The fourth experiment starts with the extended portal vein phantom. The tube mimicking the portal vein is situated in a box which mimics the human tissue. The height of the tube can be adjusted in this phantom, however, it is possible to detect lactate at almost all positions of the tube inside the body. All these experiments are performed with a 20 mM lactate concentration, which is at the high end of pathological lactate concentrations. In the fifth

experiments, the lactate concentration is lowered stepwise from pathological to physiological lactate concentrations. Although it is shown that a 2.5 mM lactate concentration is still detected, this part of the research needs follow up. For example by repetition of the same experiment with the use of a newly prepared lactate solution and/or different dilution steps.

The last two experiments of this thesis are to determine the possibility to measure lactate in a solution with other molecules. In the sixth part, lactate in fat emulsion, a lactate with lipids solution is prepared. This solution is used to see the influence of lipids on the lactate doublet in the spectrum acquired with MRS. It is know that lipids overlap the lactate peaks. However, it seems that it is possible to distinguish between lactate and lipids, for example with the use of a different program for data analysis or a different sequence. Further research is necessary to investigate the possibilities to distinguish the lactate doublet from the lipid peaks. The same applies for the last and seventh part of this study. Two measurements in healthy human test subjects are performed to see the capability of the CSI sequence to detect the portal vein and generate a spectrum. The portal vein is detectable and MRS can be performed in this portal vein, but it is not certain whether the lactate peak can be distinguished.

In conclusion, it is possible to measure pathological lactate concentrations, ranging from 2.5 to 50 mM, in a flow model comparable to the human portal blood flow. MRS is a promising diagnostic tool to diagnose CSD or CSS, but some technical hurdles need to be overcome before clinical studies are started. The aim for further research should be to investigate the options to distinguish the lactate doublet from the overlapping lipid peaks. When this becomes possible and clinical studies can be performed, it can be discussed whether or not MRS is less invasive and time consuming technology for the diagnosis of intestinal ischemia.

Abbreviations

AAW = Anterior abdominal wall
AO = Aorta
ASL = Arterial spin labeling
ASS = acute splanchnic syndrome
BIR = B_1-insensitive rotation
BMI = Body Mass Index
CA = Celiac artery
CACS = Celiac artery compression syndrome
cm = centimeter
CO_2 = Carbon dioxide
CSD = Chronic splanchnic disease
CSI = Chemical shift imaging
CSS = Chronic splanchnic syndrome
CT = Computed tomography
CTA = Computed tomography angiography
DSA = Digital subtraction angiography
GET = Gastric exercise tonometry
Hz = Hertz
IMA = Inferior mesenteric artery
IMV = Inferior mesenteric vein
g/mol = gram per moles
jMRUI = Java-based magnetic resonance user interface
kg = kilogram
kPA = Kilopascal
L = Liter
M = Molarity
ml/min = millilitre per minute
mM = millimolarity
mm = millimeter
mmol/L = millimoles per liter
MRA = Magnetic resonance angiography
MRI = Magnetic resonance imaging
MRS = Magnetic resonance spectroscopy
NMR = Nuclear magnetic resonance
NOMI = Non occlusive mesenteric ischemia
PACE = Prospective acquisition correction
PAW = Posterior abdominal wall
pCO_2 = Carbon dioxide tension
ppm = parts per million
PTA = Percutaneous transluminal angioplasty
PV = Portal vein
RF = Radio frequency

SMA = Superior mesenteric artery
SMAS = Superior mesenteric artery syndrome
SMV = Superior mesenteric vein
SV = Splenic vein
T= Tesla
TE = Echo time
TR = Repetition time
VOI = Volume of interest

1. Introduction

1.1 Background information

Chronic abdominal pain can be the symptom of different gastrointestinal or systemic disorders. Among all causes, one of the most difficult to recognize is chronic ischemia of the splanchnic vascular system (Sreenarasimhaiah 2005). Three major aortic branches supply the gastrointestinal tract, the celiac artery (CA), the superior mesenteric artery (SMA) and the inferior mesenteric artery (IMA). Further elaboration on these arteries is necessary to understand the background of the disorder for this study.

The CA arises from the aorta at the level of the median arcuate ligament of the diaphragm. The CA divides into the splenic artery, common hepatic artery and left gastric artery, which supplies the stomach, proximal duodenum, liver and spleen. The midgut receives arterial blood from the SMA. Branches of the SMA include the middle, right and ileocolic arteries as well as jejuna and ileal arteries and arterioles. These branches are responsible for the blood supply to the distal duodenum, small intestine (jejunum and ileum) and the proximal colon. The SMA arises from the aorta about 1 cm caudally from the CA. the IMA arises about 3-5 cm above the aortic bifurcation and divides into the ascending and descending branches, including the left colic, marginal and sigmoid arteries. These arteries supply the distal colon and the rectum (van Bockel, Geelkerken et al. 2001; Sreenarasimhaiah 2005; Moore and Dalley 2006; Kitselaar, Lemson et al. 2007).

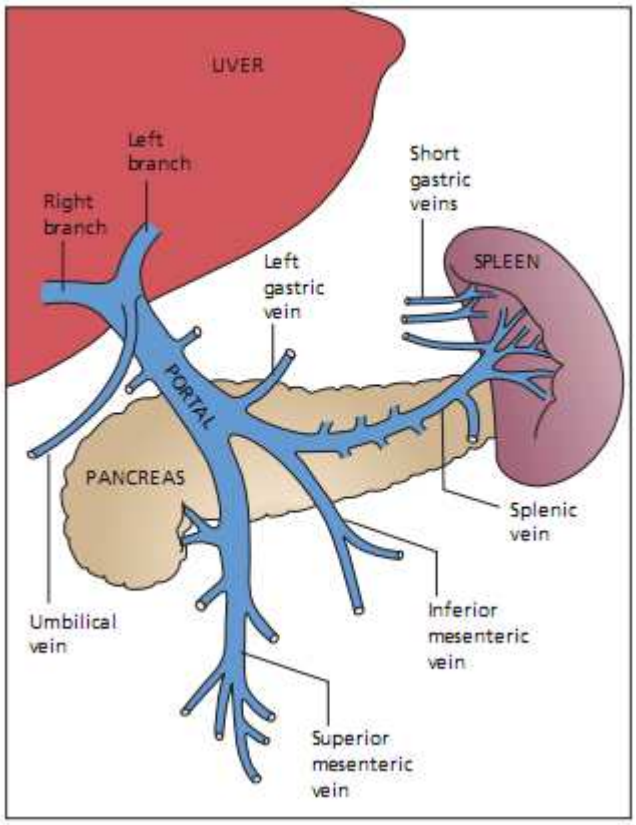


Figure 1. Anatomy of the portal venous system (Burroughs, Dooley et al. 2011).

The system for venous drainage of the splanchnic system is the portal circulation (figure 1). The portal circulation includes all veins which are involved in transporting oxygen poor blood from the abdominal organs, such as the spleen, pancreas and gallbladder (Burroughs, Dooley et al. 2011). The splenic vein (SV), superior mesenteric vein (SMV), inferior mesenteric vein (IMV), left gastric vein, right gastric vein and cystic veins drain into the portal vein. The IMV drains directly into the SV. The SV and SMV join and form the portal vein adjacent to the pancreatic head. The portal vein transports almost all nutrients from the alimentary tract and continues onward to drain blood through the porta hepatis into the sinusoids of the liver (Sreenarasimhaiah 2005; Moore and Dalley 2006). Not only the nutrients, but

also all other substances and molecules from the alimentary tract are transported through the portal vein, and a part is transported through the thoracic chylys duct. These molecules can possibly be measured to acquire knowledge about the functioning of the arteries. This is investigated in this study.

Stenosis or occlusion of the CA, SMA or IMA, most commonly caused by atherosclerosis, can lead to ischemia of the intestines (Sreenarasimhaiah 2005). Atherosclerosis is a process in which the lumen of a blood vessel becomes narrowed by cellular and extracellular substances (Ross 1993). Lesions progress mainly through three stages. In the first stage lipid filled macrophages are present in the subendothelial space, which causes fatty streak lesion. In the second stage a fibrous plaque is formed, consisting of a central acellular area of lipid covered by a fibrous cap containing smooth muscle cells and collagen. In the third and final stage plaque rupture can trigger thrombus formation and media destruction may lead to aneurysm formation (Carmeliet, Moons et al. 1998).

Significant splanchnic arterial stenosis has a high prevalence, ranging from 30 to 50%. Significant splanchnic artery stenosis causes the development of collaterals (Kolkman, Bargeman et al. 2008), which can be made visible with the use of imaging techniques. A stenosis or occlusion can cause restriction of the blood supply, and thereby inadequate blood supply and a lack of nutrition transport to tissues. With ischemia there is a shortening of the most important nutrients, like oxygen, in cells, which will reduce the cellular metabolism. Ischemia can occur in all tissues, which also includes tissue of all organs. For example ischemia of the intestine can be caused by a blood clot, atherosclerosis, or constriction of in the CA, SMA, and/or IMA. Ischemia can also be a result of (high) physical exercises (Mensink, Geelkerken et al. 2008). In this study it is important that ischemia of the intestines can cause different concentrations of some molecules. These molecules will be transported through the portal vein, as already mentioned two paragraphs above, and can be used as markers for a measurement of ischemia.

1.2 Chronic splanchnic disease and chronic splanchnic syndrome

In this thesis a new diagnostic tool to distinguish between chronic splanchnic disease (CSD) and chronic splanchnic syndrome (CSS) is investigated. CSS is defined as abdominal discomfort possibly caused by symptomatic stenosis of one or more of the splanchnic arteries, and CSD can be characterized as significant asymptomatic stenosis of one or more of the splanchnic arteries (figure 2) (van Wanroij, van Petersen et al. 2004). Intestinal ischemia caused by stenosis or occlusion in the CA or/and SMA are more common than in the IMA (Sreenarasimhaiah 2005). Brandt et al have claimed that embolic disease of the SMA accounts for 50% of all acute intestinal ischemia events. Progression of thrombotic disease in the SMA counts for an additional 10% of all cases (Brandt and Boley 2002). Other studies for the prevalence of asymptomatic mesenteric arterial stenosis were performed in patients with peripheral vascular disease and renal artery stenosis. In that study was shown that 27% of the patients had a stenosis

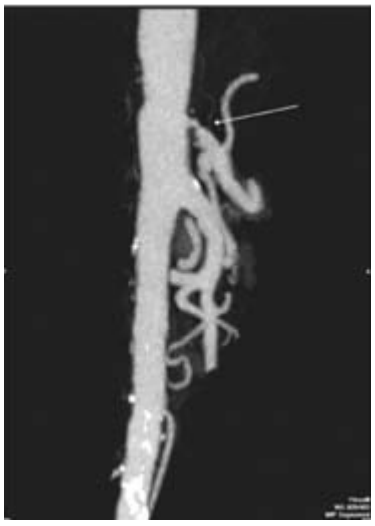


Figure 2. 3D-MDCT reconstruction which shows a stenosis of the CA (Schmitz Serpa, Tachibana et al. 2010).

greater than 50% in the CA or the SMA. In 3.4% there was a significant stenosis visible in both vessels (Valentine, Martin et al. 1991). A small number of the patients with CSD will develop CSS. Compared with CSD the true incidence of CSS is rare, but the incidence is currently unclear (Kolkman, Bargeman et al. 2008).

Atherosclerosis is the most common source for occlusion or severe stenosis of a vessel, it accounts for more than 90% of all cases (van Bockel, Geelkerken et al. 2001). Hyperlipidemia, diabetes and smoking are well known risk factors for atherosclerosis (Sreenarasimhaiah 2005). Occlusion or sever stenosis can lead to ischemia of organs or tissues. In this study ischemia of the intestine, most of the times caused by occlusion or (severe) stenosis in the CA, SMA and/or IMA, is used to build experiments.

Ischemia of the intestine can also be caused by celiac artery compression syndrome (CACS), Wilky's syndrome, superior mesenteric artery syndrome (SMAS), and non occlusive mesenteric ischemia (NOMI) (Kolkman and Mensink 2003; Kolkman, Bargeman et al. 2008; Chou, Lin et al. 2012). Constriction of celiac arterial blood flow by compression is caused by the connection of the diaphragm to the median arcuate ligament (Taylor, Moneta et al. 1995; Chou, Lin et al. 2012). This is called CACS or SMAS and has an incidence reported ranging from 12.5-24% (Chou, Lin et al. 2012). NOMI is caused by splanchnic vasoconstriction resulting from a systemic shock (van Bockel, Geelkerken et al. 2001; Kolkman and Mensink 2003; Kolkman, Bargeman et al. 2008). From all cases of acute mesenteric ischemia NOMI counts for 20-30% and has a mortality rate of 50% (Trompeter, Brazda et al. 2002).

If chronic ischemic symptoms develop, CSS will occur. This disorder will probably lead to three characteristic ischemic symptoms, called the classical triad. The symptoms are postprandial pain, loss of weight, and epigastric bruit. Postprandial pain is the most characteristic symptom and can persist for 1 to 3 hours after a meal. Due to the postprandial pain the patients have a fear of eating, they take smaller portions, often with less fat and proteins. Weight loss is almost always caused by this reduced food intake. Other clinical symptoms in patients with CSS are occasional changes in bowel habit, diarrhea, unexplained gastric ulcers, gastroparesis and non-specific abdominal pain, although those are less specific (van Bockel, Geelkerken et al. 2001; Kolkman, Bargeman et al. 2008). Patients with CSS have a considerable risk to develop acute splanchnic syndrome (ASS). The patients who develop ASS have a high risk of developing bowel infarction, which has a high mortality rate (Kolkman, Bargeman et al. 2008).

During anamneses the symptoms of the classical triad are questioned. This is the start for the diagnosis of CSS, but thereafter many different steps and techniques have to be taken after anamnesis. All results are discussed with several specialist, a vessel surgeon, gastrointestinal surgeon, and radiologist, to complete the diagnostic process. Different diagnostic steps will be described in the next sub-chapter. All these steps will take a long time, what is strenuous for the patient as well, as for the physician. This study is aimed to ease the diagnosis, but therefore the current diagnostic tools will be expanded.

1.3 Diagnosis of splanchnic ischemia

Much effort is required to distinguish between CSD or CSS, this applies to both single vessel and multi vessel stenosis. Before new diagnostic tools can be identified, current diagnostics have to be understood. Different techniques will be used to solidify the diagnosis. At first non-invasive Duplex ultrasonography is an attractive technique. It is not always possible to detect the CA and the IMA is rarely visualized, but the SMA is accessible to measure size and anatomical position (Sreenarasimhaiah 2005). A more recent study has shown that it is possible to detect a significantly higher peak systolic velocity and end-diastolic velocity in the CA or SMA when there is a single stenosis in the SMA respectively CA. The study has shown that stenosis in either CA or SMA shall increase the flow velocity in the unaffected artery, which was possibly caused by the created collaterals (van Petersen, Kolkman et al. 2014). As shown in figure 3, besides the anatomy of the artery also the flow during inspiration and expiration can be measured with Duplex (van Wanroij, van Petersen et al. 2004). Increased peak systolic velocities and end diastolic velocities are characteristic for stenosis or occlusion. There will be areas of high velocity jets, sometimes with turbulent flow, due to a significant stenosis, this coincides with an overall increased flow velocity. The velocities can be measured using Duplex, which are usually performed during fasting. With the velocity values a distinction can be made between normal vessel anatomy and stenosis (Kolkman, Bargeman et al. 2008; Mensink, Geelkerken et al. 2008). It is not possible to diagnose intestinal ischemia with only the use of ultrasound, but vascular stenosis or/and occlusion can be identified. Next to the anamneses, this can be an indication to start other diagnostic methods to diagnose CSD or CSS (van Wanroij, van Petersen et al. 2004; Sreenarasimhaiah 2005).

More information of the vessel anatomy is gained through the use of magnetic resonance angiography (MRA), computed tomography angiography (CTA) or digital substraction

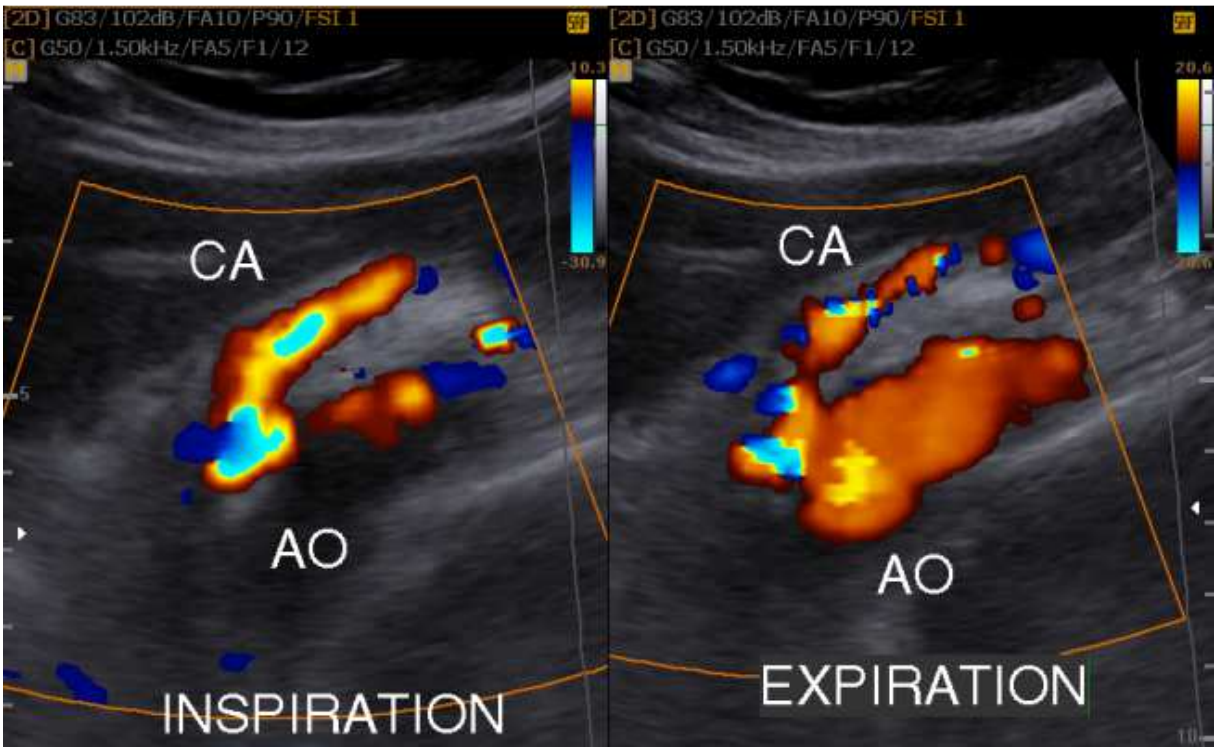


Figure 3. Color Doppler Ultrasound recording of the Celiac Artery (CA) and the aorta (AO) during inspiration and expiration. During expiration several stenosis in the CA are visualized (Youssef 2013).

angiography (DSA) (van Bockel, Geelkerken et al. 2001; van Wanroij, van Petersen et al. 2004; Kitselaar, Lemson et al. 2007). With the use of contrast MRA is it possible to identify stenosis in the arteries and collaterals (Billaud, Beuf et al. 2005). Several studies have shown that angiograms of the SMA were provided in 90%, from the CA in 75-90% and the IMA in 25% (Laissy, Trillaud et al. 2002). Another option with MRA is measuring the actual flow through the splanchnic and portal circulation. There is a relationship between flow in arteries and veins (Burkart, Johnson et al. 1993), but measuring the flow in arteries is more difficult than in veins, due to the smaller caliber and the pulsatile characteristic of arterial flow (Lycklama a Nijeholt, Burggraaf et al. 1997; Kolkman, Bargeman et al. 2008).

To visualize the abdominal arteries with CTA a multi-slice CT scanner is required. With this method it is possible to image the arteries, veins and collaterals (Iannaccone, Laghi et al. 2004). With CT it is possible to measure in inspiration and expiration, what is prerequisite if there is suspicion of CASC. The advantage of CT is the requirement of information of all structures in the abdomen. If there is no obstruction or occlusion of the arteries or veins, an alternative diagnosis can be evaluated, such as bowel obstruction, perforations, pancreatitis and abscesses (Kolkman, Bargeman et al. 2008).

The “gold standard” to classify and diagnose the stenotic lesions in patients with CSS is DSA (van Wanroij, van Petersen et al. 2004). DSA is based upon fluoroscopy and CT and the use of intravenous injections of iodinated radiographic contrast agents. Combined with digital subtraction techniques, DSA is optimal for the diagnosis of CSS (Brody, Enzmann et al. 1982). To visualize the origin of the splanchnic vessels anteroposterior and lateral abdominal aortic injections will be used (Mensink, van Petersen et al. 2006). DSA is used in interventional radiology, where it is not only possible to visualize the vessels, but also to perform endovascular therapeutic procedures at the same time. DSA has a high diagnostic accuracy and gives the therapeutic opportunity for example to place a stent in the artery with a stenosis (Kolkman, Bargeman et al. 2008). With DSA a detailed view can be obtained of the vessel anatomy, collaterals, stenosis or vascular variations of the CA, SMA and IMA (Kolkman, Mensink et al. 2004). All this information is essential for the determination of the optimal revascularization strategy (Kolkman, Bargeman et al. 2008).

To gain functional information and solidify the diagnosis of CSS, gastric exercise tonometry (GET) can be performed. The introduction of GET caused a change in diagnostic possibilities and can be used to diagnose gastrointestinal ischemia (Otte, Oostveen et al. 2001). Currently this is the most sensitive test for the diagnosis of ischemia. With tonometry the carbon dioxide level is measured. CO_2 will accumulate due to gastric mucosal ischemia. A tonometer is a nasogastric catheter with a balloon connected at the tip. This catheter will be positioned intra-gastric and connected to a modified capnograph (van Wanroij, van Petersen et al. 2004). With a moderate exercise test or/and with eating of meals during a 24 hour test, ischemia can be detected (Otte, Oostveen et al. 2001; Kolkman, Bargeman et al. 2008).

Ischemia can be provoked during exercise, which results in an increased lactate production. In patients with CSD or CSS ischemia can also be caused by ingestion of meals. The pH shall decrease which is buffered by bicarbonate causing an increase of carbon dioxide tension (pCO_2) in the tissue. GET can measure the differences between mucosal and aortic pCO_2

(Kolkman, Bargeman et al. 2008; Mensink, Geelkerken et al. 2008). If the mucosal pCO_2 is increased, compared with the pCO_2 in the circulation, there will be ischemia. This comparison can represent the level of ischemia by the pCO_2 gradient (van Wanroij, van Petersen et al. 2004). After eating, the gastric and small bowel pCO_2 gradients may increase. Physiologically the gradient can increase up to 0.8 kPa pCO_2, therefore with an increase exceeding 0.8 kPa pCO_2 there is a high chance of ischemia (van Wijck, Lenaerts et al. 2012). Extra information is gained with the 24 hour tonometry test. It has been shown that the pCO_2 increase is prolonged and in most cases in combination with abdominal pain (Kolkman, Bargeman et al. 2008).

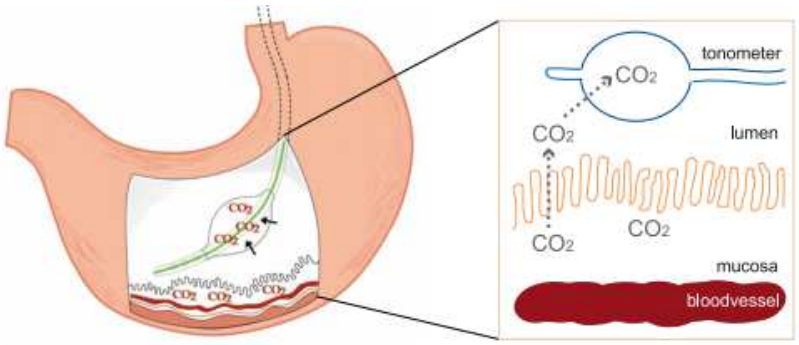


Figure 4. This figure shows the working mechanism of gastric tonometry. GET relies on the principle that there is an accumulation of CO_2 in the mucosa due to decreased transport of CO_2 via blood vessels. In the stomach the accumulated CO_2 increases the pCO_2 (CO_2 tonus). With the balloon of a tonometer catheter this pCO_2 is detected (van Wijck, Lenaerts et al. 2012).

All diagnostic tools together, from anamnesis to Duplex to CTA, MRA or DSA and GET the diagnose CSS can be determined. After the imaging techniques it can be determined if there is a single vessel or multiple vessel stenosis. The results of GET can be used to evaluate if a patient with a single vessel stenosis will benefit from revascularization, this treatment is than primarily symptom relief. In patients with multiple vessel stenosis GET is less important. For these patients revascularization is for prevention of morbidity and death (Mensink, van Petersen et al. 2006). Unfortunately GET is invasive, labor intensive and the interpretation of the GET data is difficult (Mensink, Geelkerken et al. 2008). Therefore a method that is non-invasive, easier to interpret, and usable at multiple locations is desired for diagnosis in clinical settings.

From this analysis it is clear that the diagnosis of CSD or CSS is complicated. Many steps have to be taken, leading in a time consuming process. During this whole process the patient lives in uncertainty and has to wait for the results and the next steps that have to be taken for treatment. If this process can be accelerated with less diagnostic tools or less time consuming diagnoses, that will be beneficial for the patient. The patient will know more about his current health state and can be treated in an earlier stadium. If this new diagnosis is less invasive and time consuming it is not only beneficial for the patient, but also for the physician, and thereby for the whole clinical practice.

1.4 Treatment for splanchnic ischemia

Treatment of CSD or CSS is in most cases not considered as urgent, however therapy is preferred as symptom relief and to minimize the risks for bowel infarction or other serious clinical consequences (Sreenarasimhaiah 2005). The treatment of choice is dependent on the health status of the patient. Therefore different aspects can be taken into account, such as the history of the patient, which vessels are narrowed and to what degree, is there collateral development, is there any evidence of acute ischemia, or the possibility to develop a bowel infarct (Kolkman, Bargeman et al. 2008). There are different treatment options for patients with CSS, these options

are conservative medical treatment, surgical reconstruction, and endovascular therapy. Patients with multiple vessel CSS are at considerable risk to develop ASS. For these patients conservative medical treatment for symptom relief, and surgery or endovascular treatment for revascularization shall be performed (van Bockel, Geelkerken et al. 2001).

The traditional therapy for patients with intestinal ischemia was mesenteric arterial revascularization. Transarterial and transaortic endarterectomy, reimplantation and vascular bypass are the most common surgical options. To achieve primary mesenteric revascularization in single or multiple vessel cases, transaortic endarterectomy has been used in both acute and chronic ischemia (Sreenarasimhaiah 2005; Kolkman, Bargeman et al. 2008). The majority of hospitals suggest that the antegrade autogeneous revalscularization technique of the CA and SMA offer the best results (Kolkman, Bargeman et al. 2008).

Patients with CSS traditionally underwent surgical bypasses with fairly good outcomes (Sreenarasimhaiah 2005). For a surgical bypass three different reconstructions can be performed (Mensink, van Petersen et al. 2006). The antegrade reconstruction will result in an arterial inflow arising from the thoracic or supraceliac aorta. With the retrograde reconstruction an inflow from the infrarenal aorta or the common iliac artery is created with an ileomesenteric or ileosplenic bypass graft. A single or multiple vessel reconstruction will result in outflow in the CA, SMA and possibly IMA (Park, Cherry et al. 2002; Sreenarasimhaiah 2005). Another option is the use of autogenous vascular reconstruction. This can be achieved by the use of the saphenous vein or the superficial femoral vein (Sreenarasimhaiah 2005).

Nowadays minimally invasive therapy has become the treatment of interest and became an accepted approach for stenotic or/and occlusive lesions. For patients with CSS percutaneous transluminal angioplasty (PTA) or stenting can be performed with a possible clinical benefit and 90% stent patency (Sreenarasimhaiah 2005). PTA alone has a low short-term success rate and is mostly combined with various types of stents (van Wanroij, van Petersen et al. 2004; Kolkman, Bargeman et al. 2008). Primary stenting is the preferred endovascular treatment if the patient has stenotic lesions. Due to the high radial force resistance of the stent, the best option is a balloon expandable stent (van Wanroij, van Petersen et al. 2004). Minimally invasive endovascular therapies have emerged as a viable alternative for patients with single vessel or short segment of atheroslerosis (Sreenarasimhaiah 2005).

However endovascular treatment is preferred, it is also risky because recanalisation can result in perforation and outflow dissection. For pinpoint stenosis the treatment is successful if the thinnest wire can get through the stenosis. During stenting of long stenotic lesions the surgeon should take care not to overstent branching vessels (van Wanroij, van Petersen et al. 2004). Endovascular treatment is also not eligible for patients suffering CACS. The arcuate ligament causing the extraluminal tight compression of the CA cannot durably pushed aside with a stent (van Wanroij, van Petersen et al. 2004).

The disadvantage of revascularization surgery is the inconsiderable burden for the patient (Kolkman, Bargeman et al. 2008). Comparison of operative revascularization with endovascular therapy shows the shorter long-term patency of an endovascular treatment (Atkins, Kwolek et al. 2007; Biebl, Oldenburg et al. 2007). Due to this low short-term morbidity of endovascular treatment, it will be the treatment of choice for patients with limited life

expectancy or those who are too weak to undergo an operation. The bypass reconstruction is recommended for younger patients with a normal body mass index and multiple vessel stenosis or occlusion (Kolkman, Bargeman et al. 2008). Although there are different important question among treatment techniques for patients with CSS, and probably other methods will be a better solution to treat patient, this is out of the scope for this study.

1.5 Magnetic Resonance Spectroscopy

The aim of this study is to analyse the application of Magnetic Resonance Spectroscopy (MRS) as diagnostic tool to distinguish CSD form CSS. MRS is a non-invasive and safe technology.

Like Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (MRS) is based on the principle of Nuclear Magnetic Resonance (NMR). MRS is used to analyse the presence of components in a solution, in this study different concentrations of lactate, acetate, and lipids. Although nowadays MRS is clinically mostly used for brain analysis, MRS can be an interesting technology in other parts of the human body because it is non-invasive, less expensive, and it can be integrated with an MRI examination. This non-invasive tool is ultimately useful to examine biochemical characteristics of pathophysiological processes (Duncan 1996; Gujar, Maheshwari et al. 2005).

As mentioned, MRS can be integrated with MRI and therefore performed in a similar way. There are only some additional steps needed before the data acquisition. A first important step is ensuring the homogeneity of the magnetic field (Gujar, Maheshwari et al. 2005). With the use of shimming this can be accomplished. Shimming is an automated process used for MRS, but manual adjustments are necessary in some cases (Castillo, Kwock et al. 1996; Burtscher and Holtas 2001). Due to high water concentrations within tissue, other metabolites will become almost invisible after measurement, therefore suppression of the water signal is necessary with the use of water suppression pulses (Castillo, Kwock et al. 1996).

Due to electronic, vibrational or rotational states atoms and molecules have a range of energy levels. Absorption and emission of photons is characteristic for the interaction between atoms and electromagnetic radiation. The energy level of the photons exactly matches an energy level difference in the atom. In spectroscopy the different atoms are distinguished on the basis of frequencies. The NMR signal is caused by electromagnetic induction. NMR is based on the concept of nuclear spin (de Graaf 2007). While MRI uses signals from hydrogen protons, which are used to form the anatomic images. MRS uses the acquired signals to determine other metabolite concentrations (Gujar, Maheshwari et al. 2005). An example it chemical shift imaging (CSI), which is used in this study. The CSI methodology is explained in the third chapter. The determined metabolites can be for example lipids, Glutamate N-acetyl Aspartate, Creatine, Choline, or Lactate. If the concentration of one of these metabolites differ in patient with CSD or patients with CSS, this metabolite will be the target to measure with MRS. As already mentioned in the diagnostic chapter, lactate will increase due to ischemia. In this master thesis it will be examined if MRS is an appropriate technique to measure lactate in a portal vein model.

1.6 Lactate

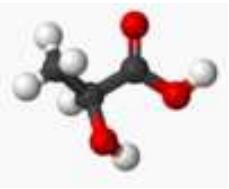


Figure 5. Ball-and-stick model, and skeletal formula of L-lactic acid.

Lactic acid is a chemical compound, carboxylic acid, with the chemical formula $C_3H_6O_3$ (see Figure 5). If lactic acid is situated in a solution it can lose a proton form the carboxyl group. The product of this loss is the lactate ion, with the chemical formula $CH_3CH(OH)COO^-$. Normally there is no increase of lactate in humans, although during normal metabolism (rest) and exercise L-lactate is constantly produced due to the metabolism in red blood cells (McArdle, Katch et al. 2010). Lactic acid has stereoisomers, L-lactic acid and D-lactic acid. The human body normally does not produce D-lactate (Murray, Gonze et al. 1994).

In multiple studies MRS is used to detect lactate concentrations in the liver. Lactate is a potential marker for ischemia, because it is generated as the end product of anaerobic glycolysis (de Graaf 2007). It is released in the circulation and mainly metabolized in the liver (60%) and the kidneys (30%). Normally the systemic blood lactate concentration can range from 0.5-2.2 mmol/L, due to hepatorenal uptake and release to the circulation. Due to ischemia, the serological lactate concentration can rise ultimately from normal concentrations to 20 mmol/L (Murray, Gonze et al. 1994; Evennett, Petrov et al. 2009; Brandis 2010). This increased lactate concentration is not only the case for patients with CSD or CSS or other conditions following a restricted blood flow. It is also observed in patients with hypoxia, tumors and in the human brain following functional activation and hyperventilation (Prichard, Rothman et al. 1991; Frahm, Kruger et al. 1996; de Graaf 2007).

All lactate (the L and D isomer) produced in the intestine is transported through the portal vein to the liver, thereafter lactate will be metabolized. The detection of lactate usually signifies the interruption of oxidative phosphorylation and the initiation of anaerobic glycolysis (Gujar, Maheshwari et al. 2005). The chemical structure of lactate contains four non exchangeable protons in the methane and methyl groups (Figure 6). In the spectrum of lactate there is a doublet resonance at 1.3 ppm due to the three equivalent methyl protons, you will see twin peaks. The single methine proton gives rise to the quartet at 4.1 ppm. Normally the total L- and D-lactic acid is in a low concentration of 0.5 mM. This concentration is already observable in a short-TE ^1H NMR spectrum. However, there are some difficulties with the detection of lactate, due to macromolecules and lipids. It is possible that the lactate peaks will be overlapped by large lipid resonances (de Graaf 2007).

For the diagnosis of patient with CSD or CSS, the lactate concentration can be measured in the intestinal circulation. As mentioned before in normal circumstances there is no production of D-lactate, and only slow metabolization of this isomer. Patients with intestinal ischemia have a bacterial overgrowth, due to break down of the mucosa. This bacterial overgrowth and their

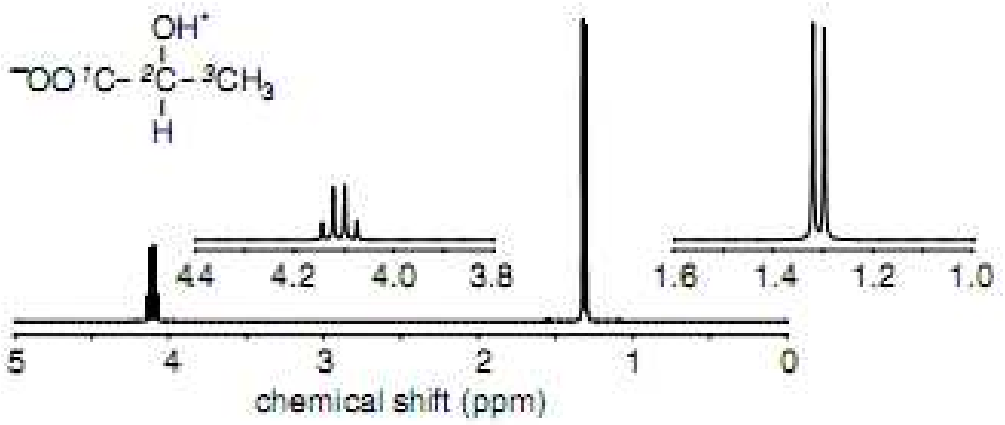


Figure 6. Chemical structure and simulation of the ^{1}H NMR spectrum of lactate (de Graaf 2007).

release of metabolites results in systemic effects, such as D-lactate acidosis. The bacteria cause release of D-lactate into the portal circulation (Murray, Gonze et al. 1994).

To avoid the effect of metabolization of lactate by the liver the ideal location for the measurement of increase lactate concentrations as a result of bowel ischemia is the portal vein. Although measurements specifically on D-lactate can be preferred, in this study there will be no distinction between D-lactate and L-lactate. With MRS both metabolites are measured together as lactate, MRS cannot discriminate between isomers. The liver parenchyma could be another location for measurements, but the lactate concentration inside the liver can differ from location to location, because of inhomogeneous liver function throughout the liver (Murray, Gonze et al. 1994). Therefore it is chosen to focus in this study on the possibility to measure lactate concentrations, which means both D- and L-lactate, in the portal vein by the use of MRS.

1.7 Phantom

For the measurements in this study a phantom is built. With this phantom, measurements can be performed to investigate the possibility to detect lactate in the blood flow. The phantom needs to mimic the properties and function of the portal vein. Therefore, the functions of the portal vein have to be known before preparation of the phantom is possible.

The normal diameter of the portal vein is 10-15 mm (Moore and Dalley 2006) and the portal venous blood flow is 864±188 ml/min (Barthelmes, Jakob et al. 2010). To mimic the portal vein a tube with a 13 mm inner diameter will be used and the flow will be generated with a pump. The idea is that for the detection of lactate a lactate solution will be prepared which can be analysed. Different concentrations of lactate will be used to evaluate the possibilities for measurement in a patient. An elaborated description of the used phantoms is described in section 3, materials and methods.

2. Aim and research question

The aim of this study is to develop a new clinical applicable, noninvasive and accurate tool to measure the increased anaerobic metabolism in the gastrointestinal tract in the presence of failing bloodflow.

2.1 Historical background

It is clear from the background that for the diagnosis of patients with CSD and CSS, there is a need for a non-invasive diagnostic test. Studies to develop a new test already stated a couple of years ago. In the whole process it was tried to measure lactate, a potential marker for ischemia, in the liver or in the portal vein. This was tried during short periods of three months during internships. All these internships led to promising results as well as new problems which had to be overcome.

In prior research lactate was defined as a marker to quantify tissue ischemia. The first experiments, in September 2009, were performed to measure lactate levels in the liver. In two subjects MRS is accomplished before and after an exercise test. The exercise test was ten minutes with a heart rate of 160 per minute, with an increased workload. After this test a second MRS was accomplished and analyzed. There was no difference with the spectra perceived during the measurements before the test (Ymker 2009). Thus after these experiments it was still unknown whether it was practically possible to measure lactate in the liver parenchyma with MRS. To overcome the practical problems different small studies followed.

In May 2010 spectroscopy spectra were made with the use of a 14.1 T Nuclear Magnetic Resonance (NMR) system from agar phantoms injected with different concentrations of lactate (20 mM – 0.1 mM). Hereafter samples made using ground bovine liver with different concentrations of lactate (20 mM – 0.1 mM) added, were measured with the use of a 1.5 T NMR system. With this study the limit of detection was probably between 5 mM and 10 mM. With the 14.1 T system there lies a limit at 1 mM. This study proves the principle of the idea, but it fails with the implementation of a more realistic model (Doppenberg 2010), another model had to be developed for further experiments.

A study in December 2010 was started with the detection of lactate concentrations in the liver. The liver was measured with the use of MRS. To perform these experiments two liver models were developed. These were a chicken liver model and a tissue mimicking model. The chicken livers were injected with different concentrations of lactate (1 M-5 mM) and embedded in an agar-solution. The tissue mimicking model was made of intralipid. This intralipid was combined with different concentrations of lactate (1 M - 5 mM). The spectroscopy spectra of lactate obtained from both models were comparable with human liver spectra. With this experiment on a 1.5 T NMR system, the limit lies above 100 mM, what is clearly too high. A new approach and further experiments were necessary (Groot Nibbelink 2010).

In a following study in November 2011, it was tried to detect physiological concentrations of lactate in whole blood. A 7 T NMR system was used to measure the spectra of

different concentrations of lactate. For the experiments blood of a healthy volunteer was combined with varying concentrations of lactate (20 mM – 0.5 mM). With this study it was shown that physiological concentrations of lactate can be measured with a 7 T system (de Jonge 2011). These experiments were static measurements. Blood in the portal vein flows with a certain velocity, therefore the next step would be to measure lactate in a solution in flowing circumstances.

In March 2012 a new model was created, a flow phantom, to measure lactate in flow. With the use of Arterial Spin Labelling the lactate concentrations were measured. The flow phantom was made of three parts, a tube, a syringe, and a syringe pump. The outer layer of the tube was closed and filled with water, and the measured solution flows through the inner layer. A solution of 50 mM lactate with 50 mM acetate was prepared and measured with an 11.7 T NMR system, with different flow velocities (0.0015 ml/min – 1.000 ml/min). With the used flow velocities there was no influence observed on the measured lactate concentrations (van Leersum 2012). However a velocity of 1.000 ml/min is not comparable with the flow velocity of blood in the portal vein.

The most important step for the following study would be to increase the flow velocity and change the 11.7 T preclinical NMR system for a clinical 3 T system. This was done in August 2012. Again a flow phantom was used and different concentrations of lactate (50 mM - 3.125 mM). It was shown that the 3.125 mM lactate concentration was detectable in the phantom without flow, but with a flow velocity above 60 ml/min not even the 50 mM lactate concentration was measurable. It was clear that the flow has obviously an influence on the measured lactate level. Therefore it was concluded that the use of a STEAM sequence to detect physiological or even pathological levels of lactate in the portal vein is not optimal (Zhou 2012).

2.2 Current study

Summarizing, previous studies already measured lactate as a diagnostic marker for ischemia using different NMR systems (ranging from 14.1 T to 1.5 T). These studies have shown that it is possible to measure physiological concentrations of lactate in static whole blood. Also we concluded that is unlikely that static MSR lactate measurements in the liver is the way to go. Although many hurdles need to be taken, MRS lactate measurement in portal flow is the most promising way to go. Therefore a ‘new’ flow phantom was developed mimicking the portal flow. In two other studies high lactate concentrations were measured in different flow velocities with an 11.7 T preclinical system and a 3 T clinical system. With the 3 T system it is shown to be possible to measure a 50 mM lactate concentration until the flow reached 60 ml/min.

To translate this diagnostic tool to the clinic the 60 ml/min has to increase. Therefore in this study it is important that the flow velocity increases and that the detection of lactate is still possible with these higher velocities. With these higher velocities, comparable to the real velocity in the portal vein, high concentration of lactate as well as lower concentrations should be still visible in the spectrum. Until now it is possible to measure lactate concentrations of 50 mM, but with that concentration is too high to occur in a patient’s blood stream. In this study the high concentration will be the starting point, but it has to decrease before this can be used in clinical settings.

In this study, the same idea for the flow phantom will be kept in mind during the construction of a new phantom that will mimic the portal vein. This new portal vein phantom will be used to measure lactate concentrations in a higher flow velocity with the 3 T clinical system. In previous studies MRS is performed with a STEAM sequence. This sequence was not found to be adequate for the purpose of measuring lactate in a flowing solution. Therefore the first step will be to evaluate a variety of sequences to receive the best results. The ultimate goal is to design and evaluate a method with the use of MRS for the diagnosis of patients with CSD or CSS. If it is possible with the flow phantom and the circumstances are translatable to humans, a new study can be conducted to measure on patients.

If it seems to be possible to measure lactate with MRS with the use of another sequence as used in previous studies, further experiments will be performed to answer the following question: *'Is it possible to measure physiological and pathological concentrations of lactate in the portal vein with the use of Magnetic Resonance Spectroscopy, to diagnose patients with Chronic Splanchnic Syndrome?'* To formulate an answer to this question and come to a conclusion, multiple steps have to be taken. Therefore multiple sub-questions have to be investigated during this study, which require different experiments that follow each other. After some disappointments during the previous studies, the first sub-question which needs to be answered is *'Which sequence is usable to measure concentrations of lactate in a flow comparable to the blood flow of the portal vein?'*

After the evaluation of different sequences and it becomes possible to measure lactate concentrations, the flow velocity has to increase and an expanded portal vein phantom has to be created. With this model multiple experiments can be performed to analyse the model and different physiological and pathological concentrations of lactate. Thereafter these findings have to be translated to clinical studies including healthy volunteers and patients with CSD and CSS. to draw definitive conclusions. To evaluate all different steps the next sub-questions need to be evaluated:

- *'Is it possible to detect a lactate-concentration in velocities comparable to portal flow?'*
- *'Which coil can be used to measure the lactate-concentration in the human body?'*
- *'Can the lactate concentration be measured inside a mimicked body?'*
- *'What is the position of the portal vein in the human body?'*
- *'Which concentration of lactate is still possible to detect and measure?'*
- *'Is the lactate peak detectable with the addition of lipids to the solution?'*
- *'What will happen when the sequence is used during 'real' measurements on humans?'*

The sub-questions follow each other logically for the experimental set-ups. This master thesis consist of three different parts with measurements. In the first part, chapter 3, the simple portal vein is used to investigate the possibility to measure lactate in higher flow velocities within a setting similar to current clinical MRI settings. In the second part, chapter 4, the measurements with the extended portal vein phantom are described. This part is to show the possibility to measure at all different heights within the human body and to measure pathological as well as physiological lactate concentrations. The third part and last part of measurements, chapter 5, a further translation to patients is made. At first, the pure lactate solution is combined with a lipid solution to investigate the influence of other molecules in the spectrum and after this some measurements on human test subjects are described.

All three parts of measurements contain the materials and methods, results, discussion, and a short conclusion for the sub-questions coherent to this part. After the three parts two more chapters will follow. In chapter 6 the recommendations for further studies is substantiated. The question *'How can we design an experiment with (healthy) human beings?'* is worked out. An overall conclusion of the work performed for this master thesis is given in chapter 7.

3. Simple portal vein phantom

Multiple experiments will be performed in an attempt to formulate an answer for the main question of this study, *'Is it possible to measure physiological and pathological concentrations of lactate in the portal vein with the use of Magnetic Resonance Spectroscopy, to diagnose patients with Chronic Splanchnic Syndrome?'* The main question is divided into multiple sub-questions to underline following set-ups of experiments and make hypotheses. In this first part of the study a simple portal vein is used to perform three different experiments. Besides that, the method for data analysis is described.

A useful sequence is determined in the first part. Different flow velocities are measured in the second part, and different coils are used in the third part. This whole subsection is necessary to investigate whether or not it is possible to measure lactate in the blood flow, the correct volumetric flow rate, and if this is possible in a setting compared to current clinical settings, similar MRI system and coils.

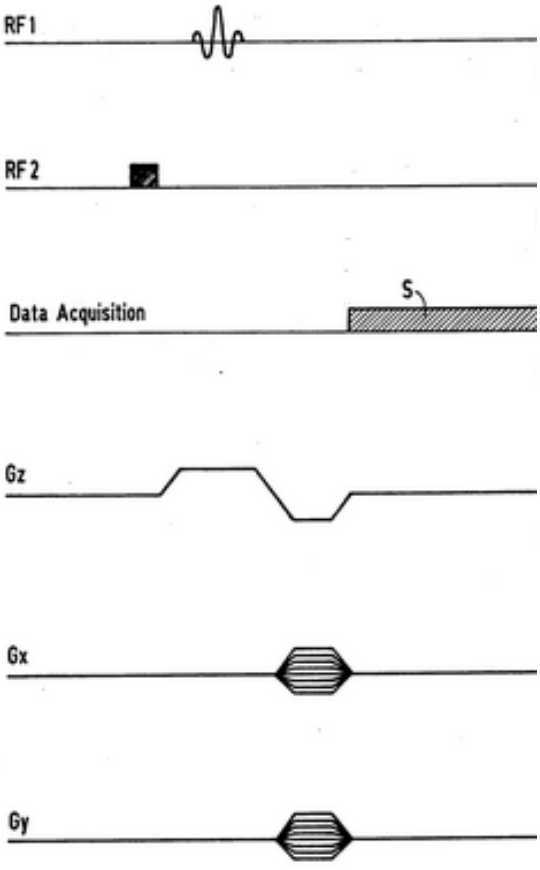


Figure 7. Schematically representation of classical CSI. Each sequence starts with the application of an RF pulse. The RF2 pulse is not spatially selective and designed to cause flip angle of 180°. RF1 is applied with slice selection gradient Gz. Together with this, two phase encoding gradients Gx and Gy are applied. (Kolem and Sauter 1993).

3.1 Materials and Method

3.1.1 Sequence determination

In previous experiments a STEAM pulse was used for the detection of lactate in a flow. With this method the signal of lactate decreased and it was not possible to detect lactate in a flow above 60 ml/min. Before more experiments could be performed the sub-question: *'Which sequence is usable to measure concentrations of lactate in a flow comparable to the blood flow of the portal vein?'* requires an answer. Before experimenting, different sequences are evaluated and the use of adiabatic pulses and Arterial Spin Labeling (ASL) are seen as the best option to measure lactate with the use of MRS.

To evaluate the hypothesis and find an answer for this question, the first step in this study is to find the best sequence. With trial and error multiple MRS sequences are tested, with different echo times and radio frequency (RF) pulses, such as adiabatic pulses. To determine the best sequence for this study a small syringe with 10 mM lactate and acetate solution is used, this is a static solution. The measurements are performed with a 3 T clinical MRI scanner (Siemens). The same scanner is used to

measure patients. The best spectroscopy sequence to detect lactate seems a Chemical Shift Imaging (CSI) method with an adiabatic BIR 45º pulse.

The selected sequence is phase encode chemical shift imaging. Finer localization within the volume of interest (VOI) is achieved with the use of CSI (Gonen, Arias-Mendoza et al. 1997). Automated procedures for ^{1}H MR Spectroscopy have become available, which causes MRS examinations to become easy and reliable. ^{1}H MR Spectra obtained by CSI is expected to facilitate the detection of spectral changes by direct comparison of all regions in the VOI (Sijens, van den Bent et al. 1997). Because a CSI obtained spectra can be voxel-shifted during postprocessing, precise placement of the VOI during the examination is unnecessary (Vigneron, Nelson et al. 1990). It makes CSI an excellent tool for the differentiation of distinct regions (Moudrakovski, Lang et al. 2000). Figure 7 represents the theory behind classical CSI schematically (Kolem and Sauter 1993; Pohmann, von Kienlin et al. 1997).

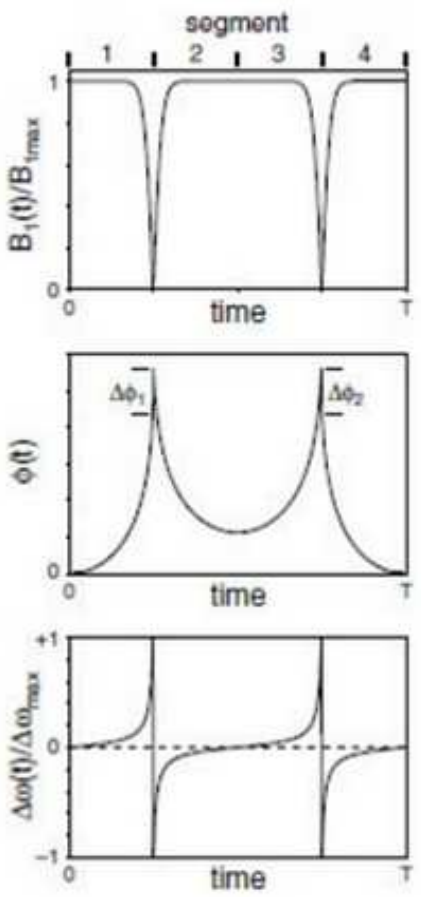


Figure 8. B_1 insensitive rotations (BIR) from adiabatic pulses. In this figure a RF amplitude B_1 (t), phase φ (t), and frequency $\Delta\omega$ (t). Between segments 1 and 2, and 3 and 4, two phase shifts are executed. In the top plot only the amplitude profile, in the middle plot the phase shift $\Delta\varphi_1$ and $\Delta\varphi_2$, and the bottom plot the frequency sweep (de Graaf 2007).

The RF pulse used during the experiments is an adiabatic pulse. In an adiabatic pulse the carrier frequency varies with time during the pulse. These frequency-swept pulses have applications ranging from *in vivo* to high resolution spectroscopy of isolated molecules (Tannus and Garwood 1997). Universal rotations can be accomplished with the class of composite adiabatic pulses known as B_1-insensitive rotation (BIR). BIR pulses can uniformly rotate all components of magnetization lying in a plane perpendicular to the rotation axis which remains constant despite changes in B_1 amplitude (Tannus and Garwood 1997). The basic principle by which excitation or refocusing can be achieved is by utilizing instantaneous reversal of the orientation of the effective B_1-field (Bendall, Garwood et al. 1987; Ugurbil, Garwood et al. 1987). The amplitude and result of executed phase shift is schematically shown in Figure 8. Most adiabatic pulses are full passage, a 180º excitation pulse, but it is possible to design an adiabatic pulse with any angle (Norris and Haase 1989). In this study a designed adiabatic pulse with an angle of 45º is used (BIR 45).

3.1.2 Increasing velocities

After sequence determination, a simple phantom is made to perform the first experiments. These first experiments are done to know whether it is possible to measure lactate concentrations in a blood flow with a velocity exceeding 65 ml/min, and answer the sub-question *'Is it possible to detect a lactate-concentration in high flow velocities?'* With the described sequence (section 3.1) it is thought that lactate could be measured in higher flow velocities

running through the phantom. The difference with the STEAM pulse in previous experiments is the shorter echo time (TE), which will be beneficial for the detection of lactate in higher volumetric flow rates.

Figure 9. Pump, Verder peristaltic 2005

The phantom consists of three parts, a 13 mm inner diameter tube (Gardena, polyvinylchloride), a pump (Figure 9 (Verder peristaltic 2005, Verder Vleuten B.V.)) and a bucket with lactate solution. For the first experiment a 40 mM lactate solution is prepared with lithium lactate ($C_3H_5O_3Li$, 96.01 g/mol, Merck-Schuchardt). This lactate solution, with similar lactate molecules to a portal vein, is pumped into the tube and measured with and without flow velocity. The pump has a capacity of 0.07 to 1460 ml/min. Different velocities, 0, 65, 160, 500, 1100 and 1350 ml/min, are used to determine at which velocity it is still possible to detect lactate. With this it is tried to come near the real portal venous blood flow, which is 864±188 ml/min (Barthelmes, Jakob et al. 2010). After the first measurements the solution is diluted to a 20 mM lactate solution and the same experiment is repeated. All measurements are performed in a 3 T clinical MRI scanner, with the previous described CSI sequence and a ^1H surface coil.

Prior to each experiment a shim is performed to adjust the homogeneity of the magnetic field. The inhomogeneity of the magnetic field is produced by the main magnet of the MRI system due to imperfections in the magnet or to the presence of external ferromagnetic metal. Shimming may involve changing the configuration of the magnet, adjust the currents in shim coils or the addition or removal of ferromagnetic metal from the magnets poles (Pearson 1991; Miner and Conover 1997). The variation of the magnetic field with position is a gradient. Gradients have different shapes and each type of gradient can produce different distortions in the NMR lines. To obtain satisfactory spectra the relatively small gradients must be shimmed out (Pearson 1991). Before the spectroscopy measurements in this study a shim is performed to acquire a NMR signal with as least as possible distortion.

3.1.3 Different coils

The surface coil, used in the previous experiments with the simple portal vein phantom, is not applicable for measurements on patients. The surface coil is too small to cover a human body, therefore other coils should be investigated for a useful experiment translatable to the clinic. The next sub-question *'Which coil can be used to measure the lactate-concentration in the human body?'* requires some experiments to answer it. In clinical settings a body with spine coil combination is mostly used during MRI diagnosis. Thus, the hypothesis becomes that this combination will be suitable in this study. Due to the fact that the body and spine coils are used in the clinic, these coils are used to repeat the experiments to measure lactate in the simple portal venous phantom.

After different measurements there is no signal detectable with this setup (the simple portal vein phantom). A probable solution is to expand the phantom. For now, this is done with an infuse bag filled with water The bag is placed over the tube in order to make it possible for the coils to detect over a larger surface, and to detect the lactate solution underneath the infuse bag. With this expansion there is a signal detectable, only the lactate is not measurable due to the large amount of air between the bag and the tube. This simple portal vein phantom, only a tube, is too small for the coils to measure. For following research an extensive phantom is built, which is described in the next chapter.

This new phantom has to make it possible to measure the 'tube', which will still mimic the portal vein, inside a mimicked 'human' body. Hereby a following sub-question is considered: *'Can the lactate concentration be measured inside a mimicked body?'* It is hypothesized that a larger measurable 'object' would make it possible to detect molecules with the body and spine coil combination, and that the mimicked body would not have an influence on the possibility to measure lactate in the tube. In the next chapter, the experiments to formulate an answer to this question are described.

3.1.4 Data analysis

All data acquired from the experiments have to be analyzed, therefore an interface that can read and use MRI data was necessary. With jMRUI (Java-based magnetic resonance user interface, version 5.0, Marie Curie Framework, France) all data is analyzed and the integrals are calculated from the different peaks. The water peak at 4.7 ppm is suppressed with the use of a water suppression preprogrammed in jMRUI. Hereafter the area under the curve from the lactate doublet at 1.3 ppm is determined for the measurement. The data from the volunteers measured for the localization of the portal vein is also visualized with the use of jMRUI.

All the analyzed data and the findings during the experiments will be used to form the results for this study. The described measurements, in this and following chapter, are all performed to formulate an answer to the main question. However, first the figures are shown and discussed in each part, after which possible recommendations are formulated. Thereafter a conclusion can be drawn for question.

3.2 Results

3.2.1 Sequence determination

The best spectroscopy sequence to use for this study is a phase encoded CSI (Chemical Shift Imaging) method with an adiabatic pulse with a 45° angle (B_1-insensitive rotation) pulse. Therefore the [csi_fid_bir45_nodec_lowres] sequence, preprogrammed in the executed computer of the used MRI scanner, is chosen for the experiments. Figure 10 shows a direct result after MRS measurement. Although this is not a picture of the first measurement to determine the sequence, the results are comparable on the basis of the same sequence, and lactate and acetate concentrations which are used in both measurements.

3.2.2 Increasing velocities

With the use of a simple phantom of the portal vein, different flow velocities are used during the measurements, to determine at which velocity it is still possible to detect lactate. With 0 ml/min

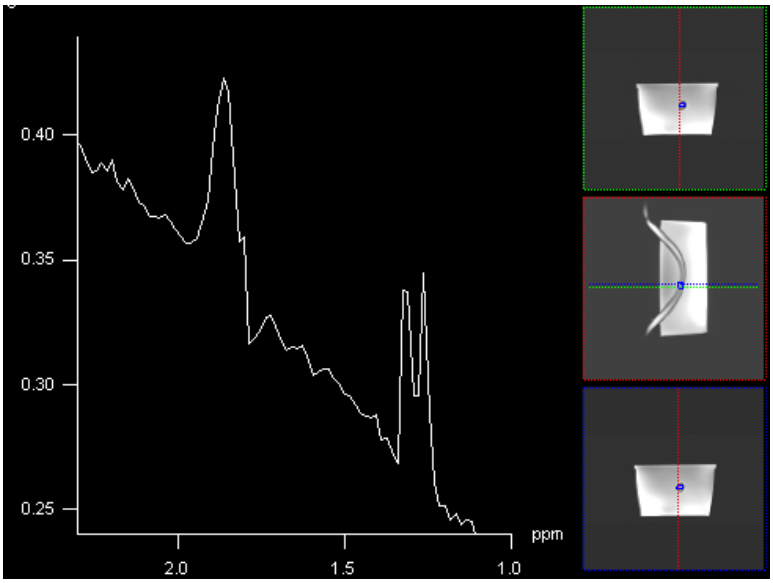


Figure 10. Direct analysis of the MRI results during the measurements. This is a measurement with the [csi_fid_bier45_nodec_lowres] sequence of 20 mM lactate in the tube and 5 mM acetate in the 'body'. After phase shift, round 1.3 ppm the lactate doublet and round 1.9 ppm the acetate peak is visible. In the right part of this picture the box (body) with the inserted tube (portal vein) are visible.

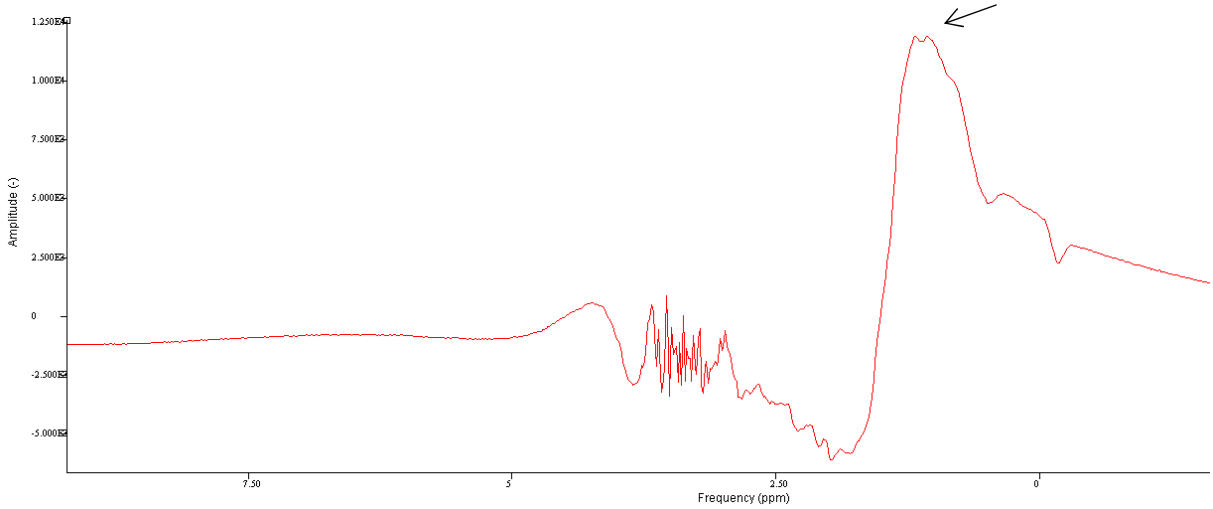


Figure 11. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from a simple portal vein phantom measurement to detect lactate with a velocity of 65ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. The lactate doublet at 1.3 ppm can be identified.

the first measurement is performed to test the set-up. After this the speed is increased to 65 ml/min, which is the velocity at which lactate was not visible during previous measurements with the STEAM sequence. With the CSI sequence, there is a shorter TE and with an increased velocity it is still possible to detect lactate (see Figure 11).

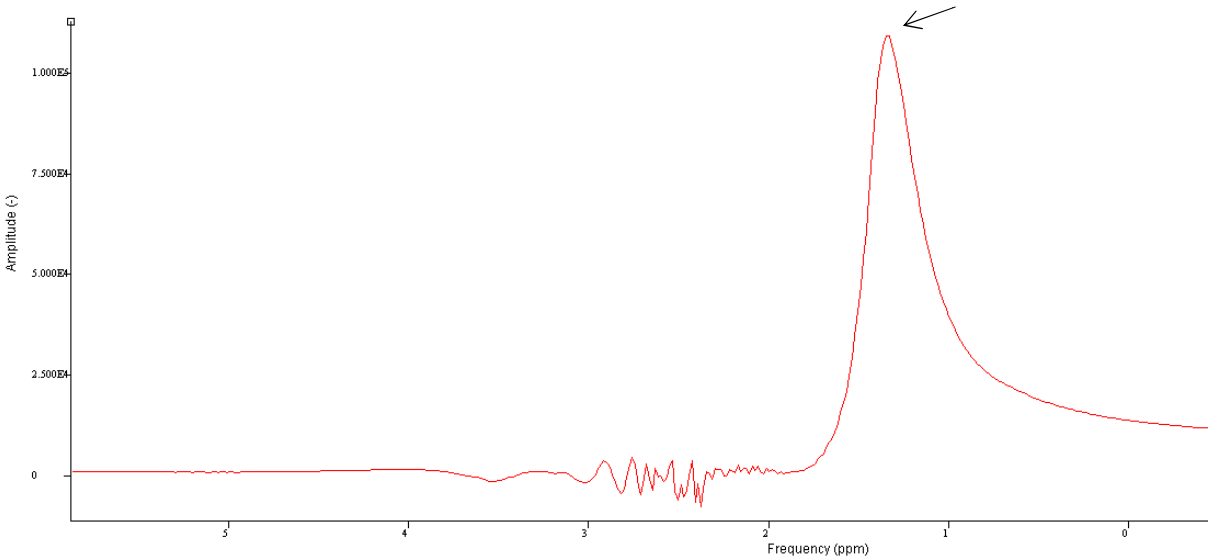


Figure 12. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from a simple portal vein phantom measurement to detect lactate with a velocity of 1350 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. The lactate (doublet) at 1.3 ppm can be identified.

The velocity is increased in multiple steps (see the results from these experiments in appendix A). The last measurement is performed with a velocity of 1350 ml/min (see Figure 12), which is higher than the normal velocity of the blood flow in the portal vein. In all these measurements lactate is detectable.

With this experiment it is presented that it is possible to measure lactate above 60 ml/min. Although with increasing flow velocities it is still possible to detect lactate with MRS, this is a solution of 20 mM lactate, which is at the high extreme of the lactate range within CSS or CSD (other velocity measurements are shown in appendix A). Besides this high lactate concentration, a peculiar finding is that the amplitude of the lactate peak/doublet changes with an increased flow velocity. The position of the peak is similar in all measurements (1.3 ppm), but the height differs. This is normal in the case of a short repetition time (TR) with a longer T1, what is the case in this sequence. Besides that the idea is that the lactate should be fresh, what is not certainly the case during all experiments. The velocity measurements were performed after each other, thus the same, 'new' batch of lactate is used so the lactate is not influenced by storage. A follow up on this finding is presented in the discussion (Figure 24).

3.2.3 Different coils

The experiments to detect lactate with different flow velocities are performed with the use of a surface coil. This is a very small coil situated just above a tube. This coil is usable in the case of the simple portal vein phantom, which just contained of a single tube with a pump. However the human body, at the location where the portal vein normally is situated, has a larger surface and needs a larger coil to cover the surface. To become able to translate this research to clinical settings, it is important to stay as close as possible to the clinical situation and the normal situation during MRI measurements. Therefore the use of a body with spine coil combination is used for the detection of lactate in the simple portal vein phantom. However, it is not possible to detect lactate due to the large amount of air detected by the coils. To make it possible to use the body with spine coil combination an extensive phantom of the portal vein is built.

3.3 Discussion

3.3.1 Sequence determination

The first sub-question needed to be investigated before further measurements are possible is: *'Which sequence is usable to measure concentrations of lactate in a flow comparable to the blood flow of the portal vein?'* The hypothesis is that adiabatic pulses or Arterial Spin Labeling (ASL) seems to be the preferred/best options to fulfill the needs. Although they were promising during the first experiments, a higher flow velocity made ASL useless as well compared with the STEAM pulse used in previous studies to measure lactate in a portal vein phantom. It is again not possible to measure lactate in a high flow velocity.

The hypothesis can be partly rejected, because the use of a Chemical Shift Imaging (CSI) seems to be the best option for further experiments, combined with an adiabatic BIR 45º pulse. The [csi_fid_bir45_nodec_lowres] sequence, preprogrammed in the executed computer of the used MRI scanner, is chosen for the experiments. In the third chapter, materials and method, more specifications of this sequence are described (Gonen, Arias-Mendoza et al. 1997; Tannus and Garwood 1997). During the follow up experiments this sequence is used.

3.3.2 Increasing velocities

Now a sequence is determined which is usable to detect lactate-concentrations in a flow with a velocity above 65 ml/min the following sub-question *'Is it possible to detect a lactate-concentration in high flow velocities?'*, can be discussed. With the use of the simple portal vein phantom and the [csi_fid_bir45_nodec_lowres] sequence, it is thought that it will be possible to measure lactate in a flow velocity comparable to the blood flow velocity in the portal vein.

From the results it is possible to confirm this hypothesis. With the use of a 20 mM lactate solution, it is possible to detect lactate in a flow with a velocity above 65 ml/min. During the experiments the velocity is stepwise increased to 1350 ml/min, which is higher than the normal portal venous blood flow of 864±188 ml/min (Barthelmes, Jakob et al. 2010). In all flow experiments the 20mM lactate concentration is still detectable.

Although the hypothesis of this sub-question can be confirmed, the used and measured lactate concentration is 20 mM. This concentration is too high compared with physiological and pathological concentrations (Evennett, Petrov et al. 2009). Therefore the lactate concentration has to decrease to investigate if it is possible to detect physiological concentrations.

Besides the high concentration, also the amplitude is different with different flow velocities. Fortunately, the lactate peak/doublet still appears at the same position in the spectrum (± 1.3 ppm). Therefore it can be concluded that in all cases the lactate was detected and measured appropriately, however, due to the fact that with an increased velocity, the amplitude of the peak is higher (Figure 24). So, there is a difference in the height of the peaks, which cannot correlate with the fact that the lactate concentration is the same. This has an influence on the calculated lactate concentration when applied as a diagnostic tool. This phenomenon is further discussed in the following chapter.

3.3.3 Different coils

As mentioned in the previous chapters, the used surface coil is not applicable for the human body. Some small experiments with coils are performed to evaluate the sub-question: *'Which coil can be used to measure the lactate-concentration in the human body?'* To stay as close to clinical settings as possible it is hypothesized that a body and spine coil combination is suitable for measurements.

It is not possible to detect the lactate-concentration in the tube of the simple portal vein phantom. This is probably caused by the large surface area covered by the coils. The tube of the simple phantom is too small to detect by the coils, and with the addition of infusion bags with saline solution, a cavity with air is created. Although there is an air cavity, the use of the infusion bags makes it possible to measure something, in contrast to the tube alone. However, due to the air cavity between the bag and the tube it is not possible to detect the lactate in the tube with MRS. A new extended flow phantom is built to overcome this problem. This phantom consists of a body containing a liquid with the tube inside. This eliminates the air cavity introduced by the infusion bags. Already before the first measurements of this phantom it is hypothesized that this coil combination is suitable for further experiments and this hypothesis is confirmed during the first measurements with the extensive portal vein phantom.

3.4 Conclusion

This part consists of three different experiments. First, sequence determination, in which a suitable sequence is determined for the detection of lactate in flow velocities above 65 ml/min. It seems that this is possible with the preprogrammed [csi_fid_bir45_nodec_lowres] sequence, consisting of Chemical Shift Imaging (CSI) and B_1-insensitive rotation (BIR). The goal in the second experiment is to detect lactate in a velocity similar or above human blood flow, around 1000 ml/min. The velocity is stepwise increased and it becomes visible that in a velocity of 1350 ml/min lactate is still detectable with MRS. A surface coil is used in the previous two experiments, which is not suitable to use for a human body. From the third experiment, where different coils are used, it is concluded that an extended version of the portal vein phantom is necessary to use a body with spine coil combination. This combination is closest to real clinical settings and therefore desirable.

4. Extended portal vein phantom

As mentioned in the conclusion of the previous part, the simple portal vein phantom is not sufficient, therefore the phantom has to be expanded. In this second part of this study, an extended portal vein phantom is build and used for two additional experiments stemming from the results discussed in the previous chapter. It is shown that lactate can be measured in the same velocity as a human blood flow with the use of MRS. Although this seems possible, the position of the portal vein in the body is not directly under the coil, and the lactate concentration will differ. The investigation of these two important clinical issues is described in this part.

4.1 Materials and Method

4.1.1 Different position in the body

To extend the previously used portal vein phantom a box (10 x 25 x 16 cm, polyethylene terephthalate) is added, mimicking the body of a human. To mimic bodily tissue, the box is filled with 4 L of a 2% cellulose solution (hydroxyethyl-cellulose, Fluka). To this solution 5 mM acetate (sodium acetate, 82.03 g/mol, Sigma) is added for detection with spectroscopy and 145 mM sodium chloride (58.5 g/mol, Sigma) is added as an ionic compound. The tube is inserted through the lid of the box, whereby it is possible to adjust the height of the tube in the box (Figure 13).

With the first measurements the tube is positioned 2 cm above the spine coil, with a distance of approximately 14 cm to the body coil. The experiments are performed with the 20 mM lactate solution. The measurements are performed with and without flow velocity. The chosen velocity is about 1000 ml/min, comparable with the venous portal blood flow in humans (Brown, Halliwell et al. 1989). With the expansion of the phantom at each 2 cm a marking is made on the box and the tube is movable. After finishing the first measurements, the tube is moved 2 cm above and at each position the experiment is repeated. With these results the influence of the height of the tube from the coils could be compared and evaluated. It should be possible to measure the lactate concentration in the phantom comparable with the position of the portal vein in a human. The question following is *'What is the position of the portal vein in the human body?'* The anatomical position of the portal vein is known, but the exact distances between posterior abdominal wall and the portal vein and the anterior abdominal wall and the portal vein of a person has to be

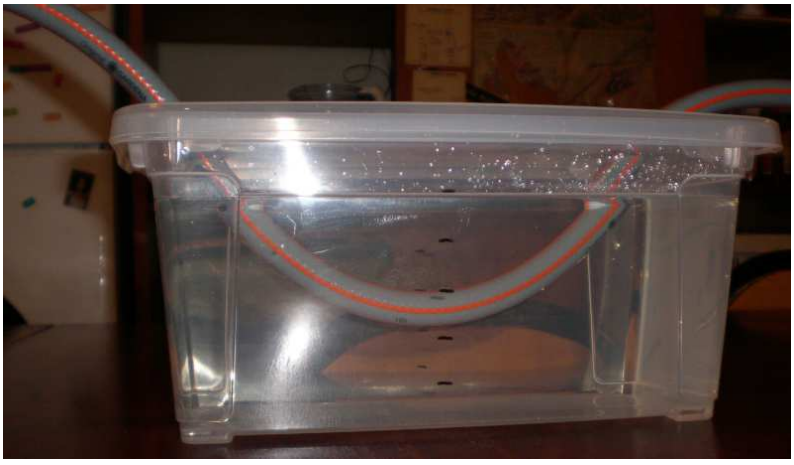


Figure 13. Extended phantom of the portal vein within the body. The box filled with cellulose solution. Height of the tube can be adjusted.

investigated.

The average position and diameter of the portal vein is determined using CT scans withdrawn from the vascular registry of Medisch Spectrum Twente, Enschede. Sixteen different CT scans, ten men with an average age of 73 ± 6.7 and six women with an average of 76 ± 7.6 years are measured. In men the diameter of the portal vein is 11.9 ± 0.759 mm and in women 9.41 ± 1.24 mm. The mean distance between the portal vein and the anterior abdominal wall is 112 ± 14.4 mm in men and 61.6 ± 7.45 mm in women. The average distance from the portal vein to the posterior abdominal wall is 152 ± 20.4 mm in men and 121 ± 16.4 mm in woman.

For these experiments the same CSI sequence with BIR 45º pulse is used as described for the experiments in the previous chapter, with the simple portal vein phantom. A shim is performed before each spectroscopy measurement. For the shimming process the whole model is used, the tube as well as the whole content of the box. Thereafter the spectroscopy is performed and the different concentrations of lactate in the tube and acetate in the box are measured.

4.1.2 Pathological and physiological lactate concentrations

Until now all experiments are performed with a high concentration of lactate, 20 mM. As mentioned in the introduction chapter, normal systemic blood lactate concentrations range from 0.5-2.2 mM. Pathological lactate concentrations (in the portal vein) can rise ultimately to 20 mM due to ischemia (Murray, Gonze et al. 1994; Evennett, Petrov et al. 2009; Brandis 2010). However the used 20 mM corresponds to very severe ischemia and more experiments are necessary to know if lower concentrations of lactate are detectable with this same method. The following described experiments are used to consider the next sub-question: *'Which concentration of lactate is still possible to detect and measure?'* Due to previous studies it is thought that it would not be possible to detect low concentrations of lactate inside the tube of the portal vein phantom.

With the extended portal vein phantom measurements are performed with the use of different lactate concentrations. The tube is positioned in the middle of the box, at approximately 7 cm above the spine coil, and again the flow velocity of 1000 ml/min is used. Each concentration is measured with and without 50 Hz water suppression. The experiments started with the 20 mM solution, thereafter the solution was diluted to 10, 5 and 2.5 mM and the same measurements are performed for each concentration. The 2.5 mM concentration is also measured without flow, to compare this spectrum with the obtained spectrum from the measurement with flow.

4.1.3 Data analysis

JMRUI is used for visualization of the lactate peak at 1.3 ppm and possibly the lactate peak at 4.1 ppm. However, with the concentration measurement the measured concentration is compared with the real concentration in the tube. The concentration measurements started with 20 mM and ended with 2.5 mM. To determine the measured concentration a calculation is necessary (equation 1). First the lactate peak amplitude, doubled due to the double peak, is divided with the water peak amplitude:

$$x\ amplitude = \frac{\text{2x amplitude lactate peak}}{\text{amplitude water peak}} \quad (1)$$

Hereafter the amount of protons is used. Water has two protons and the lactate at 1.3 ppm has three protons. The sum (2/3) is used for the measurement and compensated with water, 55 M. Therefore the total calculation becomes:

$$x\ concentration = x\ amplitude * \frac{2}{3} * 55 \quad (2)$$

For each real concentration the measured concentration is calculated (equation 2) and a concentration curve is drawn with Microsoft Excel.

4.2 Results

4.2.1 Different position in the body

The extensive portal vein phantom contained a 'body' in which the tube (portal vein) is situated. With the use of the extensive portal vein phantom it is possible to adjust the height of the portal vein within the mimicked body solution, and determine the influence of the position of the portal vein. For all measurements a 20 mM lactate solution is pumped through the tube with a flow velocity of 1000 ml/min.

The first measurement is performed with the tube 2 cm above the spine coil. Figure 14 shows that there is a lactate doublet visible at 1.3 ppm. This measurement is performed with the tube inside the box 2 cm above the spine coil, and a solution of 20 mM lactate with a velocity of 1000 ml/min.

Hereafter the tube inside the box is highered to 4 cm and 6 cm above the spine coil. The 6 cm above the spine coil position is approximately the middle of the box. This is assumed to be

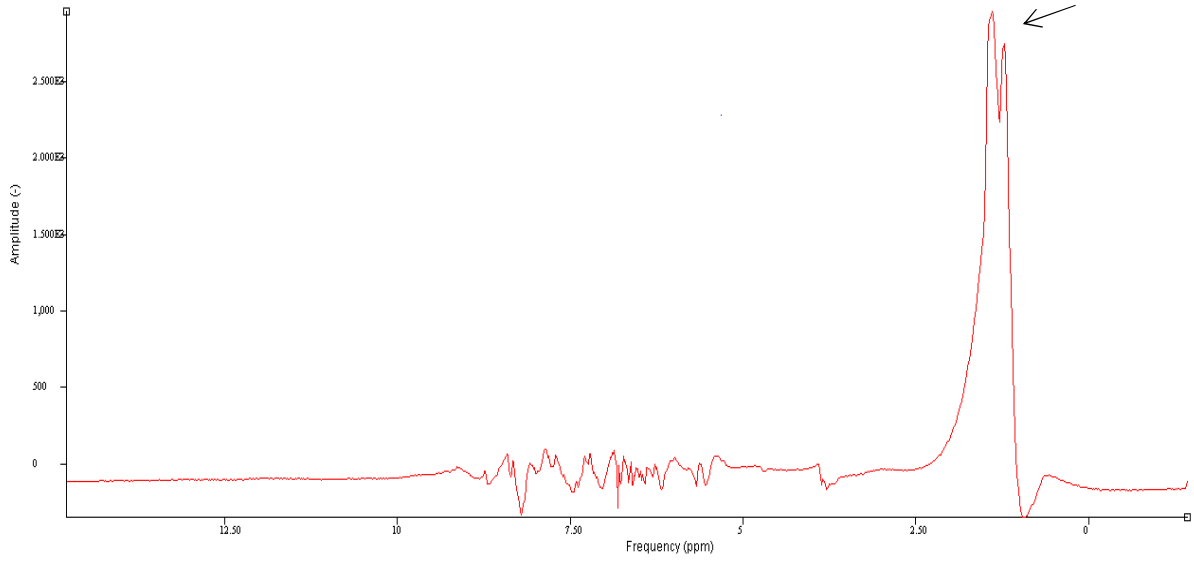


Figure 14. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from the extended portal vein phantom measurement to detect lactate in the tube 2 cm above the spine coil. This measurement was performed with 20 mM lactate solution and a velocity of 1000 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. The lactate (doublet) at 1.3 ppm can be identified.

the most difficult to still detect lactate there. However it is the most important to measure because the portal vein is generally somewhere in the middle of the body. At this height it is still possible to detect a lactate doublet (see Figure 15), what is a positive finding for the further experiments.

The last experiment of this series is performed 10 cm above the spine coil, which is at the surface of the mimicked body fluid in the box. It is visible in Figure 16 that at this height lactate is not detected. The data from the spine coil is used for analysis, so that can be a reason for more difficulty to detect lactate. If the body coil will be used to analyze it could be beneficial for the lactate doublet. Repetition of the data analysis or experiment can show this.

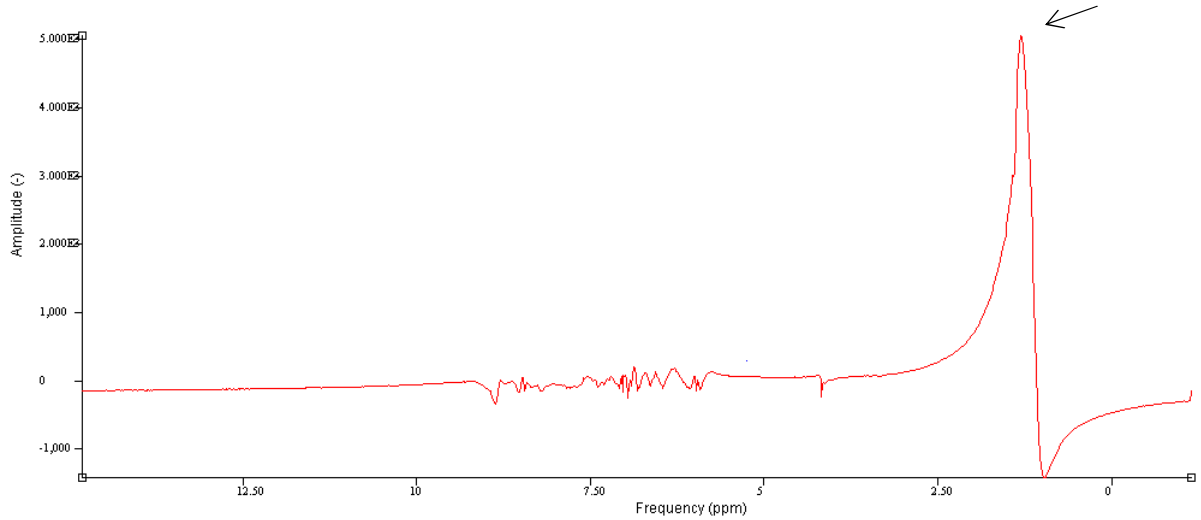


Figure 15. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from the extended portal vein phantom measurement to detect lactate in the tube 6 cm above the spine coil. This measurement was performed with 20 mM lactate solution and a velocity of 1000 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. A lactate peak at 1.3 ppm can be identified.

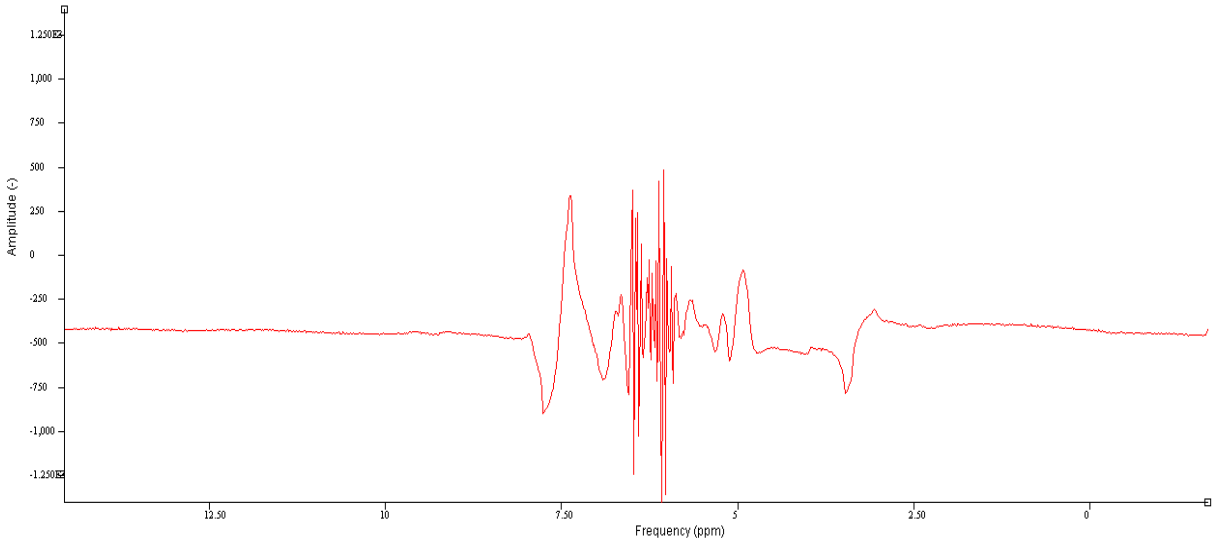


Figure 16. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from the extended portal vein phantom measurement to detect lactate in the tube 10 cm above the spine coil. This measurement was performed with 20 mM lactate solution and a velocity of 1000 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. A lactate peak at 1.3 ppm cannot be identified at this height.

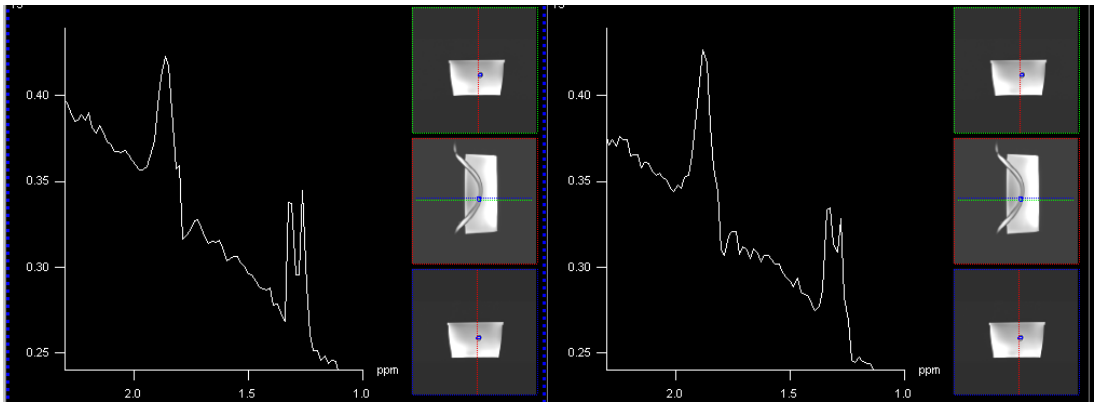


Figure 17. Direct analysis of the MRS results during the measurements. This is a measurement with 20 mM lactate in the tube and 5 mM acetate in the 'body'. Round 1.3 ppm the lactate doublet and round 1.9 ppm the acetate peak is visible in both measurements. In the left picture was during a measurement with a velocity of 1000 ml/min and the right picture without flow.

The measurements with the tube approximately in the middle of the 'body' are repeated, because it is possible that the results are influenced by the velocity. The results without and with a 1000 ml/min flow velocity can be compared, due to the use of the same setup and similar shimming (see Figure 17). It can be concluded that lactate can be measured in the tube of the extended portal vein phantom at different heights with or without flow (other results are shown in appendix B).

As already mentioned, it is assumed that the portal vein is somewhere in the middle of the human body, as regards to height. To gain more knowledge about the average diameter and distances of the portal vein within the human body, 16 CT scans from the patient of the Medisch Spectrum Twente database were used (see appendix C for all data). The average age, diameter of the portal vein, distance between portal vein (PV) and anterior abdominal wall (AAW), and distance between portal vein and posterior abdominal wall (PAW) are calculated (see table 1).

From the above presented figures it can be concluded that the height of the tube within the portal vein phantom has some influence on the detection of lactate. In a normal human body the distance between the portal vein and the back or the abdominal wall is larger than in the experimental set-up, but different between man and women (see table 1). However, this larger distance is no problem, because the results from the height adjusted measurements show similar results independent on the height of the tube in the phantom.

4.2.2 Pathological and physiological lactate concentrations

Previous measurements are all performed with the use of 20 mM. As before mentioned, this concentration of lactate is higher than the physiological lactate concentration as well as the pathological concentration range of lactate in the blood circulation. It is researched if it is also possible to measure lactate in lower concentrations (all graphs are shown in appendix D). During these experiments the lactate concentration is lowered from 20 mM towards 2.5 mM. The data acquired during all experiments is processed with jMRUI to calculate the concentrations. Although the lactate peak is the best detectable and measurable with 20 mM (see Figure 18), it is still visible at 2.5 mM (see Figure 19).

Parameter	Man (10)	Women (6)
Age (year)	73 ± 6.7	76 ± 7.6
Diameter PV (mm)	11.9 ± 0.759	9.41 ± 1.24
Distance PV-AAW (mm)	112 ± 14.4	61.6 ± 7.45
Distance PV-PAW (mm)	152 ± 20.4	121 ± 16.4

Table 1. In this table the average with standard deviation calculations from measurements of 16 CT scans (10 man and 6 women). PV = portal vein, AAW = anterior abdominal wall, and PAW = posterior abdominal wall.

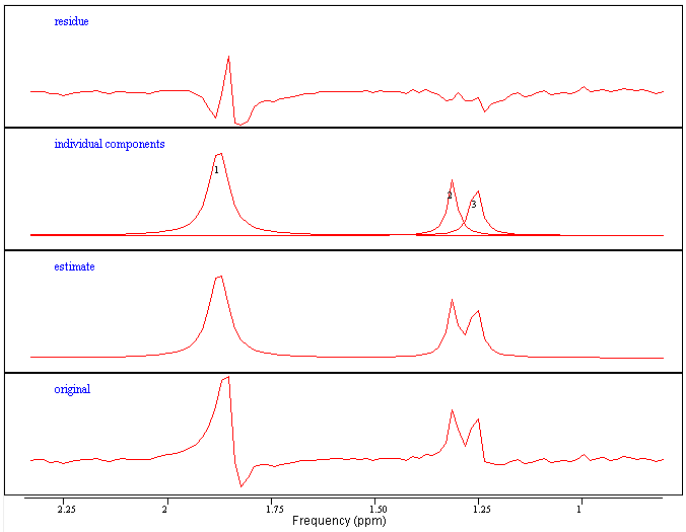


Figure 18. Processed data with jMRUI after measurement with a 20 mM lactate concentration spectrum. The lactate doublet visible at 1.3 ppm, and water peak at 4.7 ppm after peak remover. In the upper picture the residue after analysis. In the second graph are the individual components shown of the peaks. In the third picture the estimated doublet is calculated. The spectrum below is the original lactate doublet.

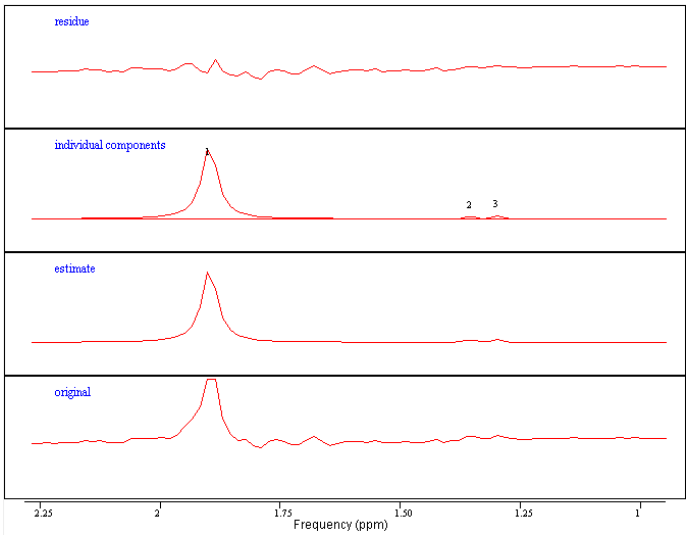


Figure 19. Processed data with jMRUI after measurement with a 2.5 mM lactate concentration spectrum. The lactate doublet visible at 1.3 ppm, and water peak at 4.7 ppm after peak remover. In the upper picture the residue after analysis. In the second graph are the individual components shown of the peaks. In the third picture the estimated doublet is calculated. The spectrum below is the original lactate doublet.

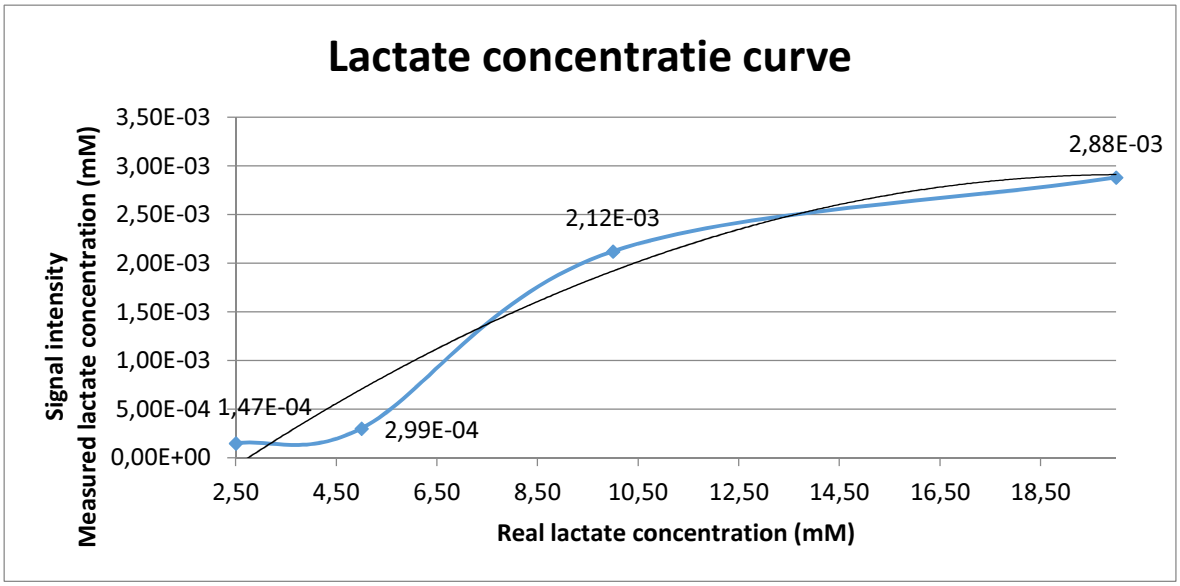


Figure 20. The lactate concentration curve after measurements of 20 to 2.5 mM lactate. The x-axis shows the real lactate concentrations and the y-axis shows the lactate concentrations measured with 3 T MRI.

After data processing it is possible to calculate the measured lactate concentration with the use of equation (1) and (2) given in materials and method section 4.1.3. With the calculated concentrations of all measurements a concentration curve is formed (see Figure 20).

In figure 21 it is visible that the lactate concentration drops fast below 10 mM. Although it is still possible to measure the lactate concentration of 2.5 mM, the calculated concentration (for all measurements) is lower than the actual concentration in the phantom. This is not only the case for 2.5 mM, but also for all other concentrations. Overall it can be concluded that the measured lactate concentration is lower than the actual lactate concentration used during the experiments. This phenomenon is evaluated in the discussion. The actual numbers of the amplitudes and calculated lactate concentrations from this series of experiments can be found in appendix D.

4.3 Discussion

4.3.1 Different position in the body

An expansion on the simple portal vein phantom is made. The new phantom consists of a mimicked body in which the tube is inserted for the experiments. The first question to answer is *'Can the lactate concentration be measured inside a mimicked body?'* It is thought that with the use of the body and spinal coil it will be possible to measure the tube with the lactate concentration when it is inserted in a 'body' of liquid. Thereby is hypothesized that the molecules in the solution of the box do not influence the measurements of the tube.

The trial is performed with the tube approximately in the middle of the box. It is concluded that it is possible to acquire a lactate peak/doublet at 1.3 ppm and an acetate peak at 1.9 ppm. However, to gain the best results the voxel grid is adjusted for these experiments. The voxel grid used in experiments on the simple portal vein phantom contained half the amount of voxels. For the experiments with the extensive portal vein phantom the grid covers the whole box. This causes an increase in voxels as well as an increase in measuring time from 8 to 15 minutes. This

longer measuring time is no problem, but the voxel inside the tube is smaller now (see Figure 21). An acetate peak is visible due to the acetate in the solution of the mimicked body. After the trial measurement, following measurement are performed to compare the results between the bottom and the top position of the tube inside the box. The experiments start at 2 cm above the spine coil (bottom position), and higher with steps of 2 cm, until 10 cm above the spine coil (top position).

During the measurements and from the first pictures acquired with MRS, it seems that it is possible to detect lactate at all heights within the mimicked body (see Figure 22). However, after data analysis it is not possible to detect lactate in the measurements of the portal vein at 10 cm above the spine coil. At all other heights, lactate is detected. Why it is not possible to detect lactate at the top position measurements after data analysis, is not clear. Although it is not sure if this will become possible, it provides no difficulty for further research. From portal vein analysis in CTA data, it is clear that the portal vein is not that close to the anterior abdominal wall, but more a couple mm above the middle between posterior and anterior abdominal wall.

The middle position shows the best detectable results, also in comparison with the peaks of molecules in the surrounding. The influence of acetate was higher in lower positions of the tube (see Figure 22, upper right). Although the reason for this influence of other molecules is not determined, it is possible to elaborate on the origin of this. As explained some paragraphs above, in these experiments the voxel grid was enlarged to cover the whole box. This smaller voxel is necessary to eliminate the amount of 'body' that the voxel covers (see Figure 22, lower right). Although the voxel is smaller and should not cover the 'body', it is possible that is detects some molecules at the corners of the voxel. This amount of detected lactate and acetate probably varies due to the position of the tube and the used voxel for detection.

Another program to read and analyze MRS data can be beneficial. It is possible to investigated if that will solve some problems to detect and calculate the lactate concentrations. The program of first choice is 3DiCSI, but it was not possible to use. JMRUI is used instead, because it works on the computer used to analyze the data. It can be beneficial for CSI acquired MRS data to analyze with the use of another program, such as 3DiCSI. It is possible to read MRS data with JMRUI, but 3DiCSI is specialized to read and visualize CSI data. Thus the use of 3DiCSI can be beneficial for the data analysis of the acquired data in this study.

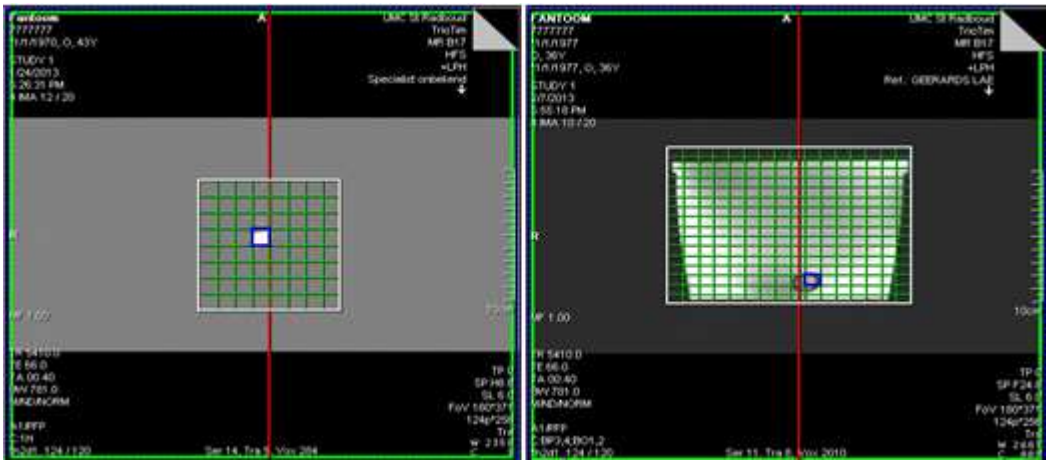


Figure 21. The voxel grid (green) of the MRI measurement of the simple portal vein phantom (left) and extensive portal vein phantom (left), with chosen voxel inside the tube (blue).

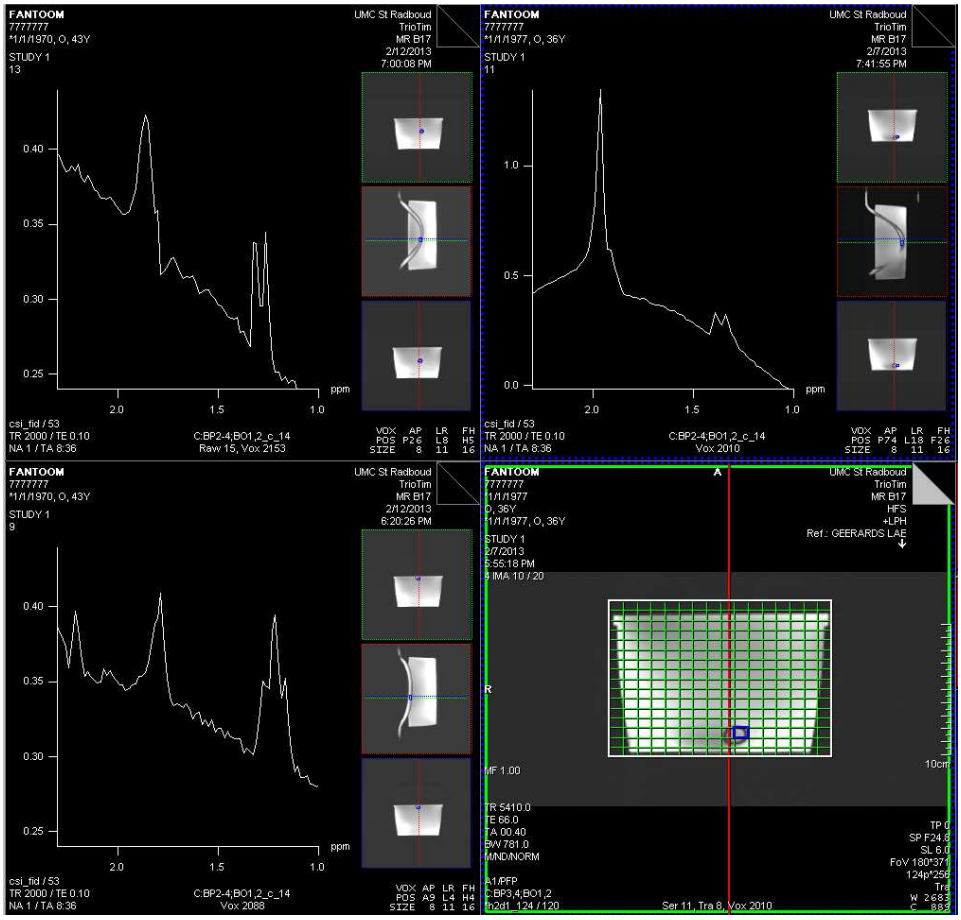


Figure 22. Direct analysis of the MRS results during the measurements. This is a measurement with 20 mM lactate in the tube (with a flow velocity of 1000 ml/min) and 5 mM acetate in the 'body'. Round 1.3 ppm the lactate doublet and round 1.9 ppm the acetate peak is visible in both measurements. In the upper left picture was during a measurement with the tube 6 cm above the spine coil, the upper right picture is during a measurement with the tube 2 cm above the spine coil (bottom position), the lower left picture is during a measurement with the tube 10 cm above the spine coil (top position), and the lower right picture is the voxel grid (green) of the MRI measurement of the box with tube 2 cm above the spine coil, with chosen voxel inside the tube (blue).

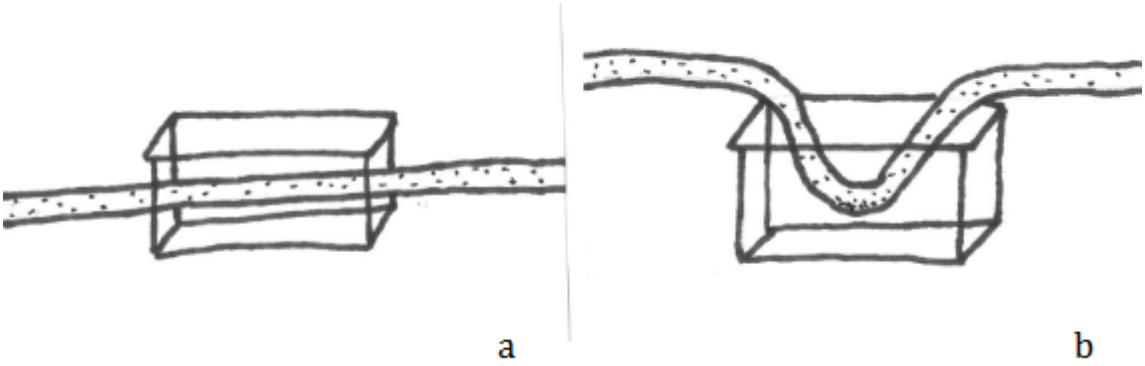


Figure 23. This figure shows two schematic representation of the tube inside the box ('body'). Drawing a shows the ideal situation in which the vein in situated in the body without any curve. Drawing b shows the actual situation during the measurements, the tube is inserted through the lid of the box, which causes a curve in the tube. The lactate concentration is measured in the curve.

Another point of discussion is the curve at the bottom of the tube. Due to the fact that the tube is inserted into the box trough the lid, there is a curve in the tube. This curve can have an influence on the flow through the tube. It is thought that due to the curve some lactate molecules can stick into the bottom of curve attached to the wall of the tube (Figure 23, b). The tube used in these experiments seems to have a smooth inner surface, but it is possible that this surface causes cleavage of lactate molecules to it. In that case, lactate is always measured. The experiments of the height differences are spread over three days, in between which the tube is rinsed, therefore this discussion can possibly be rejected. Although between the different days of experiments the set-up is cleaned, it is still possible that the curve has influence on the detected amount of lactate. More experiments are necessary to know the effect of the curve.

In this study it is tried to know the effect of the curve. Besides this extensive portal vein phantom another, more extensive phantom, is build (Figure 23, a). Although the idea behind this model was to acquire more reliable results, there are no measurements possible with this model, and no gain of results. MRS shows no signal in this new phantom. The reason for this is not certain, but it can be caused by the glue used to build the phantom. To eliminate the curve a straight tube is inserted and glued in the box. The glue is used to fix the tube and make the box watertight. Some glue attaches the tube inside the box, which can have caused the signal problems with these measurements. The shimming phase is already troublesome, and with the actual measurement there is no good signal detectable from the whole phantom. This phantom is not used for other experiments, but it can be an idea for the future to build a portal vein phantom through which the tube is straight.

To conclude this section, for the extensive portal vein model the tube is measured at all different heights in the phantom. This has some but no major influence on the possibility to detect lactate. To translate these results to the clinic, the real position of the portal vein inside the human body needs to be known. The question following is *'What is the position of the portal vein in the human body?'* Therefore 16 CT scans are measured, from which the results are shown in table 1 and appendix C. The CT portal vein data is not gathered from patients with intestine ischemia. Therefore the Body Mass Index (BMI) could be different between these patients and patients in the database. Although the BMI can be higher in the patients from the database compared to patients with intestinal ischemia, this difference can be neglected on the average. To know this with more certainty it is possible to determine the average distances in more CT scans of patients with intestinal ischemia.

4.3.2 Pathological to physiological concentration

All the previous experiments are performed with the use of 20 mM lactate concentration, which is too high. As a following step in this research, the concentration is lowered to answer the next sub-question: *'Which concentration of lactate is still possible to detect and measure?'* It is hypothesized that it is difficult (even impossible) to detect a lactate concentration of 10 mM or lower with the use of the same set-up.

For each measurement in this part of the research, the lactate concentration is diluted. It has to be possible to detect concentrations below 20 mM to continue the study with this set-up and MRS approach. For each measurement the lactate concentration is halved, so each measurement is performed with a different solution. To have a start measurement again the 20

mM is used, and thereafter lactate peaks in 10, 5, and 2.5 mM lactate concentrations are detected. Although it is probable that lactate is still detectable with a concentration of 2.5 mM, the peak is decreased to a minimum. This raises the possibility that the result is not caused by the lactate signal, but caused by noise. A lower concentration is probably not detectable with the set-up of this research.

A peculiar finding is the fast drop of lactate between 10 and 5 mM (Figure 20). It is not clear what the reason is for this drop. To evaluate this finding, the same experiment needs to be repeated. It is possible that there went something wrong during the experiments, e.g. the dilution. However, it is just speculative to discuss this at the moment and a reproduction of the experiment would be helpful. Probably the finding is just a one-time result, but it is only possible to show that with a repetition of the measurements. When this concentration experiment is repeated twice or more, the resulting curves can be compared. This can give more information about the drop. Besides that, it is recommended for further research to start with a different method of data analysis.

The calculated lactate is much lower than the real lactate which flows through the tube (Figure 20). The particle size can have an influence on the measured concentration of the molecule, in this case lactate (Weber, Rambau et al. 1993; Yang, Yang et al. 2013). If that is the case in this research, more experiments are necessary to acquire knowledge on the influence of particle size. This can give difficulty in measuring lactate in the human body. A possibility is to investigate and compare the blood molecules of healthy humans and patients with CSD or CSS, thereby taking into account the difference of L-lactate and D-lactate. In this analysis the lactate molecules used for the solutions can be determined and compared as well.

When the particle size remains similar, the same molecule can have an effect on the concentration measurement with a higher velocity (Weber, Rambau et al. 1993). As shown in Figure 24, when the velocity increases, the detected lactate peak increases as well. It can be

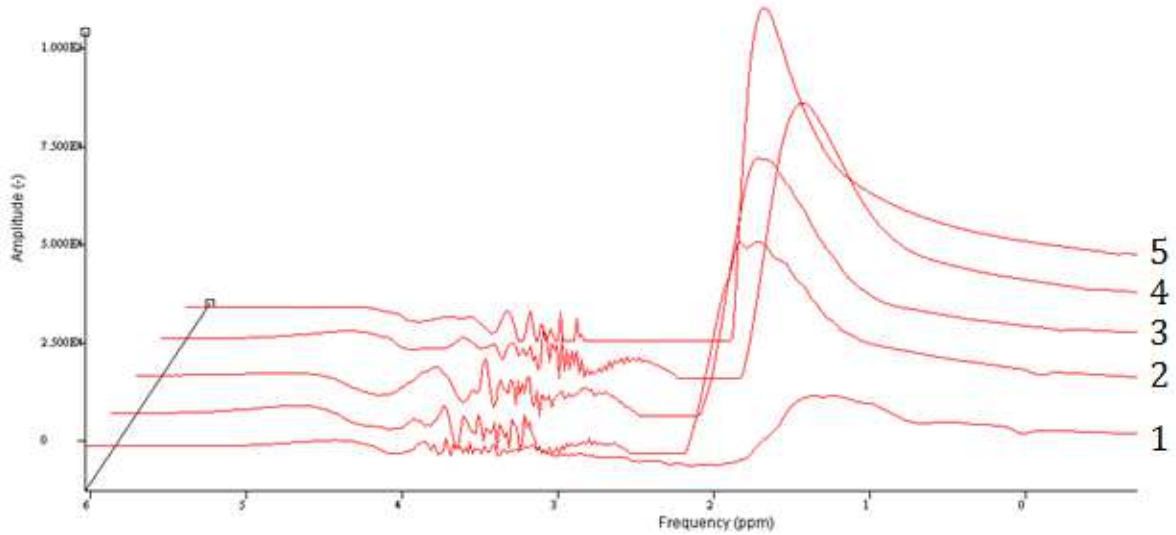


Figure 24. This pictures shows the spectra from the processed data with jMRUI. 1) Is the spectrum from a simple portal vein phantom measurement to detect lactate with a velocity of 65ml/min, 2) a velocity of 160 ml/min, 3) a velocity of 500 ml/min, 4) a velocity of 1000 ml/min, and 5) a velocity of 1350 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover, for all spectra. The lactate (doublet) at 1.3 ppm can be identified.

argued that the velocity has an influence on the lactate molecules (Weber, Rambau et al. 1993). It is possible that the molecules are easier to detect due to the flow, but it is also possible that lactate molecules displace with more ease in a higher flow velocities, and that more molecules displace which leads in more detected lactate molecules. This will change when other molecules are added to the solution, compared to the human blood. In the human blood flow, the molecules will flow differently from a pure lactate solution, and can affect the lactate peak. The discussion on the influence of other molecules, e.g. lipids, will continue in the next chapter.

As mentioned in the previous section, the curve in the tube can have an influence on the detection of molecules. This curve cannot have an influence on the measurements performed with the simple portal vein phantom. Thereby, with the extended portal vein phantom all measurements are performed with an approximate velocity of 1000 ml/min. The pump with voltage-meter is used with the same settings during all measurements, so the velocity can slightly differ, but that can have no large influence on the detected lactate molecules. So, although the velocity can have an influence on the calculated lactate concentration between different velocities, the same velocity will not induce that amount of change between measurements. The same velocity cannot explain the large drop in calculated/detected concentration between 10 and 5 mM.

Besides velocity, an explanation of the concentration can be based on the storage of the solution between days of experiments. According to Sigma-Aldrich, the optimal storage condition of L-lactate as well as for DL-lactate is 2-8 °C in a glass bottle (Sigma-Aldrich 2015; Sigma-Aldrich 2015). The lactate solutions are stored at a workspace in a laboratory in a plastic can. This could have an influence on the concentration used during follow up experiments (Caner, Vergano et al. 1998). Although the experiments to detect different concentrations were performed during the same day, the concentration used was 'old'. The 20 mM starting solution was already used in previous experiments. This storage of the solution could be a possible explanation of the difference between calculated concentration and 'actual' used concentration. An option to overcome this storage problem in further experiments is the use of alanine. It is possible to store alanine and it has the same signal in the spectrum.

To conclude and to answer the sub-question of this section *'Which concentration of lactate is still possible to detect and measure?'*, the concentration that is possible to measure is 2.5 mM. This is possible even a lower concentration, so it is not possible to give a definite conclusion about the lowest possible concentration to detect. However, the hypothesis can be rejected, because it the hypothesis that it will be difficult (even impossible) to detect a lactate concentration of 10 mM or lower with the use of the same set-up, is false.

4.4 Conclusion

This second part of the study starts with the extended portal vein phantom. The tube mimicking the portal vein is situated in a box which mimics the human tissue. From the experiments with the adjustable height, it is shown that it is possible to detect lactate at almost all positions of the tube inside the body. All previous experiments are performed with a 20 mM lactate concentration, which is at the high end of pathological lactate concentrations. The possibility to measure lower concentrations is necessary for clinical practice. In the second experiments of

this part, form pathological to physiological lactate concentrations, the concentration is lowered stepwise. Although it is shown that a 2.5 mM lactate concentration is still detected, this part of the research needs follow up. The same experiment can be repeated with the use of a newly prepared lactate solution and/or different dilution steps.

5. Human portal vein

The last and third part of this study is a further translation from experimental settings towards clinical settings. The set-up is already translatable to the clinic, and it is possible to measure lactate in a blood flow velocity at different heights in a human body. And although it seems possible to detect low concentrations of lactate, this is still a pure lactate in water solution. The human blood consists of more molecules, therefore more experiments are necessary. The pure lactate solution will be adjusted in this part, and finally measurements on human test subjects are performed to evaluate the used sequence.

5.1 Materials and Method

5.1.1 Lactate in a fat emulsion

Besides lactate, human blood contains a lot more molecules. In the experiments of the previous chapters, only lactate is used in the prepared solution. Molecules in the human blood such as proteins, lipids, lipoproteins, cholesterol, metabolites and ions, vary in size, mobility and concentration. With the use of spectroscopy the blood profile can be visualized and used in the clinic for diagnosis (Jupin, Michiels et al. 2013). In normal individuals the profile can be similar, but under pathological conditions the content can vary (Csonska 1918; Jupin, Michiels et al. 2013). For the detection of lactate in the human blood, the peak is visualized at 1.3 ppm, but unfortunately a peak belonging to lipids can overlap the lactate peak (table 2). Thereby comes also the fact that with normal scaling the lipid and water peaks would be that huge, that other metabolites cannot be visible (Hesselink 2014).

The possible overlap of lipids and lactate can become a problem with the measurements of the lactate concentration in the blood. To investigate this, the following described set-up is used to elaborate on the sub-question *'Is the lactate peak detectable with the addition of lipids to the solution?'* Therefore an experiment with the use of intralipid (intralipid 20%, Fresenius Kabi B.V., 's-Hertogenbosch), obtained from Fresenius Kabi B.V., is performed. This intralipid emulsion contained purified soybean oil, purified egg phospholipids, glycerol, sodium and water. For the experiment a 4 L solution with 5 mM lactate is prepared. To this solution the 20% intralipid solution is used to add lipids. To mimic the concentration in the human blood a 0.2% lipid solution is prepared. After the first measurement there is a large peak visualized within the

Metabolite	ppm
Lipids	0.9 – 1.4
Lactate	1.3
NAA	2.0
Glutamine/GABA	2.2 – 2.4
Creatine	3.0
Choline	3.2

Table 2. Observable proton metabolites in MRS (Hesselink 2014).

1.2-1.4 ppm region. Possibly there are two small peaks at the left side of the peak caused by the lactate. To know this with more certainty the lipid concentration is halved and the experiment is repeated.

For these experiments a single voxel STEAM sequence is used. A short TE, long TE, and water suppression switched off are compared. With the longer TE the lactate peak was inverted, so the lipids and lactate could cancel each other. After these first experiments with the lipid-lactate solution (0.1% lipid, 5 mM lactate) in a stationary situation and the single voxel sequence, the solution is added into the extended phantom. With the use of the same CSI sequence with BIR 45º pulse as described for previous experiments the lipid-lactate solution (0.1% lipid, 5 mM lactate) is measured. A shim is performed before spectroscopy. For the shimming process the whole model is used, including tube and whole content of the box. Thereafter the spectroscopy is performed with the use of short and long echo times. For these experiments the high flow velocity, 1000 mL/min, is used.

5.1.2 Localization of portal vein; in vivo measurements

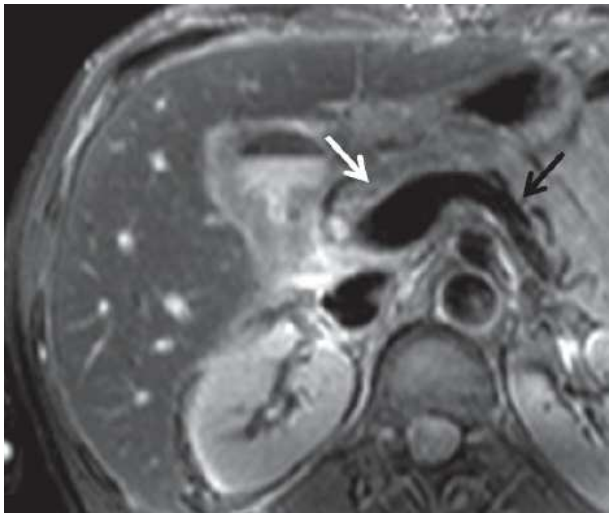


Figure 25. MRI axial T2-weighted image, with the main portal vein (white arrow) and the confluence of splenic vein (black arrow) and superior mesenteric artery (Roberts, Mazzariol et al. 2011).

MRI is useful to create a map of the anatomy involving the portal venous system (Figure 25). The portal venous system comprises all the veins draining the abdominal part of the digestive tract (Lupescu, Masala et al. 2006). For this study at least the portal vein should be localized in the patient to determine the VOI which can be used for the spectroscopy. Therefore is it necessary that the portal vein is visible in the images acquired with MRI. The first measurements are performed with a healthy small woman, 55 kg. The volunteer is in supine position. Different sequences are used and evaluated for the detection of the portal vein, the aorta, the inferior cava vein and the bifurcation in the liver. A prospective acquisition correction (PACE) is tested to overcome the artifacts due to respiration.

Artifacts caused by respiratory motion are one of the major problems. These artifacts can produce image blurring, ghosting, loss of signal intensity, and misregistration, and thereby obscuring the interesting anatomical structures (Kim, Kim et al. 2008). Various techniques have been used to reduce respiratory artifacts. In the PACE technique, virtual respiratory monitoring with navigator echoes is used to trace the movement of the diaphragm during the respiratory cycle (Asbach, Klessen et al. 2005). PACE is a real-time gradient-recalled echo fast low-angle shot navigator sequence for monitoring diaphragmatic movement (Low, Alzate et al. 1997; Kim, Kim et al. 2008). This technique allows the patient to breathe freely during the measurement. It results in an acquisition of images free of motion artifacts in the abdomen without the need for apnea, which is useful in uncooperative patients and in prolonged experiments (Basaran, Agildere et al. 2008).

A second experiment is performed with a healthy male volunteer, 70 kg. He had a prone position. The sequences with the best results after the first measurements with the women are used. After localization of the portal vein a next measurement with the CSI sequence is performed for spectroscopy. To perform a measurement of the total abdomen volume this experiment took almost 50 minutes. Before measurement it is tried to achieve a reasonable shim, in spite of the abdominal movements. These two experiments are the first tryouts to explore whether or not it is possible to use the selected sequence for measurements on patients, to diagnose. In the discussion part of this chapter, these experiments are used to elaborate on the sub-question *'What will happen when the sequence is used during 'real' measurements on humans?'*

5.2 Results

5.2.1 Lactate in a fat emulsion

From literature research it is known that near to the lactate peak at 1.3 ppm possible lipid peaks are detectable in the human blood stream. With the use of the lipid with lactate solution, prepared as described in materials and method, it is investigated whether lactate is still detectable or not. At first the lipid concentration seems too high, but with 0.3% lipid there is a lactate peak possibly visible with a single voxel sequence. With a dilution to 0.04% lipid in lactate, there are clear lactate peaks visible (see Figure 26).

From these results it can be concluded that with a physiological lipid concentration it is difficult to detect the lactate peak, but if the lipid concentration is lowered the lactate doublet is easily detectable. This will be the main problem to overcome during further research, the possible solutions to this have to be investigated.

5.2.2 Localization of portal vein; in vivo measurements

The portal vein is located in two test persons with the use of a 3 T MRI scanner. After

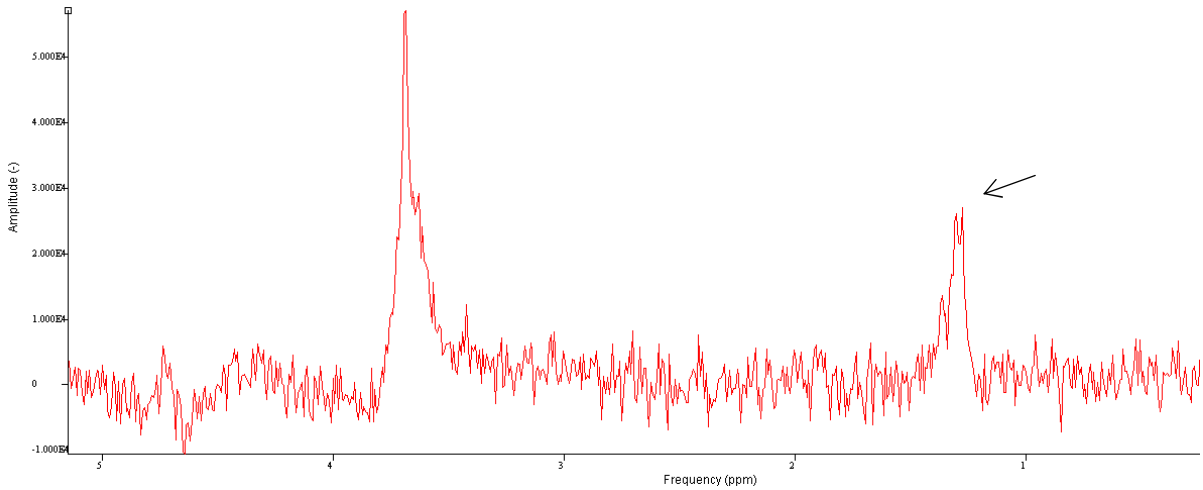


Figure 26. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from a simple portal vein phantom measurement to detect 5 mM lactate and 0.04% intra-lipid, with a velocity of 1000 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. The lactate (doublet) at 1.3 ppm can be identified, and a large lipid peak at 3.7 ppm.

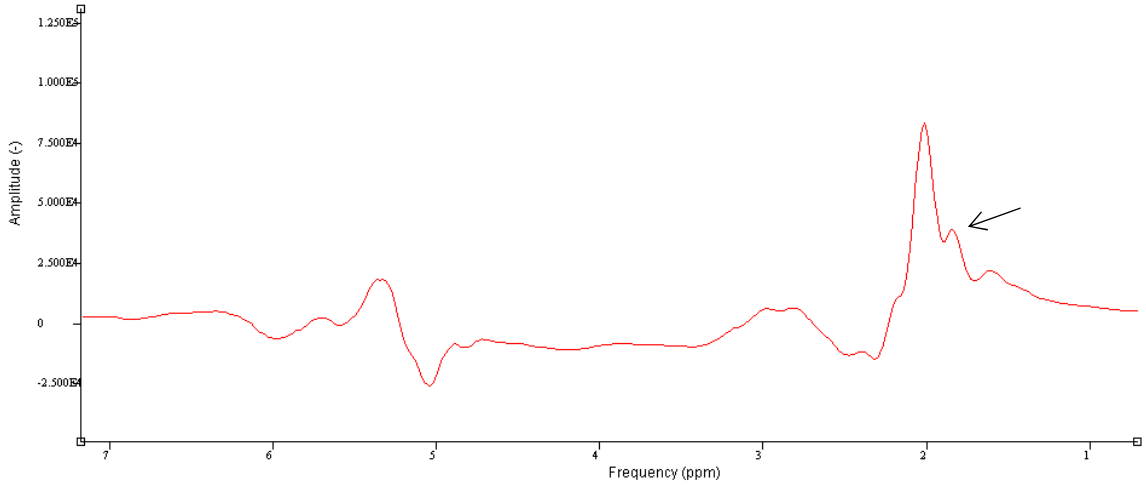


Figure 27. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from an in vitro measurement to locate the portal vein and measure lactate. The water peak at 5 ppm is removed with the use of peak-remover. The lactate doublet at 1.5 ppm can be identified, next to probably a lipid peak.

localization the same sequences, which was used during the previous experiments, is now used to measure lactate in the portal vein. In the same range as lactate, 1.3 ppm, there are lipid peaks detectable in the normal blood flow. With the use of jMRUI and the function peak remover, it was possible to remove the water peak and make the possible lactate peak visible (see Figure 27).

From Figure 27 and Figure 28 it can be concluded that it is possible to detect lactate in the portal vein of a living human being. This was a normal, healthy human being in rest, so there is probably no increased lactate concentration. However, the distinction between lipids and lactate is difficult to make at this moment, as already mentioned in section 4.6.

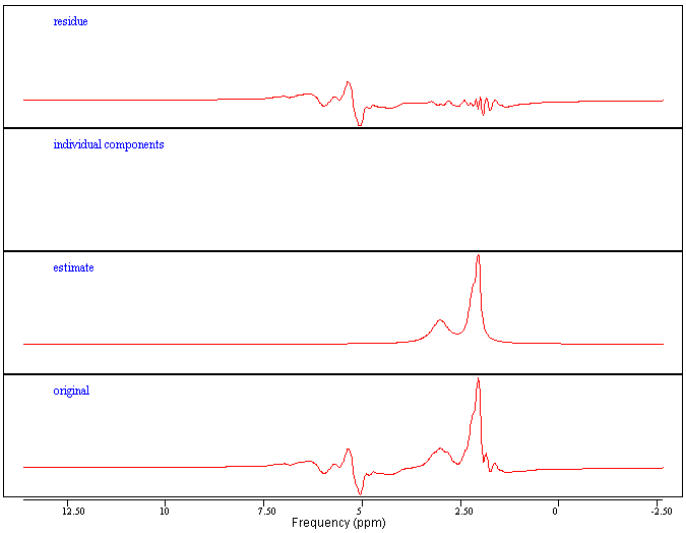


Figure 28. Processed data with jMRUI after in vivo measurement to locate the portal vein and detect lactate. The lactate doublet is not visible at 1.3 ppm. In the upper picture the residue after analysis. In the second graph are the individual components shown of the peaks. In the third picture the estimated doublet is calculated. The spectrum below is the original spectrum.

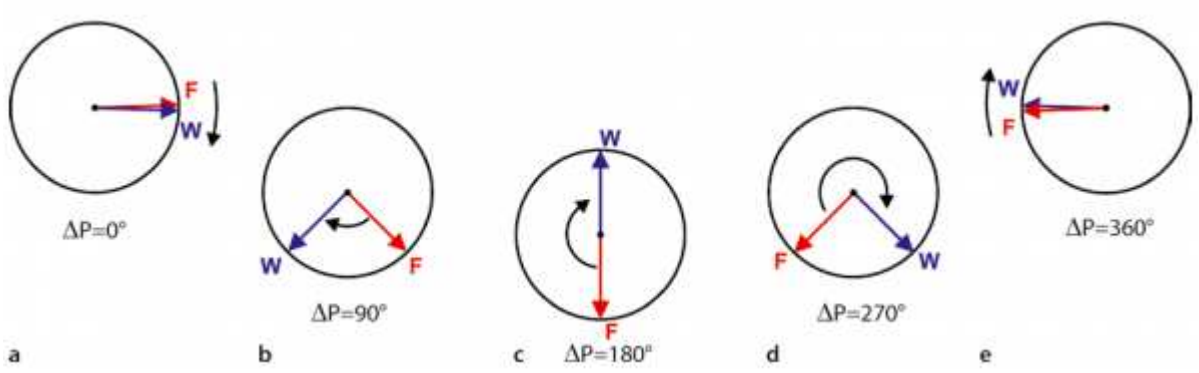


Figure 29. Signal loss due to chemical shift. The water and lipid protons are excited simultaneously with a non-selective RF pulse with a different phase difference (stepwise form 0º to 360º in figure a) to e) respectively). ΔP = phase difference, F = fat, and W = water (Chrysikopoulos 2009).

5.3 Discussion

5.3.1 Lactate in fat emulsion

The human blood contains a diversity of molecules, including lactate. Most of these different molecules appear at a different position (ppm) in the spectroscopy spectrum of the human blood, however the doublet of lactate at 1.3 ppm can be overlapped by a peak acquired from lipids. This has a negative outcome for the measurements performed in this study. It is thought that the lipids will overlap the lactate doublet, but to evaluate this the sub-question *'Is the lactate peak detectable with the addition of lipids to the solution?'* requires an answer.

The use of lipids makes it harder to detect lactate. However, with the use of a single voxel sequence it is possible to distinguish between lactate and lipids round 1.3 ppm. The best option at this moment to discern lactate form lipid is with the use of a sequence that would invert the lactate peak. Although it is possible to distinguish between lipid and lactate with the use of single voxel sequence, it is not possible to make a distinction between the two peaks with the use of the [csi_fid_bir45_nodec_lowres] sequence. Sequence building is necessary to have a combination of the different ideal sequences to detect lactate and discern lactate from lipids. It seems that CSI is the optimal sequence to detect lactate in high concentrations as well as low concentrations and in low flow velocity as well as in high velocity, but this sequence is not optimal to distinguish lactate in a fat emulsion. There are lactate editing techniques, but these are out of the scope of this research. Follow up study should start with a focus on these techniques.

A suggestion to solve the lipid-lactate problem for measurements with real human blood, is shown in Figure 29. The vectors start in phase, but will diverge and go through interference that is repetitively destructive and constructive. This phenomenon between fat and water protons, is a consequence of the difference in the Larmor frequency. In this way periodical overlap and opposition can be predicted, in phase or out of phase (Chrysikopoulos 2009). In further research it can be evaluated if this method is applicable for the distinction between lipids and lactate. With the use of intermediate TE (1.35 s), the lactate peak will inverts, but the lipid peaks do not invert (Figure 30).

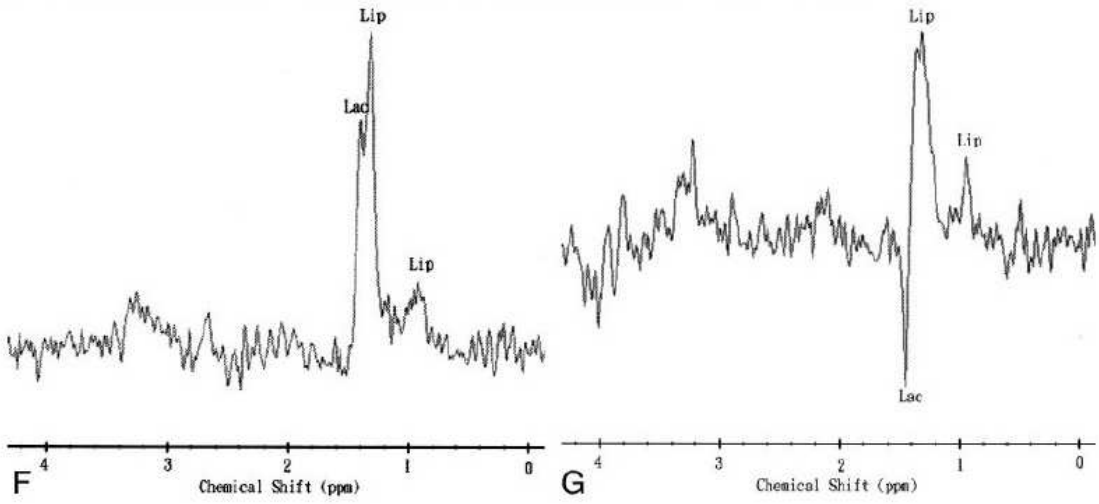


Figure 30. Two different spectra acquired from measurements in a pig. Data acquired with a short TE are shown in figure G. In figure G an intermediate TE (1.35 s) is applied which result in an inverted lactate peak (Lac), but the lipid peaks (Lip) are not inverted (Vijayan 2012).

The hypothesis of the sub-question can be confirmed, because the peaks by lipids and lactate overlap. Although this seems a problem, it has to be possible to differentiate between the two. Further research is necessary to gain knowledge about the possibilities to distinguish between fat and lactate.

5.3.2 Localization of the portal vein; in vivo measurements

Until now, the sequence is only used to measure lactate, acetate, or lipids in a phantom. Beside some problems which are possible to overcome, the sequence seems usable. It is hypothesized that this sequence is also of use in MRS measurements with humans. The following sub-question is evaluated for further discussion, *'What will happen when the sequence is used during 'real' measurements on humans?*'

During this study three different healthy human test subjects are measured. Each subject is measured with a different method. The first human subject is placed in the MRI in supine position. In this test subject, only the portal vein position is measured. The second subject is placed in prone position, again to determine the position of the portal vein as well as watch what happens when the [csi_fid_bir45_nodec_lowres] MRS sequence is applied in this area. Although the latter two test subjects are both measured with the use of this sequence, the first is shortly measured with the use of a small voxel grid and the latter is measured with a larger voxel grid covering a large part of the body. The large grid resulted in a measuring time of almost 50 minutes.

The portal vein is easily detectable with the use of 'normal' MRI measurements as well as with the use of the CSI sequence used for this study. In prone position it was less difficult to apply the CSI sequence. With the use of the MRS voxel grid just a small part of the body was covered. In the second test subject this grid is enlarged to see the influence of the surrounding tissues. As already mentioned in the previous section, the lipid peak and lactate peak stand in the same region after spectroscopy measurements.

The lactate solution in the portal vein of healthy human subjects is in the range of 0.5 to 2.2 mmol/L. This is even lower than the 5 mM used in the lactate-fat emulsion. Further experiments with human test subjects should be performed, but a solution for the distinction between lactate and lipids has to be investigated before more experiments are performed on human subjects. More measurements are necessary, but the contribution of subjects to this research will only have an added value when we can use the results. If it appears that it is necessary to use another sequence to distinguish, the data of the test subjects measured with the other sequence is useless. So, at this moment it can be argued whether or not the hypothesis should be rejected. The sequence is useful to measure in human test subjects, but it is not possible to discern lactate in the spectrum.

5.4 Conclusion

The last part of this study contains two different experiments, which are designed to determine the possibility to measure lactate in a solution with other molecules. Lactate is measured in fat emulsion with the use of a prepared lactate with lipids solution. This solution is used to see the influence of lipids on the lactate doublet in the spectrum acquired with MRS. It is know that lipids can or will overlap the peaks that belong to lactate. It is important to overcome this difficulty before MRS can become a diagnostic tool. Further research is necessary to investigate the possibilities to discern the lactate doublet form the lipid peaks. The same applies for the last part of this study. Two measurements of healthy human test subjects are performed to see the capability of the CSI sequence to detect the portal vein and generate a spectrum. The portal vein is detectable an MRS can be performed in this portal vein, but it is not certain whether the lactate peak can be distinguished.

Overall, it seems a promising technology, but especially for this part of the study, further research is necessary. The main concerns resulting from the experiments in this study is the required short TE, induced with the CSI sequence, to have a lactate signal in high flow velocities. In contrast to the long TE time necessary to invert the lactate peak in the spectrum, as an opportunity to distinguish it from the lipid peak. This TE antithesis and possible other lactate editing techniques have to be investigated, possibly with the development of a specialized sequence in further research.

6. Recommendations

More basic research need to be done before the glimpse on the horizon, a noninvasive, universally applicable, accurate, short-term diagnostic test to distinguish patients with CSS from patienst with CSD, become within clinical reach. In this chapter a summary of the recommendations is given and future perspectives are discussed.

First, the program to analyze the data has to be evaluated with the use of a different data analysis. In this way, some of the findings described in this study must be reevaluate. The program of choice is 3DiCSI to analyze the data acquired with a CSI sequence, such as the one used in this study. As already mentioned, due to technological difficulties this was not possible during this study, therefore jMRUI is chose which is also a suitable program to analyze MRS data. However, it can be useful to compare the data of some experiments to investigate whether or not this 3DiCSI, or even other programs to analyze MRS data, give different results.

Second, a point for discussion is from which vein or area the MRS sample should be taken. Why the portal vein? In the introduction it is mentioned that the portal vein is the vein through which all 'waste' from the bowel system flows. And that the liver is already processing all 'waste' which results in different concentrations all over the organ (Murray, Gonze et al. 1994). However, Murray et al. (1994) give other arguments about the lactate levels. They argue that the portal vein preferentially receives blood from the bowel which can contain lactate. D-lactate is not metabolized in the liver, which means that the concentrations in the peripheral veins are probably closely correlate to levels in the portal vein. In a study with the use of a rat with mesenteric ischemia, they have shown that D-lactate levels in the peripheral veins are elevated at 2 hours (Murray, Gonze et al. 1994). This finding suggests that measuring the portal vein is a good option, but not the only option.

Third, following this discussion, whether or not is it possible and necessary to distinguish L-lactate from D-lactate. If D-lactate, which is the isomer of lactate that increase with CSS or CSD, is not metabolized, the peripheral veins are a better option for diagnosis. If the normal (L-)lactate concentration in the portal vein is ranging from 0.5 to 2.2 mmol/L this would not have a large influence on the increased lactate concentration due to ischemia. Thus, even if it is not possible to distinguish between the two isomers, it is probably not necessary because the influence on the total amount of lactate is negligible. Besides this, the ability to detect lactate increases with a higher concentration.

In a study by Kolkman et al. (2008), it is suggested to use contrast enhanced MRA, because it gives the possibility to measure the actual flow. It is shown in studies with healthy volunteers and patients that there is an increase in flow velocity, with a decrease in total flow, after a meal in the patients with stenosis compared with the healthy volunteers. Results from these studies are promising for the use of MRA as a method for diagnosis of patients with CSS (Kolkman, Bargeman et al. 2008). However, this study would be useful as a tool to diagnose CSS, but the use of MRS to measure lactate concentrations has the potential to produce more accurate

results. If the MRS diagnosis seems to be sensitive to diagnose CSS or CSD patients, the whole diagnostic process can possibly change. MRS can become a replacement for tonometry.

The fourth important step to take is analyze the possibility to distinguish between lactate and lipids with the use of [csi_fid_bir45_nodec_lowres], or investigate whether or not it is beneficial to create a new sequence. In previous sections the difficulties to differentiate the lipid peaks form the lactate doublet are already lined out, and it should be clear that this point needs focus in further research. When it is possible to overcome the lipid-fat problem, further research will become easier with its way towards clinical settings. Besides analyzing the sequence for the phantom also optimization of the sequence for in vivo measurement has to be taken into account. When it becomes possible to create a more sequence which is even more accurate to detect lactate in a flow, this sequence should be applicable to measure in human (test) subjects.

When it is possible to differentiate between the lipid and lactate peaks, the fifth step can be to set-up an experiment with healthy humans an eventually patients. The last sub-question of this study, *'How can we design an experiment with (healthy) human beings?'* First, the lactate concentration in healthy human beings can increase with the use of an exercise test. This will be useful for the measurement of lactate in the portal vein. The optimal settings to measure humans can be determined as well as the value of the MRS to detect lactate. The following step is to measure and evaluate the set-up in experiments with patients. Whether or not it is possible to detect lactate in the healthy subjects, it is beneficial to perform three or more measurements on patients as well before a conclusion of the usability of MRS to diagnose CSS or CSD can be drawn. After this it is possible to refine the diagnosis for patients with the use of follow-up experiments. For both experiments, with healthy test subjects and patients, a METC request is necessary. This has to be taken into consideration with the design of experiments on test subjects.

The consideration how to embed this technology into current practice has to be questioned and analyzed. An important discussion contains the impact of this technology, MRS to diagnose ischemia. The current diagnosis of CSS and CSD consists of several steps with the use of multiple non-invasive as well as invasive diagnostic tools. The best care for a patient is to diagnose without invasive tools. However, at this moment the physician is not able to give this care, so the best care from his point of view is with the use of gastric exercise tonometry (GET). Which care is demanded? Probably, the care with the use of all different diagnostic tools to have a diagnose with high sensitivity and specificity, what will lead to the highest quality of life for the patient (Beauchamp and Childress 2001). This is the most beneficial for both patient and physician. The best care will include the use of invasive technologies, such as GET, at this moment, but it is expected that these invasive tools can be dismissed with the use of MR-spectroscopy. When portal vein lactate MRS becomes clinical applicable the protocol to diagnose CSS or CSD can change, the provided best care by the physician and the sought best care by the patient may move towards one another.

In conclusion vein lactate MRS is a promising technology to diagnose intestinal ischemia. The work described in this master thesis has taken this technique a great stride towards clinical application, but further basic research is necessary before a clinical trial can be started .

7. Conclusion

Multiple experiments are performed in an attempt to answer the main question: *'Is it possible to measure physiological and pathological concentrations of lactate in the portal vein with the use of Magnetic Resonance Spectroscopy, to diagnose patients with Chronic Splanchnic Syndrome?'* An answer to this question is given in this last part of the study. The study consists of seven different part, which all together lead to the answer of the main question. Each part consist of a sub-question used for the set-up of the experiment (as described in materials and method), following results, which lead to a discussion and conclusion. The overall conclusion of the study is given in this part.

From the first part, sequence determination, it is clear that the preprogrammed [csi_fid_bir45_nodec_lowres] sequence, consisting of Chemical Shift Imaging (CSI) and B_1-insensitive rotation (BIR) is useful to detect lactate concentrations with Magnetic Resonance Spectroscopy (MRS). The second part, simple portal vein, shows that a velocity of 1350 ml/min lactate is still detectable, which is even higher than the average velocity of the human blood flow (864±188 ml/min). In the third part, different coils, it is investigated whether or not it is possible to use coils which are used in current clinical practice. It is evident that the body with spine coil can be suitable, but an extension of the phantom is necessary. However, from these first three parts it can be concluded that it is possible to measure lactate with MRS in velocities comparable to the human body. The next step is to expand the phantom and mimic the human body in more detail.

With this extended portal vein phantom, the experiments of the fourth part are performed. The height of the tube, mimicking the portal vein, is adjusted in the bodily part of this phantom. From this experiment can be concluded that it is possible to detect lactate at almost all positions of the tube inside the body, but the most important is possible to detect the lactate solution in with the tube situated in the middle of the box. The first time in which the lactate concentration is lower than 20 Mm is during the fifth experiments, from pathological to physiological lactate concentrations. It is shown that a 2.5 mM lactate concentration is still detectable with MRS. However, this part of the study needs follow up, for example by repetition of same experiment or use of a newly prepared lactate solution and/or different dilution steps. The findings in these two parts are important, because it seems that MRS is suitable to detect lactate concentrations near physiological concentrations at different heights in the body. However, these experiments are performed with pure lactate solutions, and other molecules can have an influence on the detectability of lactate.

From the sixth part, lactate in a fat emulsion, can be concluded that it is not possible to distinguish between lactate and lipid peaks in this study. In the detection of lactate with lipid solution, with concentrations comparable to the human body, there is an overlap of the peaks that belong to lipids and lactate, around 1.3 ppm. Further research is necessary to investigate the possibilities to discern the lactate doublet form the lipid peaks, because it seems that it is possible to distinguish between lactate and lipids, for example during data analysis. The same applies for the last and seventh part of this study, in vivo measurements. Although it is possible

to use the CSI sequence to detect the portal vein in three healthy human test subjects and to perform MRS in this portal vein, it is not certain whether the lactate peak can be distinguished or not. From these parts can be concluded that it seems possible to measure the portal vein in human beings, but it is not evident that lactate concentrations can be measured. It is necessary to find a solution for the distinction between lactate and lipid peaks.

Overall can be concluded that it seems possible to measure physiological and pathological concentrations of lactate in the portal vein with the use of MRS, to diagnose patients with Chronic Splanchnic Syndrome. It is possible to measure lactate concentrations, ranging from 2.5 to 50 mM, in a flow velocity comparable to the human blood flow. It is possible to measure lactate at different heights within the human body, and it is possible to detect the portal vein and perform MRS with the use of the CSI sequence. Thus, portal vein lactate MRS is still a promising diagnostic tool to diagnose intestinal ischemia, but some hurdles need to be overcome in following studies. The aim for follow up research should start with the investigation of options to discern the lactate doublet from the overlapping lipid peaks. When this becomes possible, follow up research with the use of (healthy) human test subjects is the next step. MRS as a less invasive and time consuming technology for the diagnosis of intestinal ischemia is auspicious, but still several steps away.

8. Appendices

8.1 Appendix A – Results different velocity measurements simple portal vein phantom

There were measurements performed with velocities between 65 and 1350 ml/min to detect lactate in the portal vein model.

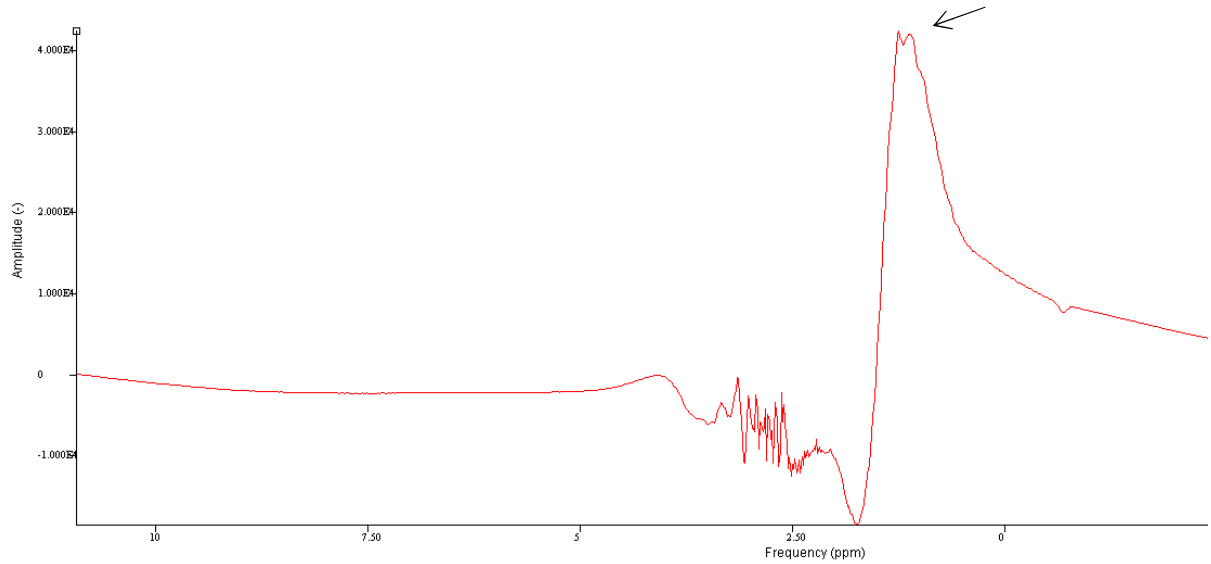


Figure 31. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from a simple portal vein phantom measurement to detect lactate with a velocity of 160 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. The lactate doublet at 1.3 ppm can be identified.

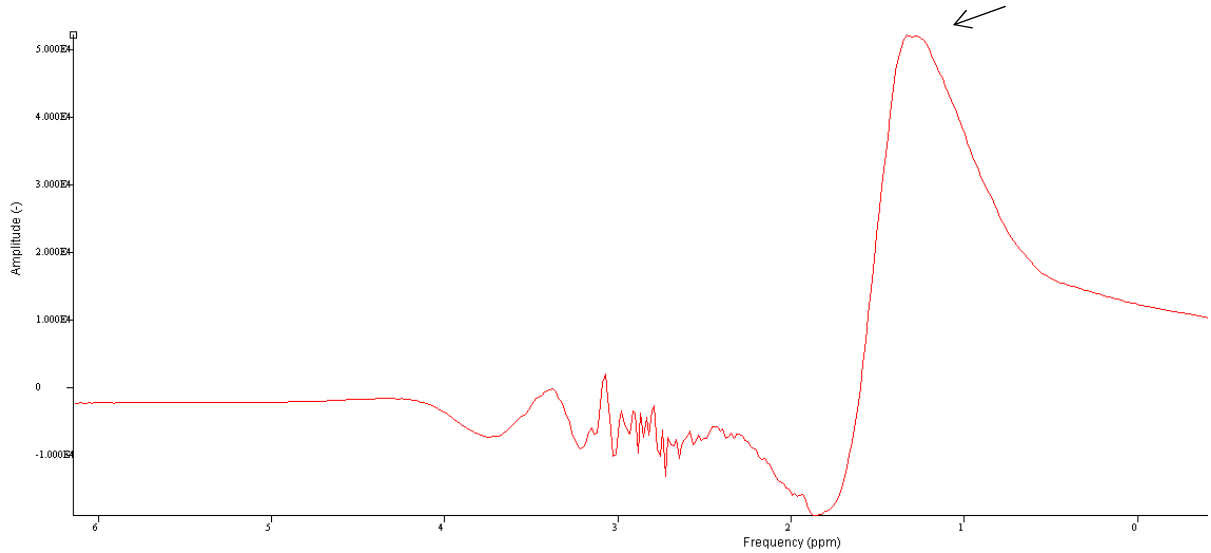


Figure 32. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from a simple portal vein phantom measurement to detect lactate with a velocity of 500 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. The lactate doublet at 1.3 ppm can be identified.

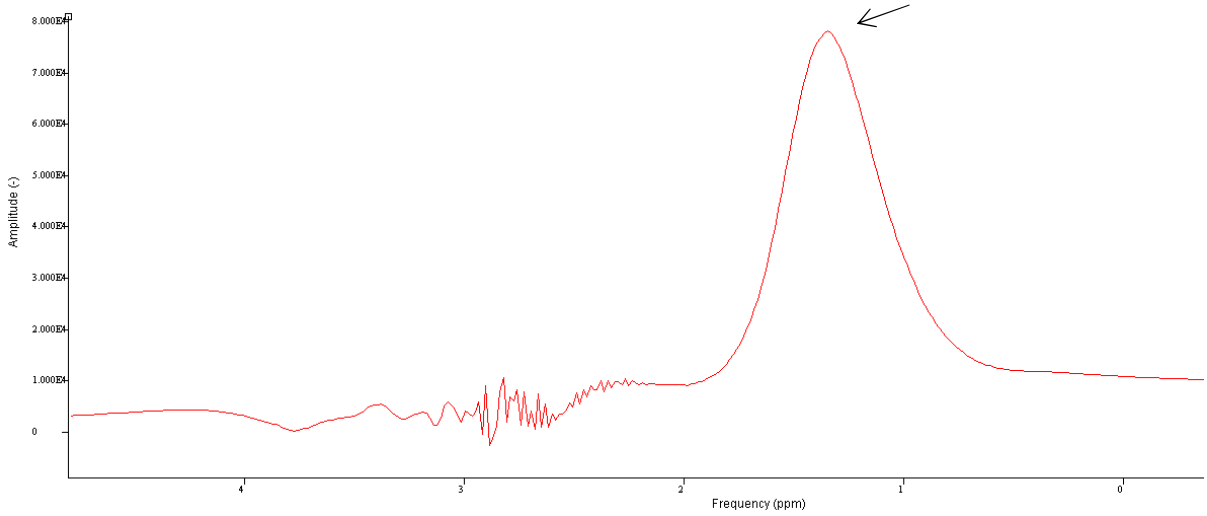


Figure 33. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from a simple portal vein phantom measurement to detect lactate with a velocity of 1000 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. The lactate (doublet) at 1.3 ppm can be identified.

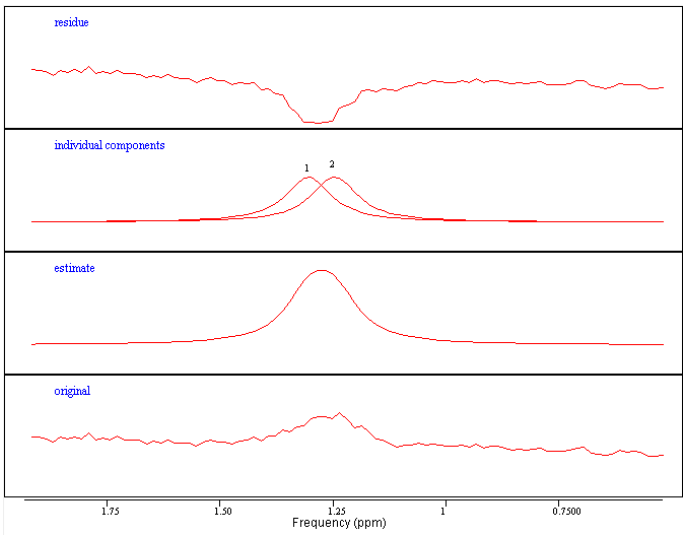


Figure 34. Processed data with jMRUI after measurement with a 20 mM lactate concentration spectrum with a flow velocity of 400 ml/min. The lactate doublet is visible at 1.3 ppm. In the upper picture the residue after analysis. In the second graph are the individual components shown of the peaks. In the third picture the estimated doublet is calculated. The spectrum below is the original lactate doublet.

8.2 Appendix B – Results different height measurements extensive portal vein phantom

In the results most of the figures acquired from the height experiments are presented, but there were some additional figures. In the figures below it is shown that 8 cm above the spine coil the doublet of lactate already disappears. Furthermore during the MRS measurement there was still lactate and acetate visible at all heights.

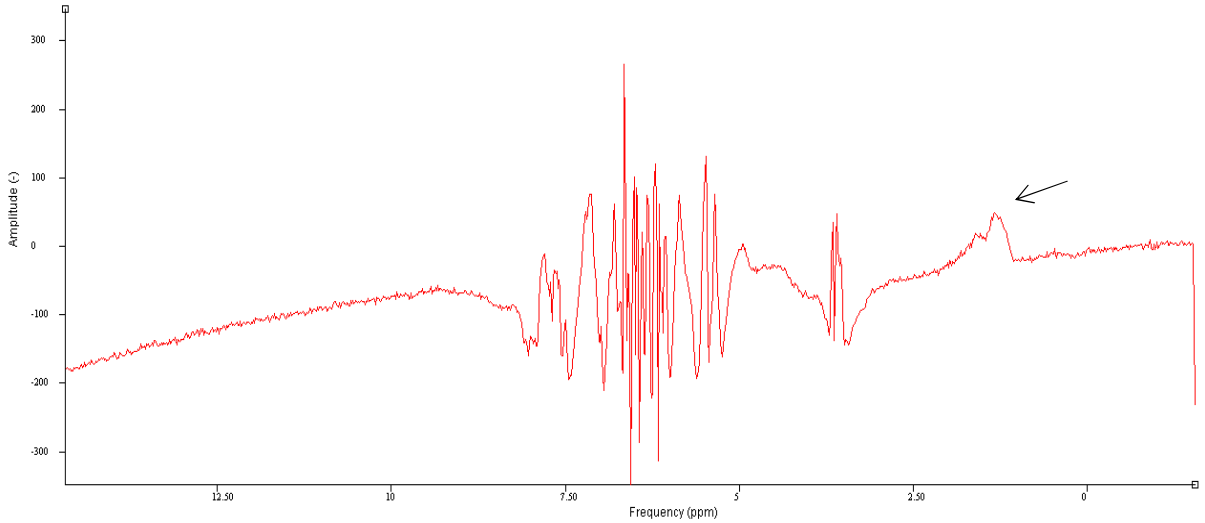
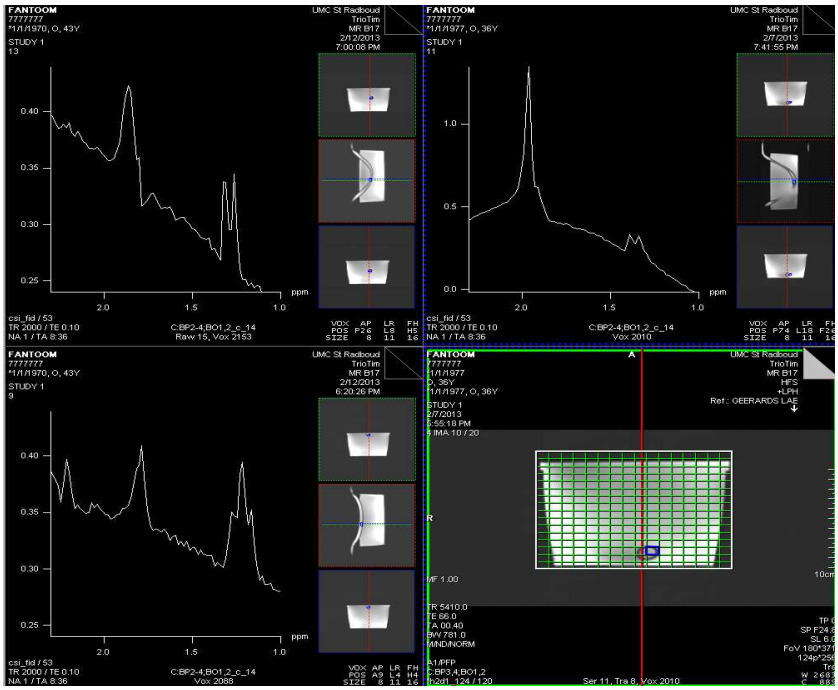


Figure 35. This pictures shows the spectrum from the processed data with jMRUI. This is the spectrum from the extended portal vein phantom measurement to detect lactate in the tube 8 cm above the spine coil. This measurement was performed with 20 mM lactate solution and a velocity of 1000 ml/min. The water peak at 4.7 ppm is removed with the use of peak-remover. A possible lactate doublet at 1.3 ppm can be identified.



Figuur 36. Direct analysis of the MRS results during the measurements. This is a measurement with 20 mM lactate in the tube and 5 mM acetate in the 'body' with a flow velocity of 1000 ml/min. Round 1.3 ppm the lactate doublet and round 1.9 ppm the acetate peak is visible in all measurements. The above left picture was during a measurement 6 cm above the spine coil, above right was 2 cm above the spine coil, below left is 10 cm above spine coil, and below right is the box with the voxel grid and the chosen voxel to analyze within the tube.

8.3 Appendix C – Portal vein data CT scans

To gain knowledge about the average diameter and distances of the portal vein within the human body, 16 CT scans from the patient database were used. The age, diameter of the portal vein (PV), distance between portal vein and abdominal wall (AW), and distance between portal vein and back (B) were measured.

Man	Age	Diameter PV	Distance PV-AW	Distance PV-B
1	78	12.71	126	182
2	76	11.18	115	151
3	67	12.24	129	189
4	79	10.77	135	152
5	77	11.55	99	137
6	67	10.71	112	139
7	70	12.63	104	137
8	84	12.04	102	167
9	74	12.27	91	141
10	62	12.53	104	129
mean	73	11.9	112	152
stdev	6.7	0.759	14.4	20.4

Table 3. This table contains all data of the 10 man measured with the use of CT scans. PV = portal vein, AW = abdominal wall, and B = back. Below the thick line the average (mean) and standard deviation (stdev) of the calculations.

Women	Age	Diameter PV	Distance PV-AW	Distance PV-B
1	82	8.51	61	109
2	64	8.56	58	119
3	85	9.82	55	152
4	74	11.74	76	108
5	72	8.72	58	113
6	79	9.11	62	124
mean	76	9.41	61.6	121
stdev	7.6	1.24	7.45	16.4

Table 4. This table contains all data of the 6 women measured with the use of CT scans. PV = portal vein, AW = abdominal wall, and B = back. Below the thick line the average (mean) and standard deviation (stdev) of the calculations.

8.4 Appendix D – Results different lactate concentrations

With the experiments to different lactate concentrations, not only 20 and 2.5 mM were measured and analyzed, but also 10 and 5 mM to complete the lactate concentration curve. For the calculations needed to make this curve the same concentrations were also measured without the use of the water suppression. Besides the graphs made during the measurements, the amplitude of the lactate peak and the amplitude of the water peak were determined to create the concentration curve.

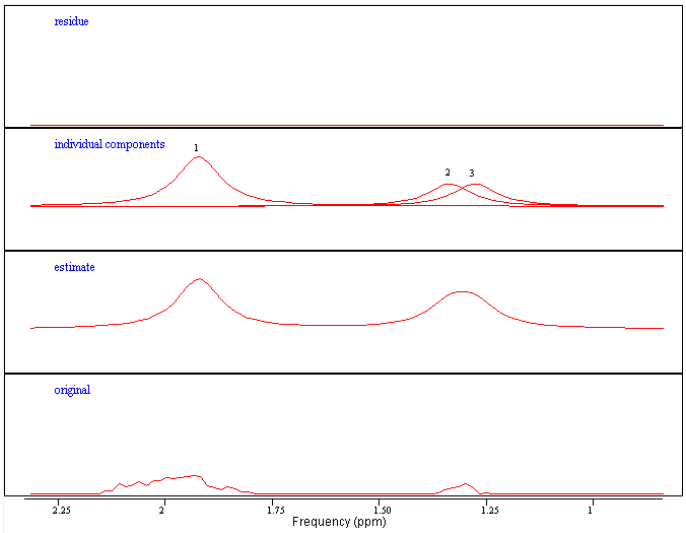


Figure 37. Processed data with jMRUI after measurement with a 10 mM lactate concentration spectrum. The lactate doublet is visible at 1.3 ppm, and water peak at 4.7 ppm after peak remover. In the upper picture the residue after analysis. In the second graph are the individual components shown of the peaks. In the third picture the estimated doublet is calculated. The spectrum below is the original lactate doublet.

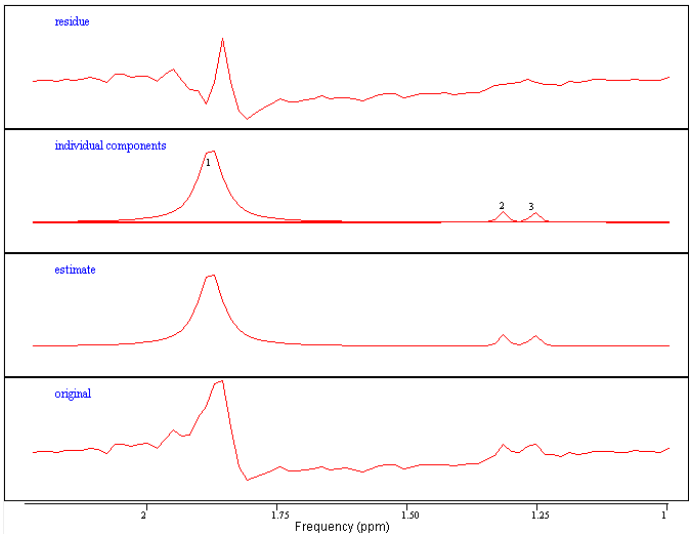


Figure 38. Processed data with jMRUI after measurement with a 5 mM lactate concentration spectrum. The lactate doublet is visible at 1.3 ppm, and water peak at 4.7 ppm after peak remover. In the upper picture the residue after analysis. In the second graph are the individual components shown of the peaks. In the third picture the estimated doublet is calculated. The spectrum below is the original lactate doublet.

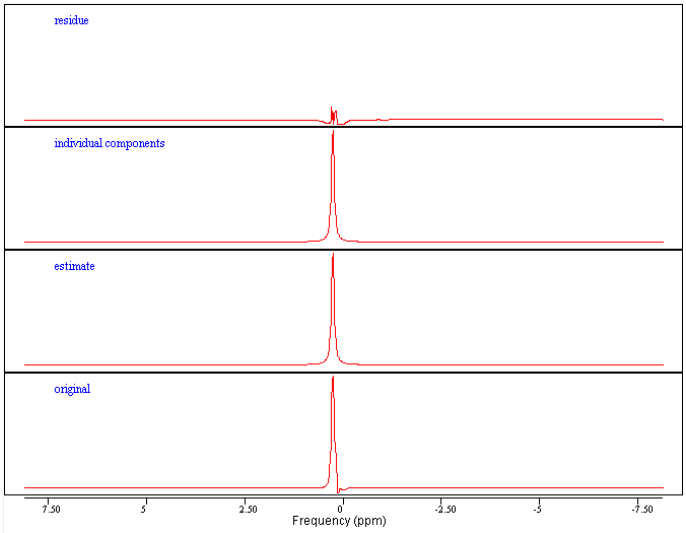


Figure 39. Processed data with jMRUI after measurement with a 20 mM lactate concentration spectrum, without water suppression. The lactate doublet is not visible at 1.3 ppm. In the upper picture the residue after analysis. In the second graph are the individual components shown of the peaks. In the third picture the estimated doublet is calculated. The spectrum below is the original lactate doublet.

Lactate concentration (mM)	Lactate peak amplitude	Water peak amplitude	Measured concentration
20	1.09	27810	2,88E-03
10	0.78	27130	2,12E-03
5	0.12	28190	0,30E-03
2.5	0.05	27020	0,15E-03

Table 5. Numbers measured from the processes jMRUI data. These numbers are used to calculate the measured lactate concentrations.

8.5 Appendix E – Single voxel and multiple voxel measurements from lactate in a fat emulsion

For the measurements of the lactate-lipid emulsion, a single voxel as well as a multiple voxel approach was used to determine and acquire the best results.

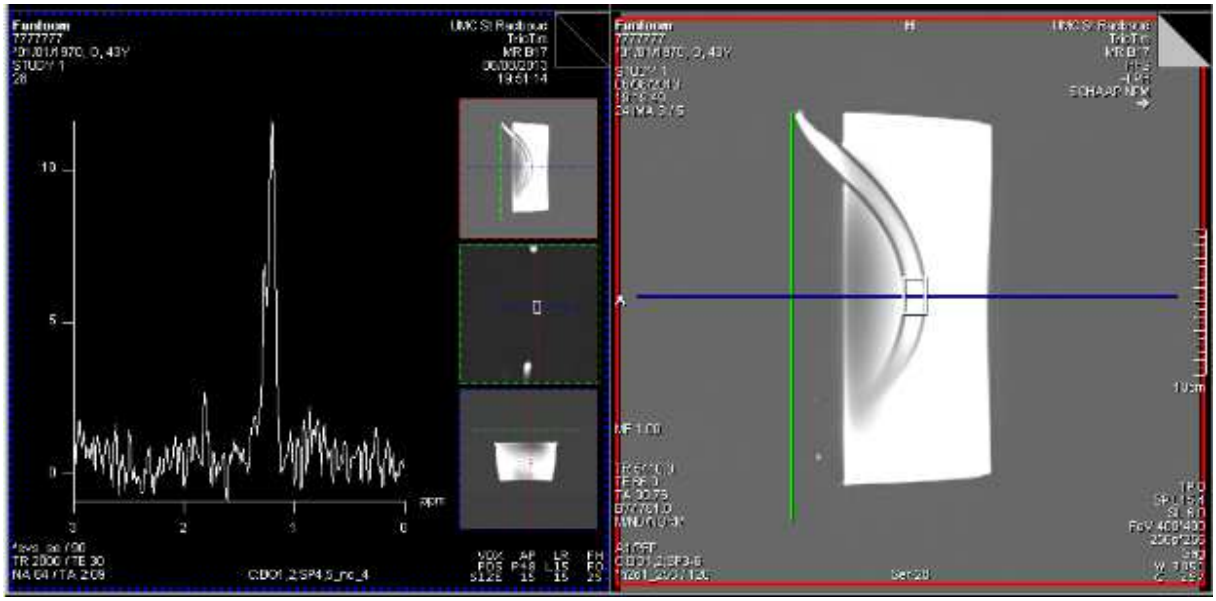


Figure 40. Direct analysis of the MRI results during the measurements. This is a single voxel measurement of 5 mM lactate with 0.04% intra-lipid. Round 1.3 ppm the lactate doublet is visible in the left diagram. In the right diagram the box (body) with inserted tube (portal vein) are visible. In the middle of the box and tube the measured voxel.

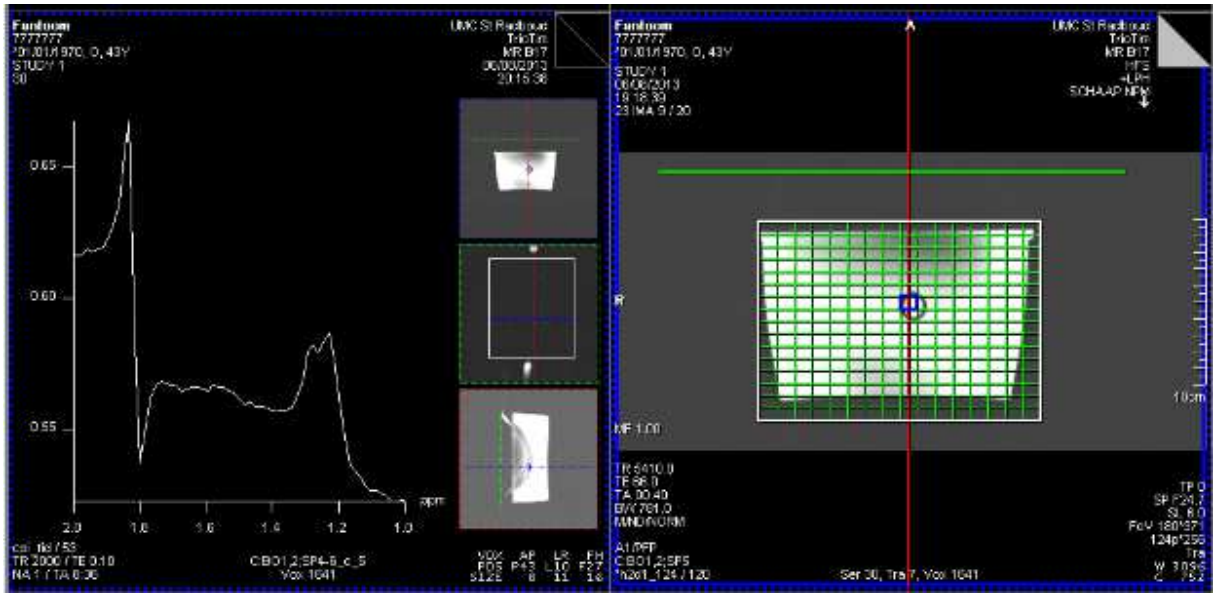


Figure 41. Direct analysis of the MRI results during the measurements. This is a multiple voxel measurement of 5 mM lactate with 0.04% intra-lipid. Round 1.3 ppm the lactate doublet is visible in the left diagram, and round 1.9 ppm a possible acetate peak. In the right diagram the box (body) with the inserted tube (portal vein) are visible. Multiple voxels covering the whole box are measured, to visualize the diagram at the left the blue squared voxel is chosen in the middle of the tube.

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