

Development of Proton Magnetic Resonance Spectroscopy in Human Heart at 3 Tesla

Belén Rial Franco



Michaelmas Term, 2010

Department of Cardiovascular Medicine
University of Oxford

To Maliló

Development of Proton Magnetic Resonance Spectroscopy in Human Heart at 3 Tesla

Belén Rial Franco

Wolfson College, University of Oxford

Abstract

Cardiovascular magnetic resonance imaging (MRI) is a well established technique in clinical cardiology. Different MRI sequences are routinely used to assess cardiac anatomy, function, viability and other parameters that aid diagnosing cardiac disease. Conversely, cardiac magnetic resonance spectroscopy (MRS), the only available method for a non-invasive study of human cardiac metabolism, has not evolved into a clinical tool yet. The combination of both techniques holds great potential to gain insight into the causality of cardiomyopathy diseases or other medical conditions with high cardiovascular risk profile, like diabetes or obesity and improve the clinical management of cardiac diseases.

Nowadays, high field clinical MR systems have the great potential of improving the low spatial and temporal resolution and reproducibility of MRS. The aim of this thesis was to develop and implement a cardiac ^1H -MRS method at 3 T that can be applied in clinical routine for the assessment of creatine and lipid levels in the human myocardium. The methodological developments to advance cardiac MRS are presented first. A robust ^1H -MRS method comprising an optimized single-voxel technique, phased-array coil combination routine, optimized water suppression, breath-hold averaging and post-processing methods were developed. First, reproducibility and feasibility of the method were validated in vivo by acquiring ^1H -MRS of the liver in almost one hundred healthy subjects. Subsequently, myocardial lipids levels were obtained in healthy volunteers by single breath-hold ^1H -MRS triggered to mid-diastole, showing good reproducibility in an acquisition time less than 12 s. The good spectral resolution achieved using this method was demonstrated by the ability to differentiate for the first time two pools of myocardial lipids in spectra from the septum of patients with suspected myocardial lipid excess. Finally, creatine levels for healthy volunteers were investigated using multiple breath-hold acquisitions. Thus, this study shows the practicality and feasibility to incorporate this rapid cardiac ^1H -MRS method into clinical studies of the human myocardium.

Word Count: 25100

Thesis submitted for the degree of Doctor Philosophy
at the University of Oxford

Michaelmas Term, 2010

Acknowledgments

First of all, I would like to express my sincere gratitude to my supervisor Dr Jürgen Schneider for his great support and guidance. Since the day he encouraged me to get on and study for a PhD, he has made this work possible. I would like to thank Dr Matthew Robson for his constant support, endless enthusiasm and help with coding. I feel very fortunate to have had the opportunity to study with both of them. Thanks for their knowledge, patience and tireless reading of this thesis. My deep thanks to Professor Stefan Neubauer for supporting this privileged opportunity to develop my PhD in his group.

Thanks BMRU, for useful, entertaining and sometimes MR related conversations: Kiterie, my MRS-partner, Mahon, Lee-Anne, Kyle, Vicky and also Hannah and Patrick (and biscuits). Thanks OCMR, for early and cheerful mornings, for teaching me the medical aspects of this project: Banjo, thanks for all the scanning time together, ideas and even singing, Joe, Pete, Cameron, Chris, Erica, Saira and Vanessa. Also, a big thanks to the Basic Science group, always ready to help.

Special thanks for all volunteers who have contributed to the results of this thesis. Thank you so much for coming so early in the morning and always being happy to collaborate.

I cannot forget Mamá and Papá and my family who have always been there with their endless support and invaluable wisdom. Gracias.

Finally, I particularly thank Fran, my husband, for his interminable encouragement to always do my best and pursuit of higher goals. Thanks for walking this path with me, even 10000 km away.

This study was financially supported by the British Heart Foundation grant PG/05/115.

Related Publications

1. **Rial B**, Neubauer S, Robson MD, Schneider JE (2008). Non-invasive quantification of fat in normal liver with 3.0 Tesla Proton Magnetic Resonance Spectroscopy. Scientific Meeting International Society for Magnetic Resonance in Medicine, British Chapter, Newcastle.
2. **Rial B**, Neubauer S, Robson MD, Schneider JE (2009). Metabolite quantification in normal liver with 3.0 Tesla Proton Magnetic Resonance Spectroscopy at short echo times. 26th Scientific Meeting European Society of Magnetic Resonance in Medicine and Biology, Antalya.
3. **Rial B**, Neubauer S, Robson MD, Schneider JE (2010). Myocardial Fat Content: Single Breath-Hold ¹H-MR Spectroscopy at 3 T. 19th Scientific Meeting, International Society for Magnetic Resonance in Medicine, Stockholm.
4. Suttie J, Dass S, **Rial B**, Banerjee R, Cox P, Cochlin L, Francis J, Karamitsos T, Forfar C, Hilton-Jones D, Robson M, Schneider J, Petersen S, N. WHS (2010) Cardiovascular magnetic resonance imaging (CMR) detects myocardial fibrosis, impaired circumferential strain, abnormal ³¹P energetics and myocardial lipidosis in patients with dystrophinopathies even in the presence of normal left ventricular ejection fraction; AHA Scientific Sessions, Chicago.
5. Banerjee R, **Rial B**, Suttie J, Cox P, Lewandowski A, Johnson A, Francis J, Holloway C, Rider O, Schneider J, Robson MD, Clarke K, Leeson P, Neubauer S (2011) Cardiac steatosis is associated with excess body weight in otherwise healthy adults. SCMR/Euro CMR Joint Scientific Sessions, Nice.
6. Suttie JJ, Dass S, **Rial B**, Banerjee R, Cox. P, Cochlin L, Francis JM, Karamitsos TD, Robson MD, Clarke K, Forfar C, Hilton-Jones D, Schneider JE, Petersen SE, Watkins H, Neubauer S (2011). Dystrophinopathies are characterised by impaired cardiac metabolism, contractile dysfunction and fibrosis in patients with and without Cocksackie B3 exposure. SCMR/Euro CMR Joint Scientific Sessions, Nice.
7. **Rial B**, Neubauer S, Robson MD, Schneider JE, (2010) Rapid quantification of myocardial lipid content in humans using single breath-hold ¹H-MRS at 3 T. (Accepted for publication in Magnetic Resonance in Medicine).

Contents

Acknowledgments	iii
Related Publications	iv
List Of Figures	ix
List Of Tables	xi
List Of Abbreviations	xii
0 INTRODUCTION	1
Motivation and Background	1
Objectives	5
Outline	5
References	8
1 CARDIAC ^1H-MRS AT 3 T: METHODOLOGICAL DEVELOPMENT	11
1.1 ^1H -MRS Theory Overview	11
1.2 Technical Challenges for Cardiac ^1H -MRS	13
1.2.1 Low Metabolite Concentration	15
1.2.2 Phased-Array Coils	16
1.2.3 Water Suppression	17
1.2.4 Spectral Analysis and Quantification	18
1.2.5 Motion: Respiration and Heart Beat	19
1.3 Localized Spectroscopy: STEAM and PRESS	20
1.3.1 Single Voxel Localization Techniques	20
1.3.2 Peak B_1 Limitation and Chemical Shift Displacement	25

1.3.3	Echo Time: TE	26
1.3.4	Flip Angle Effects and Spatial Variation of the B_1 Field	26
1.4	Signal Reception: Phased-Array Coils	30
1.5	Water Suppression	33
1.6	Quantification and Post-Processing	36
1.7	Motion Compensation	40
1.7.1	Frequency Correction	42
1.7.2	Phase Correction	44
1.7.3	Water Suppression Calibration	47
1.8	Discussion	47
1.9	References	51
2	LIVER ^1H-MRS AT 3 TESLA	56
2.1	Introduction	56
2.2	Liver ^1H -MRS Overview	58
2.3	Methods	60
2.3.1	Liver ^1H -MRS Validation in Healthy Volunteers	60
2.3.2	Necessity of Water Suppression in Liver ^1H -MRS	62
2.3.3	Liver Fat Assessment in Pre- and Post- Menopausal Women	63
2.3.4	Spectral Quantification	63
2.3.4	Statistical Analysis	65
2.4	Results	66
2.4.1	Liver ^1H -MRS Validation in Healthy Volunteers	66
2.4.1.1	Within-Days Reproducibility	66
2.4.1.2	Between-days reproducibility	73

2.4.2	Necessity of Water Suppression in Liver ^1H -MRS	75
2.4.3	Liver Fat Assessment in Pre- and Post- Menopausal Women	80
2.5	Discussion	82
2.5.1	^1H -MRS Validation in Healthy Volunteers	82
2.5.2	Necessity of Water Suppression in Vivo	86
2.5.3	Liver Fat Assessment in Pre- and Post- Menopausal Women	86
2.5	Conclusion	87
2.6	References	88
3	CARDIAC METABOLISM OF THE HUMAN HEART	92
3.1	Overview	92
3.1.1	Myocardial Lipid Metabolism	93
3.1.2	Myocardial Creatine Metabolism	94
3.2	Preliminary Studies	95
3.2.1	Cardiac Triggering	95
3.2.2	Blood Contamination	99
3.3	Single Breath-Hold ^1H -MRS Cardiac Lipid Assessment	100
3.3.1	Methods	101
3.3.1.1	Subjects	101
3.3.1.2	^1H -MRS Technique	101
3.3.1.3	Validation of Single Breath-Hold Lipid Content Measurement	102
3.3.1.4	Spectral Postprocessing	103
3.3.1.5	Statistical Analysis	105
3.3.2	Results	105
3.3.3	Discussion	109
3.4	Identification of Two Myocardial Lipid Pools	112
3.4.1	Methods	113
3.4.2	Results	115

3.4.3	Discussion	119
3.5	Creatine: Baseline Values in Normal Hearts	120
3.5.1	Methods	121
3.5.2	Results	122
3.5.3	Discussion	124
3.6	Conclusions	127
3.7	References	129
4	CONCLUSIONS	135

List of Figures

Figure 1.1 STEAM.	22
Figure 1.2 PRESS.	23
Figure 1.3 STEAM vs PRESS.	24
Figure 1.4 Echo amplitude plotted as a $\sin^3$ function.	27
Figure 1.5 B_1 inhomogeneities.	28
Figure 1.6 Water amplitude as function of the pulse voltage.	29
Figure 1.7 90° RF voltage.	29
Figure 1.8 Project combination scheme.	31
Figure 1.9 Combination routines comparison	32
Figure 1.10 Combination comparison, low SNR case.	32
Figure 1.11 WET.	33
Figure 1.12 WET and DRYSTEAM.	34
Figure 1.13 Off-resonance effects on water suppression.	35
Figure 1.14 WET effect on spectra.	35
Figure 1.15 Cr/Water ratio at different locations in the magnet.	38
Figure 1.16 T_1 and T_2 relaxation.	39
Figure 1.17 Free-breathing liver spectra.	41
Figure 1.18 Water frequency variation within a breath-hold.	43
Figure 1.19 Inter breath-hold water frequency variation.	44
Figure 1.20 Intra breath-hold phase variation.	45
Figure 1.21 Inter breath-hold phase variation.	46
Figure 2.1 Voxel location in liver.	61
Figure 2.2 Liver ^1H -MR spectrum.	66
Figure 2.3 Repeated L_{CH_3} % measurements, Bland-Altman plots.	67
Figure 2.4 Repeated L_{CH_2} % measurements, Bland-Altman plots.	68
Figure 2.5 Repeated $L_{\text{CH}_2\text{-CH}}$ % measurements, Bland-Altman plots.	69
Figure 2.6 Repeated $L_{\text{CholineComponents}}$ % measurements, Bland-Altman plots.	70
Figure 2.7 Repeated $L_{\text{Glycogene+Glucose}}$ % measurements, Bland-Altman plots.	71
Figure 2.8 Liver ^1H -MRS reproducibility plot.	73

Figure 2.9 Between-Days reproducibility.	74
Figure 2.10 Scheme of liver ^1H -MR spectra analyzed.	75
Figure 2.11 WSOFF vs WSON liver spectra.	76
Figure 2.12 High-quality liver ^1H -MR spectrum.	77
Figure 2.13 WSOFF vs WSON: Bland-Altman plots.	79
Figure 2.14 BMI vs liver lipid levels.	80
Figure 2.15 Hepatic liver content distribution with age.	81
Figure 2.16 Liver lipid levels in pre- and post menopausal woman.	81
Figure 3.1 Cardiac triggering.	96
Figure 3.2 Water amplitude at different point in the R-R interval.	97
Figure 3.3 FID of a cardiac ^1H -MRS experiment.	98
Figure 3.4 STEAM black blood properties.	100
Figure 3.5 Single breath-hold acquisition scheme.	102
Figure 3.6 Reproducibility of single breath-hold ^1H -MRS measurements.	106
Figure 3.7 Linear regression: single vs multiple breath-holds.	107
Figure 3.8 Bland-Altman plot: single vs multiple breath-holds.	107
Figure 3.9 Myocardial lipid levels correlation with BMI.	108
Figure 3.10..Voxel localization.	114
Figure 3.11 Myocardial IMCL and EMCL resonances.	115
Figure 3.12 IMCL an EMCL fitting in muscular dystrophy patients.	116
Figure 3.13 IMCL an EMCL fitting in obese subjects.	117
Figure 3.14 IMCL and EMCL levels.	119
Figure 3.15 Cardiac ^1H -MR spectra showing creatine.	123

List Of Tables

Table 1.1	Chemical shift displacement.	25
Table 1.2	T_1 and T_2 relaxation times in a creatine solution phantom.	40
Table 2.1	Technical and biological obstacles of ^1H -MRS.	57
Table 2.2	Liver ^1H -MRS publications at 3 T.	59
Table 2.3	Liver prior knowdlege.	64
Table 2.4	Summary of within-day reproducibility.	72
Table 2.5	Summary of between-days reproducibility.	74
Table 2.6	Descriptive of the groups.	77
Table 2.7	Descriptive of groups.	80
Table 3.1	Subject characteristics.	113
Table 3.2	Soft constrains used for cardiac spectral fitting.	114
Table 3.3	EMCL and IMCL FWHM.	118
Table 3.4	Creatine concentrations.	122

Abbreviations

ADC	Analog-to-Digital Converter
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CHESS	Chemical Shift Selective
CK	Creatine Kinase
CR	Total creatine
CRLBs	Cramer-Rao Lower Bounds
CRSD	Cramer-Rao Standard Deviation
CSD	Chemical Shift Displacement
ECG	Electrocardiography
EMCL	Extramyocellular Lipids
FID	Free Induction Decay
HCM	Hypertrophic Cardiomyopathy
HLSVD	Hankel Lanczos Single Value Decomposition
IMCL	Intramyocellular Lipids
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
NAFLD	Non-alcoholic Liver Disease
PCr	Phosphorylated Creatine
ppm	parts per million
PRESS	Point Resolved Spectroscopy
RF	Radiofrequency
SNR	Signal to Noise Ratio
STEAM	Stimulated Echo Acquisition Mode
T	Tesla
T ₁	Spin-lattice relaxation time
T ₂	Spin-spin relaxation time
T2DM	Type 2 Diabetes Mellitus
TE	Echo Time
TM	Mixing Time
TMA	Trimethylammonium Compounds
TR	Repetition Time
WET	Water suppression Enhanced through T ₁ effects

0

INTRODUCTION

Motivation and Background

This work is motivated by the need for precise measurements of fat, creatine and other cardiac metabolites in the studies of cardiac disease. Magnetic Resonance Spectroscopy (MRS) is the only technique that provides a non-invasive window into the metabolism of living heart. It does not require any radiation or external contrast, and allows for the investigation of energy metabolism (^1H - and ^{31}P -MRS), substrate supply (^1H -MRS), substrate metabolism (^1H - and ^{13}C -MRS) or ion homeostasis (^{23}Na -MRS). While technically challenging, the development and implementation of these approaches on clinical scanners will provide research clinicians with essential tools to help understand, and diagnose heart disease. This thesis aims to advance ^1H -MRS to non-invasively measure fat and creatine content in the heart. It is set out to use the latest technologies available on clinical MRI scanners, i.e. high field (3 Tesla), phased-array RF coils, and

advanced processing algorithms in order to implement an optimised technique that can be used in clinical routine.

The acquisition of high-quality cardiac proton spectra is technically demanding. The signal-to-noise ratio (SNR) for myocardial metabolites is low because of their low concentrations and due to the significant distance from the RF coils. Nowadays clinical 3 T scanners with phased-array receive coils are increasingly common, with the advantages of increased SNR (1) and higher spectral resolution. However, high-field MR spectroscopy suffers from increased magnetic field inhomogeneities (2) that pose further challenges for the water signal suppression, essential in order to detect the weak metabolite signals. Moreover, cardiac and respiratory motion results in changing voxel location and can therefore affect shimming and water suppression. ECG gating is generally adequate for compensating cardiac motion. Various approaches have been proposed to reduce the influence of respiratory motion, including respiratory gating (3-5) and navigator gating with volume tracking (6-8). Despite the progress of cardiac ^1H -MRS as a research tool, only few studies so far have examined cardiac metabolism in humans using this tool at 3 T (6,8,9), with the vast majority of work being performed at 1.5 T.

Recent advances in cardiac ^1H -MRS have enabled the non-invasive study of myocardial lipid metabolism in humans (4,5,7) typically requiring a 4-10 minute acquisition. Initial studies in humans have confirmed findings from animal research, suggesting that cardiac lipid levels may be considered as a potential biomarker for myocardial dysfunction (10-15).

¹H-MRS respiratory gating based on the ECG signal performed in insulin-resistant subjects showed increased myocardial lipid levels despite normal left ventricular ejection fraction, suggesting that cardiac steatosis could be an early feature of impaired cardiac function in the insulin-resistant state, leading to type 2 diabetes mellitus (T2DM) (16). Also using the same method, a study reported that a 6 month pioglitazone treatment improved glycemic control and reduced myocardial lipid levels in patients with T2DM (17).

Respiratory triggering by navigator scans has been used more often to study the effects of nutritional interventions on myocardial lipid metabolism and cardiac function in healthy and T2DM patients. After a very low-calorie diet, myocardial lipid levels increased and left ventricular diastolic function decreased in lean men (18,19) and T2DM patients (15), suggesting that the decrease in visceral adipose tissue contributes to high levels of circulating fatty acids and probably to the myocardial lipid storage excess. In a later study, it was demonstrated that these effects were independent of age, body mass index (BMI), heart rate and diastolic blood pressure in T2DM patients (14). However, when the T2DM cohort was treated with acipimox during the diet no changes were observed in myocardial lipid levels (15). The same research group found that myocardial lipid levels increased with aging and was associated with age-dependent decline in diastolic function independent of BMI and blood pressure (20). Very recently, they confirmed the improvement in left ventricular function in T2DM patients after a pioglitazone treatment. However no changes on myocardial lipid levels were observed on myocardial lipid levels (21). Another recent study, evaluated the effect of a 12-week physical training in obese subjects, demonstrating myocardial lipid levels reduction and left ventricular ejection fraction improvement (22).

All the studies described above were performed at 1.5 T, only two studies have implemented navigator gating in ^1H -MRS at 3 T. The first study demonstrated the feasibility of acquiring high quality cardiac spectra using respiratory motion correction based on navigator echoes (6). Assuming a 50% navigator efficiency, 64 spectra were acquired in about 4 minutes during free-breathing. However, neither reproducibility nor metabolite quantification were reported in this study. More recently, Liu et al reported a 5-10 minute acquisition time to obtain a cardiac spectrum with 32 average using ^1H -MRS with navigator gating at 3 T (9).

The representative spectra in the previous studies showed a clear lipid resonance, however the spectral resolution was not sufficient to reliably resolve myocardial creatine levels. In fact, only two research groups have reported myocardial creatine concentrations obtained by ^1H -MRS at 1.5 T. Bottomley et al (23) showed for first the time the potential of ^1H -MRS to study cardiac metabolism by reporting a 65% reduction of creatine levels in the infarcted region of the heart of patients with a history of myocardial infarction compared to that from non-infarcted tissue and from healthy volunteers. Nakae et al (24-29) published several studies, all very similar, reporting low creatine concentration in patients with ischemic, hypertrophic and dilated cardiomyopathies compared to normal. Their results suggested that myocardial creatine indicates the severity of heart failure, regardless the physiologic pathway leading to myocardial dysfunction. Then again, the reduced resolution and low SNR characteristic of the spectra displayed in the studies of both research groups, could compromise the estimation of metabolite concentrations.

All these studies indicate that a further technologic development in ^1H -MRS, by improving the sensitivity of the technique to augment spatial resolution and metabolite detection will

give valuable insight in the non-invasive study of human cardiac metabolism with emphasis on common clinical conditions such as diabetes, myocardial ischemia, hypertrophy and heart failure.

Objectives

The specific objectives of this thesis are:

- To develop and optimize a proton magnetic resonance spectroscopy technique, and to establish it as a clinical tool for the non-invasive characterization of normal and diseased human myocardium in vivo at 3 T.
- To apply this technique to the hearts of normal volunteers to establish optimum spectral, temporal and spatial resolution and lowest variability, for the measurement of ^1H -MRS detectable metabolites in vivo.

Outline

Chapter 1 describes the methodological development of a robust and highly reproducible ^1H -MRS technique at 3 T for the non-invasive characterization of human myocardium in vivo. The work in this chapter is mainly performed on phantoms. Firstly, a brief overview of the most relevant parts of proton NMR spectroscopy is detailed. Next the technological challenges for cardiac ^1H -MRS are outlined and then addressed one by one: including the optimization of a single voxel localization method; the implementation of an algorithm to combine individual signals acquired with phased-arrays ensuring optimal sensitivity to increase the signal-to-noise of the experiment; the evaluation of water suppression methods with respect to their stability and degree of water suppression in phantom creatine

solutions; the acquisition and quantification methods are tested by estimating the creatine concentration present in the phantom and finally an algorithm for averaging in vivo ^1H -MR spectra acquired over consecutive breath-holds is presented as a method for respiratory motion correction.

Chapter 2 evaluates the reproducibility and feasibility of the developed spectroscopic method in vivo. Liver metabolism is investigated as a second methodological development step towards cardiac ^1H -MRS. The liver provides many of the challenges of cardiac imaging but has less motion than the heart. Further, ^1H -MRS in the liver is an important technique that adds information regarding cardiovascular risk. The first section of Chapter 2 gives a general overview of liver ^1H -MRS. Then a study in seven healthy volunteers addresses questions of reproducibility by assessing within-day and between-weeks variability for several liver metabolites. Moreover, based on previous ^1H -MRS studies of liver metabolism that are performed without water suppression, a comparison between water-unsuppressed and water-suppressed spectra is given and sufficient spectral resolution and accuracy of data fitting is evaluated for both cases. Finally, Chapter 2 describes a study investigating the relationship between menopausal condition and hepatic lipid content in healthy women using the ^1H -MRS liver methods developed in this thesis. This clinical project was performed with Dr Rajarshi Banerjee as part of a clinical collaboration with the Oxford Centre for Diabetes, Endocrinology & Metabolism.

Chapter 3 evaluates the spectroscopic localization method, initially developed in phantoms and further optimized in liver studies, on cardiac ^1H -MRS measurements at 3 T. A preliminary study investigates the optimal cardiac phase for triggering ^1H -MRS acquisitions by evaluating myocardial water content reproducibility acquired at different

points during the R-R interval. Also the ‘black-blood’ property attributed to single voxel localization methods is studied. The following section evaluates the effects of compensating artefacts produced by cardiac and respiratory motion on ^1H -MRS measurements. It is hypothesized that myocardial lipid content can be accurately measured within a single breath-hold of less than 15 s. Furthermore, this chapter investigates whether it is possible to differentiate two myocardial lipid pools in patients with suspected high myocardial lipid levels. This work is done in collaboration with Dr. Joseph J. Suttie to study cardiac function in patients diagnosed with muscular dystrophy. To conclude, myocardial creatine levels are examined in 14 healthy volunteers. This study investigates the feasibility to establish creatine baseline values in normal hearts using ^1H -MRS at 3 T for the first time.

Finally, Chapter 4 summarises the work that has been developed from this project.

References

1. Tyler DJ, Hudsmith LE, Clarke K, Neubauer S, Robson MD. A comparison of cardiac (31)P MRS at 1.5 and 3 T. *NMR Biomed* 2008;21(8):793-798.
2. Noeske R, Seifert F, Rhein KH, Rinneberg H. Human cardiac imaging at 3 T using phased array coils. *Magn Reson Med* 2000;44(6):978-982.
3. Felblinger J, Jung B, Slotboom J, Boesch C, Kreis R. Methods and reproducibility of cardiac/respiratory double-triggered (1)H-MR spectroscopy of the human heart. *Magn Reson Med* 1999;42(5):903-910.
4. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *American journal of physiology* 2005;289(5):E935-939.
5. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003;49(3):417-423.
6. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. *Magn Reson Med* 2004;51(6):1091-1095.
7. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of 1H-MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007;245(1):251-257.
8. Gabr RE, Sathyanarayana S, Schar M, Weiss RG, Bottomley PA. On restoring motion-induced signal loss in single-voxel magnetic resonance spectra. *Magn Reson Med* 2006;56(4):754-760.
9. Liu CY, Redheuil A, Ouwerkerk R, Lima JA, Bluemke DA. Myocardial fat quantification in humans: Evaluation by two-point water-fat imaging and localized proton spectroscopy. *Magn Reson Med* 2010;63(4):892-901.
10. Zhou YT, Grayburn P, Karim A, Shimabukuro M, Higa M, Baetens D, Orci L, Unger RH. Lipotoxic heart disease in obese rats: implications for human obesity. *Proc Natl Acad Sci U S A* 2000;97(4):1784-1789.
11. Young ME, Guthrie PH, Razeghi P, Leighton B, Abbasi S, Patil S, Youker KA, Taegtmeyer H. Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart. *Diabetes* 2002;51(8):2587-2595.
12. Sharma S, Adroge JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeyer H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *Faseb J* 2004;18(14):1692-1700.
13. Szczepaniak LS, Victor RG, Orci L, Unger RH. Forgotten but not gone: the rediscovery of fatty heart, the most common unrecognized disease in America. *Circ Res* 2007;101(8):759-767.
14. Rijzewijk LJ, van der Meer RW, Smit JW, Diamant M, Bax JJ, Hammer S, Romijn JA, de Roos A, Lamb HJ. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol* 2008;52(22):1793-1799.
15. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A, Romijn JA, Smit JW. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *American journal of physiology* 2008;295(3):E714-718.

16. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, Levine BD, Raskin P, Victor RG, Szczepaniak LS. Cardiac steatosis in diabetes mellitus: a ¹H-magnetic resonance spectroscopy study. *Circulation* 2007;116(10):1170-1175.
17. Zib I, Jacob AN, Lingvay I, Salinas K, McGavock JM, Raskin P, Szczepaniak LS. Effect of pioglitazone therapy on myocardial and hepatic steatosis in insulin-treated patients with type 2 diabetes. *J Investig Med* 2007;55(5):230-236.
18. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007;56(12):2849-2853.
19. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW, Romijn JA. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008;93(2):497-503.
20. van der Meer RW, Rijzewijk LJ, Diamant M, Hammer S, Schar M, Bax JJ, Smit JW, Romijn JA, de Roos A, Lamb HJ. The ageing male heart: myocardial triglyceride content as independent predictor of diastolic function. *Eur Heart J* 2008;29(12):1516-1522.
21. van der Meer RW, Rijzewijk LJ, de Jong HW, Lamb HJ, Lubberink M, Romijn JA, Bax JJ, de Roos A, Kamp O, Paulus WJ, Heine RJ, Lammertsma AA, Smit JW, Diamant M. Pioglitazone improves cardiac function and alters myocardial substrate metabolism without affecting cardiac triglyceride accumulation and high-energy phosphate metabolism in patients with well-controlled type 2 diabetes mellitus. *Circulation* 2009;119(15):2069-2077.
22. Schrauwen-Hinderling VB, Hesselink MKC, Meex R, van der Made S, Schar M, Lamb H, Wildberger JE, Glatz J, Snoep G, Kooi ME, Schrauwen P. Improved Ejection Fraction after Exercise Training in Obesity Is Accompanied by Reduced Cardiac Lipid Content. *J Clin Endocrinol Metab* 2010;95(4):1932-1938.
23. Bottomley PA, Weiss RG. Non-invasive magnetic-resonance detection of creatine depletion in non-viable infarcted myocardium. *Lancet* 1998;351(9104):714-718.
24. Nakae I, Mitsunami K, Omura T, Yabe T, Tsutomoto T, Matsuo S, Takahashi M, Morikawa S, Inubushi T, Nakamura Y, Kinoshita M, Horie M. Proton magnetic resonance spectroscopy can detect creatine depletion associated with the progression of heart failure in cardiomyopathy. *J Am Coll Cardiol* 2003;42(9):1587-1593.
25. Nakae I, Mitsunami K, Matsuo S, Matsumoto T, Morikawa S, Inubushi T, Koh T, Horie M. Assessment of myocardial creatine concentration in dysfunctional human heart by proton magnetic resonance spectroscopy. *Magn Reson Med Sci* 2004;3(1):19-25.
26. Nakae I, Mitsunami K, Yabe T, Inubushi T, Morikawa S, Matsuo S, Koh T, Horie M. Detection of metabolic abnormality in asynergic regions of ischemic human myocardium using ³¹P and ¹H magnetic resonance spectroscopy. *J Cardiovasc Magn Reson* 2004;6(3):685-696.
27. Nakae I, Mitsunami K, Matsuo S, Inubushi T, Morikawa S, Tsutomoto T, Koh T, Horie M. Myocardial creatine concentration in various nonischemic heart diseases assessed by ¹H magnetic resonance spectroscopy. *Circ J* 2005;69(6):711-716.
28. Nakae I, Mitsunami K, Matsuo S, Horie M. Creatine depletion and altered fatty acid metabolism in diseased human hearts: clinical investigation using ¹H magnetic resonance spectroscopy and ¹²³I BMIPP myocardial scintigraphy. *Acta Radiol* 2007;48(4):436-443.

29. Nakae I, Mitsunami K, Yoshino T, Omura T, Tsutamoto T, Matsumoto T, Morikawa S, Inubushi T, Horie M. Clinical features of myocardial triglyceride in different types of cardiomyopathy assessed by proton magnetic resonance spectroscopy: comparison with myocardial creatine. *J Card Fail* 2010;16(10):812-822.

1

CARDIAC ^1H -MRS AT 3 T: METHODOLOGICAL DEVELOPMENT

1.1 ^1H -MRS Theory Overview

Magnetic resonance spectroscopy (MRS) is based on the scientific property that nuclei with a magnetic dipole moment can be excited by an external electromagnetic field. Different atomic nuclei, like ^1H , ^{13}C , ^{19}F and ^{31}P , can be used in MR as long as they have magnetic moment, and thereby behave like little magnets. In an MRS experiment, when a sample is placed into a external static magnetic field B_0 , the nuclear dipole gyroscopically precess about the external field axis at a constant rotational velocity, called the Larmor frequency:

$$\omega_0 = \gamma B_0 \quad (1.1)$$

where γ is the gyromagnetic ratio characteristic of the isotope (42.6 MHz/T for ^1H).

If a radio frequency (RF) pulse is applied, the nucleus will absorb energy only if the frequency of irradiation is equal to the Larmor frequency.

The magnetization of a large ensemble of spins, which have the same Larmor frequency (isochromat) at equilibrium ($\mathbf{M} = M_0 \hat{z}$), determines the amplitude of the detected NMR signal. Using classical mechanics, the net magnetization M_0 can be approximated as:

$$M_0 = \frac{n(\gamma\hbar)^2}{4kT} B_0 \quad (1.2)$$

Here n is the number of nuclei, γ is the gyromagnetic ratio, $\hbar$ is Planck's constant, k is the Boltzmann constant and T is the sample temperature. Note that the equilibrium magnetization, therefore the NMR signal strength, is directly proportional to the number of nuclei in the sample. Also a higher intensity will be obtained at higher B_0 . Note also that at room temperature and practical magnetic field strengths the net magnetization is very small compared to the total possible magnetization of the sample, i.e. the sample is very weakly polarised.

When the magnetization $\mathbf{M}$ is perturbed from its equilibrium state it will return to that state through a process known as relaxation. Relaxation is a complex phenomenon, which can be described by two simplified exponential processes. The first process, called spin-lattice or longitudinal relaxation, describes the recovery of the z-component of magnetization

characterized by the time constant T_1 . This mechanism requires energy dissipation from the nuclei to the surroundings. The second process, called spin-spin or transverse relaxation, describes the loss of the transverse (x and y) components of the magnetization, and is characterized by the time constant T_2 .

Following an RF pulse that rotates M_0 by 90° into the transverse plane, the magnetization precesses about B_0 at the Larmor frequency and induces an emf in the receive coil. This sampled signal is called the free induction decay (FID) and corresponds to an exponentially decaying sinusoid in the time domain. These data are converted to frequency-domain by Fourier transformation. In this spectrum the localisation of peaks indicate the chemical identity of a species and the area underneath the peaks signal (amplitude of the FID at time zero) indicates the amount of that species. The linewidth of each peak is inversely proportional to the decay time in the time domain for this metabolite.

1.2 Technical Challenges for Cardiac ^1H -MRS

The implementation of ^1H -MRS methods in human heart in vivo is technically challenging not only due to the low metabolite concentrations, but also due to the dominating water signal, which needs to be suppressed sufficiently in order to detect the weak metabolite signals. Both, water suppression and separation of the metabolites require a good homogeneity of the static magnetic field, which is especially difficult to achieve in heterogeneous tissues and is further hampered by the effects of motion (cardiac and respiratory motion and patient movement). ^1H -MRS is in general a very insensitive technique, making the detection of low concentration metabolites a compromise between time resolution and signal-to-noise ratio (SNR). Due to these significant methodological

difficulties, limited pilot feasibility studies on ^1H -MRS in human heart in vivo were reported until 2006 (1-10), some of which demonstrated the feasibility of detecting the creatine resonance at 1.5 T. For example, Nakae et al demonstrated a correlation between creatine content and ejection fraction in patients with dilated or hypertrophic cardiomyopathy (5). Bottomley et al showed reduced creatine content in infarcted myocardium (2). However, all these studies can only be considered as preliminary due to the poor SNR, spectral baseline problems and poor spectral resolution. These difficulties have prohibited an accurate quantitative analysis of the spectroscopic data and are the reason for the large variability reported (5,6).

Since Schar et al introduced sophisticated motion correction methods such as navigation and volume tracking in ^1H -MRS (11) more cardiac metabolism studies using ^1H -MRS have been reported, mainly focusing on myocardial lipid metabolism (12,13,14 ,15-24).

Clinical MR-systems with higher static magnetic field strength B_0 (≥ 3 T) are particularly beneficial for MRS, because they not only improve the SNR, but also the spectral resolution. So far, very few studies had reported preliminarily results on the feasibility of cardiac ^1H -MRS at 3 T. Two studies showed that good quality cardiac spectra could be obtained at 3 T by using motion correction, one based on navigator echoes for double triggering with volume tracking (11) and the other using constructive averaging (12). Whereas, another study published myocardial lipid levels in healthy volunteers for the first time (23). If correction for the increased susceptibility artefact at this field strength is achieved, suppression of the dominant water signal in ^1H -MRS, separation and identification of neighbouring resonances will all benefit, and, therefore, the accuracy of quantitative analysis of ^1H spectra should be substantially improved. Furthermore, the

latest generation of clinical MR-systems are equipped with multiple receive coils which are more sensitive and provide a higher SNR. Sophisticated motion correction methods such as navigation and volume tracking (11) benefit from increased computational power. Further benefits in system stability and powerful pulse programming environments make challenging techniques more feasible in the clinic. These advances in MR technology will be crucial for the development and application of new clinical cardiac ^1H -MRS methods.

1.2.1 Low Metabolite Concentration

It is perhaps surprising that ^1H -MRS, which is the form of MRS that is theoretically the most informative and with the highest resolution, has so far only minimally been explored in the human heart. The proton nucleus is the most sensitive nucleus for MR. Since it has a very high gyromagnetic ratio and protons are present in nearly all metabolites, ^1H -MRS may in the long term have the greatest potential for clinical application to observe, identify and quantify biologically relevant compounds in tissue (25).

In vivo ^1H -MR spectra, both from a beating mouse heart and from human heart show a resonance at 3 ppm, corresponding to creatine and commonly assigned to total creatine (both phosphorylated (PCr) and unphosphorylated creatine). The concentration of creatine in normal human myocardium measured using ^1H -MRS was about 25 μmol per mg left ventricular mass (2). Knowledge of creatine levels is important for the understanding of cardiac pathophysiology. The total creatine content determines the capacity of the creatine kinase/phosphocreatine (CK/PCr) energy storage and transport system. A loss of creatine is characteristic for the failing heart (26,27) and has been postulated as one major mechanism leading to contractile dysfunction due to energetic derangement (26,28).

^1H -MR spectra also exhibit resonances for the $-\text{CH}_2-$ and CH_3- groups of lipids between 0.85-2.2 ppm. Long-chain fatty acids are important oxidizable substrates for the heart under normal physiological conditions, and several resonances are detectable by ^1H -MRS even in healthy myocardium. In the myocardium of healthy individuals, the triglyceride concentrations have been reported to range between 5 and 20 μmol per mg left ventricular mass (4). Many forms of heart disease, such as diabetes, cardiac hypertrophy or heart failure are associated with chronic alterations of fatty acid utilization (29). Thus, detection and quantification of the myocardial lipid content should be a sensitive marker for assessing the physiological condition of myocardial tissue.

1.2.2 Phased-Array Coils

MRI acquisitions have benefited significantly from the development of phased-array coils. Phased-array coils provide improvements in SNR and decrease the sensitivity to coil positioning that are inherent to single coil systems. More than two decades ago, Roemer et al described widely accepted methods for implementing phased-array coils including combining data from multiple receiving elements (30). However, phased-array coils for MRS studies have not been widely used until more recently, primarily because spectra from each voxel required individual processing for each coil element. Several studies presented new approaches for the combination for MRS data acquired using phased-array coils. (31-35).

When the work for this thesis started, all the published studies on cardiac ^1H -MRS had used single coils for signal reception. It was only very recently that proton cardiac spectra were acquired using multiple phased-arrays (22,23). Based on a preliminary solution available on the MR system, one of the aims of this thesis was to improve on the methods

that can efficiently combine the signals from the different receivers. In particular, the combined signal $S_{comb}(t)$ can be obtained through a weighted combination of the individual signals $S_i(t)$ in the time domain (the coil number indexed with i). The weighting factors wf_k to maximise the signal to noise ratio can be calculated from the unsuppressed water signal $A_{W,k}$ (32).

1.2.3 Water Suppression

Despite its high sensitivity, in vivo ^1H -MRS fell behind the use of other nuclei and was feasible only when efficient water suppression (WS) methods were presented (36). The water signal is three to five orders of magnitude larger than metabolite signals. Although nowadays analog-to-digital converters (ADCs) have sufficient resolution to digitize the low metabolite resonances in the presence of a large water resonance without degrading the metabolite SNR, other problems result from gradient-induced artifact signals (sidebands), severe baseline distortion, and spectral overlap, and hence water suppression is still desirable when performing proton spectroscopy. Both, chemical shift selective (CHESS, (36)) pulses followed by crusher gradients and WET scheme (Water suppression Enhanced through T_1 effects (37)) have commonly been used for water suppression and were available for STEAM and PRESS on the scanner. The aim was to investigate other suppression schemes such as a combination of CHESS with STEAM localization (DRYSTEAM, (38)) and the performance of the WET method at 3 T.

1.2.4 Spectral Analysis and Quantification

Metabolite quantification follows data acquisition. To estimate the metabolites from ^1H -MR spectra, the resonance areas are calculated. In proton MRS, one of the most challenging aspects of spectral quantification is to define the residual water signal (and associated rolling baseline) following imperfect water suppression. Incomplete definition of the baseline affects assessment of the error in the global fit and leads to incorrect convergence of the minimisation function. Movement and shimming issues in myocardium lead to broader spectral lines and increase this challenge.

Quantification of spectral peaks using prior knowledge can be performed in the time domain with jMRUI (39) which includes the AMARES (40) program; in the frequency domain (LCModel, (41)) and others (42). AMARES and LCModel have already been compared for quantification of brain (43) and skeletal muscle (44) spectra, finding similar reliability for both methods. The frequency domain LCModel approach is a robust technique commonly applied in brain MRS, which synthesises the spectrum from a linear combination of basis spectra of metabolite solutions. However, this method is constrained by the completeness of the basis set (45), which is currently undefined for myocardium.

The natural goal of in vivo MRS is to produce a quantitative measurement of the amount of certain chemical in the sample. In some circumstances, a qualitative or semi-quantitative analysis of the MRS data may be sufficient. This involves either the presence or absence of a particular resonance in the MR spectrum (for example, the detection of lactate as a marker for anaerobic metabolism), or the calculation of metabolite ratios (such as PCr/ATP in ^{31}P -MRS). In general, however, both for clinical research and for later application in clinical practice, an absolute-quantitative approach is desirable. This

requires the use of a concentration standard (internal or external), a quantitative analysis (i.e. fitting) of the MR data and the knowledge of several parameters relevant for absolute quantification, such as MR relaxation times, RF coil sensitivity and B_1 field characteristics. To begin with, the practical goal of quantifying cardiac ^1H -MR spectra is to produce measurements, which can be reliably repeated and compared with other measurements. Initially, the metabolite signals will be referenced to the unsuppressed water signal obtained from the same location within the heart (2,5). Using constant sequence timing (i.e. TE and TR) in all experiments, this approach will allow addressing the basic requirements such as feasibility and stability measurements and the assessment of reproducibility.

1.2.5 Motion: Respiration and Heart Beat

The heart is probably the most challenging target for in vivo ^1H -MR spectroscopy, because the heart's shape and position depend on cardiac and respiratory motion. Cardiac motion can be compensated for by synchronizing the MR scan to the heart beat using the ECG-signal and acquiring the data at the same position within the cardiac cycle, such as in the diastolic phase of the heartbeat. Residual variations of the zero-order phase in the MRS data caused by motion, which lead to a destructive averaging, can be corrected in post-processing (12). The influence of respiratory motion on the field inhomogeneity increases with the B_0 field (46) and is difficult to compensate for in humans.

Since den Hollander et al (1) observed for the first time myocardial lipids using ^1H -MRS, successive studies primarily focused on the development of respiratory motion correction techniques. It has been shown that respiratory and cardiac double triggering based on the ECG signal (47) provides improved spectral quality and reproducibility. More recently,

another technique for respiratory motion compensation based on navigator echo signals commonly used in cardiac MR imaging (48) has been incorporated to cardiac ^1H -MRS at 1.5 T (13) and at 3 T (11). The majority of the myocardial lipid metabolism studies published to date performed respiratory motion correction within long acquisitions using one of the two previous methods.

Only one study used breath-holding for respiratory motion correction (10), reporting an increased in myocardial lipid deposition in obese subjects and suggesting a fatty acid overflow to the myocardium owing to ectopic fat excess. Spectra were acquired during 10 breath-holds and averaged in post processing. This study at 1.5 T did not use water suppression; hence the intense water signal hampered accurate lipid quantification.

The higher field strength used in this study should improve spectral resolution and signal to noise ratio compared to 1.5 T. One of the objectives of this work was to assess the feasibility of single and multiple breath-hold ^1H -MRS acquisitions for myocardial metabolite quantification at 3 T, including a dedicated post processing routine for data averaging.

1.3 Localized Spectroscopy: STEAM and PRESS

1.3.1 Single Voxel Localization Techniques

Localized in vivo ^1H -MR spectra of human tissue are commonly acquired using Stimulated Echo Acquisition Mode (STEAM) (49) or Point Resolved Spectroscopy (PRESS) (50) localization pulse sequences, which provide localization of a selected volume in a single scan. Better magnetic field homogeneity can be achieved when the

sampling volume is small. This high field homogeneity is a requirement for water suppression, and aids spectral resolution and line shapes, which will benefit quantitative analysis. The single voxel approach is suitable for investigating metabolism including normal or global metabolic alterations. Furthermore, it could potentially be used for assessing basic parameters such as transverse or longitudinal relaxation times, which are required for absolute quantification of metabolite concentrations.

Both sequences, PRESS and STEAM were available on the 3 T MR system in basic forms, but needed substantial modifications to be applicable for cardiac MRS. This section summarizes the findings of basic sequence development performed to define the optimal sequence for human cardiac ^1H -MRS. Finally, the sequence of choice was equipped with adequate water suppression and motion compensation schemes as discussed in section 1.5 and section 1.7 of this chapter.

The second focus of sequence development was to address the major problem of how to homogenize the static magnetic field over a defined region of interest, which is known as shimming. Practically, the most important parameter determining the spectral quality is the magnetic field homogeneity across the region of interest. High homogeneity is required to improve the water suppression as well as to separate neighbouring resonances. In order to separate creatine resonance at 3 ppm from TMA and carnitine at 3.2 ppm and 3.4 ppm, the homogeneity needs to be better than 0.18 ppm or 22 Hz for 3 T. Initially, the 3 T Siemens Trio scanner was equipped with an automatic shimming procedure that generated a map of the field distortions in 3D, and provided an excellent shim in phantoms and the static brain. However, this approach was impractical for cardiac application, because the sequence neither had implemented motion compensation correction nor could the phase information

reliably be extracted in flowing blood. Before any work was carried out to develop a shimming routine, the 3 T system was equipped with a new shim method by the vendor (Works-in-Progress package CV_shim_450). This shim adjustment was based on a multi-slice 2D field map obtained from a gradient double echo acquisition and very importantly for cardiac applications, acquisition could be ECG cardiac gated and performed within a single long breath-hold (18-22 s).

The stimulated echo (51) sequence STEAM (Figure 1.1) for localized NMR spectroscopy consists of three orthogonal slice selective 90° RF pulses (52-54). The first pulse excites

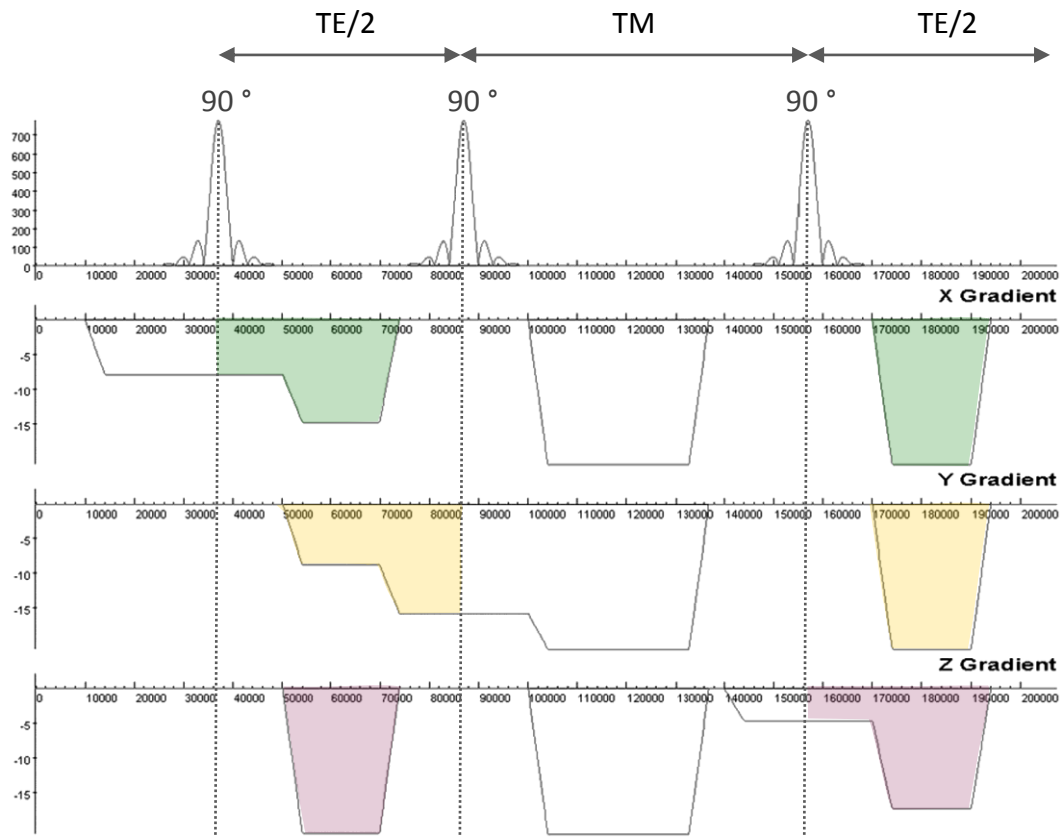


Figure 1.1 Pulse sequence diagram for Stimulated Echo Acquisition Mode (STEAM). The RF pulse train (top trace), and the 3 gradient axes used for encoding position and spoiling unwanted magnetization are shown.

the spins in a slice. During the first $\text{TE}/2$ period the spins dephase in the transverse plane by a crusher gradient (coloured gradients in Figure 1.1). The second 90° RF pulse is applied at $t=\text{TE}/2$, distributing the spins in a plane along the $\pm Z$ -axis and perpendicular to the axis along with the second 90° RF pulse was given. Half of the signal is stored along the Z axis, whereas the other half will dephase in the presence of a crusher gradient during the mixing time TM . After this time a third 90° RF pulse will be applied followed by a rephasing crusher gradient (coloured gradients in Figure 1.1) resulting in a stimulated echo at a time $\text{TE}/2$ after this last 90° RF pulse.

The double spin echo sequence PRESS (Figure 1.2) uses a 90° RF excitation pulse followed by two refocusing 180° RF pulses (50,55). By combining these RF pulses with

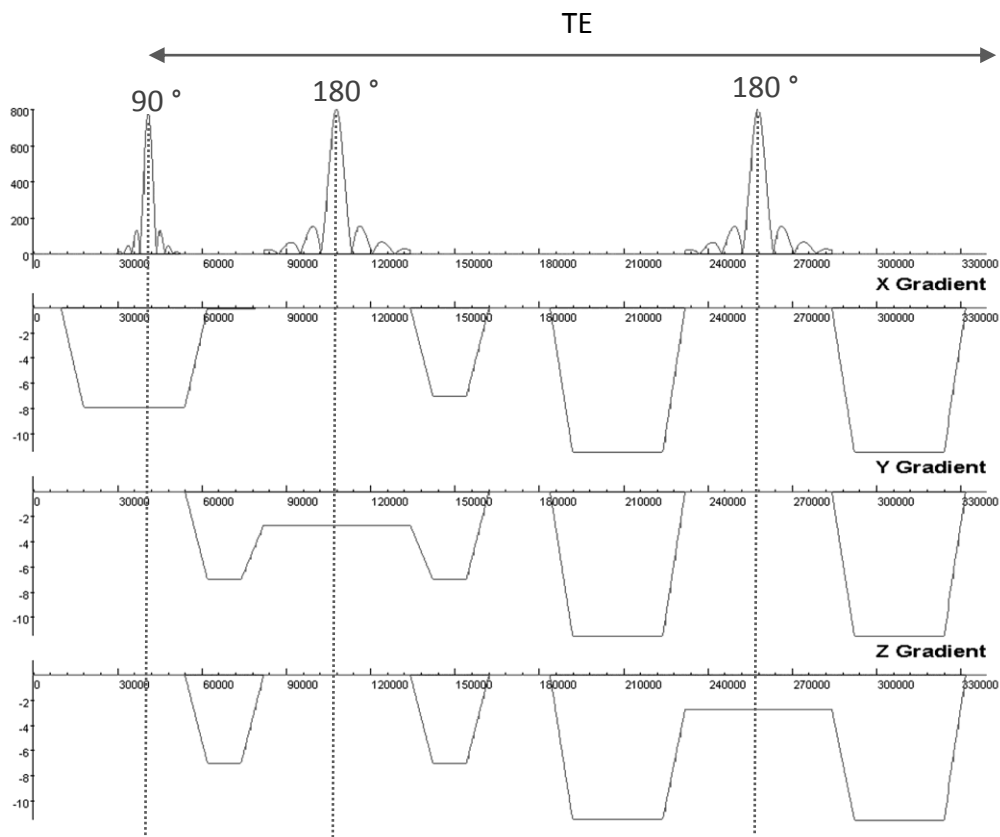


Figure 1.2 Pulse sequence diagram for Point Resolved Spectroscopy (PRESS). The RF pulse train (top trace), and the 3 gradient axes. Note the pairs of gradients around the 180° pulses to remove unwanted magnetization.

three orthogonal magnetic field gradients, the first excitation will only affect spins in a particular slice. A 180° RF pulse applied simultaneously with a magnetic field gradient orthogonal to the first gradient will refocus spins in a second slice, resulting in a spin echo signal from the cross section of the two RF pulses. Finally, a third pulse combined with a magnetic field gradient orthogonal to the other two gradient directions will result in a spin echo signal from the intersection of all three slices. PRESS preserves all initial magnetization, which is subject to T_2 decay during the entire echo time.

Both sequences were available on the 3 T clinical MR-system, but needed significant modifications to be suitable for cardiac MRS.

The loss of half of the potential signal is the most evident drawback of the STEAM localization technique in contrast with PRESS where the complete signal is acquired offering a 2-fold SNR increase (Figure 1.3).

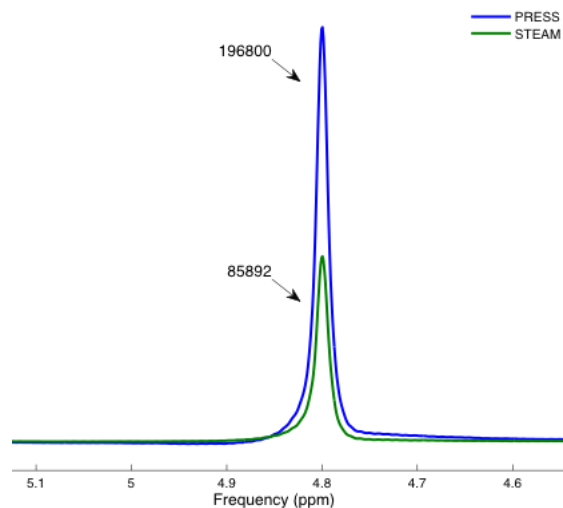


Figure 1.3 Water ^1H -MRS spectra obtained with STEAM (green line) and PRESS (blue line) in a $2 \times 2 \times 2$ cm voxel in a phantom with 15mM creatine in aqueous solution. Amplitude values (196800 and 85892) are in arbitrary units.

However, to select one localization method over the other, more factors have to be considered, like peak B_1 limitations, shortest TE attainable, sensitivity to spin displacement, actual size of the selected volume and dependence on B_1 inhomogeneities.

1.3.2 Peak B_1 Limitation and Chemical Shift Displacement

STEAM sequence was used for this work because at the peak B_1 limits of the scanner the spectral width of the 90° RF pulses used by STEAM is considerably narrower than that for 180° RF pulse. Weaker gradients associated with the wider bandwidth of the 180° pulse will introduce larger spectral offsets, impacting on the localization of metabolites with different chemical shift.

As an example of the chemical shift effect, the following table (Table 1.1) lists the voxel sizes used in the cardiac experiments for STEAM localization, the gradient strength needed for localization and the chemical shift displacement observed depending on frequency resonance of the sinc RF pulses.

Voxel Size (mm)	G (mT/m)	Water-Fat	Water-Creatine	Fat-Creatine
		CSD mm	CSD mm	CSD mm
RL	12	6.59	1.5	0.7
	19	4.16	2.4	1.2
FH	32	2.47	4.0	2.0
	36	2.20	4.5	2.2
AP	22	3.59	2.7	1.4

Table 1.1 RL right to left, AP anterior to posterior, FH feet to head, CSD chemical shift displacement

The largest chemical shift displacement will be observed in feet-to-head direction and is 4.5mm between water and fat. When performing signal localization in areas close to epicardial fat, the chemical shift displacement artifact can lead to severe spectral lipid contamination. If a PRESS sequence were to be used, all these CSD values would be doubled, resulting in very poorly localised spectra.

1.3.3 Echo Time: TE

STEAM sequence has shorter TE than PRESS, which needs considerable gradient spoiling to dephase unwanted signals created by the slice-selective refocusing pulses. A shorter TE may prevent signal loss for spins with short T_2 values, reduce motion sensitivity and, therefore improve the reliability of quantification. The minimum TE of the STEAM sequence was reduced by reprogramming the pulse sequence from 20 to 10 ms. Frequency selective RF pulses, TE and TM crusher gradients were all adjusted to find the best compromise between optimising the quality of the slice profile and the need to minimise motion sensitivity by shortening echo times. TE and TM crusher gradient strengths were evaluated on a phantom to ensure the complete elimination of unwanted coherences and to achieve accurate localization.

1.3.4 Flip Angle Effects and Spatial Variation of the B_1 Field

Using conventional sinc RF pulses the volume selected by STEAM is about 65% higher than that selected by PRESS (56,57). PRESS requires optimised 180° refocusing pulses to ensure a well-defined slice profile. It is well known that the signal amplitude depends upon well adjusted 90° or 180° RF pulses, and that the loading from different subjects and exact subject position alter this scaling. Hence, it is very important to set the RF transmit power

levels accurately to achieve the required RF pulse flip angle (58). The generally accepted method for setting the correct RF power levels to achieve 90° and 180° RF pulses is to attain the maximum echo amplitude of an RF spin-echo sequence. The echo amplitude for this α - 2α sequence is proportional to $\sin^3\alpha$. In order to assess the influence of the automatic transmitter reference voltage adjustment, the amplitude of the stimulated echo for three 90° RF pulses was fitted to a $\sin^3$ curve.

In the 3 T scanner used for data acquisition in this thesis, the reference amplitude was the input voltage to the transmitter required to produce a 180° rotation for a 1 ms rectangular pulse. All RF pulses were scaled to this reference amplitude. The adjustment did not refer to the adjustment volume, but rather to a range at the magnet isocentre. The reference amplitude was determined automatically by the system. The accuracy of the transmitter reference voltage was assessed by calculating the maximum of the $\sin^3$ curve described by the amplitude of the stimulated echo when the pulse voltage was varied manually from 100 to 1000 V. The theoretical $\sin^3$ dependency is (Figure 1.4):

$$Ampl = Ampl_{90} \cdot \sin^3\left(\frac{\pi}{2} \cdot \frac{Voltage}{Voltage_{90}}\right) \quad (1.3)$$

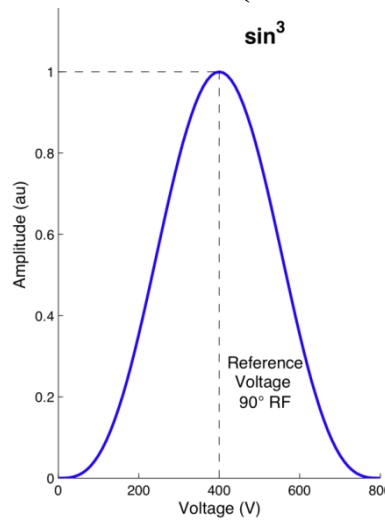


Figure 1.4 Echo amplitude plotted as a $\sin^3$ function of the RF pulse voltage.

Fitting the measured values, the theoretical quantities Ampl_{90} and Voltage_{90} can be determined.

A spherical phantom filled with a 0.1M solution of lithium acetate and sodium acetate was placed in the centre of the magnet. An 8mL voxel was located at the isocentre. The pulse voltage was varied manually from 100 to 1000 V and the corresponding signal was measured without water suppression using the body coil for transmit and 15 elements from the Siemens “Body-Matrix” (6 elements) and “SpineMatrix” (9 elements) receive array coils operating in triple. To investigate B_1 inhomogeneities, the voxel position was changed by 20 mm in six directions (x,y,z,-x,-y,-z). The coil load and adjustment parameters were the same in all directions (Figure 1.5)

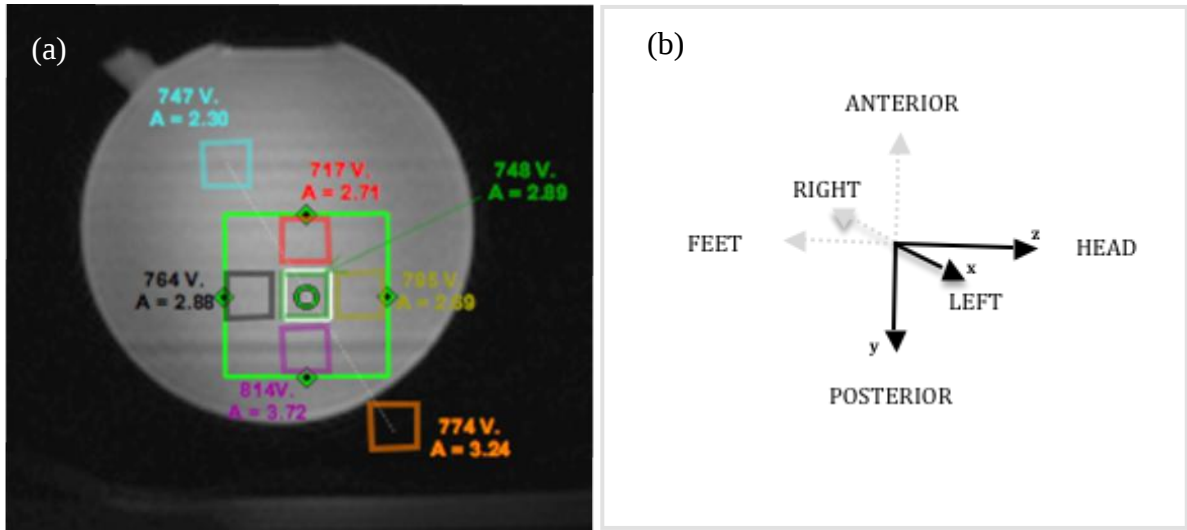


Figure 1.5(a) Sagittal view of the phantom showing different voxels positions used for the investigation of B_1 inhomogeneities (coloured squares) Amplitude (A value) and reference voltage values obtained from the $\sin^3$ fitting are also plotted. (b) Principal gradient axes scheme clarifying that sagittal images are perpendicular to the x direction.

The experimental variation of total water signal as a function of the applied RF voltage is plotted in Figure 1.6. The maximum water signal was found in the y-direction at the

position close to the receiver coil. At this location, the reference 90° RF voltage (814 V) determined by the MR system matched the fitted value (Figure 1.6 purple data set).

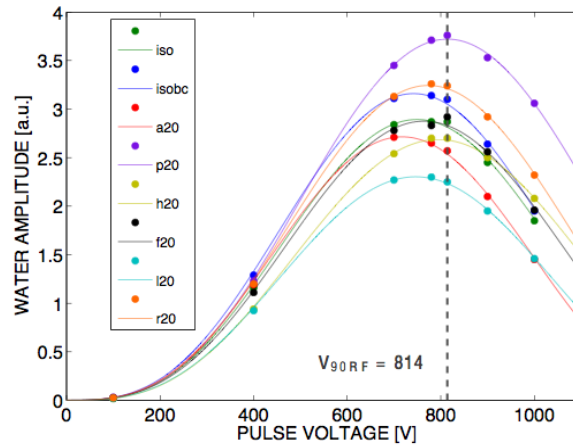


Figure 1.6 Water amplitude as function of the pulse voltage. Measured amplitude values (coloured dots) were fitted to a sinusoidal curve for each voxel location. The dashed vertical line shows the reference voltage (814 V) for a 90° RF pulse.

Figure 1.7 shows the percentage change between the automatically adjusted 90° RF voltages and the theoretical fitted value for the different locations. A flip angle of 90° was achieved for only one voxel position; flip angles were $> 90^\circ$ (2-12% higher) at the other voxel locations. The signal still followed a $\sin^3$ dependence (Figure 1.4) but the signal amplitude was decreased (1-6% lower), indicating that the adjustment of the RF flip angle was slightly influenced by inhomogeneous RF field distribution of the transmitter coil.

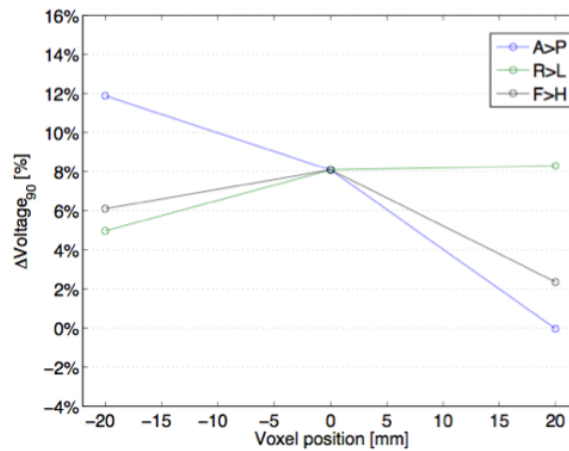


Figure 1.7 Difference between automatically adjusted and fitted 90° RF voltage

1.4 Signal Reception: Phased-Array Coils

The routine available in the system for combining MRS signals from individual coil elements (dubbed *system combination*) used the phase of the first-time point of the free induction decay (FID) to represent the phase of the time-domain spectra. If the spectrum had poor SNR or good water suppression it might be difficult to obtain an accurate phase value and unreliable spectra were seen, hence a combination routine was developed (dubbed *project combination*).

The *project combination* algorithm was based on the theory of the phased array coil described by Roemer et al (30). Several assumptions were made. The combination was performed without detailed knowledge of the coil magnetic fields, using the amplitude of the time domain signal without water suppression to represent the weighting factors required for weighted signal summation (32). In MR spectroscopy, the relative height of a particular spectral peak can be used as an approximation to flux density magnitudes at the location of the voxel from each coil. Furthermore, the correlation coefficients matrix of the noise detected by individual coils showed only small correlations between coils for the specific equipment used in this work.

Both, water unsuppressed and suppressed data were required in order to use the combination algorithm (Figure 1.8). First the phase and amplitude of the individual signals were calculated using jMRUI and weighting factors were deduced from these values. Then water-suppressed individual signals were phased using the previously calculated water phase, correcting for any possible eddy current effects (59). Only coils that contribute more than 10% to the signal ($\text{wf}_k \geq 0.1$) were used in the combination, since the rejected

data had poor SNR and it might be difficult to obtain an accurate measure of the phase, which can result in erroneous signal combination.

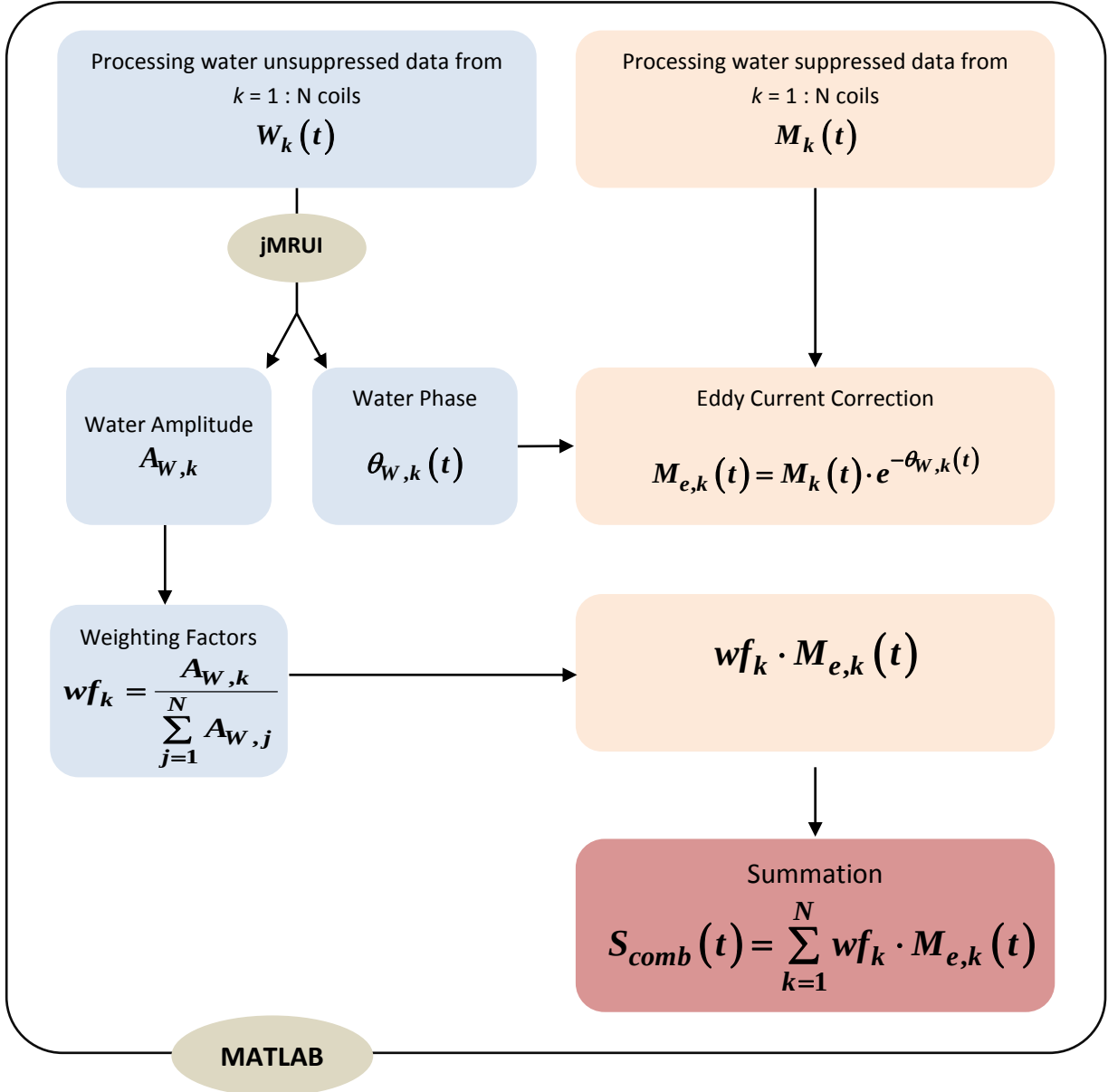


Figure 1.8 Project combination scheme. k : coil element; N : total number of coil elements, W_k : signal acquired without water suppression with element k ; M_k : signal acquired with water suppression with element k ; $A_{w,k}$: water amplitude acquired with element k , $\theta_{w,k}$, water phase acquired with element k ; $M_{e,k}$: signal with eddy current correction acquired with element k ; wf_k : weighting factor of element k ; S_{comb} : combined signal

The combination solution available in the system, *system combination* was compared to this routine acquiring an averaged ^1H -MR spectrum using the body coil for transmit and 15

elements from the Siemens “Body-Matrix” (6 elements) and “SpineMatrix” (9 elements) receive array coils operating in triple mode in a phantom (15mM creatine solution). Figure 1.9 shows that both routines provided very similar results when water-unsuppressed spectra were obtained.

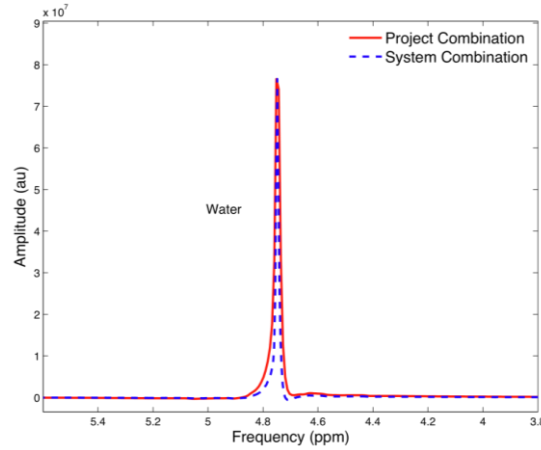


Figure 1.9 Combination comparison, high SNR case: water-unsuppressed spectra. The SNR of the water peak was only 2% higher using the *system combination* (3854, red line) than using the *project combination*. (3791, dash-blue line)

However, when the spectrum had low SNR, the *project combination* method combined the signals from the different receivers more efficiently, providing a 23% increase in SNR (Figure 1.10).

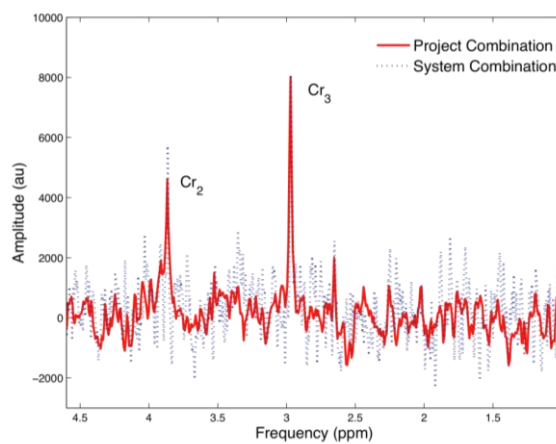


Figure 1.10 Combination comparison, low SNR case: water-suppressed spectra. *Project combination*, in red, resulted in an improved creatine SNR (0.69) compare to *system combination*, in blue (0.53). The spectra were scaled according to the creatine resonance at 3ppm for visualisation purposes.

1.5 Water Suppression

“Water suppression Enhanced through T1 effects” (WET) (37) was the water suppression technique investigated. The water suppression sequence was placed in the preparation period, before the localization sequence (see Figure 1.11). It consisted of three frequency-selective Gaussian-modulated RF pulses of equal duration (17.9 ms, bandwidth = 50 Hz) set at the water resonance (4.7 ppm) followed by a delay period containing a gradient dephasing pulse. Flip angles of 89.2° , 83.4° and 160.8° degrees, with an interpulse period $t_D = 60$ ms were used.

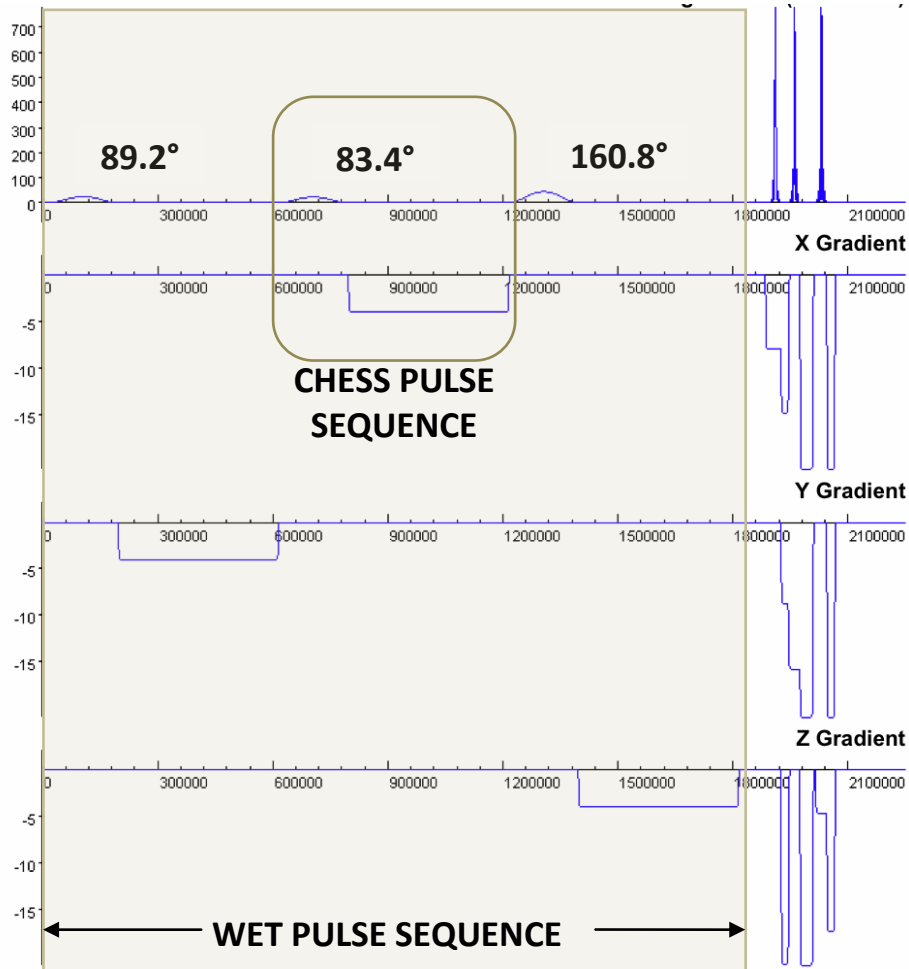


Figure 1.11 WET and CHES suppression schemes preceding STEAM localization pulse sequence.

A frequency selective 90° RF pulse followed by a gradient dephasing pulse is defined as CHES water suppression (36). In theory, WET shows improvements in water suppression

by adjusting the flip angle in three repeated CHESS cycles and it has reduced T_1 - and B_1 -sensitivity when compared a suppression scheme that uses $3 \times 90^\circ$ pulses. The RF flip angles were obtained from Bloch equation analysis of the longitudinal magnetization for $400 \leq T_1 \leq 2000$ ms and B_1 within $\pm 10\%$ of the nominal value as described by Ogg et al (37).

WET was compared to a 3-CHESS cycle to investigate the degree of water suppression in a 15mM Cr phantom. Careful consideration was given to verify that the applied magnetic field gradients did not lead to complete refocusing of transverse magnetization (38). The WET scheme preceding the STEAM localization sequence suppressed the water by a factor 300, as opposed to a factor 50 for 3-CHESS cycle (Figure 1.12).

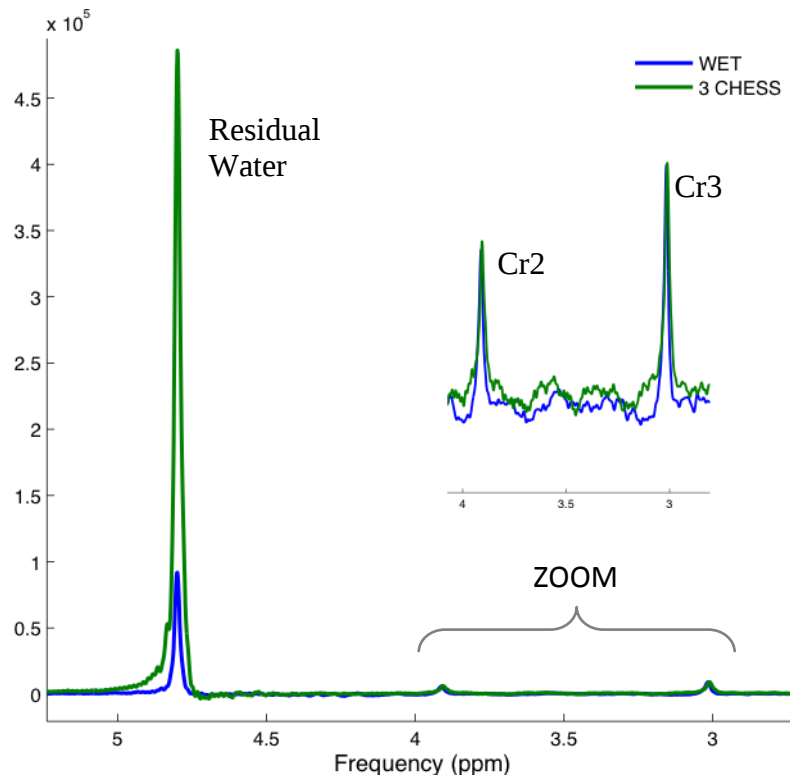


Figure 1.12 WET -3 CHESS comparison methylene (Cr2) and methyl (Cr3) protons

Furthermore, the frequency profile of water suppression was investigated to eliminate any direct effect on the metabolites. Figure 1.13 shows measured values of the residual water

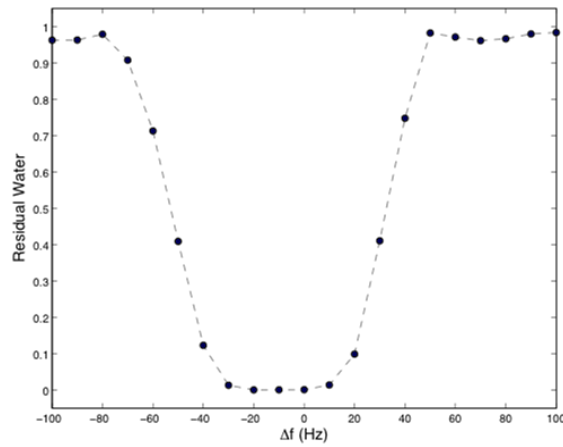


Figure 1.13 Off-resonance effects on water suppression

versus off-resonance increments (Δf) using WET with Gaussian RF pulses (bandwidth: 35 Hz). At frequency offsets within ± 20 Hz of the resonance frequency of the sample, the water suppression was very uniform.

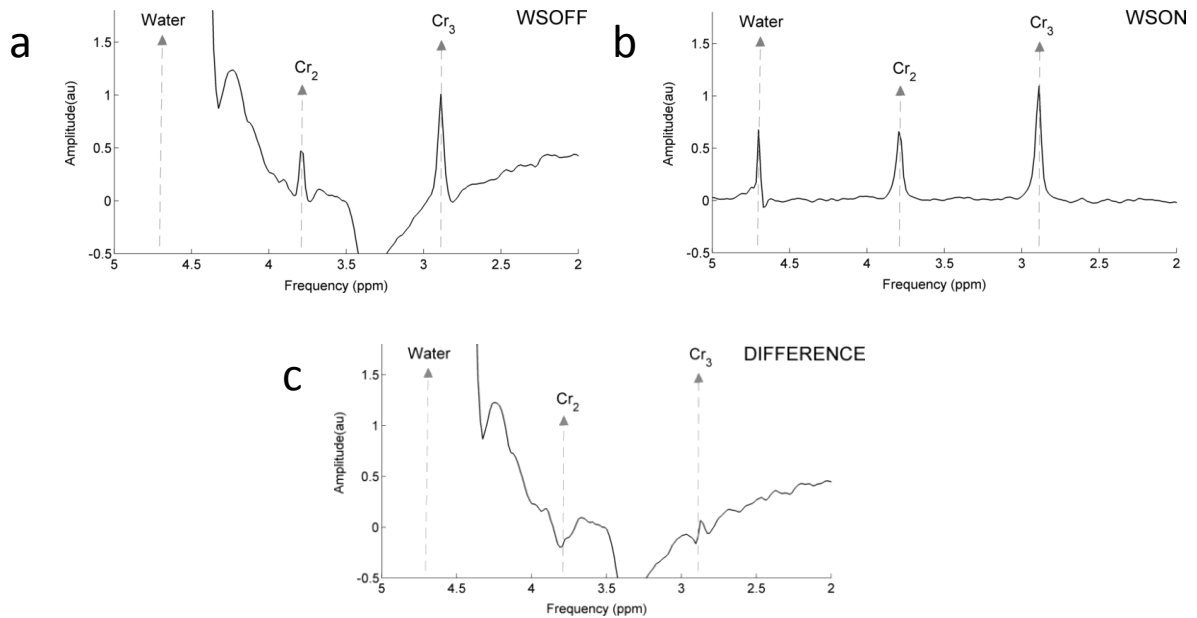


Figure 1.14 ^1H -MRS spectra from the 15mM creatine phantom. Observed resonances: water at 4.7 ppm, Cr_2 at 3.8 ppm and Cr_3 at 2.9 ppm. Spectrum (a) was acquired without water suppression (WSOFF), spectrum (b) with WET water suppression (WSON) and the difference spectrum (c) demonstrates that WET did not affect the creatine resonances

Figure 1.14 shows spectra from a 15mM creatine solution acquired without (a), with water suppression (b) and the difference between them (c), confirming that the peaks of the metabolites of interest close to the water (Cr peaks resonate at 100 and 210 Hz relative to the water peak at 3T) did not suffer loss of intensity. Spectrum (a) shows clear baseline distortions, which are consistent with the presence of the large water resonance.

1.6 Quantification and Post-Processing

All spectral quantification in this thesis was performed in the time domain, using the AMARES algorithm included in the MRUI package (V 3.0) (40).

As previously mentioned, the unsuppressed water peak amplitude at 4.7 ppm was used to calculate the percentage of metabolite contents (M) relative to water as the amplitude of metabolites (A_M) divided by the amplitude of the water amplitude (A_W), and multiplied by 100.

$$M = \frac{A_M}{A_W} \times 100 \quad (1.4)$$

The absolute concentrations of ^1H -MR spectra, expressed in mmol/L or $\mu\text{mol/g}$ of a specific metabolite (M), were calculated according to the following equations (60):

$$[M] = [W] \times \left(\frac{N_W}{N_M} \right) \times \left(\frac{A_M}{A_W} \right) \times F_{M/W} \times E_{M/W} \quad (1.5)$$

$$F_{M/W} = \left[1 - \exp(-TR/T_{1W}) \right] / \left[1 - \exp(-TR/T_{1M}) \right] \quad (1.6)$$

$$E_{M/W} = \exp(TE/T_{2W}) / \exp(TE/T_{2M}) \quad (1.7)$$

where, [W] is the concentration of water in tissue (e.g. [W] in myocardial tissue is 55 mol/kg times 72.7 % (% myocardial water content by weight (61,62)); the ratio N_W/N_M represents the protons on water (2) divided by protons on the metabolite (e.g. 3 in total creatine and 68 in lipids (4),63); $F_{M/W}$ is the spin-lattice (T_1) correction factor; and $E_{M/W}$ is the spin-spin correction (T_2) factor.

A further evaluation of the spectral quantification was based on the Cramer-Rao standard deviation (64) (CRSD) provided by the AMARES fitting algorithm as the standard deviation of the amplitude. Also, an estimate of the SNR of a metabolite under investigation was obtained by dividing the CRSD of the metabolite peak by the lipid peak amplitude and converted to a percentage (rCRSD %) (65).

$$rCRSD\% = \frac{CRSD}{A_M} \times 100 \quad (1.8)$$

Initial studies using the short echo time STEAM sequence were performed in a spherical phantom containing 15 mM creatine solution. The spatial homogeneity of Cr/Water quantification was examined by shifting multiple VOI (8 ml) along each axis until reaching the borders of the phantom. Cr/Water values showed no systematic dependency on the position of the VOI within a range of 80mm (Figure 1.15).

Furthermore, to assess the accuracy of the STEAM method, the absolute creatine concentration in a cylindrical phantom with known concentration (30mM/L) was calculated using Eq 1.5-1.7 A total of 4 scans were acquired without water suppression with a TR= 4 s and 35 water-suppressed scans with TR = 2 s. These relaxation times were chosen to match future in vivo measurements. Other scan parameters were, TE/TM=10/7

ms, 1024 points sampled with a spectral width of 2000 Hz. Two different voxel sizes and orientations were selected: a 20x20x20 mm tranverse voxel and a 32x18x22 mm double-oblique voxel. Spin-lattice and spin-spin relaxation times of water and creatine were measured first.

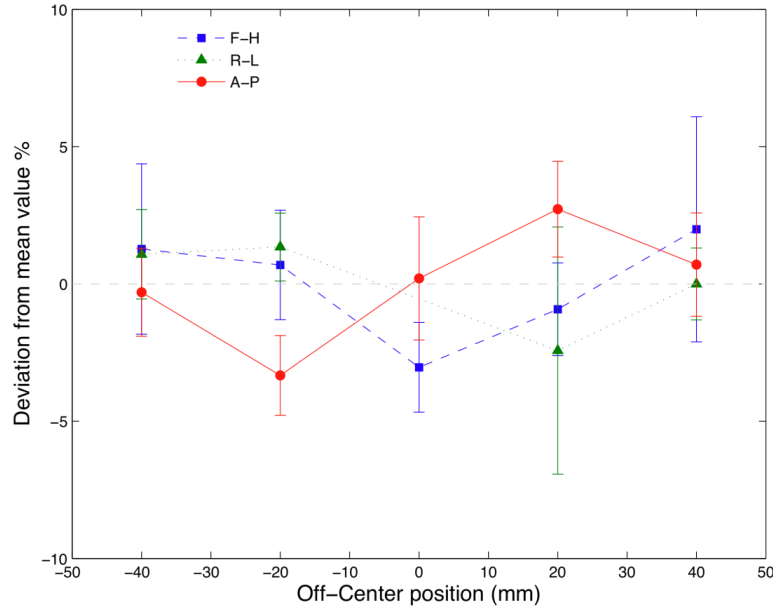


Figure 1.15 Deviation of the Cr/Water ratio from the mean value as a function of VOI position in right to left (R-L), anterior to posterior (A-P) and feet to head (F-H) direction. Error bars indicate the accuracy of the spectral fitting (CRSD).

For the assessment of the spin-lattice relaxation time of creatine and water, unaveraged STEAM data were acquired with and without water suppression, respectively, with constant TE (10 ms) and TR varied from 0.8 s to 20 s. In addition, one spectrum was acquired after leaving the sample in the scanner without any experiments performed during five minutes to estimate equilibrium magnetization (A_∞). T_1 values were calculated from linear regression of semilogarithmic plots of ($A_\infty - A_{TR}$) vs TR:

$$\ln(A_\infty - A_{TR}) = -\frac{TR}{T_1} + C_1 \quad (1.9)$$

The signal intensities for the T_2 calculation were obtained from the spectra measured at 8 different TE values: 10, 20, 40, 60, 80, 100, 150 and 200 ms with a TR = 20 s. T_2 values were then calculated from linear regression of:

$$\ln(A_{TE}) = -\frac{TE}{T_2} + C_2 \quad (1.10)$$

T_1 and T_2 measurements were performed in a 20x20x20 mm voxel positioned in the centre of the phantom. The relaxation behaviour of the water and creatine in the phantom is shown in Figure 1.16.

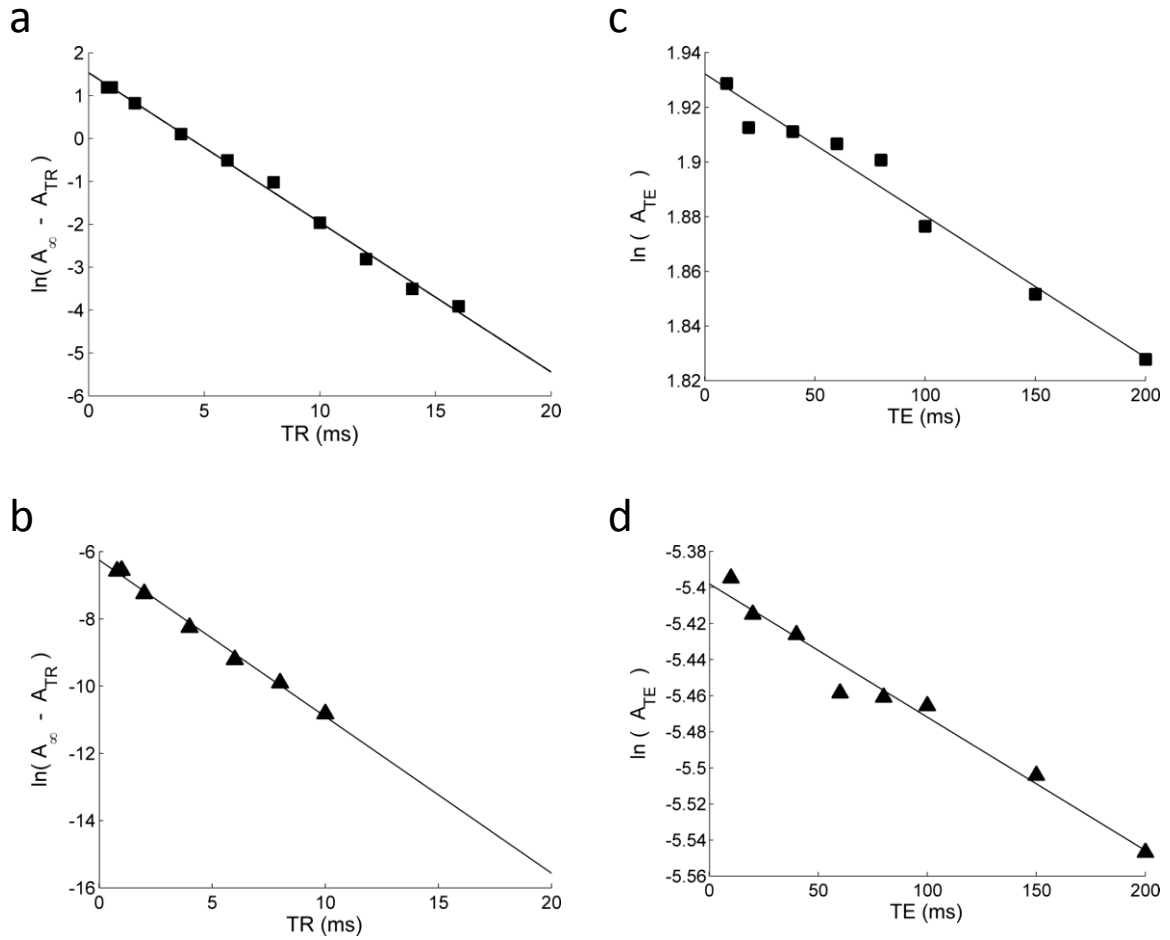


Figure 1.16 (a-b) Spin-lattice and (c-d) spin-spin relaxation behaviour. Water (squares) and creatine (triangles) signals in a creatine solution phantom.

Linear regression of semilogarithm plots for T_1 relaxation of water and creatine gave excellent results with $R^2 = 0.998$ and 0.997 respectively. The analysis of T_2 relaxation with semilogarithm plots also lead to excellent results, with $R^2=0.985$ and 0.986 for water and creatine signals, respectively. Relaxation times obtained from the fit are shown in Table 1.2.

	T_1 (s)	T_2 (s)
Water	2.87 ± 0.06	1.93 ± 0.13
Creatine	2.15 ± 0.07	1.35 ± 0.19

Table 1.2 T_1 and T_2 relaxation times in a creatine solution phantom. Relaxation time errors were calculated based on the standard errors of the best-fit values of the slope.

Using these relaxation times and Eq. 1.5-1.7 the creatine concentration obtained from an 8 mL transverse (30.2 ± 2.2 mM/L) and a 12.7 mL double-oblique volume (29.56 ± 1.9 mM/L) was almost identical to the real phantom concentration (30 mM/L creatine solution). Errors were calculated based on the Cramer-Rao standard deviation of the AMARES fitting and standard error propagation formulae. Thus, accurate quantification of total creatine concentrations in solution is feasible using ^1H -MRS, provided that T_1 and T_2 relaxation times are also known.

The peaks and prior knowledge used to fit in vivo ^1H -MR spectra will be specified in the methods section of each specific study.

1.7 Motion Compensation

Abdominal and thoracic ^1H -MR spectrum quality suffers from breathing motion, which causes organ displacements similar to the size of the localized volume and leads to outer volume contamination and considerable variations of lineshape, phase and amplitude. The

increased SNR at 3 T can reduce examination times, so the averaging of breath-hold data could be an approach to overcome the artifacts of respiratory motion while keeping a tolerable acquisition time for the patient.

The need for averaging individual spectra acquired in multiple measurements is depicted in Figure 1.17. Although the frequency is stable within a single breath-hold, there is a variation between breath-holds. An 8 ml voxel was placed in the liver and localized STEAM ^1H -MR spectra were acquired during free breathing. The first spectrum consisted of 128 averages. In the second scan, 64 consecutive, non-averaged measurements were acquired. In the averaged spectrum (pink trace), the motion in the presence of field gradients changed the phase of the echo signal resulting in destructive interference when the 128 consecutive signals were averaged. In this case, the water signal intensity was lower compared to a dedicated post-processing summation. The spectrum shown by the green trace was obtained by individually phase-correcting the 64 signals (12) and a better spectral resolution was demonstrated when a frequency alignment algorithm was applied before summation (blue trace).

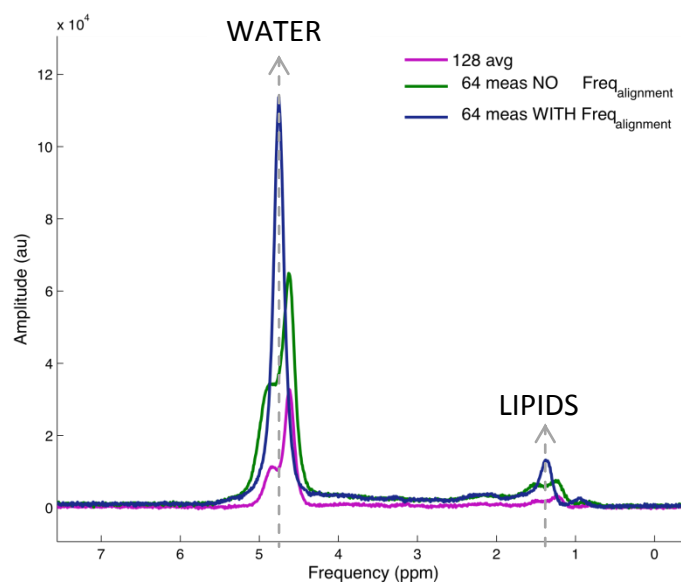


Figure 1.17 Liver spectra acquired during free breathing showing the benefits of postprocessing correction methods.

Importantly, the lineshape of the green and pink spectra reflected the absence of frequency alignment correction during averaging. The blue spectrum shows a substantial improved lineshape due to phase and frequency correction before summation of the individual measurements. Hence, in vivo ^1H -MRS was performed during breath-hold to minimize motional degradation and ensure the localization of the same tissue volume between scans. The multiple breath-hold summation consisted of the following steps: all combined spectra were phased individually first. The spectral peak with the largest amplitude was then automatically selected and the phased spectra were frequency aligned using this peak as a reference. Finally, the corrected spectra were averaged. Next, the effect of frequency alignment and phase correction on the data was investigated.

1.7.1 Frequency Correction

The frequency alignment algorithm first zero-fills and applies an exponential filter to increase SNR of the individual data; second, it finds the location of the residual water peak in a region centred at 4.7 ppm and any other prominent peaks that could be under investigation, for example lipids in a region centre at 1.3 ppm, in the spectrum in the frequency domain; third, aligns all the spectra to the chemical shift of the peak with the largest amplitude. This frequency shift correction algorithm was applied before averaging all measurements acquired during several breath-holds. To quantify the influence of cardiac motion and changes between breath-holds, the frequency of the water resonance (f) acquired in the first measurement during the first breath-hold was selected as reference. 15 volunteers underwent cardiac ^1H -MRS and 5 breath-holds with 7 measurements per breath-hold were acquired (a total of 35 measurements).

The intra breath-hold frequency variation was calculated as:

$$\Delta f_i^{\text{intra}} = \left(\sum_{j=1}^m f_{ij} - f_{11} \right) / m \quad (1.11)$$

Whereas, inter breath-hold frequency variation:

$$\Delta f^{\text{inter}} = \overline{\Delta f_i^{\text{intra}}} \quad (1.12)$$

Where $i = 1:b$ ($b = 5$, number of breath-holds) and $j = 1:m$ ($m = 7$, number of measurements per breath-hold).

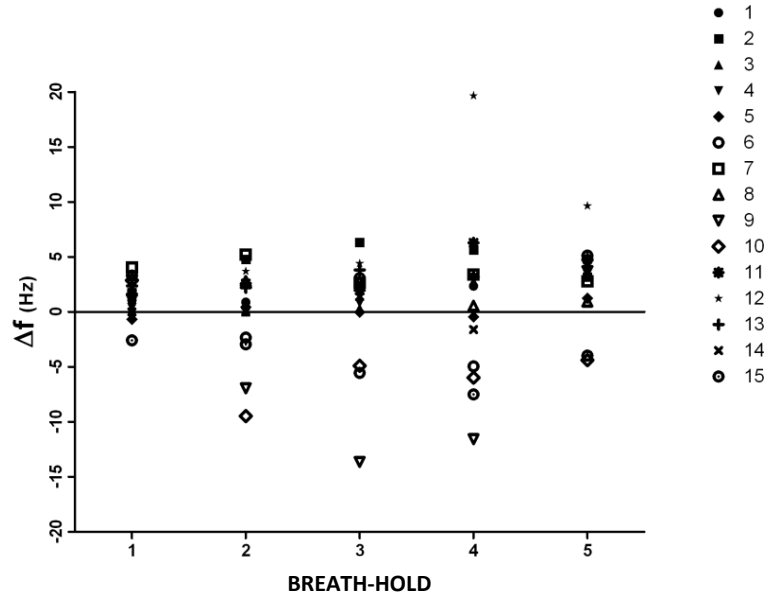


Figure 1.18 Water frequency variation within a breath-hold.

The frequency variation obtained in 7 measurements acquired within a breath-hold is depicted in Figure 1.18. Data acquired in five repeated breath-holds is shown for each volunteer. The mean frequency variation within a breath-hold for all 15 volunteers was 1.1 ± 1.8 Hz.

Figure 1.19 shows the inter breath-hold frequency variation for the 15 volunteers. Error bars represent the SD of the phase shift of 34 signals acquired during 5 breath-holds with respect to the first measurement in the first breath-hold. The mean frequency shift of the water signal over 7 breath-holds was 1.1 ± 3.4 Hz in 15 volunteers.

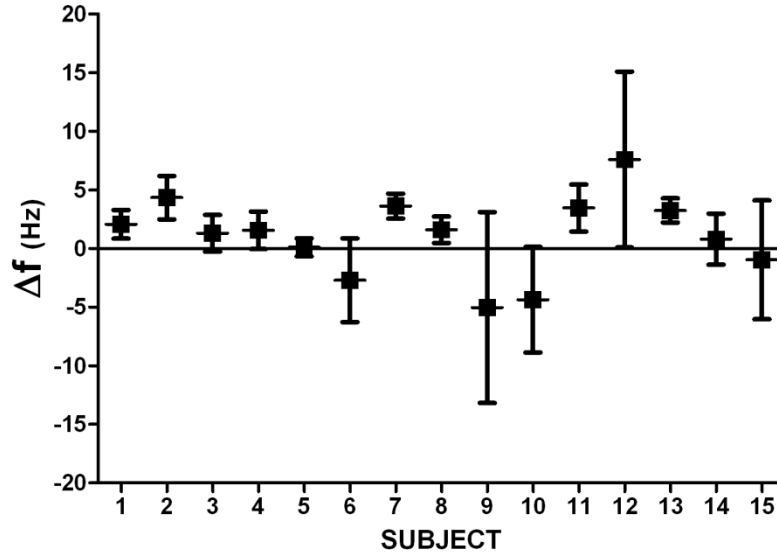


Figure 1.19 Inter breath-hold water frequency variation measured in 34 signals acquired over 5 breath-holds in 15 volunteers. The first signal measured during the first breath-hold was chosen as reference. Error bars represent the standard deviation of the frequency between measurements

1.7.2 Phase Correction

Phase correction was applied to constructively average all the measurements ($N = m \times b = 7 \times 5 = 35$) to prevent SNR reduction. The magnitude of the phase correction was calculated based on the phase of the resonance with the largest mean amplitude in the spectrum (either the residual water or the lipid resonance) to have sufficient SNR for a reliable determination of the phase. It was assumed that the phase shifts due to motion in all acquisitions were random and equally distributed in the phase range $[\theta_1, \theta_2]$. The importance of the phase variation between the N measurements can be estimated as:

$$\Delta\theta = \frac{(\theta_2 - \theta_1)}{2} \quad (1.13)$$

and the standard deviation:

$$\sigma_\theta = \frac{\Delta\theta}{\sqrt{3}} \quad (1.14)$$

The phase variation within the same breath-hold (intra) was calculated as:

$$\Delta\theta_i^{\text{intra}} = \frac{(\theta_{i2} - \theta_{i1})}{2} \quad (1.15)$$

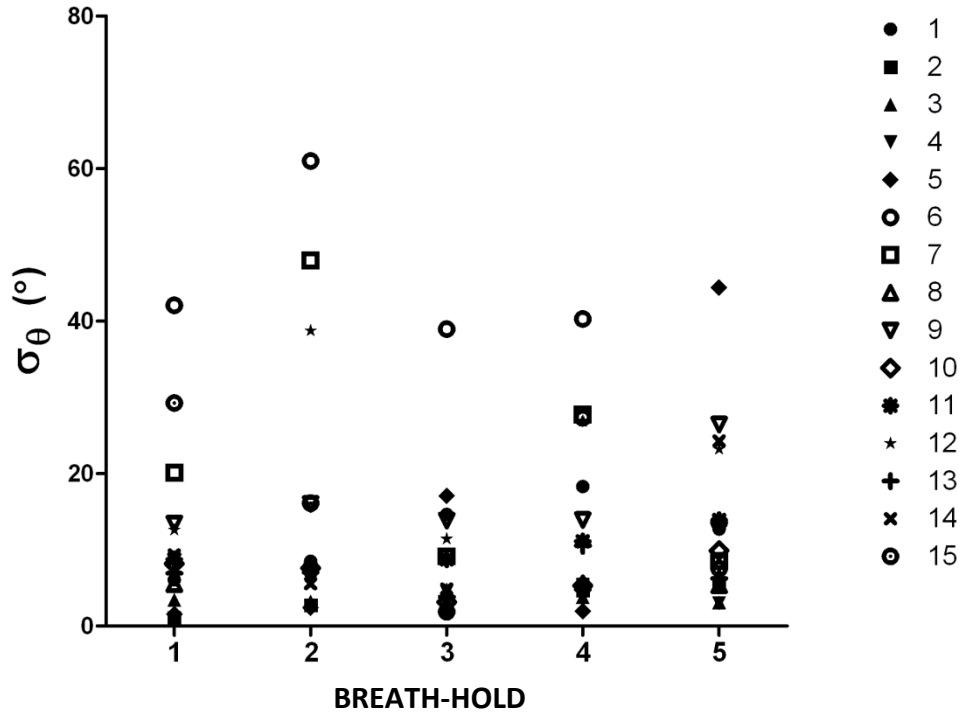


Figure 1.20 Intra breath-hold phase variation during the acquisition of 7 measurement per breath-hold. Data from 5 consecutive breath-holds were acquired in 15 volunteers.

The mean phase variation of the 7 measurements acquired in each breath-hold for all subjects was $\sigma_{\theta}^{\text{intra}} = 12.9^{\circ} \pm 12.6^{\circ}$ (mean $\pm$ SD, all subjects) (Figure 1.20). There was no difference in the phase variation in between breath-holds for each subject (two-way anova, $p=0.38$). However, the phase variation between subjects was significantly different, $p < 0.0001$.

For all $N=35$ averages, the inter breath-hold phase variation was obtained by:

$$\Delta\theta_N^{\text{inter}} = \frac{(\theta_2 - \theta_1)}{2} \quad (1.16)$$

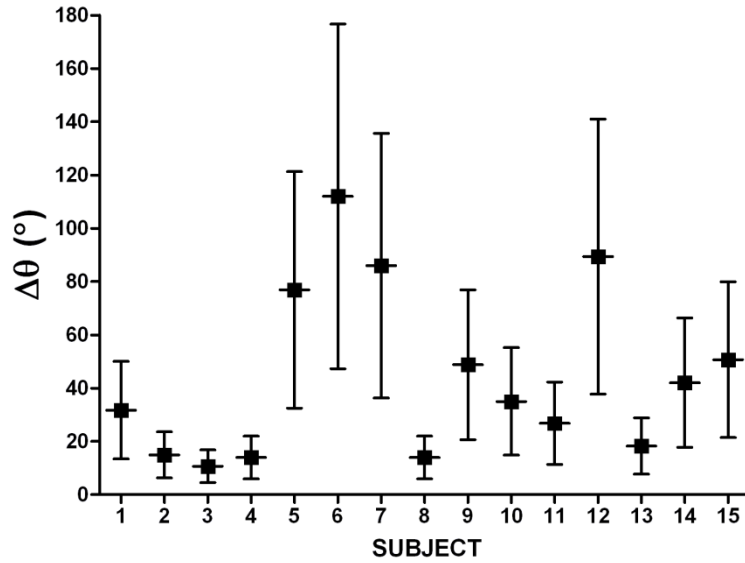


Figure 1.21 Inter breath-hold phase variation obtained in the constructive averaging of 35 signals from 15 volunteers Error bars represent

Figure 1.21 shows the phase variation in 35 cardiac spectra acquired during 7 breath-holds.

$\Delta\theta_N^{\text{inter}} = 44.7^{\circ} \pm 32.2^{\circ}$ and a phase variation of $\sigma_{\theta}^{\text{inter}} = 25.8^{\circ} \pm 18.6^{\circ}$ was found in the

constructive average of 35 cardiac signals (data are mean $\pm$ SD, all subjects). This value is in concordance with the phase variation published by Garb et al: $\sigma_\theta = 30.3^\circ$ from the constructive averaging of 128 cardiac double gated signals (12).

1.7.3 Water Suppression Calibration

WET water suppression used long, low power Gaussian RF pulses. The transmitter gain of these RF pulses needed to be scaled by a factor for low-flip angle RF pulses, due to the non-linear behaviour of the RF amplifier in this regime. This adjustment was performed automatically by the system, giving good results in phantom experiments. However, cardiac triggering was not available during the automatic adjustment in the Siemens MRI product software and very long breath-hold periods were required for respiratory motion correction, which made the automatic calibration unreliable for in vivo ^1H -MRS in the myocardium. Therefore, a new approach was developed. The STEAM localization pulse sequence was implemented with the additional functionality to acquire cardiac gated water-suppressed spectra using a range of different correction factors with cardiac gating and during a single breath-hold. The correction factor with the most effective water-suppression was then chosen by the operator and applied in the following experiments. This approach was implemented and found to perform successfully.

1.8 Discussion

The first part of this thesis involved substantial work focused on basic sequence development to overcome the technical problems associated with ^1H -MRS in human heart at 3 T.

Preliminary ^1H -MRS results obtained in phantoms suggested several advantages of STEAM localization method for the quantification of myocardial metabolites over PRESS, although all studies reported so far in cardiac ^1H -MRS but two (2,12) used single voxel PRESS. The limited peak B_1 that RF amplifiers provided for body imaging at 3 T was one of the main reasons to select STEAM as single voxel technique for localization. Additionally, STEAM uses 90° RF pulses instead of 180° RF pulses minimizing non ideal slice profiles (56,57). Furthermore, the minimum TE using STEAM was successfully halved to 10 ms by taking in consideration the available RF power and the effect of shortening RF pulses on their frequency profiles. It is noteworthy that MRS with echo time $TE = 10$ ms diminishes the effects of T_2 on metabolites-to-water amplitude ratio measurements (66,67) and results in a more consistent quantification for a clinical interpretation. Hence, future cardiac ^1H -MRS data in this thesis will benefit from the use of short echo times compared with previous studies, where the TE ranged from 15 ms to 40 ms.

Another achievement was the improvement of the method to efficiently combine signals from different receivers. The combination method developed, based on the Roemer theory for phased-array combination (30) and using the water resonance to weight the spectra, provided an increase in SNR of 23 % over the available one when applied to ^1H -MRS in phantom experiments. In this method computational demand is relatively low. On the other hand, the approach required manual intervention to obtain the weighting factors by quantification of the water amplitude and phase using AMARES, which may introduce operator variability. The use of a default prior knowledge data set was introduced to minimize errors.

Sufficient water suppression was verified using the available WET suppression scheme. The superiority of WET over CHESS confirmed the T_1 and B_1 insensitivity of WET at 3 T achieved by optimizing the nutation angles of the CHESS elements and optimizing the interpulse delay. Also, in the phantom experiments the reduction in the water resonance did not affect the baseline. Moreover, it was demonstrated that the methyl-resonance of creatine was not affected by the WET module. A spectroscopic measurement of the frequency profile of the WET module showed a narrow excitation band centred at the water resonance. In addition, phantom experiments with and without WET module showed no difference in the creatine resonance.

The excellent agreement between the creatine concentration in a phantom and the concentration calculated from the STEAM spectrum using the water as internal reference and T_1 and T_2 values calculated in other experiments with the same STEAM protocol, reflects the good field homogeneity, degree of water suppression and localization accuracy of the method.

Finally, motion correction was investigated for in vivo ^1H -MRS. This included the implementation of a new ECG-triggered protocol for an optimum scaling of the RF pulses used for water suppression performed within single breath-hold (12-17 s). In addition, a dedicated post-processing routine consisting of constructive averaging of cardiac ^1H -MRS acquired during consecutive breath-holds combined with a frequency alignment showed good preliminary results. The phase variation between 35 cardiac ^1H -MR spectra acquired over 7 breath-holds (25.8°) was very similar to the phase variation reported by Gabr et al (30.3°) in 128 cardiac ^1H -MR spectra acquired using navigator echo triggering(12).

In conclusion, a novel proton magnetic resonance technique has been developed, including an optimized short echo time STEAM single voxel localization method combined with a water suppression strategy modified for in vivo measurements, taking full advantage of the improved SNR offered by phased array coils. Furthermore, a post-processing routine was developed for correction of motion-induced dephasing of in vivo ^1H -MRS data acquired during breath-holding.

1.9 References

1. den Hollander JA, Evanochko WT, Pohost GM. Observation of cardiac lipids in humans by localized ¹H magnetic resonance spectroscopic imaging. *Magn Reson Med* 1994;32(2):175-180.
2. Bottomley PA, Weiss RG. Non-invasive magnetic-resonance detection of creatine depletion in non-viable infarcted myocardium. *Lancet* 1998;351(9104):714-718.
3. Felblinger J, Jung B, Slotboom J, Boesch C, Kreis R. Methods and reproducibility of cardiac/respiratory double-triggered (1)H-MR spectroscopy of the human heart. *Magn Reson Med* 1999;42(5):903-910.
4. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003;49(3):417-423.
5. Nakae I, Mitsunami K, Omura T, Yabe T, Tsutamoto T, Matsuo S, Takahashi M, Morikawa S, Inubushi T, Nakamura Y, Kinoshita M, Horie M. Proton magnetic resonance spectroscopy can detect creatine depletion associated with the progression of heart failure in cardiomyopathy. *J Am Coll Cardiol* 2003;42(9):1587-1593.
6. Walecki J, Michalak MJ, Michalak E, Bilinska ZT, Ruzyllo W. Usefulness of ¹H MR spectroscopy in the evaluation of myocardial metabolism in patients with dilated idiopathic cardiomyopathy: pilot study¹. *Academic Radiology* 2003;10(10):1187-1192.
7. Nakae I, Mitsunami K, Matsuo S, Matsumoto T, Morikawa S, Inubushi T, Koh T, Horie M. Assessment of myocardial creatine concentration in dysfunctional human heart by proton magnetic resonance spectroscopy. *Magn Reson Med Sci* 2004;3(1):19-25.
8. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *American journal of physiology* 2005;289(5):E935-939.
9. Nakae I, Mitsunami K, Matsuo S, Inubushi T, Morikawa S, Tsutamoto T, Koh T, Horie M. Myocardial creatine concentration in various nonischemic heart diseases assessed by ¹H magnetic resonance spectroscopy. *Circ J* 2005;69(6):711-716.
10. Kankaanpää M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Iozzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006;91(11):4689-4695.
11. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. *Magn Reson Med* 2004;51(6):1091-1095.
12. Gabr RE, Sathyanarayana S, Schar M, Weiss RG, Bottomley PA. On restoring motion-induced signal loss in single-voxel magnetic resonance spectra. *Magn Reson Med* 2006;56(4):754-760.
13. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of

- myocardial triglyceride content: reproducibility of ¹H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007;245(1):251-257.
14. van der Meer RW, Hammer S, Smit JW, Frolich M, Bax JJ, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Lamb HJ. Short-term caloric restriction induces accumulation of myocardial triglycerides and decreases left ventricular diastolic function in healthy subjects. *Diabetes* 2007;56(12):2849-2853.
15. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW, Romijn JA. Progressive caloric restriction induces dose-dependent changes in myocardial triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008;93(2):497-503.
16. van der Meer RW, Hammer S, Lamb HJ, Frolich M, Diamant M, Rijzewijk LJ, de Roos A, Romijn JA, Smit JW. Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men. *J Clin Endocrinol Metab* 2008;93(7):2702-2708.
17. van der Meer RW, Rijzewijk LJ, Diamant M, Hammer S, Schar M, Bax JJ, Smit JW, Romijn JA, de Roos A, Lamb HJ. The ageing male heart: myocardial triglyceride content as independent predictor of diastolic function. *Eur Heart J* 2008;29(12):1516-1522.
18. Lamb HJ, Smit JW, van der Meer RW, Hammer S, Doornbos J, de Roos A, Romijn JA. Metabolic MRI of myocardial and hepatic triglyceride content in response to nutritional interventions. *Curr Opin Clin Nutr Metab Care* 2008;11(5):573-579.
19. Hammer S, van der Meer RW, Lamb HJ, de Boer HH, Bax JJ, de Roos A, Romijn JA, Smit JW. Short-term flexibility of myocardial triglycerides and diastolic function in patients with type 2 diabetes mellitus. *American journal of physiology* 2008;295(3):E714-718.
20. Rijzewijk LJ, van der Meer RW, Smit JW, Diamant M, Bax JJ, Hammer S, Romijn JA, de Roos A, Lamb HJ. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol* 2008;52(22):1793-1799.
21. van der Meer RW, Rijzewijk LJ, de Jong HW, Lamb HJ, Lubberink M, Romijn JA, Bax JJ, de Roos A, Kamp O, Paulus WJ, Heine RJ, Lammertsma AA, Smit JW, Diamant M. Pioglitazone improves cardiac function and alters myocardial substrate metabolism without affecting cardiac triglyceride accumulation and high-energy phosphate metabolism in patients with well-controlled type 2 diabetes mellitus. *Circulation* 2009;119(15):2069-2077.
22. Schrauwen-Hinderling VB, Hesselink MKC, Meex R, van der Made S, Schar M, Lamb H, Wildberger JE, Glatz J, Snoep G, Kooi ME, Schrauwen P. Improved Ejection Fraction after Exercise Training in Obesity Is Accompanied by Reduced Cardiac Lipid Content. *J Clin Endocrinol Metab* 2010;95(4):1932-1938.
23. Liu CY, Redheuil A, Ouwerkerk R, Lima JA, Bluemke DA. Myocardial fat quantification in humans: Evaluation by two-point water-fat imaging and localized proton spectroscopy. *Magn Reson Med* 2010;63(4):892-901.
24. Krssak M, Winhofer Y, Gobl C, Bischof M, Reiter G, Kautzky-Willer A, Luger A, Krebs M, Anderwald C. Myocardial lipids and myocardial function in insulin resistant population. 2010; *Proceedings of ISMRM Stockholm*. p 174.
25. Neubauer S. Cardiac magnetic resonance spectroscopy. In: Lardo AC FZ, Chronos NAF, Fuster V., editor. *Cardiovascular magnetic resonance: established and emerging applications*: London: Martin Dunitz; 2003. p 39-60.
26. Neubauer S, Horn M, Naumann A, Tian R, Hu K, Laser M, Friedrich J, Gaudron P, Schnackerz K, Ingwall JS, et al. Impairment of energy metabolism in intact

- residual myocardium of rat hearts with chronic myocardial infarction. *J Clin Invest* 1995;95(3):1092-1100.
27. Nascimben L, Ingwall JS, Pauletto P, Friedrich J, Gwathmey JK, Saks V, Pessina AC, Allen PD. Creatine kinase system in failing and nonfailing human myocardium. *Circulation* 1996;94(8):1894-1901.
 28. Neubauer S, Clarke K. Cardiac energy metabolism in heart failure. In: Marber M, editor. *Cardiac Metabolism and ischemic cardiomyopathy*: Puteaux: Wolters Kluwer Health; 2003. p 45-61.
 29. Szczepaniak LS, Victor RG, Orci L, Unger RH. Forgotten but not gone: the rediscovery of fatty heart, the most common unrecognized disease in America. *Circ Res* 2007;101(8):759-767.
 30. Roemer PB, Edelstein WA, Hayes CE, Souza SP, Mueller OM. The NMR phased array. *Magn Reson Med* 1990;16(2):192-225.
 31. Brown MA. Time-domain combination of MR spectroscopy data acquired using phased-array coils. *Magn Reson Med* 2004;52(5):1207-1213.
 32. Natt O, Bezkorovaynyy V, Michaelis T, Frahm J. Use of phased array coils for a determination of absolute metabolite concentrations. *Magn Reson Med* 2005;53(1):3-8.
 33. Dong Z, Peterson B. The rapid and automatic combination of proton MRSI data using multi-channel coils without water suppression. *Magnetic resonance imaging* 2007;25(8):1148-1154.
 34. Bydder M, Hamilton G, Yokoo T, Sirlin CB. Optimal phased-array combination for spectroscopy. *Magnetic resonance imaging* 2008;26(6):847-850.
 35. Rodgers CT, Robson MD. Receive array magnetic resonance spectroscopy: Whittened singular value decomposition (WSVD) gives optimal Bayesian solution. *Magnetic Resonance in Medicine* 2010;63(4):881-891.
 36. Haase A, Frahm J, Hanicke W, Matthaei D. ^1H NMR chemical shift selective (CHESS) imaging. *Phys Med Biol* 1985;30(4):341-344.
 37. Ogg RJ, Kingsley PB, Taylor JS. WET, a T_1 - and B_1 -insensitive water-suppression method for in vivo localized ^1H NMR spectroscopy. *J Magn Reson B* 1994;104(1):1-10.
 38. Moonen CT, van Zijl PC. Effective Water Suppression for in vivo proton NMR Spectroscopy (DRYSTEAM). *Journal of Magnetic Resonance* (1969) 1990;88(1):28-41.
 39. Naressi A, Couturier C, Castang I, de Beer R, Graveron-Demilly D. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. *Comput Biol Med* 2001;31(4):269-286.
 40. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997;129(1):35-43.
 41. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993;30(6):672-679.
 42. Slotboom J, Boesch C, Kreis R. Versatile frequency domain fitting using time domain models and prior knowledge. *Magn Reson Med* 1998;39(6):899-911.
 43. Kanowski M, Kaufmann J, Braun J, Bernarding J, Tempelmann C. Quantitation of simulated short echo time ^1H human brain spectra by LCModel and AMARES. *Magn Reson Med* 2004;51(5):904-912.

44. Weis J, Johansson L, Ortiz-Nieto F, Ahlstrom H. Assessment of lipids in skeletal muscle by LCModel and AMARES. *J Magn Reson Imaging* 2009;30(5):1124-1129.
45. Hofmann L, Slotboom J, Jung B, Maloca P, Boesch C, Kreis R. Quantitative ¹H-magnetic resonance spectroscopy of human brain: Influence of composition and parameterization of the basis set in linear combination model-fitting. *Magn Reson Med* 2002;48(3):440-453.
46. Wood ML, Mark Henkelman R. The magnetic field dependence of the breathing artifact. *Magnetic Resonance Imaging* 1986;4(5):387-392.
47. Felblinger J, Kreis R, Boesch C. Effects of physiologic motion of the human brain upon quantitative ¹H-MRS: analysis and correction by retro-gating. *NMR Biomed* 1998;11(3):107-114.
48. Wang Y, Rossman PJ, Grimm RC, Riederer SJ, Ehman RL. Navigator-echo-based real-time respiratory gating and triggering for reduction of respiration effects in three-dimensional coronary MR angiography. *Radiology* 1996;198(1):55-60.
49. Frahm J, Bruhn H, Gyngell ML, Merboldt KD, Hanicke W, Sauter R. Localized high-resolution proton NMR spectroscopy using stimulated echoes: initial applications to human brain in vivo. *Magn Reson Med* 1989;9(1):79-93.
50. Ordidge RJ, Bendall MR, Gordon RE, Connelly A. Volume selection in in-vivo spectroscopy. In: Govil G KC, Saran A, editor. *Magnetic resonance in biology and medicine*. New Delhi: Tata McGraw-Hil; 1985.
51. Hahn LE. Spin Echoes. *Phys Rev* 1950;80(4):580-594.
52. Frahm JM, K.D. Hanicke, W. Localized proton spectroscopy using stimulated echoes. *Journal of Magnetic Resonance* 1987;72:502-508.
53. Frahm J, Michaelis T, Merboldt KD, Hanicke W, Gyngell ML, Chien D, Bruhn H. Localized NMR spectroscopy in vivo. Progress and problems. *NMR Biomed* 1989;2(5-6):188-195.
54. van Zijl PC, Moonen CT, Alger JR, Cohen JS, Chesnick SA. High field localized proton spectroscopy in small volumes: greatly improved localization and shimming using shielded strong gradients. *Magn Reson Med* 1989;10(2):256-265.
55. Bottomley PA. Spatial localization in NMR spectroscopy in vivo. *Ann N Y Acad Sci* 1987;508:333-348.
56. Moonen CT, von Kienlin M, van Zijl PC, Cohen J, Gillen J, Daly P, Wolf G. Comparison of single-shot localization methods (STEAM and PRESS) for in vivo proton NMR spectroscopy. *NMR Biomed* 1989;2(5-6):201-208.
57. Ryner LN, Ke Y, Thomas MA. Flip angle effects in STEAM and PRESS-optimized versus sinc RF pulses. *J Magn Reson* 1998;131(1):118-125.
58. Perman WH, Bernstein MA, Sandstrom JC. A method for correctly setting the rf flip angle. *Magn Reson Med* 1989;9(1):16-24.
59. Klose U. In vivo proton spectroscopy in presence of eddy currents. *Magn Reson Med* 1990;14(1):26-30.
60. Bottomley PA, Lee Y, Weiss RG. Total creatine in muscle: imaging and quantification with proton MR spectroscopy. *Radiology* 1997;204(2):403-410.
61. Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH. Report of the Task Group on Reference Man. International Commission on Radiological Protection. Volume 23: Pergamon Press, Oxford; 1984. p pp. 280-285, p. 234, p. 116.
62. Bottomley PA, Atalar E, Weiss RG. Human cardiac high-energy phosphate metabolite concentrations by 1D-resolved NMR spectroscopy. *Magn Reson Med* 1996;35(5):664-670.

63. Thomassen MS, Strom E, Christiansen EN, Norum KR. Effect of marine oil and rapeseed oil on composition of fatty acids in lipoprotein triacylglycerols from rat blood plasma and liver perfusate. *Lipids* 1979;14(1):58-65.
64. Cavassila S, Deval S, Huegen C, van Ormondt D, Graveron-Demilly D. Cramer-Rao bounds: an evaluation tool for quantitation. *NMR Biomed* 2001;14(4):278-283.
65. Lamb HJ, Doornbos J, den Hollander JA, Luyten PR, Beyerbach HP, van der Wall EE, de Roos A. Reproducibility of human cardiac ^{31}P -NMR spectroscopy. *NMR Biomed* 1996;9(5):217-227.
66. Machann J, Thamer C, Schnoedt B, Stefan N, Haring HU, Claussen CD, Fritsche A, Schick F. Hepatic lipid accumulation in healthy subjects: a comparative study using spectral fat-selective MRI and volume-localized ^1H -MR spectroscopy. *Magn Reson Med* 2006;55(4):913-917.
67. Sharma P, Martin DR, Pineda N, Xu Q, Vos M, Anania F, Hu X. Quantitative analysis of T2-correction in single-voxel magnetic resonance spectroscopy of hepatic lipid fraction. *J Magn Reson Imaging* 2009;29(3):629-635.

2

Liver ^1H -MRS at 3 Tesla

2.1 Introduction

The majority of clinical applications of ^1H -MRS are related to the study of normal and pathophysiological metabolism in human brains. One of the reasons being that brain biopsy is generally considered by clinicians as a procedure of last resource, while other organs are more accessible for biopsy sampling. Moreover, localized ^1H -MRS of brain metabolites in vivo has overcome the original technical difficulties over the last two decades becoming a well-established clinical tool (1). There are many potential clinical applications for ^1H -MRS of other organs such as liver and heart, but it has been a challenge to implement this technique as a diagnostic tool for clinical routine use. Several factors affect the general clinical non-practicability: signal distortions as a result of cardiac and respiratory motion, lack of peak resolution, long acquisition times or complex processing protocols.

To characterize the performance of the method described in the previous chapter, liver metabolites were quantified first as an intermediate step towards cardiac application. The aim

was to validate the STEAM technique initially developed in phantom studies and to implement respiratory motion correction for future cardiac ^1H -MRS acquisitions. Liver ^1H -MRS shares some technical challenges with cardiac ^1H -MRS (Table 2.1) such as respiratory motion and B_1 field inhomogeneities arising due to wavelength interference effects (the RF wavelength at 3 T becomes comparable to the human trunk dimensions). On the other hand, liver is easier to study as it eliminates cardiac motion, provides better B_0 homogeneity, it is a larger structure than the ventricular wall, and good quality water suppression and shimming are easier to obtain.

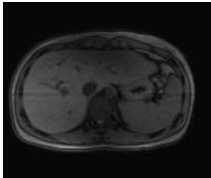
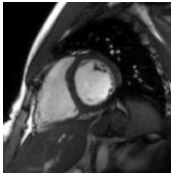
	BRAIN	LIVER	HEART
			
CHALLENGES			
ORGAN MOTION	no	no	yes
SNR	good	poor	poor
BREATHING ARTIFACTS	negligible	important	important
B_1 INHOMOGENEITIES	low	high	high

Table 2.1 Technical and biological obstacles of ^1H -MRS that need to be overcome to acquire liver and cardiac spectrum compared to brain spectrum.

The following section of this chapter will provide an overview of ^1H -MRS in the liver. The section does not attempt to describe all the studies published to date. Instead, there is a description of the potential diagnostic value of hepatic ^1H -MRS, as well as the solutions applied in previous studies performed at 3 T to minimize the influence of respiratory motion.

The next sections will focus on the validation of the ^1H -MRS technique previously developed in phantoms to investigate liver metabolism in healthy volunteers. Furthermore, given that most of the ^1H -MRS studies of liver fat metabolism only acquired water-unsuppressed spectra, the methylene lipid amplitude (1.2 ppm) in the liver was quantified using ^1H -MRS without water suppression and compared to ^1H -MRS with water suppression. In addition, preliminary results of the application of hepatic ^1H -MRS in a clinical research study are reported. This study investigates hepatic lipid content in pre- and post-menopausal women with relation to risk factors for cardiovascular disease

2.2 Liver ^1H -MRS Overview

Proton spectroscopy has become a common technique to investigate the mechanisms underlying hepatic diseases (2). The majority of the studies are focused on the quantification of liver fat content (3,4), due to the high SNR of the lipid methylene resonance in vivo. Hence, this peak is appropriate for diagnostic purposes. Fatty liver is one of the most common forms of liver disease and is increasingly recognised as a major health burden. The accumulation of fat in the liver cells is usually associated with a high intake of alcohol, although it also occurs in patients who do not abuse alcohol, commonly known as non-alcoholic fatty liver disease (NAFLD) (5). NAFLD is associated with obesity, insulin resistance and heart disease (6-8) and as the incidence of obesity and the metabolic syndrome increases, NAFLD may progress into more severe hepatocellular damage with increasing risk for the development of fibrosis and its complications. However, its pathogenesis is poorly understood. Liver biopsy is the gold standard for confirming the diagnosis in patients with clinical features of NAFLD. Although this technique can also provide information about other liver abnormalities, liver biopsy can cause discomfort and medical complications. On the other hand, localized proton magnetic resonance provides a reliable non-invasive

assessment of liver fat content (3,9,10). However, it still remains as a clinical research technique.

At the time this work was written, a total of 25 studies had been published on liver ^1H -MRS at 3 T (11-34). Table 2.2 shows an overview of the techniques used and the targeted metabolites.

Table 2.2 Liver ^1H -MRS publications at 3 T

		Water Suppression		Coils		Breathing Correction			Metabolites	
		WSOFF	WSON	Single	Multiple	No	BH	Belt&BH	Lipids	Lipids and CCC
STEAM	4	4	0	3	1	1	3	0	4	0
PRESS	21	15	6	9	12	16	2	3	17	4
Total	25	19	6	12	13	17	5	3	21	4

WSOFF: water-unsuppressed scans; WSON: water-suppressed scans; BH: breath-holding; CCC: choline containing compounds.

Four papers used STEAM and 15 used PRESS without water suppression, whereas PRESS with water suppression was used in 6 papers. Single receive coils and multiple elements phased coil arrays were evenly used. 17 papers did not perform respiratory motion correction and the remaining acquired ^1H -MR spectra during breath-holding or in combination with an abdominal compressor belt. Liver lipid metabolism was studied in 21 papers and only 4 papers reported values of CCC levels in liver.

As discussed in section 1.3, STEAM has shorter TE than PRESS, decreasing signal loss due to T_2 relaxation. Also the slice profile in STEAM is better because it is easier to produce a 90° pulse with a sharp slice profile than a 180° . Slice profile is important when investigating focal lesions, like tumours. Also, a study performed at 1.5 T comparing PRESS and STEAM localized ^1H -MRS in liver (35) found that PRESS gave higher estimates in liver fat compared

to STEAM. They suggested that this difference was due to the different sensitivity of STEAM and PRESS to J coupling, shown in high-resolution spectra of a fat phantom acquired at 3 T.

Surprisingly, only 8 (32%) of 25 studies applied respiratory motion correction and it is well known that respiratory motion will cause phase shifts in the spectra resulting in signal loss (36). Moreover, MR signal can originate from different locations within the liver due to the movement of liver associated to respiratory motion (37). Although liver fat distribution in healthy volunteers has been shown to be homogeneous (38) and similarly, liver histological results from patients with suspected NAFLD has shown that fat was diffused and equally distributed (39), signal could originate from outside the liver or from a large vessel leading to inaccurate metabolite quantification. This suggests the need for respiratory correction during liver ^1H -MRS, and even more at 3 T since breathing artifacts are exacerbated by high magnetic fields (40).

This study investigated the feasibility of water-suppressed STEAM MRS at 3 T during breath-holding for the study of liver metabolism.

2.3 Methods

2.3.1 Liver ^1H -MRS Validation in Healthy Volunteers

Seven healthy volunteers (three females, four males, age = 21-41 years; BMI = 23.4 ± 2.7 kg/m²) without prior medical history of liver disease, obesity or alcohol abuse participated in this study. Written consent was obtained before all the examinations.

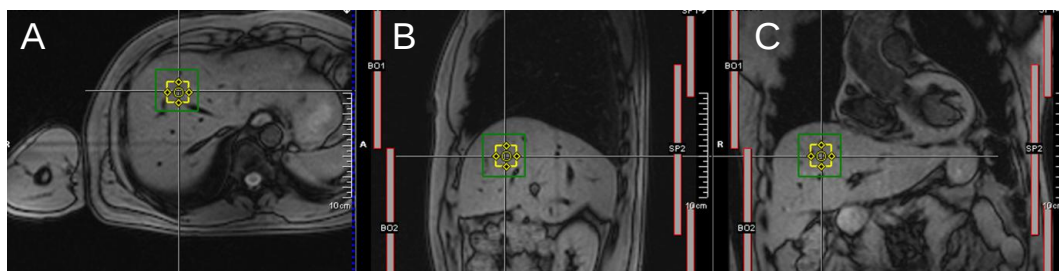


Figure 2.1 Voxel position (2x2x2 cm yellow box) in a liver at an axial (A), sagittal (B) and coronal (B) slice. The green box represents the shim voxel.

All experiments were performed on a Siemens 3 T Magnetom Tim Trio system using the anterior and posterior phased-array surface coils (15 elements) for receiving in combination with a body coil in transmit mode. Subjects arrived early in the morning following overnight fasting, lay supine and the anterior phased-array coil was placed over the abdomen. Anatomical reference images were acquired during breath-hold in end-expiration using a turbo-FLASH sequence with dark blood preparation. A voxel of 2x2x2 cm was positioned in the right hepatic lobe (Figure 2.1). Shim adjustment was performed using the automatic active 3D shimming procedure available in the system. All ^1H -MRS acquisitions were ECG-triggered to the R wave. Spectra were acquired using the STEAM sequence with the WET water suppression scheme, both previously described in Chapter 1 (sections 1.3 and 1.5). Spectroscopic parameters included a TE of 10 ms, a mixing time of 7 ms, 1024 points and a bandwidth of 2000 Hz. Effective repetition times of at least 4 and 2 s were chosen to ensure complete relaxation of the water and lipid signals, respectively (41).

Localized MR measurements consisted of one breath-hold acquisition without water suppression, which allowed for four averages followed by ten consecutive breath-hold water-suppressed acquisitions (total of 70 averages, seven acquisitions per breath-hold). The breath-hold duration was at least 14 seconds, depending on the heart rate. Each breath-hold scan was

stored as a separate file. The water-unsuppressed breath-hold file included 60 (4 measurements x 15 coils) FIDs and the water-suppressed breath-hold files included 105 (7 measurements x 15 coils) FIDs. Furthermore, the scan frequency was set at 4.7 ppm during water-unsuppressed acquisitions, while a -3.5 ppm frequency shift was applied during water-suppressed acquisitions, to reduce the 2.5 mm chemical shift displacement between the lipid and water peaks when using a sinc pulse with a 3.4 kHz bandwidth to select a 20x20x20 mm voxel.

Two aspects of reproducibility were investigated: (i) within-day reproducibility; three repeated measurements were collected per session to quantify the repeatability of the ^1H -MRS results. Volunteers remained on the scanner table in between measurements. However, adjustments of the scanner frequency and reset of shim currents were enforced in between measurements. (ii) Long-term reproducibility (between-days); all volunteers were examined twice within 2-3 weeks of the first session to assess reproducibility between weeks.

2.3.2 Necessity of Water Suppression in Liver ^1H -MRS

A total of 86 liver ^1H -MRS data sets were analyzed. Spectra acquired with and without water suppression were used to investigate the differences between metabolite quantification between both techniques. The 86 subjects (age = 41 ± 12 years, BMI = 26.1 ± 7.2 kg/m², 60 females and 26 males) were participants of other ongoing studies using liver ^1H -MRS in the department.

^1H -MR spectra were acquired as described in the previous section (2.3.1). Water unsuppressed scans consisted of 4 averages. For the aim of this section, 4 randomly chosen water-suppressed spectra out of 7 acquired during one breath-hold were considered for

constructive averaging. Thus, the SNR of the spectra with and without water suppression will be comparable. Also, post-acquisition water removal through the use of the Hankel Lanczos single value decomposition method, HLSVD (42), available in jMRUI was investigated.

2.3.3 Liver Fat Assessment in Pre- and Post- Menopausal Women

As part of an ongoing study, which aims to investigate the role of menopausal status in relation to risk factor for cardiovascular disease, liver fat was measured by ^1H -MRS in 31 women (18 pre- and 13 post-menopausal). Subjects were studied in the morning after overnight fasting. All subjects gave written informed consent.

Liver ^1H -MRS was performed as described in the previous section (2.3.1), with the difference that shim adjustment was performed on a field map obtained from a gradient double echo acquisition within a single breath-hold (18-22 s). Briefly, hepatic ^1H -MRS was obtained from a 2x2x2 cm voxel located in the liver (Figure 2.1). Spectroscopic data acquisition was triggered with ECG triggering during breath-holding to minimize motion artifacts. Water-suppressed spectra to measure liver lipid content was acquired in 3 consecutive breath-holds (total of 21 averages) and spectra without water suppression were acquired in one breath-hold (total of 4 averages) and used as internal reference.

2.3.4 Spectral Quantification

Combination of signals from individual coil elements was performed as described in section 1.4. The multiple ^1H -MRS acquisitions (i.e. four during water-unsuppressed scans and 70, 21 or four during water-suppressed scans) were averaged using the methods described in section

1.7. A total of six peaks were specified to fit the metabolite contributions in the water-suppressed spectra by using a Lorentzian linewidth. Table 2.3 shows the chemical shift starting values and the soft constrains (lower and upper bounds) applied for chemical shifts and linewidths (43,44) during AMARES quantification.

Table 2.3 Chemical shifts for ¹H-containing liver metabolites and soft constrains used for chemical shift and linewidth parameters during AMARES quantification

Metabolite	Group	Chemical Shift Starting Values (ppm)	Chemical Shift Constrains (ppm)	Linewidth Constrains (Hz)
Lipid Methyl	CH ₃	0.9	0.7 - 1.1	2 - 40
Lipid Methylene	CH ₂	1.3	1.2 - 1.4	2 - 40
Lipid Allylic	CH ₂ -CH=CH-	2.0	2.0 - 2.1	2 - 40
Choline Components	N(CH ₃) ₃	3.2	3.1 - 3.4	2 - 40
Glucose & Glycogen		3.7	3.6 - 3.9	2 - 40
Residual Water	H ₂ O	4.7	4.5 - 4.9	2 - 40

The unsuppressed water peak amplitude at 4.7 ppm was used as internal reference to calculate the percentage of a specific metabolite content relative to water as the amplitude of the metabolite divided by the amplitude of the water amplitude, and multiplied by 100.

$$\text{Metabolite} = \frac{\text{Metabolite Amplitude}}{\text{Water Amplitude}} \times 100\% \quad (2.1)$$

A further evaluation of the spectral quality in the liver ¹H-MRS validation study was based on the Cramer-Rao lower bounds (CRLBs) provided by the AMARES fitting algorithm as the

standard deviation of the amplitude. An estimate of the SNR was obtained by dividing the CRSD of the metabolite signal by the metabolite amplitude and multiple by 100 (rCRSD %)
(45)

No T_1 correction was necessary, as a TR of 4s and 2s, both water and lipids will be fully relaxed (T_1 water = 809 ms; T_1 fat = 382 ms (46)). Although water and lipid resonances have different T_2 values (T_2 water = 35.2 ms; T_2 $\text{CH}_2=\text{CH}_2$ = 39.2 ms; T_2 CH_2 = 75.5 ms and T_2 CH_3 = 45 ms reported at 1.5 T (35), which are assumed to be slightly higher at 3 T (47)), fitted amplitudes were not corrected for T_2 relaxation, as spectra were collected with short TE = 10 ms, which minimized the error due to T_2 relaxation. Moreover, all spectra were acquired with the same TE, thus omitting the T_2 -correction leads to a systematic error.

2.3.5 Statistical Analysis

The method proposed by Bland-Altman (48) was used to assess within- and between-days reproducibility. Also a repeatability coefficient (RC) was calculated by multiplying the standard deviation (SD) of the mean difference between measurements by 1.96. According to the definition of repeatability coefficient given by the British Standards Institute (49), the mean difference must not be significantly different from zero and 95% of the differences are expected to be within a range -1.96SD to +1.96SD of the mean. In addition, results from both sessions were compared using paired t-tests, and $p < 0.05$ was considered to indicate a statistical significant difference. Coefficients of variation (CV) were calculated as well by dividing the standard deviation of the mean difference between measurements by the mean metabolite content of all measurements. Also, a two-way ANOVA was used to investigate the main effects of pre- and post-menopausal condition and BMI in the liver lipid levels. Statistical analyses were performed using statistical software (SPSS, version 16.0 for Mac).

2.4 Results

2.4.1 Liver ^1H -MRS Validation in Healthy Volunteers

An example of hepatic ^1H -MR spectrum is shown in Figure 2.1. Five resonances out of seven were successfully quantified in all 7 volunteers obtained in two different sessions.

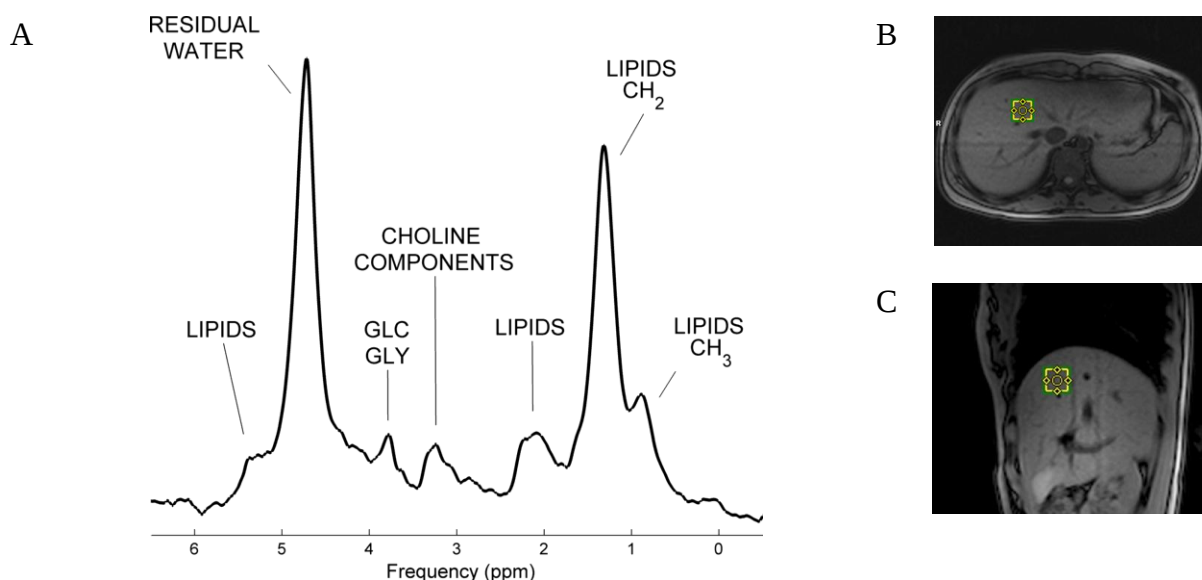


Figure 2.2 ^1H -MR spectrum (A) from an 8ml voxel located in the liver (B, C yellow box). Resonances were assigned to lipids CH_3 at 0.9 ppm, lipids CH_2 at 1.1-1.4 ppm, lipids $\text{CH}_2=\text{CH}-\text{CH}_2$ at 2.0-2.2 ppm, choline containing compounds at 3.2 ppm, Glucose +Glycogen (GLC, GLY) at 3.6- 3.8 ppm, residual water at 4.7 ppm and lipids $\text{CH}=\text{CH}$ at 5.3 ppm (not detectable in all data sets)

2.4.1.1 Within-Days Reproducibility

Figure 2.3 - Figure 2.7 show lipids CH_3 , lipids CH_2 , lipids CH_2-CH , choline components and Glycogen+Glucose content obtained in three repeated measurements on day 1 (panel A) and day 2 (panel D). Also metabolite levels are represented in Bland-Altman plots to assess reproducibility between first and second measurements and between first and third measurements obtained during day 1 (panels B and C) and day 2 (panels E and F). The central line of each Bland-Altman plot represents the mean of the difference between measurements and the dashed lines represent the 95% confidence interval. All the data points of repeated metabolite measurements during the same day are contained within the 95%

Figure 2.3 Repeated L_{CH_3} % measurements during day 1 (A) and day 2 (D). Bland-Altman plots of reproducibility between measurements during day 1 (B,C) and day 2 (E,F).

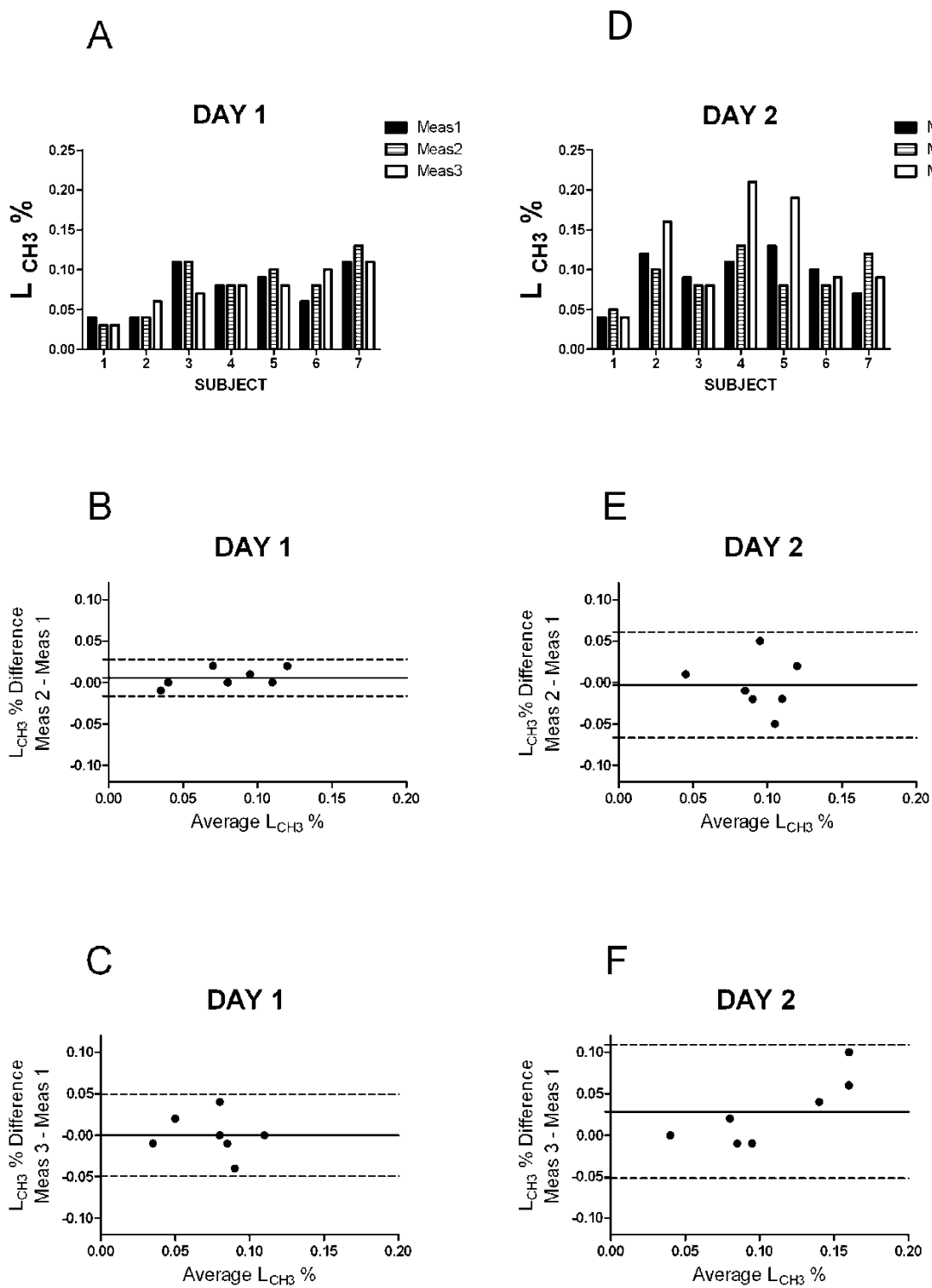


Figure 2.4 Repeated L_{CH2} % measurements during day 1 (A) and day 2 (D). Bland-Altman plots of reproducibility between measurements during day 1 (B,C) and day 2 (E,F).

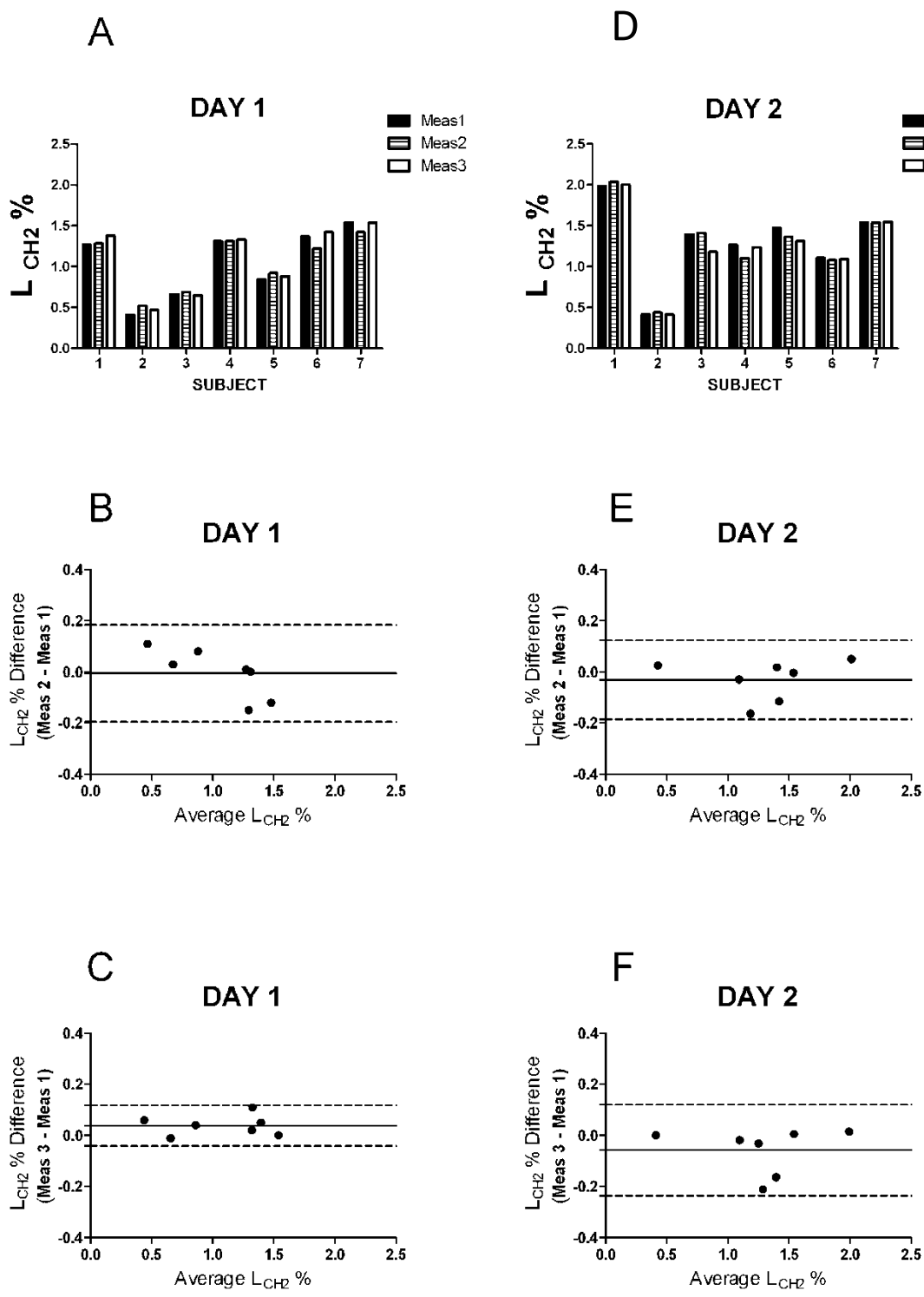


Figure 2.5 Repeated $\text{L}_{\text{CH}_2\text{-CH}}$ % measurements during day 1 (A) and day 2 (D). Bland-Altman plots of reproducibility between measurements during day 1 (B,C) and day 2 (E,F).

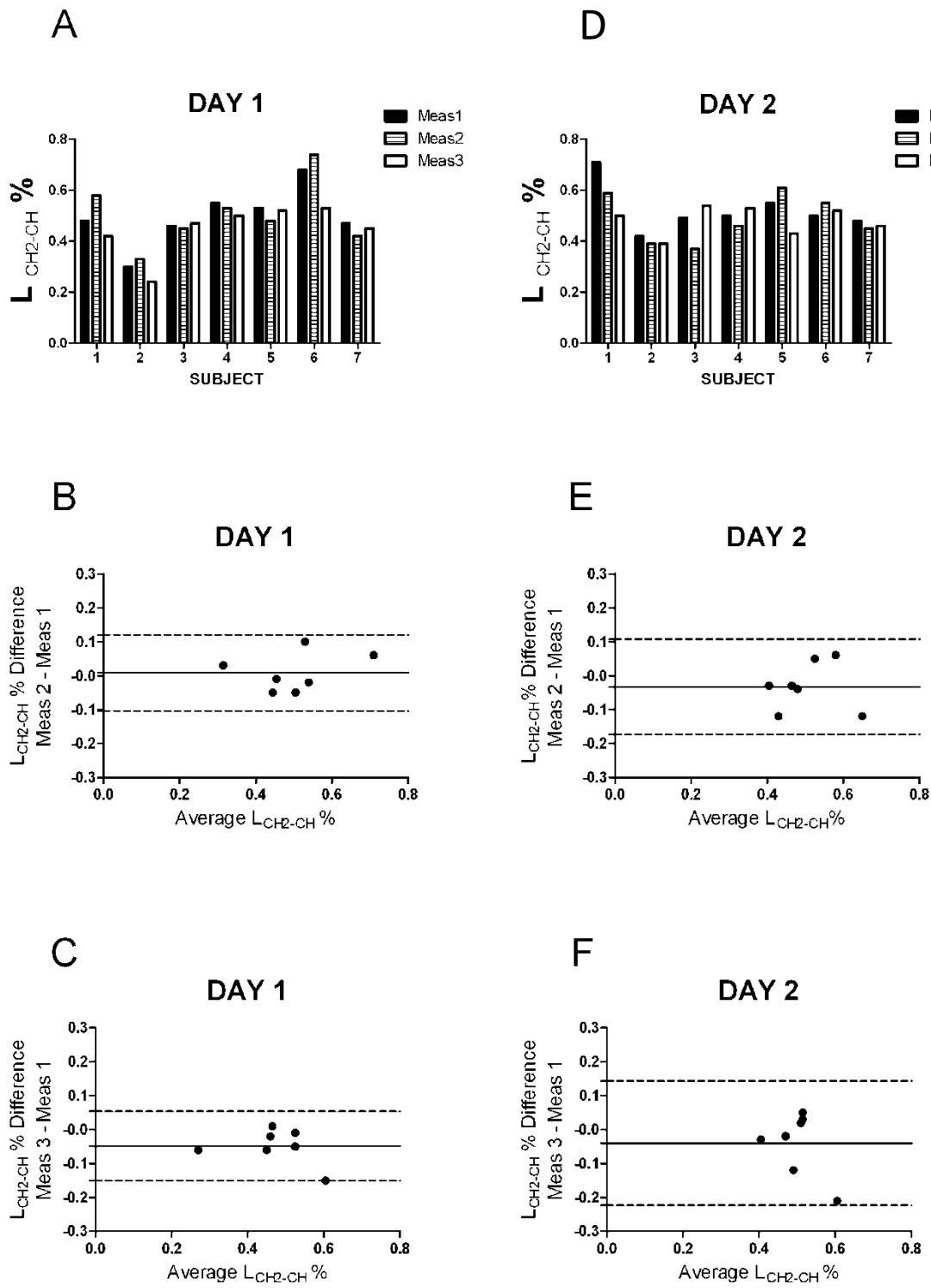


Figure 2.6 Repeated L_{CholineComponents} % measurements during day 1 (A) and day 2 (D). Bland-Altman plots of reproducibility between measurements during day 1 (B,C) and day 2 (E,F).

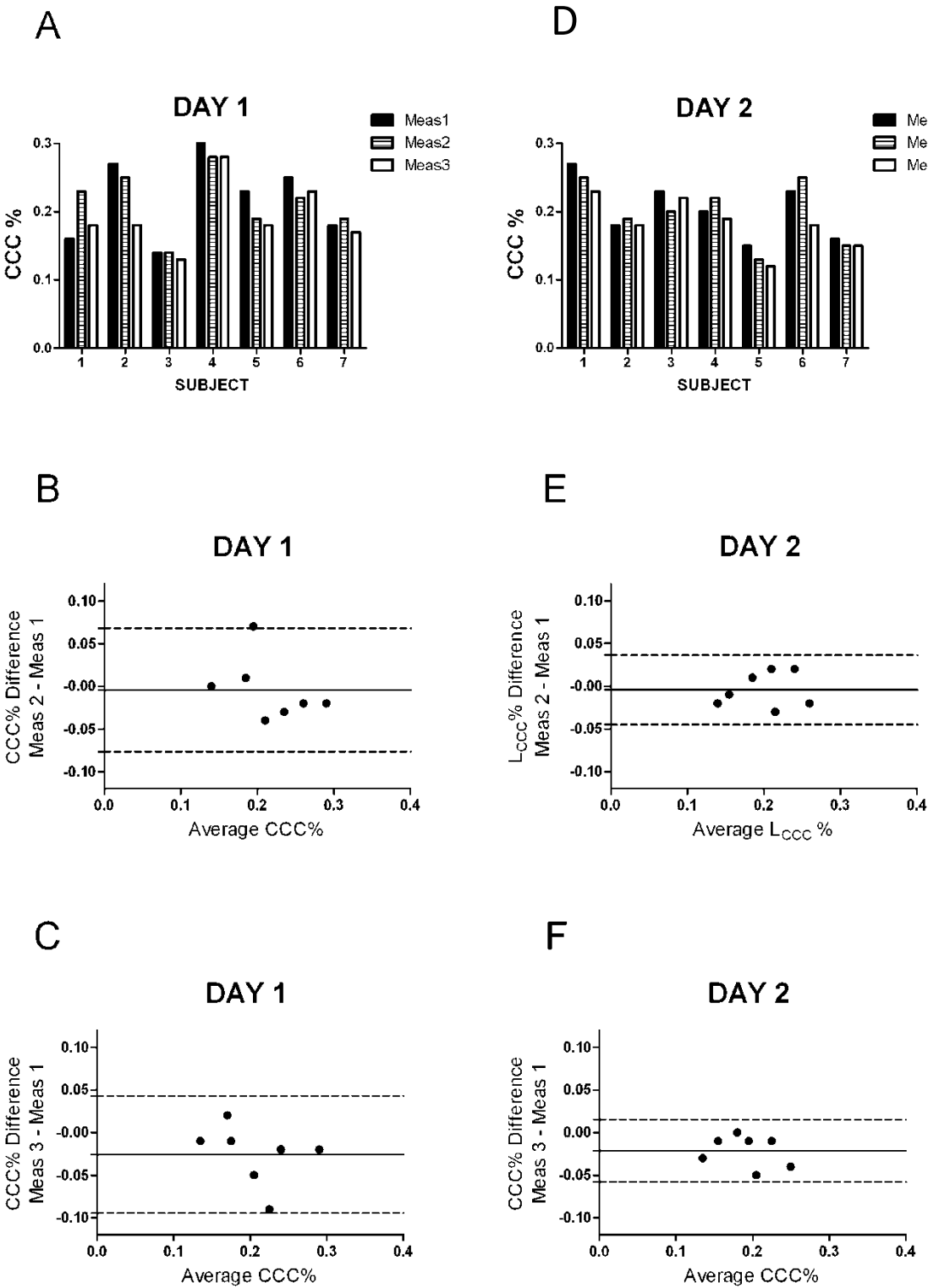
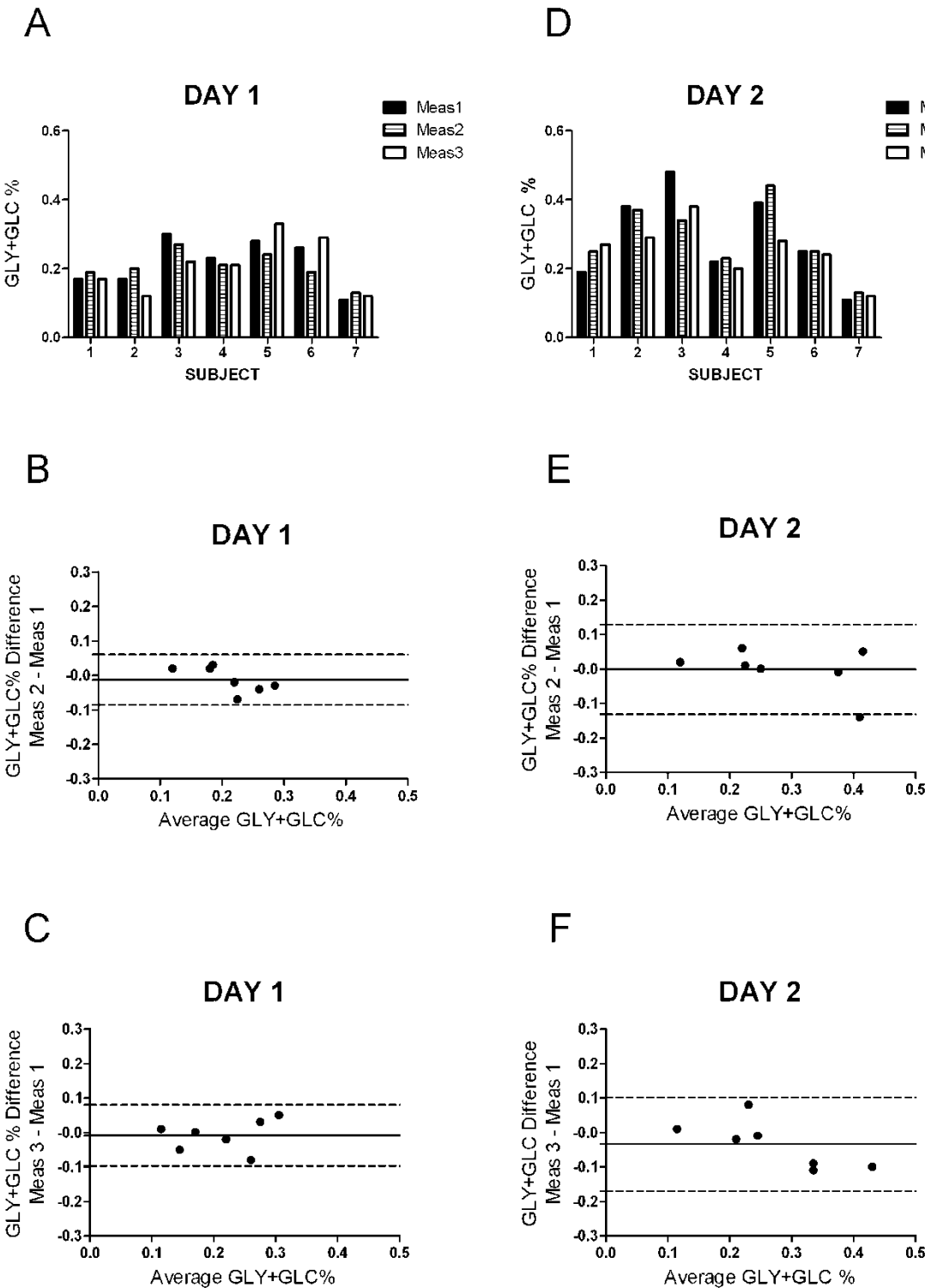


Figure 2.7 Repeated L_{GLYCOGEN+GLUCOSE} % measurements during day 1 (A) and day 2 (D). Bland-Altman plots of reproducibility between measurements during day 1 (B,C) and day 2 (E,F).



confidence intervals. Also, the average difference (bias: $-0.008 \pm 0.023\%$, mean $\pm$ SD of the five groups of resonances) between repeated metabolite content measurements was close to zero. Table 2.4 summarises the within day reproducibility statistics for all measured liver metabolites.

Table 2.4 Summary of within-day reproducibility

	CV	RC	rCRSD
Lipids CH_3	$19.3\% \pm 4.8\%$	$0.06\% \pm 0.02\%$	$10.6\% \pm 2.3\%$
Lipids CH_2	$4.8\% \pm 0.8\%$	$0.15\% \pm 0.03\%$	$1.2\% \pm 0.4\%$
Lipids $\text{CH}_2\text{-CH}$	$9.9\% \pm 0.6\%$	$0.13\% \pm 0.04\%$	$3.4\% \pm 0.4\%$
Choline Components	$9.3\% \pm 1.6\%$	$0.06\% \pm 0.02\%$	$8.6\% \pm 0.2\%$
Glycogen+Glucose	$13.4\% \pm 0.9\%$	$0.11\% \pm 0.04\%$	$9.2\% \pm 1.9\%$

CV = coefficient of variation; RC = repeatability coefficient; rCRSD= relative Cramer Rao Standard Deviation

For the group as whole, the mean CV for repeated Lipids CH_2 measurements acquired in the same day was the lowest at 4.8% , followed by Choline Components at 9.3%, Lipids $\text{CH}_2\text{-CH}$ at 10%, Glycogen+Glucose at 13.4% and Lipids CH_3 at 19.3%. Repeatability analysis is also shown in Table 2.4. In case of Lipids CH_2 , 95 % of two repeated measurements differed only by 0.15%, which could be considered as clinically irrelevant, since this value is much lower than the 5% cut-off value for liver steatosis diagnosis. The mean rCRSD of the different metabolites was lower than 11%. This indicates that ^1H -MRS spectra provide sufficient SNR to allow for a reliable quantification of hepatic metabolite content.

As an example of intra-subject reproducibility, Figure 2.8 illustrates the ^1H -MR spectra of liver from separate measurements in one volunteer (female, BMI = 21.1 kg/m^2) collected 30 minutes apart (blue and green solid lines, Lipids CH_2 = 0.52% and 0.47%, respectively) and 2 weeks later (grey and purple dashed lines, Lipids CH_2 = 0.44% and 0.41%, respectively).

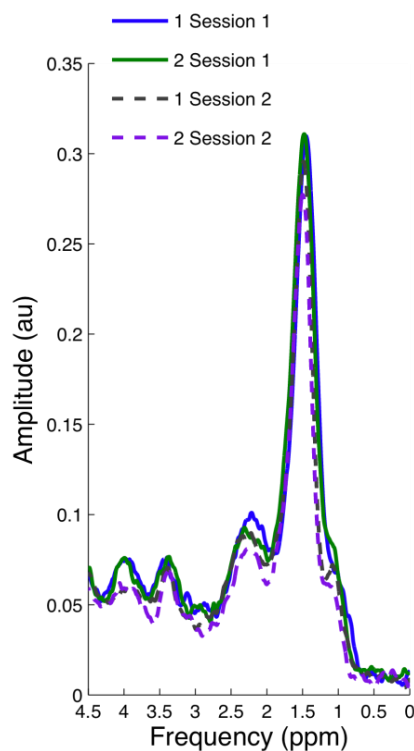


Figure 2.8 Reproducibility of liver ^1H -MRS measurements within and between sessions in one healthy volunteer. Spectra were normalized to water content.

2.4.1.2 Between-days reproducibility

The mean metabolite contents measured by ^1H -MRS in day1 did not differ from the ^1H -MRS measurement in day2 (Table 2.5). Although glycogen measurements were approaching statistic difference ($p=0.06$). This was supported by the Bland-Altman plots for metabolite content measurements obtained between-days, with the bias between measurements close to zero (Figure 2.9).

Table 2.5 Summary of reproducibility of between-days liver metabolite ^1H MRS measurements. Values are mean $\pm$ standard deviation; $p < 0.05$ was considered significant; CV = coefficient of variation; RC = repeatability coefficient

	Day 1	Day 2	p	CV	RC
Lipids CH_3	$0.08\% \pm 0.03\%$	$0.10\% \pm 0.04\%$	0.14	19.7%	0.07%
Lipids CH_2	$1.07\% \pm 0.39\%$	$1.28\% \pm 0.48\%$	0.20	12.9%	0.77%
Lipids $\text{CH}_2\text{-CH}$	$0.48\% \pm 0.11\%$	$0.50\% \pm 0.60\%$	0.65	2.1%	0.18%
Choline Components	$0.21\% \pm 0.05\%$	$0.19\% \pm 0.04\%$	0.57	5.1%	0.12%
Glycogen+ Glucose	$0.21\% \pm 0.06\%$	$0.28\% \pm 0.10\%$	0.06	19.4%	0.14%

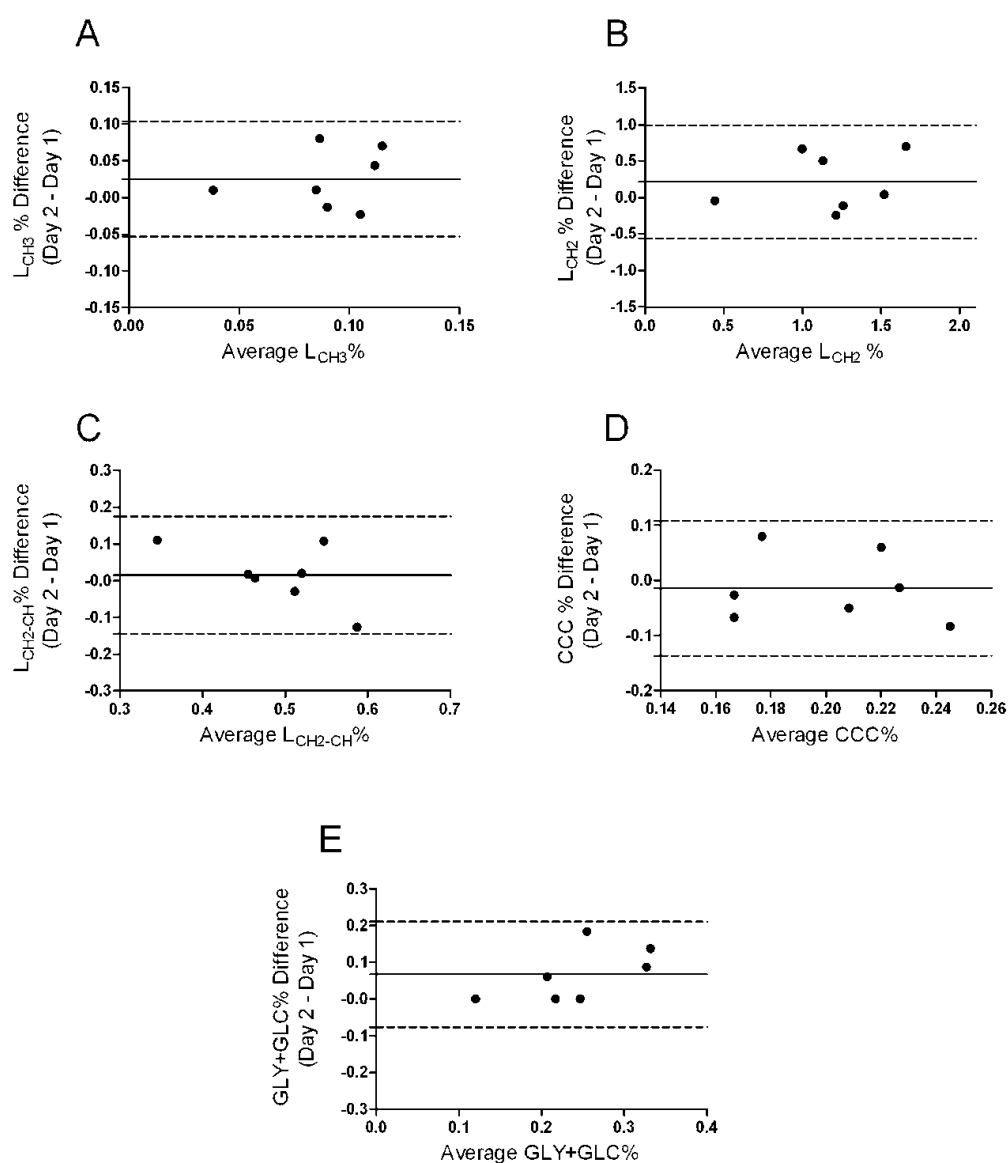


Figure 2.9 Overview of liver metabolite contents acquired in two different days to determine reproducibility between days. Bland-Altman plots for (A) Lipids CH_3 , (B) Lipids CH_2 , (C), Lipids $\text{CH}_2\text{-CH}$, (D) Choline containing components and (E) Glycogen+Glucose.

2.4.2 Necessity of Water Suppression in Liver ^1H -MRS

The flowchart in Fig. 2.10 summarizes the investigation on the benefits / necessity of water suppression. Out of 86 subjects recruited, data could not be obtained in 2 subjects due to technical problems with the scanner and a third subject (age = 15 years) had difficulties with

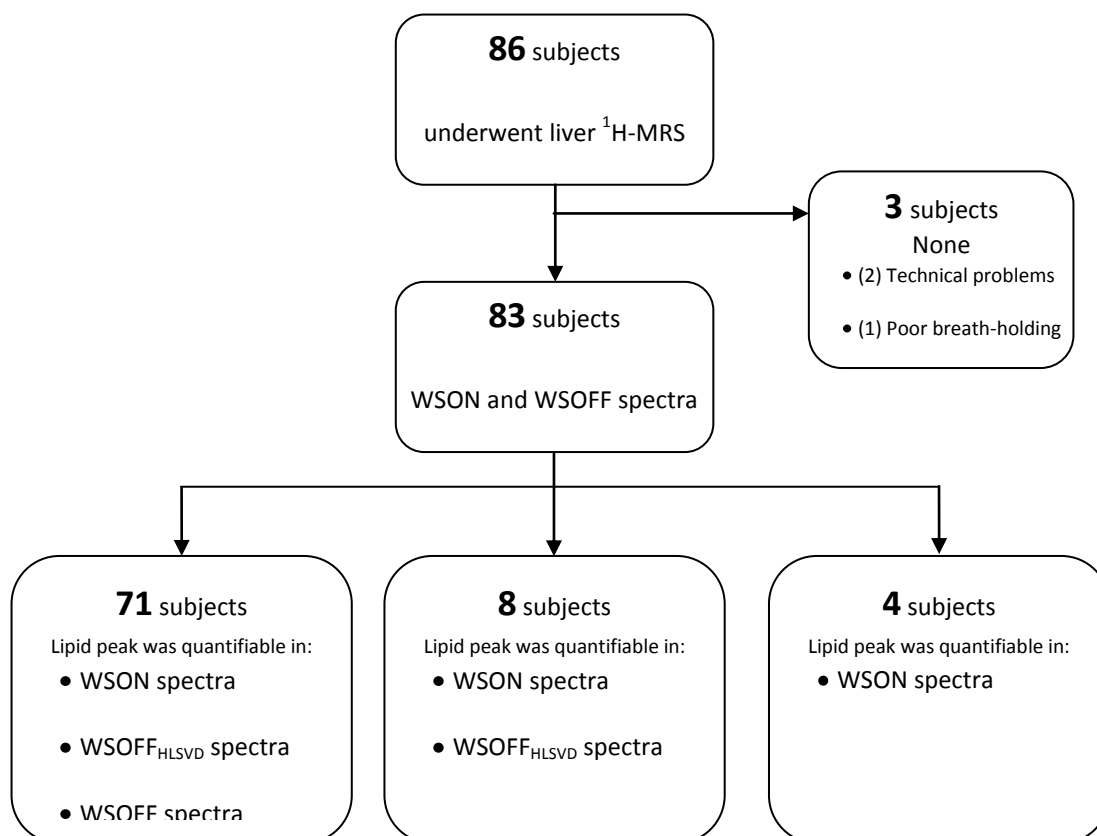


Figure 2.10 Liver ^1H -MR spectra analyzed. WSOFF: water unsuppressed spectra; WSOFF_{HLSVD}: water unsuppressed spectra with water removed by HLSVD method; WSON: water suppressed spectra.

breath-holding. Water unsuppressed and water suppressed spectra were acquired in the remaining subjects ($n=83$). The lipid CH_2 resonance at 1.2 ppm could only be reliably quantified in 71 water unsuppressed spectra (Figure 2.11 a-b) and in 79 water unsuppressed spectra with water removed by using the HLSVD method (Figure 2.11 c). In particular, the cases in which the lipid CH_2 content could not be quantified from the water-unsuppressed spectra were related to poor shimming conditions (linewidth of the water peak larger than 30

Hz) and low lipid CH_2 content ($< 0.8\%$). On the other hand, the lipid CH_2 content was quantified in all 83 water-suppressed spectra acquired.

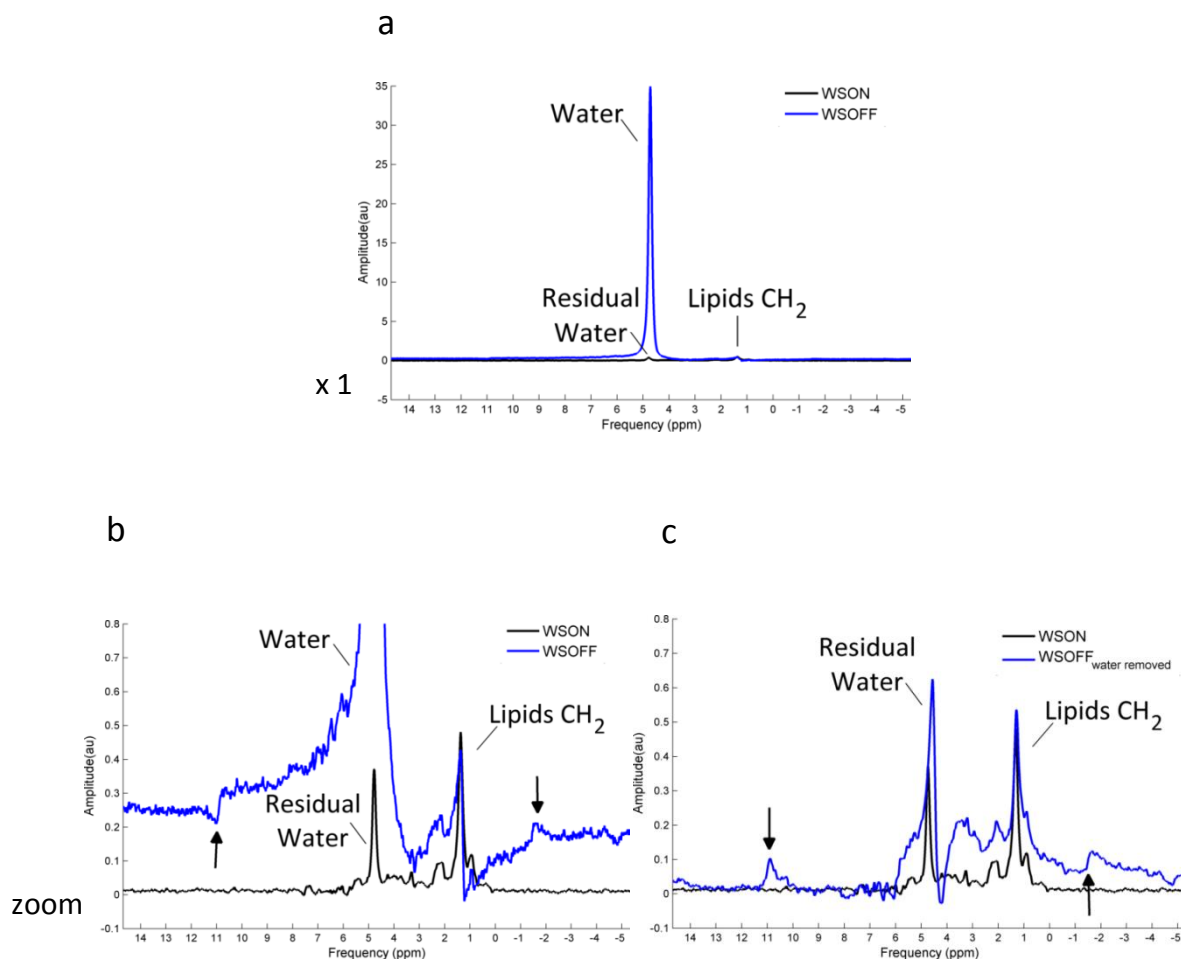


Figure 2.11(a) A single water resonance dominates in the liver ^1H -MR spectrum from a healthy volunteer. (b) Water baseline and sidebands make quantification difficult (blue line) compared to water-suppressed spectrum (black line). (c) A similar problem persists after removing the water using the HLSVD method. Localizations of prominent sidebands are indicated by the arrows.

Additionally, other resonances were identified in the spectra acquired with water suppression (Figure 2.12), including several lipid resonances, a signal from choline containing compounds and a resonance from glucose and glycogen. However, in water-unsuppressed spectra without and with water signal removed, the water baseline and sidebands made the detection and quantification of these resonances unreliable.

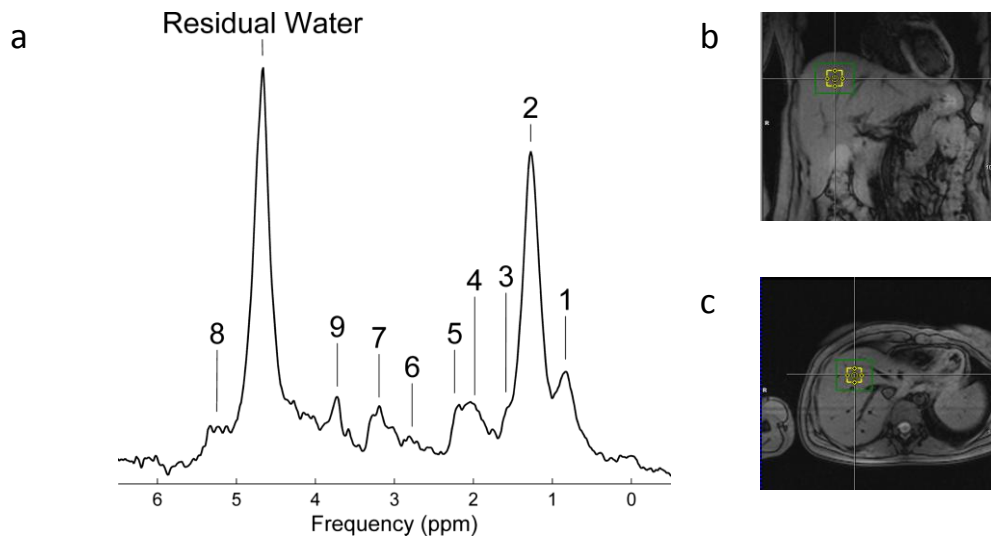


Figure 2.12 Example of water-suppressed ^1H -MR spectrum (a) from a 8 ml voxel located in a healthy liver at a coronal (b) and axial (c) slice. **1**, lipid CH_3 peak at 1.1 ppm; **2**, lipid CH_2 peak at 1.2 ppm; **3**, lipid $\text{CH}_2\text{CH}_2\text{CO}$ -peak at 1.65 ppm; **4**, lipid $\text{CH}_2\text{CH}_2\text{CH}=\text{CH}$ peak at 1.9-2.1 ppm; **5**, lipid $\text{CH}_2\text{CH}_2\text{CO}$ peak at 2.2ppm; **6**, lipid $\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}$ peak at 2.7 ppm; **7**, choline containing compounds peak at 3.2 ppm; **8**, lipid $-\text{CH}=\text{CH}-$ peak at 5.4ppm; **9**, glucose and glycogen peak at 3.6-3.9 ppm.

The ^1H -MRS lipid CH_2 content was assessed in 71 subjects by comparing the value obtained from water unsuppressed spectra, water unsuppressed spectra with water removal and water-suppressed spectra ($2.2\% \pm 3.6\%$, $2.5\% \pm 4.0\%$ and $2.1\% \pm 2.8\%$ respectively). Based on the definition of hepatic steatosis of $\geq 5\%$ hepatic fat content by ^1H -MRS (3), the group was divided in two: lipids $\text{CH}_2 < 5\%$ and lipids $\text{CH}_2 > 5\%$. Table 2.6 shows the results. 62 of 71 (87%) of subjects had normal liver fat content, whereas 9 of 71 (13%) of subjects had hepatic steatosis.

Table 2.6 Descriptive of the groups

	Total	Age (years)	BMI (kg/m^2)	WSOFF	WSOFF _{HLSVD}	WSO _N	p
L%	71 (48F/23M)	42 $\pm$ 12	26.6 $\pm$ 7.4	2.2% $\pm$ 3.6%	2.5% $\pm$ 4.0%	2.1% $\pm$ 2.8%	0.83
L% < 5%	62 (41F/21M)	41 $\pm$ 12	25.7 $\pm$ 6.6	1.2% $\pm$ 1.0%	1.3% $\pm$ 1.1%	1.2% $\pm$ 0.9%	0.84
L% > 5%	9 (7F/2M)	47 $\pm$ 10	33.0 $\pm$ 9.2	9.5% $\pm$ 6.2%	10.5% $\pm$ 7.0%	8.2% $\pm$ 3.9%	0.73

Values are mean $\pm$ SD. M: male and F: Female.

Bland-Altman plots were used to assess the degree of agreement between lipids CH_2 content quantified from the water-unsuppressed spectra, the water-unsuppressed spectra with water removed and the water-suppressed spectra (Figure 2.13). Including all subjects, Bland-Altman analysis indicated that the 95% limits of agreement between lipid quantification from spectra with and without water suppression ranged from -1.96% to 2.21% (Figure 2.13a). This range was slightly lower than the clinically important 5% cut-off value for hepatic steatosis. Similar limits of agreement were found for the lipid content obtained from the water removed spectra compared to the water-suppressed spectra (Figure 2.13b). If the study included only subjects with normal liver lipid levels ($L < 5\%$, Figure 2.13d-f), the quantification based on both water-unsuppressed and water-suppressed spectra provided very similar lipid values, with a 95% confidence interval between quantifications ranging from -0.67% to 0.59%. Again, this difference can be considered not clinically significant. In the case of assessing lipid levels in subjects with liver steatosis ($L > 5\%$, Figure 2.13g-i), the 95% confidence interval of the difference between lipid quantification using the water-unsuppressed spectra and the water-suppressed spectra varied from -4.02% to 6.62%, which could be considered clinically significant. There is, however, an obvious outlier (Figure 2.13, red point), the removal of which reduced the 95% confidence interval to a clinically unimportant range (-1.9% to 2.9%). This data point corresponded to a lipid content of 25.4% quantified from the water-unsuppressed spectrum and 15.5% from the spectrum with water suppression. (subject: female, age= 42 years, BMI = 46.4 kg/m^2). In all groups the lipid levels quantified using both water-unsuppressed and water-unsuppressed with water removed spectra were in very good agreement (Figure 2.13c,f,i). Bland-Altman plots showed very narrow limits of agreement and the mean difference between methods was almost zero.

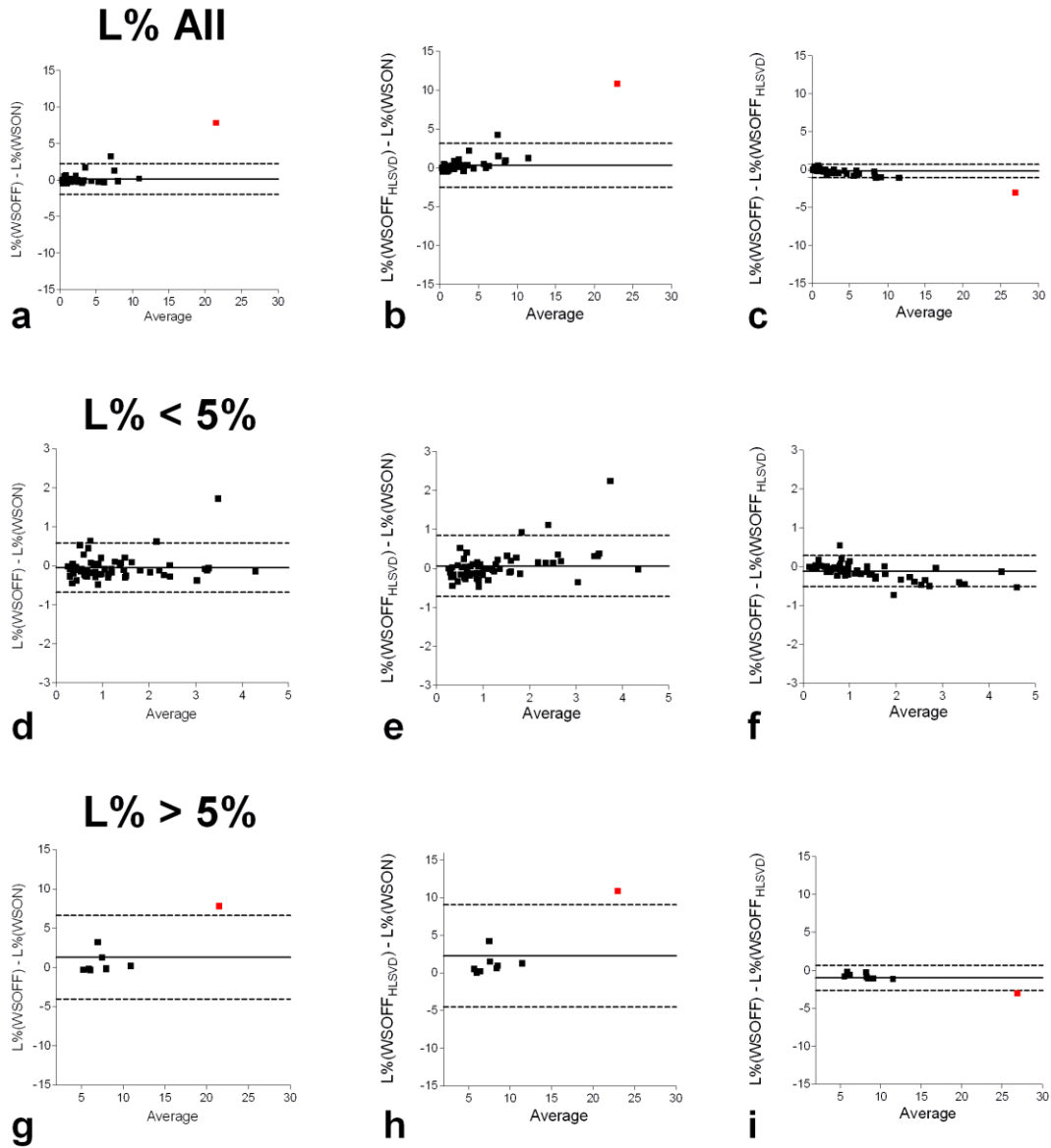


Figure 2.13 Bland-Altman plots of agreement of liver fat assessment in vivo between water-unsuppressed and water-suppressed ^1H -MRS (left column); between water-unsuppressed with HLSVD water removed and water-suppressed ^1H -MRS (middle column) and between water-unsuppressed and water-unsuppressed with HLSVD water removed ^1H -MRS (right column). The mean of each pair of measurements is plotted against their difference. (a-c) shows the results for all subjects. (d-f) investigates normal liver lipid levels, $L\% < 5\%$. (g-i) investigates fatty livers, $L\% > 5\%$. Dashed lines represent the 95% confidence intervals; solid line is the mean value and the red data point is an outlier.

For the group as a whole, the lipids CH_2 content increased linearly with BMI, independently of the spectra (WSOFF, $\text{WSOFF}_{\text{HLSVD}}$ or WSON) used for lipid quantification. As an example, Figure 2.14 shows the correlation between lipids CH_2 content obtained from the water-suppressed spectra and BMI ($r^2 = 0.32$; $p < 0.0001$).

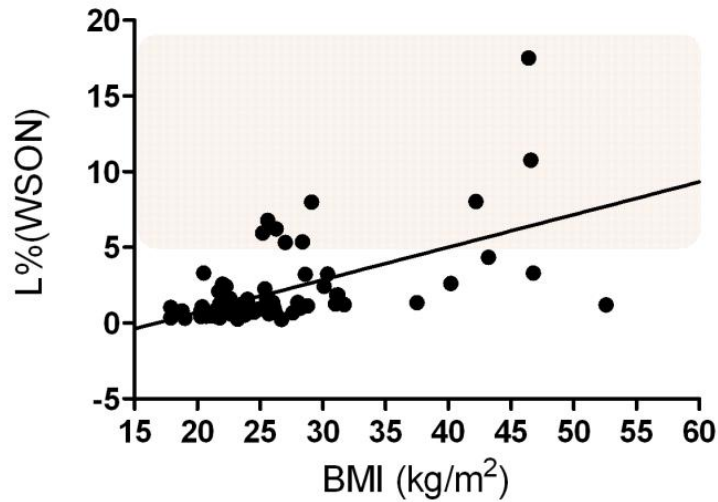


Figure 2.14 Liver lipid CH_2 content correlated positively with BMI ($L\% = 0.21 \times \text{BMI} - 3.6$; $R^2 = 0.32$). Liver steatosis is indicated in grey ($L\% > 5\%$).

2.4.3 Liver Fat Assessment in Pre- and Post- Menopausal Women

So far, a total of 31 females underwent liver ^1H -MRS in this still ongoing study. Table 2.7 shows the details of the group. Acquisition time, including localizers, shim and water suppression adjustments and spectroscopy acquisition was under 15 minutes. Figure 2.15 shows the preliminary liver lipid levels obtained from pre- and post-menopausal women as a function of age. The two groups could be clearly distinguished by age.

Table 2.7 Characteristics of the subjects depending on the menopausal state and BMI

	Condition			
	PRE		POST	
	Lean n=14	Overweight n=4	Lean n=4	Overweight n=9
Age (years)	41 ± 3	39 ± 4	58 ± 2	58 ± 2
BMI (kg/cm^2)	22.0 ± 1.1	28.0 ± 2.3	22.6 ± 1.6	27.0 ± 1.4
Lipids	$0.60\% \pm 0.28\%$	$1.36\% \pm 0.94\%$	$1.01\% \pm 0.71\%$	$2.98\% \pm 2.51\%$

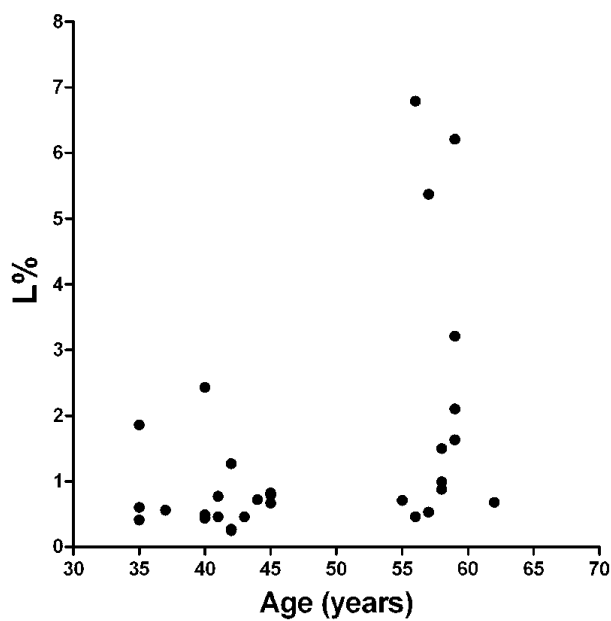


Figure 2.15 Hepatic liver content distribution with age.

A two-way ANOVA found a main effect of BMI, $F(1,26) = 4.96$, $p \leq 0.05$, indicating that overweight females had higher liver lipid levels than lean females. However, the effect of pre- or post-menopausal condition in the liver lipid levels ($F(1,26) = 2.93$, $p = 0.09$) or the interaction condition*BMI $F(1,26) = 0.93$, $p > 0.05$ (Figure 2.16) have not yet reached significance.

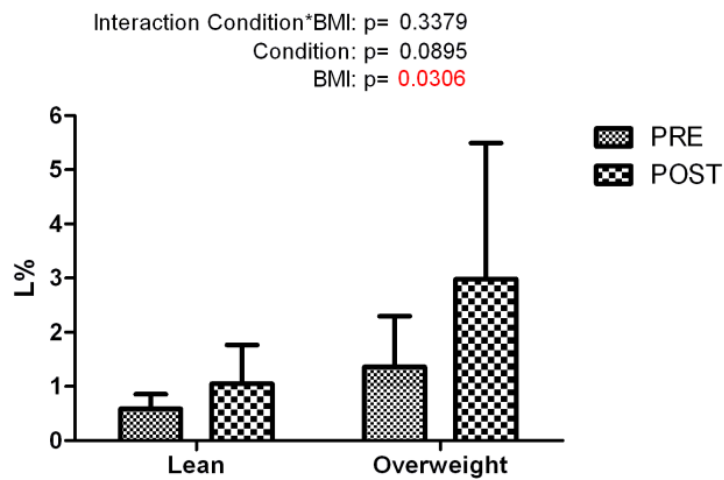


Figure 2.16 Liver lipid levels in pre- and post menopaual woman. Bars represent mean $\pm$ SD. Red text indicates statistical significance.

2.5 Discussion

2.5.1 ^1H -MRS Validation in Healthy Volunteers

Liver ^1H -MRS acquired during breath-holding in healthy volunteers has shown acceptable within day reproducibility, not only for quantifying lipids CH_2 , but other lipid resonances (CH_3 and $\text{CH}_2=\text{CH}$), choline containing compounds and Glycogen+Glucose resonances. Likewise, good reproducibility for measuring liver metabolites was achieved in a second ^1H -MRS acquisition performed at least two weeks apart.

A CV = 4.8% was found for repeated lipid CH_2 measurements within the same day. This is in concordance with CV% previously published at 3 T. Orlacchio et al reported a CV=5% in three repeated measurements of liver lipids during free-breathing in a group of 6 healthy volunteers and 26 patients with chronic hepatitis C (17). Van Werven et al found a CV=4.5% in 12 obese subjects (21). Bredella reported that the mean difference between same day scans in 6 healthy volunteers using breath-holding was -0.29% with a 95% confidence interval ranging from -1.46 to 2.05% (24). In the present study a narrower 95% confidence interval was found for repeated measurements of lipids in the same day, also using breath-holding (-0.18% to 0.12%). Another study assessing liver lipid content during breath-holding in subjects with chronic hepatitis C also reported a higher CV = 10% (27).

The evaluation of liver ^1H MRS reproducibility over time is also important, as for example the evaluation of liver lipid levels in response of to a drug treatment (34) or to a diet intervention (50) requires longitudinal application of this technique. In this study, the CV% of liver lipid measurements acquired at least 2 weeks apart in healthy subjects was about 13%. This is slightly higher than the between weeks CV of 9.5% found by Van Werven et al (21). A

difference of 0.21% between two liver lipid measurements acquired within a month with a 95% confidence interval from -0.56% to 0.99% is clinically not relevant. Liver lipid content greater than 5.56% is considered abnormal (3) and the cut-off value for the diagnosis of NAFLD. The coefficient of variation of measurements within day was lower than the coefficient of variation of measurements over time, which includes technique variability and physiological changes. This also confirms the applicability of the technique to longitudinal studies, since a small change of lipids CH_2 levels of 0.21% could be detected.

Reproducibility of ^1H -MRS to measure other lipid resonances (CH_3 and $\text{CH}_2=\text{CH}_2$) in the liver at 3 T has also been shown in this study for the first time. Typically, previous studies have suffered from low spectral resolution and only the methylene resonance was investigated, owing to the high concentration of methylene protons in liver fat (3,9,10). A recent ^1H -MRS study performed in mouse models of progressive fatty liver (51), suggested quantification of unsaturated ($\text{CH}_2\text{-CH}$ at 2.0 ppm and CH-CH at 5.3 ppm) and saturated (CH_3) fatty acids in the liver fat as a potential marker to monitor the progression of NAFLD. In particular, during the development of liver steatohepatitis, they found an increase in the unsaturated to saturated lipid ratio, which could be interpreted as cell death. Also, another study exploited the spectral resolution benefits of ^1H -MRS at 3 T to investigate the unsaturated composition of lipids in patients with NAFLD (32). The methine resonance (CH=CH) at 5.3 ppm was not studied in the reproducibility study because it could only be reliably quantified in 4 out of 7 subjects. The low content of hepatic lipids in these healthy volunteers could explain the limited SNR of this peak, which also was potentially influenced by water suppression. However, the method described in this work holds great potential to quantify unsaturated fatty acids in subjects with fatty liver. In fact, in the 71 healthy subjects studied for the assessment of lipid quantification without water suppression, nine had fatty

liver, with fat content $> 5.5\%$ and the spectra acquired had enough spectral quality to assess the unsaturated CH-CH lipid resonance.

Choline containing components (3.2 ppm) including choline, phosphocholine, glycerophosphocholine and taurine were included in the reproducibility study. An acceptable CV=9.3% was found in within-day measurements, whereas measurements taken more than two weeks apart show a lower CV=5.1%. This suggests that liver CCC levels in healthy volunteers are quite stable over time and the variability is associated with the technique. Fischbach et al observed a CV = 17% in CCC measurements repeated within a week apart (15). The inclusion of patients with hepatic tumours in the repeatability study might account for this larger CV%, reflecting possible biochemical changes in the tumour over time. The CCC levels have been identified as biomarker in cancer (52) and ^1H -MRS has been used to get biochemical information on brain and breast tumours (21,24-26,28-31,53,54). However, only a few studies have investigated the use of in vivo ^1H -MRS to characterize focal hepatic lesions. Kuo et al were the first to apply ^1H -MRS at 3 T on large focal hepatic lesions in 38 patients (11). The mean CCC to lipid ratio was not different between malignant tumours and normal liver from eight healthy volunteers. The poor spectral resolution shown in their results might result in underestimated CCC-to-lipid ratios. Also ^1H -MRS data were acquired during free-breathing for 6 minutes and only water-unsuppressed scans were performed. On the contrary, Fischbach et al acquired water-suppressed data using an abdominal compressor belt to correct for respiratory motion. Using water as internal reference, CCC and lipid levels showed no significant difference between malignant lesions (n=55) and normal liver tissue (n=39). Further studies are required in order to investigate the potential clinical utility of ^1H -MRS to characterize liver lesions. These studies will benefit for the increased SNR at 3 T in combination with respiratory motion correction during water-suppressed ^1H -MRS. Phased

array coils, as used in the present work, will improve the SNR for a better spectral quantification of CCC. The CCC rCRSD% obtained from a 35 average spectrum acquired over five 15 s-breath-holds was lower than 10% (n=7).

Furthermore, the potential of ^1H -MRS for Glycogen+Glucose quantification in healthy liver was shown. A larger CV% in between weeks (19%) compared to within day measurements (13%) was found. Glycogen synthesis and break down plays an important role in systemic glucose metabolism in health and diseases such as diabetes mellitus. ^{13}C MRS has been used to detect glycogen in vivo (C1 resonance) (55,56). The glycogen H1 resonance (5.4 ppm) is very close to water, has short T_2 and usually is overlapped by lipid proton peaks. Chen et al used NOE to observe glycogen H1 resonance in liver rats *in vivo*. (57). ^1H -MRS from liver extracts has shown a dense cluster of resonances from glycogen moieties (H2-H5) between 3.4-4.0 ppm (43) and also resonances from α - and β -glucose moieties between 3.2-4.0 ppm (58). The spectral resolution in the present study did not allow for resolving glycogen and glucose resonances. However, the acceptable reproducibility for measuring Glycogen+Glucose content in liver has the potential to assess hepatic glycogen metabolism, and warrants further investigations. The results of liver ^1H -MRS were not compared to biochemical analysis, since this procedure has to be considered unethical to perform in healthy volunteers.

This method has benefit from a dedicated summation of individual spectra acquired during breath-holding, resulting in good spectral resolution and reproducibility in ^1H -MRS of the liver to assess lipids and other metabolites.

2.5.2 Necessity of Water Suppression in Vivo

The majority of the ^1H -MRS studies at 3 T in liver metabolism had been performed without water suppression (21,24-26,28-31). While this study demonstrates that liver lipid levels obtained using water-unsuppressed ^1H -MRS were not statistically significantly different from those levels obtained using water-suppressed ^1H -MRS at 3 T in healthy subjects, only lipid CH_2 levels could be reliably quantified by ^1H -MRS without water suppression. Even when the water signal was removed using the HLSVD algorithm available in jMRUI, the only metabolite peak that could be detected with accuracy was the lipid resonance at 1.3 ppm. Losing the ability to quantify metabolites other than lipid- CH_2 represents a major limitation for this otherwise versatile technique.

The use of a water suppression technique was essential to obtain reliable quantification of more hepatic resonances, like choline containing compounds, lipids $\text{CH}=\text{CH}$, lipids CH_3 and even Glycogen+Glucose. Modern analog-to-digital converters are able to digitize the low metabolite resonances in the presence of a large water peak without degrading the metabolite SNR. However, the presence of a large water resonance leads to baseline distortions and sidebands that make the detection of metabolite unreliable.

Importantly, the method was applied to investigate liver metabolism with a success rate of 83(96%) out of 86 scans.

2.5.3 Liver Fat Assessment in Pre- and Post- Menopausal Women

The applicability of the ^1H -MRS method developed in this work into a clinical research study of liver metabolism was demonstrated. As part of the assessment of hepatic fatty acid partitioning in pre- and post-menopausal women in relation to risk factors for cardiovascular

disease, a total of 31 women successfully underwent liver ^1H -MRS over a one year period. Importantly, the total scanning time for the acquisition of high quality ^1H -MR spectra from an 8ml voxel in the liver was on average less than 10 minutes, illustrating the value of this technique as an add-on tool for other MR-investigations.

The preliminary results showed that BMI had a significant effect on hepatic lipid levels, but the menopausal condition was not quite significant. However, the groups did not have the same number of subjects, so as the number of participant increases, the menopausal condition might reach statistical significance.

2.6 Conclusion

The sequence development based on phantom experiments was successfully validated in vivo by applying ^1H -MRS on the liver of healthy volunteers. The study showed that the use of water-suppressed ^1H -MR spectroscopy to quantify lipids, choline containing compounds and glycogen in the liver over time was feasible at 3 T with phased array coils, by employing breath-holding, short TE to minimise the effects of motion and T_2 relaxation and dedicated spectral processing to combine the signals from the different coil elements. Importantly, the total acquisition time was under 10 minutes, with typically breath-hold durations of 16-18 s, without demanding requirements for the volunteers, which makes it easy to integrate into clinical studies. In addition, the possibility to quantify glycogen content in vivo with better sensitivity and without the disadvantages of ^{13}C -MRS would provide a sensitive, non-invasive tool to study glycogen metabolism.

2.7 References

1. Ross B, Bluml S. Magnetic resonance spectroscopy of the human brain. *Anat Rec* 2001;265(2):54-84.
2. Fischbach F, Bruhn H. Assessment of in vivo ^1H magnetic resonance spectroscopy in the liver: a review. *Liver Int* 2008;28(3):297-307.
3. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *American journal of physiology* 2005;288(2):E462-468.
4. Bohte AE, van Werven JR, Bipat S, Stoker J. The diagnostic accuracy of US, CT, MRI and ^1H -MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. *Eur Radiol* 2010.
5. Angulo P. Nonalcoholic Fatty Liver Disease. *New England Journal of Medicine* 2002;346(16):1221-1231.
6. Marchesini G, Moscatiello S, Di Domizio S, Forlani G. Obesity-associated liver disease. *J Clin Endocrinol Metab* 2008;93(11 Suppl 1):S74-80.
7. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002;87(7):3023-3028.
8. Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? *Diabetologia* 2008;51(11):1947-1953.
9. Thomas EL, Hamilton G, Patel N, O'Dwyer R, Dore CJ, Goldin RD, Bell JD, Taylor-Robinson SD. Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 2005;54(1):122-127.
10. Longo R, Ricci C, Masutti F, Vidimari R, Croce LS, Bercich L, Tiribelli C, Dalla Palma L. Fatty infiltration of the liver. Quantification by ^1H localized magnetic resonance spectroscopy and comparison with computed tomography. *Invest Radiol* 1993;28(4):297-302.
11. Kuo YT, Li CW, Chen CY, Jao J, Wu DK, Liu GC. In vivo proton magnetic resonance spectroscopy of large focal hepatic lesions and metabolite change of hepatocellular carcinoma before and after transcatheter arterial chemoembolization using 3.0-T MR scanner. *J Magn Reson Imaging* 2004;19(5):598-604.
12. Li CW, Kuo YC, Chen CY, Kuo YT, Chiu YY, She FO, Liu GC. Quantification of choline compounds in human hepatic tumors by proton MR spectroscopy at 3 T. *Magn Reson Med* 2005;53(4):770-776.
13. Ishii M, Yoshioka Y, Ishida W, Kaneko Y, Fujiwara F, Taneichi H, Miura M, Toshihiro M, Takebe N, Iwai M, Suzuki K, Satoh J. Liver fat content measured by magnetic resonance spectroscopy at 3.0 Tesla independently correlates with plasminogen activator inhibitor-1 and body mass index in type 2 diabetic subjects. *Tohoku Journal of Experimental Medicine* 2005;206(1):23-30.
14. Fischbach F, Thormann M, Rieke J. ^1H -Magnetresonanzspektroskopie (MRS) der Leber und von Lebermalignomen bei 3,0 Tesla. *Der Radiologe* 2004;44(12):1192-1196.

15. Fischbach F, Schirmer T, Thormann M, Freund T, Ricke J, Bruhn H. Quantitative proton magnetic resonance spectroscopy of the normal liver and malignant hepatic lesions at 3.0 Tesla. *Eur Radiol* 2008;18(11):2549-2558.
16. O'Regan D, Callaghan M, Fitzpatrick J, Naoumova R, Hajnal J, Schmitz S. Cardiac T2* and lipid measurement at 3.0 T-initial experience. *European Radiology* 2008;18(4):800-805.
17. Orlacchio A, Bolacchi F, Angelico M, Mancini A, Cozzolino V, Cadioli M, Simonetti G. In vivo, high-field, 3-Tesla 1H MR spectroscopic assessment of liver fibrosis in HCV-correlated chronic liver disease. *La Radiologia Medica* 2008;113(2):289-299.
18. Orlacchio A, Bolacchi F, Cadioli M, Bergamini A, Cozzolino V, Angelico M, Simonetti G. Evaluation of the severity of chronic hepatitis C with 3-T1H-MR spectroscopy. *AJR Am J Roentgenol* 2008;190(5):1331-1339.
19. Guiu B, Loffroy R, Petit J-M, Aho S, Ben Salem D, Masson D, Hillon P, Cercueil J-P, Krause D. Mapping of liver fat with triple-echo gradient echo imaging: validation against 3.0-T proton MR spectroscopy. *European Radiology* 2009;19(7):1786-1793.
20. Guiu B, Petit JM, Loffroy R, Salem DB, Aho S, Masson D, Hillon P, Krause D, Cercueil JP. Quantification of liver fat content: Comparison of triple-echo chemical shift gradient-echo imaging and in vivo proton MR spectroscopy. *Radiology* 2009;250(1):95-102.
21. van Werven JR, Hoogduin JM, Nederveen AJ, van Vliet AA, Wajs E, Vandenberg P, Stroes ES, Stoker J. Reproducibility of 3.0 Tesla magnetic resonance spectroscopy for measuring hepatic fat content. *J Magn Reson Imaging* 2009;30(2):444-448.
22. Zhong L, Chen JJ, Chen J, Li L, Lin ZQ, Wang WJ, Xu JR. Nonalcoholic fatty liver disease: quantitative assessment of liver fat content by computed tomography, magnetic resonance imaging and proton magnetic resonance spectroscopy. *J Dig Dis* 2009;10(4):315-320.
23. Bredella MA, Torriani M, Thomas BJ, Ghomi RH, Brick DJ, Gerweck AV, Miller KK. Peak growth hormone-releasing hormone-arginine-stimulated growth hormone is inversely associated with intramyocellular and intrahepatic lipid content in premenopausal women with obesity. *J Clin Endocrinol Metab* 2009;94(10):3995-4002.
24. Bredella MA, Ghomi RH, Thomas BJ, Ouellette HA, Sahani DV, Miller KK, Torriani M. Breath-hold 1H-magnetic resonance spectroscopy for intrahepatic lipid quantification at 3 tesla. *Journal of Computer Assisted Tomography* 2010;34(3):372-376.
25. Bydder M, Shiehorteza M, Yokoo T, Sugay S, Middleton MS, Girard O, Schroeder ME, Wolfson T, Gamst A, Sirlin C. Assessment of liver fat quantification in the presence of iron. *Magnetic Resonance Imaging* 2010;28(6):767-776.
26. Hu HH, Kim H-W, Nayak KS, Goran MI. Comparison of Fat-Water MRI and Single-voxel MRS in the Assessment of Hepatic and Pancreatic Fat Fractions in Humans. *Obesity* 2010;18(4):841-847.
27. Krssák M, Hofer H, Wrba F, Meyerspeer M, Brehm A, Lohninger A, Steindl-Munda P, Moser E, Ferenci P, Roden M. Non-invasive assessment of hepatic fat accumulation in chronic hepatitis C by 1H magnetic resonance spectroscopy. *European Journal of Radiology* 2010;74(3):e60-e66.
28. Lee SS, Park SH, Kim HJ, Kim SY, Kim M-Y, Kim DY, Suh DJ, Kim KM, Bae MH, Lee JY, Lee S-G, Yu ES. Non-invasive assessment of hepatic steatosis: Prospective comparison of the accuracy of imaging examinations. *Journal of Hepatology* 2010;52(4):579-585.

29. Roldan-Valadez E, Favila R, Martinez-Lopez M, Uribe M, Rios C, Mendez-Sanchez N. In vivo 3T spectroscopic quantification of liver fat content in nonalcoholic fatty liver disease: Correlation with biochemical method and morphometry. *J Hepatol* 2010;53(4):732-737.
30. Schreuder TC, Marsman HA, Lenicek M, van Werven JR, Nederveen AJ, Jansen PL, Schaap FG. The hepatic response to FGF19 is impaired in patients with nonalcoholic fatty liver disease and insulin resistance. *Am J Physiol Gastrointest Liver Physiol* 2010;298(3):G440-445.
31. Sevastianova K, Hakkarainen A, Kotronen A, CornÅ©r A, Arkkila P, Arola J, Westerbacka J, Bergholm R, Lundbom J, Lundbom N, Yki-JÃ¼rvinen H. Nonalcoholic Fatty Liver Disease: Detection of Elevated Nicotinamide Adenine Dinucleotide Phosphate with in Vivo 3.0-T 31P MR Spectroscopy with Proton Decoupling1. *Radiology* 2010;256(2):466-473.
32. van Werven JR, Schreuder TC, Nederveen AJ, Lavini C, Jansen PL, Stoker J. Hepatic unsaturated fatty acids in patients with non-alcoholic fatty liver disease assessed by 3.0T MR spectroscopy. *Eur J Radiol* 2010;75(2):e102-107.
33. van Werven JR, Marsman HA, Nederveen AJ, Smits NJ, ten Kate FJ, van Gulik TM, Stoker J. Assessment of Hepatic Steatosis in Patients Undergoing Liver Resection: Comparison of US, CT, T1-weighted Dual-Echo MR Imaging, and Point-resolved 1H MR Spectroscopy1. *Radiology* 2010;256(1):159-168.
34. Visser ME, Akdim F, Tribble DL, Nederveen AJ, Kwoh TJ, Kastelein JJP, Trip MD, Stoes ESG. Effect of apolipoprotein-B synthesis inhibition on liver triglyceride content in patients with familial hypercholesterolemia. *J Lipid Res* 2010;51(5):1057-1062.
35. Hamilton G, Middleton MS, Bydder M, Yokoo T, Schwimmer JB, Kono Y, Patton HM, Lavine JE, Sirlin CB. Effect of PRESS and STEAM sequences on magnetic resonance spectroscopic liver fat quantification. *Journal of Magnetic Resonance Imaging* 2009;30(1):145-152.
36. Katz-Brull R, Rofsky NM, Lenkinski RE. Breathhold abdominal and thoracic proton MR spectroscopy at 3T. *Magnetic Resonance in Medicine* 2003;50(3):461-467.
37. Noworolski SM, Tien PC, Merriman R, Vigneron DB, Qayyum A. Respiratory motion-corrected proton magnetic resonance spectroscopy of the liver. *Magnetic resonance imaging* 2009;27(4):570-576.
38. Machann J, Thamer C, Schnoedt B, Stefan N, Haring HU, Claussen CD, Fritsche A, Schick F. Hepatic lipid accumulation in healthy subjects: a comparative study using spectral fat-selective MRI and volume-localized 1H-MR spectroscopy. *Magn Reson Med* 2006;55(4):913-917.
39. Merriman RB, Ferrell LD, Patti MG, Weston SR, Pabst MS, Aouizerat BE, Bass NM. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. *Hepatology* 2006;44(4):874-880.
40. Wood ML, Mark Henkelman R. The magnetic field dependence of the breathing artifact. *Magnetic Resonance Imaging* 1986;4(5):387-392.
41. Stanis GJ, Odrobina EE, Pun J, Escaravage M, Graham SJ, Bronskill MJ, Henkelman RM. T1, T2 relaxation and magnetization transfer in tissue at 3T. *Magn Reson Med* 2005;54(3):507-512.
42. Laudadio T, Mastronardi N, Vanhamme L, Van Hecke P, Van Huffel S. Improved Lanczos algorithms for blackbox MRS data quantitation. *J Magn Reson* 2002;157(2):292-297.

43. Bell JD, Cox IJ, Sargentoni J, Peden CJ, Menon DK, Foster CS, Watanapa P, Iles RA, Urenjak J. A ^{31}P and ^1H -NMR investigation in vitro of normal and abnormal human liver. *Biochim Biophys Acta* 1993;1225(1):71-77.
44. Martinez-Granados B, Monleon D, Martinez-Bisbal MC, Rodrigo JM, del Olmo J, Lluch P, Ferrandez A, Marti-Bonmati L, Celda B. Metabolite identification in human liver needle biopsies by high-resolution magic angle spinning ^1H NMR spectroscopy. *NMR Biomed* 2006;19(1):90-100.
45. Lamb HJ, Doornbos J, den Hollander JA, Luyten PR, Beyerbach HP, van der Wall EE, de Roos A. Reproducibility of human cardiac ^{31}P -NMR spectroscopy. *NMR Biomed* 1996;9(5):217-227.
46. de Bazelaire CM, Duhamel GD, Rofsky NM, Alsop DC. MR imaging relaxation times of abdominal and pelvic tissues measured in vivo at 3.0 T: preliminary results. *Radiology* 2004;230(3):652-659.
47. Duewell SH, Ceckler TL, Ong K, Wen H, Jaffer FA, Chesnick SA, Balaban RS. Musculoskeletal MR imaging at 4 T and at 1.5 T: comparison of relaxation times and image contrast. *Radiology* 1995;196(2):551-555.
48. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res* 1999;8(2):135-160.
49. British Standards Institution. Precision of Test Methods 1: Guide for the Determination and Reproducibility for a Standard Test Method (BS 597, Part 1). BSI: London, 1975
50. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H. Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005;90(5):2804-2809.
51. Corbin IR, Furth EE, Pickup S, Siegelman ES, Delikatny EJ. In vivo assessment of hepatic triglycerides in murine non-alcoholic fatty liver disease using magnetic resonance spectroscopy. *Biochim Biophys Acta* 2009;1791(8):757-763.
52. Ackerstaff E, Glunde K, Bhujwala ZM. Choline phospholipid metabolism: a target in cancer cells? *J Cell Biochem* 2003;90(3):525-533.
53. Howe FA, Opstad KS. ^1H MR spectroscopy of brain tumours and masses. *NMR Biomed* 2003;16(3):123-131.
54. Katz-Brull R, Lavin PT, Lenkinski RE. Clinical utility of proton magnetic resonance spectroscopy in characterizing breast lesions. *J Natl Cancer Inst* 2002;94(16):1197-1203.
55. Jue T, Rothman DL, Tavitian BA, Shulman RG. Natural-abundance ^{13}C NMR study of glycogen repletion in human liver and muscle. *Proc Natl Acad Sci U S A* 1989;86(5):1439-1442.
56. Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI. Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with ^{13}C NMR. *Science* 1991;254(5031):573-576.
57. Chen W, Avison MJ, Bloch G, Shulman RG. Proton NMR observation of glycogen in vivo. *Magn Reson Med* 1994;31(5):576-579.
58. Bollard ME, Garrod S, Holmes E, Lindon JC, Humpfer E, Spraul M, Nicholson JK. High-resolution (^1H) and (^1H) - (^{13}C) magic angle spinning NMR spectroscopy of rat liver. *Magn Reson Med* 2000;44(2):201-207.

3

CARDIAC METABOLISM OF THE HUMAN HEART

3.1 Overview

From all MR-detectable nuclei, protons have the highest MR sensitivity (1). Hence, ^1H -MRS is the most frequently used together with ^{31}P -MRS due to the importance of phosphorous in energy metabolism (2). However, the potential of ^1H -MRS in the study of cardiac metabolism has been minimally explored in vivo compared to the investigations of the brain, where this technique is extensively used (3-5). The study of isolated perfused hearts using ^1H -MRS has confirmed that resonances from creatine and lipids are detectable (6,7). Cardiac ^1H -MRS has also been successfully applied in pilot studies on animal in vivo (8). The first ^1H -MRS spectra of the human heart (9) showed lipids and creatine resonances. Lipids and creatine

levels are two key components of substrate and energy metabolism, hence the detection and quantification of these molecules opens a new path to the metabolism of the heart.

3.1.1 Myocardial Lipid Metabolism

Myocardial lipid stores are regulated by dietary lipid intake, levels of lipids in plasma, non-esterified fatty acids and myocardial fatty acid uptake and oxidation. Myocardial lipids as such are most likely inert. Over nutrition and sedentary life has lead to increased obesity and type 2 diabetes (T2DM) in the developed countries (10), which also increases the risk of suffering heart failure (11). The caloric excess is normally stored in adipocytes in form of fat (triglycerides), but when the adipocytes reach maximum storage, plasma free fatty acid levels increase, and a subsequent accumulation of lipids in nonadipose tissues gradually occurs, including myocardium. The accumulation of lipids in the myocardium (cardiac steatosis) is believed to damage the heart (12,13), but it has not been clarified whether or not lipid oversupply to cardiac cells is a basic mechanism for obesity-related heart diseases in humans (14). Therefore, a further characterization of the pathological role of myocardial lipid deposits may benefit from the application of a non-invasive tool such as ^1H -MRS.

The studies presented in this thesis aim to develop a robust, reproducible, simple and quick ^1H -MRS tool at 3 T to clarify the relevance of myocardial lipid overstorage on myocardial function firstly in healthy subjects and subsequently in patients. Next, a brief overview of myocardial metabolism is discussed and is followed by preliminary results in the application of the developed STEAM technique to cardiac spectroscopy. Then the feasibility of single breath-hold ^1H -MRS for myocardial lipid levels assessment is demonstrated, along with results suggesting the ability of ^1H -MRS to discern two lipid pools in the human

myocardium. Finally, preliminary myocardial creatine levels acquired by ^1H -MRS in healthy volunteers at 3 T are presented.

3.1.2 Myocardial Creatine Metabolism

Total creatine, the sum of phosphorylated (PCr) and unphosphorylated creatine, appears at 3 ppm and 3.9 ppm in ^1H -MR spectra of the heart. Creatine plays an important role in the creatine kinase/phosphocreatine (CK/PCr) energy storage and transport system (15). A loss of creatine is a characteristic of the failing heart (16,17) and it has been postulated that impaired contractile function may be a consequence of this energetic imbalance (16,18,19).

The combined use of ^{31}P MRS, ^1H -MRS in conjunction with routine cardiac MRI techniques would yield insight in clinical conditions such as myocardial ischaemia, viability, hypertrophy and heart failure. Previous studies have shown reduced PCr/ATP ratios in HCM (20,21) but it is intensely debated and unknown whether or not this is due to limitation of oxygen supply with subsequently reduced PCr but unchanged creatine levels, or whether in HCM, the total creatine pool is depleted, thereby limiting the energy reserve of the heart (independent from mechanisms of reduced oxygen supply). Nakae et al found reduced levels of creatine in HCM patients compared to healthy volunteers (22); however the study did not describe in detail the ^1H -MRS method applied. Also, data were apparently acquired without respiratory motion correction, which lead to lower spectral resolution. Other study by the same group suggested a link between creatine depletion and impaired fatty acid metabolism in HCM (23). Impaired fatty acid metabolism might lead to myocardial fat excess (24). Hence, the expected increase in SNR at 3 T over scans at 1.5 T may enable ^1H -MRS with improved spectral resolution enabling reliable quantification of myocardial lipid and creatine levels to clarify cardiac dysfunction.

3.2 Preliminary Studies

The ^1H -MRS method developed showed good results in human liver at 3 T (Chapter 2), however cardiac motion compensation was required for the application in human heart. In this section the selection of the acquisition window throughout the cardiac cycle was investigated for the acquisition of cardiac ^1H -MR spectra with sufficient SNR and spectral resolution. Also the possible influence of ventricular blood in myocardial metabolite quantification was evaluated.

3.2.1 Cardiac Triggering

Three components constitute the motion of the heart: motion from the cardiac cycle, respiratory motion and patient motion. The latter component relies on patient cooperation and the longer the scanning protocol is, the more likely it occurs. Breath-holding was the method used in this study since it is easily implemented and has shown good preliminary results when applied to liver ^1H -MRS, where studies were completed with sufficient SNR and resolution to investigate hepatic metabolites during multiple breath holds. ECG gating remains the most commonly used technique for dealing with motion from the heart. In this section the optimum selection window for ^1H -MRS was investigated. Cardiac motion was compensated for by synchronizing the MR scan to the heart beat using the ECG trigger and acquiring water unsuppressed data at the same position in the cardiac cycle ($\text{TE}/\text{TM} = 10/7$ ms; TR of at least 4 s, 1024 points, 2000 Hz). A series of 10 consecutive breath-holds were acquired when R-wave was detected, at the beginning of diastole and another series 100 ms later in five healthy volunteers (age = 30 ± 4 years; BMI = 23.2 ± 2.6 kg/m²; 3 males/2 females, VOI = 5.4-7.2 ml). Although previous studies suggested empirical formulas for the onset of diastole (25), the trigger delay was chosen based on the duration of previous R-R intervals

observed in mid-ventricular short-axis and long-axis cine imaging. Figure 3.1 shows the typical voxel location (a) and the three points in the cardiac cycle selected for water-unsuppressed ^1H -MRS acquisitions (b) The corresponding water amplitude values obtained during consecutive breath-holds are plotted in Figure 3.1c.

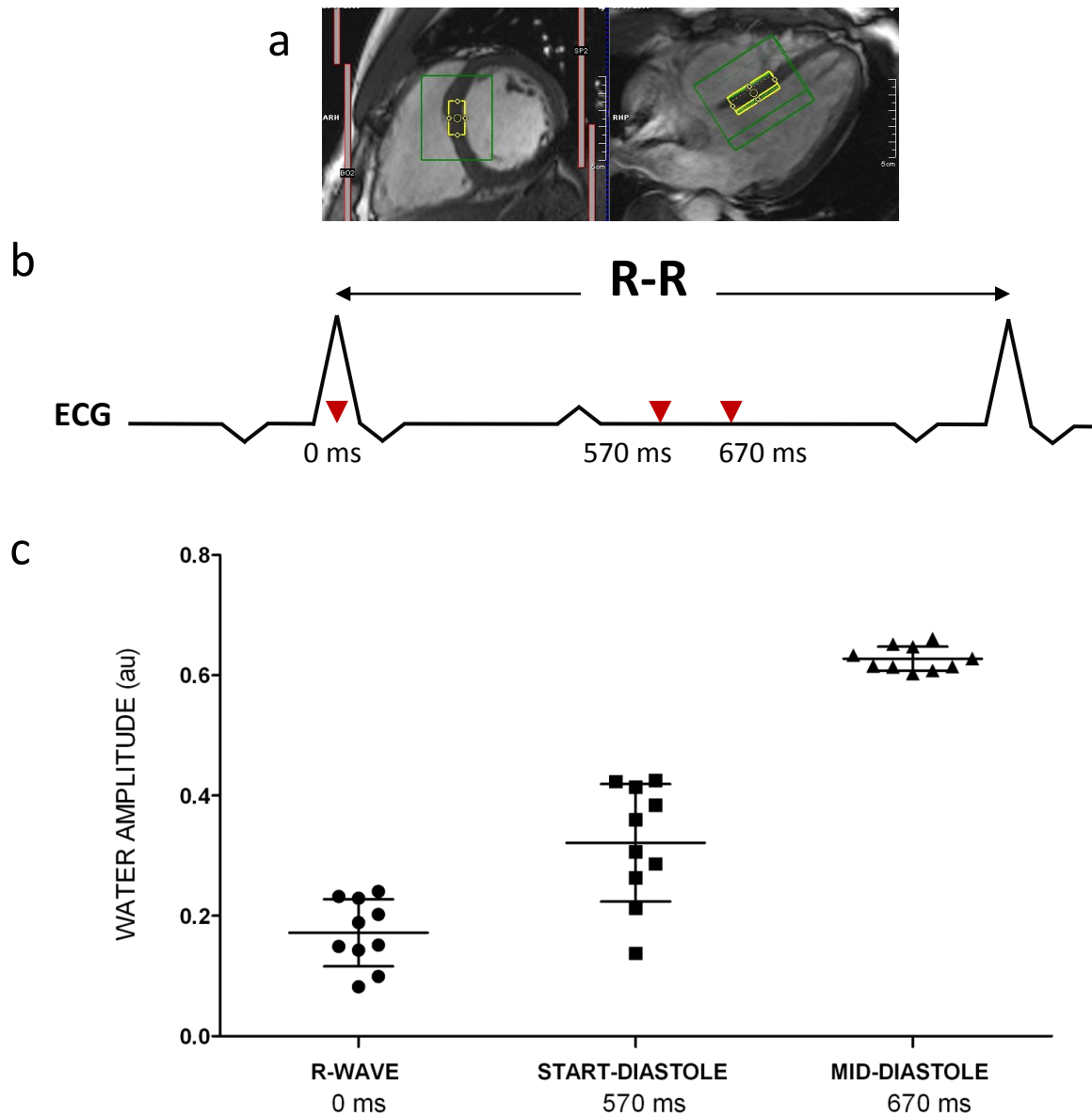


Figure 3.1 Influence of the different temporal phases of the cardiac cycle (R-R interval) in myocardial water amplitude acquired in a 6.4 ml voxel during consecutive breath-holds (BH) in a volunteer. (a) Location of the voxel (yellow) in short-and four-chamber long axis views. (b) ^1H -MRS was acquired at different temporal positions throughout the cardiac cycle. From left to right: data acquired at R-wave detection, after 570 ms of R-wave detection, data acquired after 670 ms of R-wave (c) Water amplitude variation between consecutive breath-holds for the different set ups. Error bars represent the standard deviation of the 10 consecutive breath-holds.

For the five volunteers the coefficient of variation of the water amplitude in one series of 10 consecutive breath-held unsuppressed-water scans acquired at the beginning of diastole was on average $70 \pm 13\%$ higher than at mid-diastole. Also, acquisition at mid-diastole resulted in a 3-fold increase in water amplitude and, therefore, SNR (Figure 3.2).

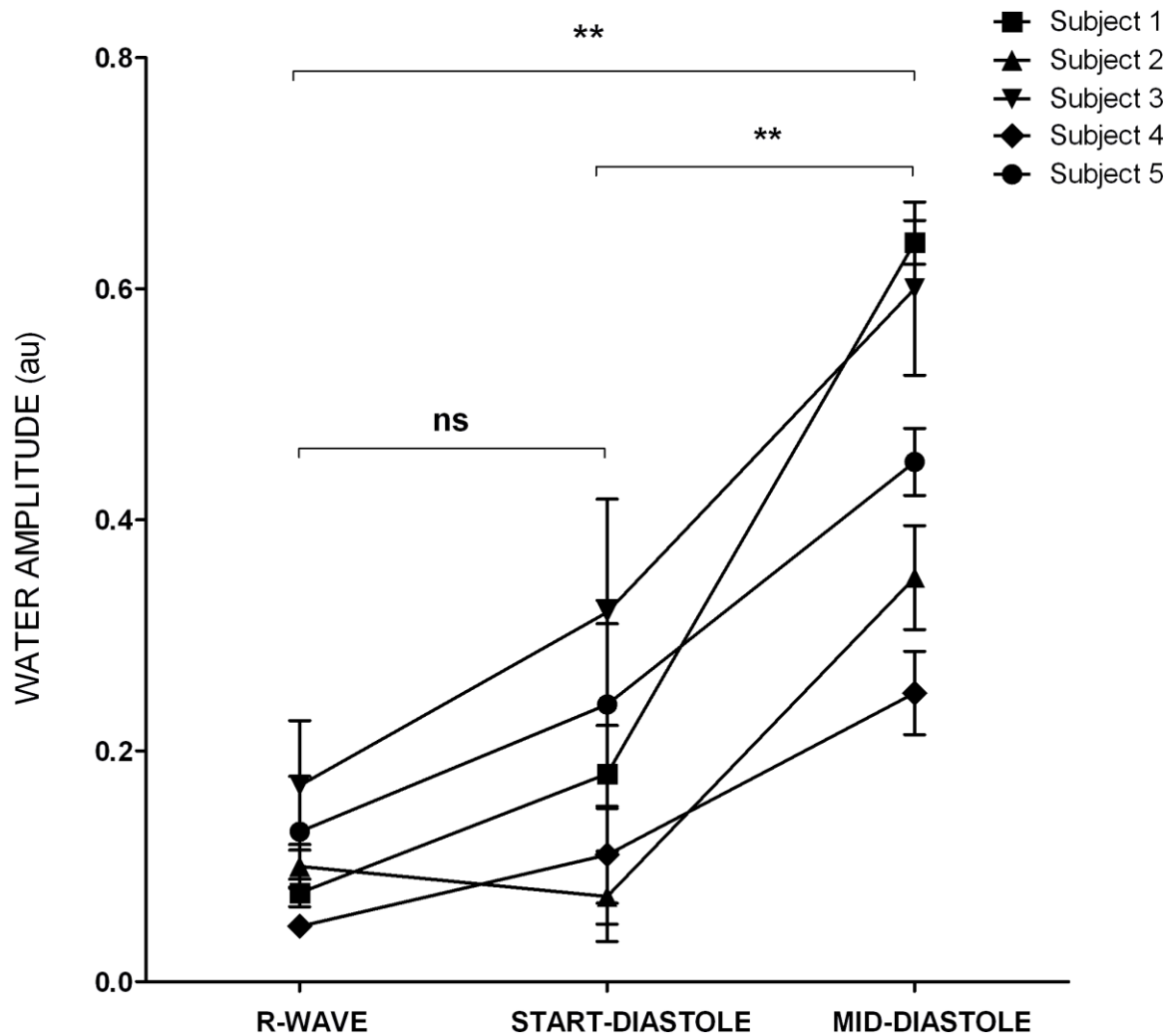


Figure 3.2 Water amplitude obtained at different acquisition windows using breath-held, ECG gated ^1H -MRS. Water amplitude was increased for scans gated to mid-diastole compared to start-diastole and R-wave detection in all five subjects. Data points are mean $\pm$ standard deviation of 10 consecutive breath-holds per subject.

These results confirmed that mid-diastole is the optimum acquisition window for cardiac ^1H -MRS. Several studies applying STEAM for cardiac imaging (26,27) reported that the

contraction of the cardiac muscle results in dephasing of the magnetization, which do not give rise to a stimulated echo. Frahm et al (26) recommended late diastole triggering for optimum results during STEAM imaging, since the movements of the heart wall during early diastole resulted in complete loss of myocardial signal. Although the FID was long (acquisition time = 512 ms) compared to the remaining time in the R-R interval, the motion during the next R-wave did not compromise the spectral quality of the data. While this long acquisition was required for phantom experiments, the in vivo signal had decayed down to noise level after 100 ms (Figure 3.3). Importantly, no adverse effect on the spectral analysis has to be expected, as data were fitted in the time domain.

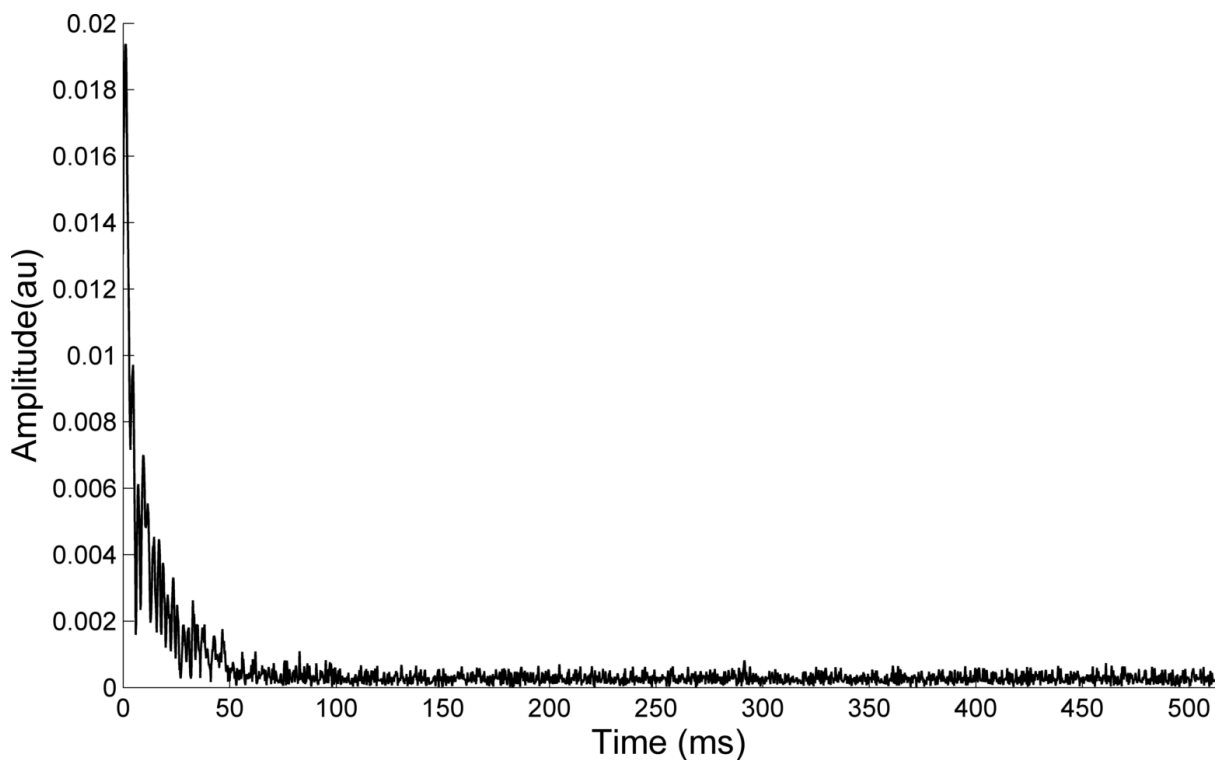


Figure 3.3 Exponential decay FID of a cardiac ^1H -MRS experiment. Signal decayed to noise level after 100 ms.

3.2.2 Blood Contamination

The black blood properties of STEAM and other localized spin-echo MR methods have already been described in several studies (8,22,28). The water signal from blood is dephased due to blood movement in the presence of the gradients used in the localized MRS method, which leads to substantial loss of water signal from blood. To verify the black blood properties of STEAM, a series of breath-held water-unsuppressed scans was acquired by positioning the voxel (VOI = 12.7 ml) in the septum, in the blood pool adjacent to the septum and also with the voxel placed in the middle of the ventricle. Furthermore, to confirm that indeed the water signal does not increase with increasing voxel size beyond a certain width, three more water scans were obtained by increasing the voxel size in the septal direction from 18 mm over 22 mm to 26 mm (VOI from 12.7 ml over 15.5 ml to 18.3 ml).

Figure 3.4 shows the results. Placing the voxel next to the septum (Figure 3.4A) or into the middle of the ventricle (Figure 3.4B) yielded only negligible ($\leq 4\%$) water signal (traces A and B, relative to spectrum C). Figure 3.4C-E shows the change in voxel size trans-septally from 18 mm over 22mm to 26 mm, which resulted in 22% (D) and 44% (E) increment of the voxel volume compared to C. The water signal, however, increased only by 7% (D) and 15% (E). Both experiments verified that blood contamination did not represent a problem as long as the voxel dimensions were chosen sensibly.

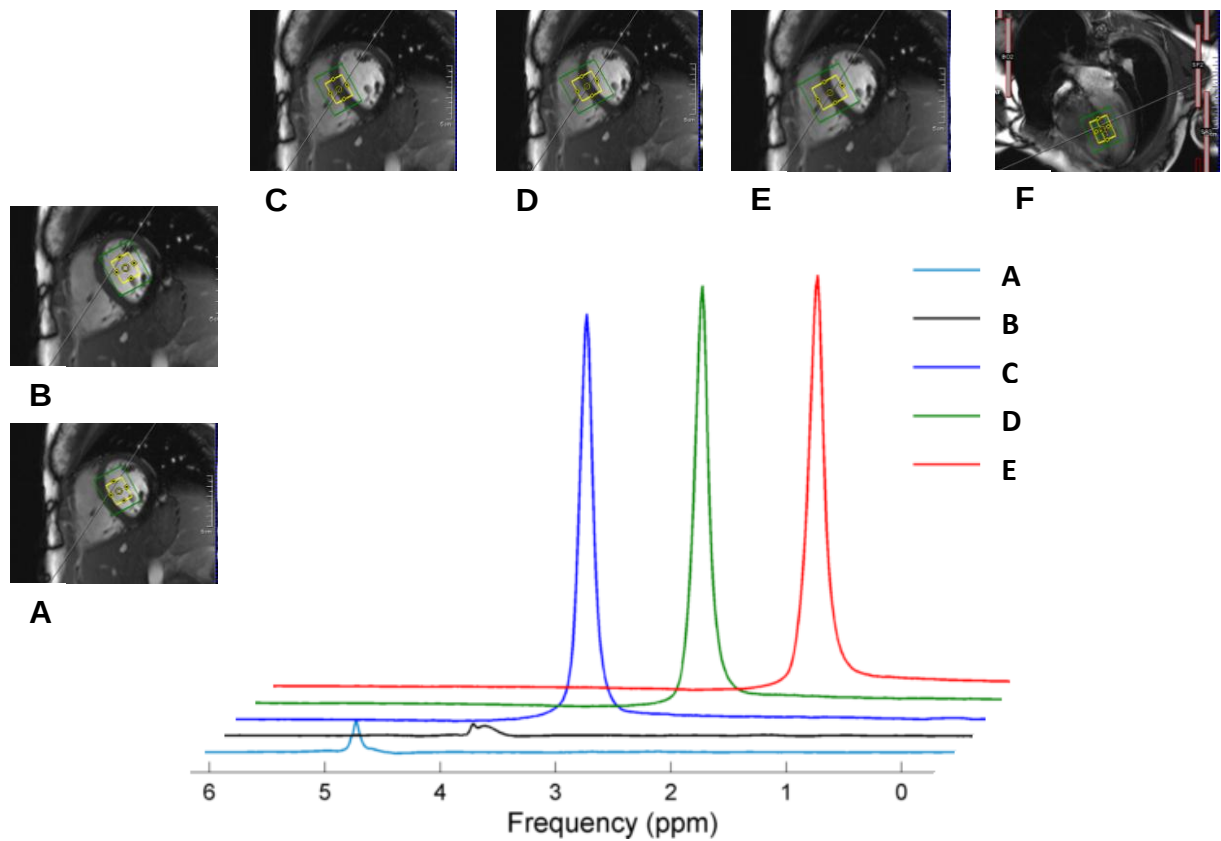


Figure 3.4 STEAM black blood properties. A 12.7 ml voxel located adjacent to the septum(A) and in the middle of the ventricle (B) did not lead to significant water signal as when voxel was placed over the septum (C); traces A,B and C respectively. Owing to the black blood properties of the sequence, an increase in voxel volume to include more blood from 12.7 ml (C) to 15.5 ml (D) and 18.3 ml (E) did not result in substantial water signal increase (traces D and C respectively). (F) Long axis MR image shows the location of the 12.7 ml STEAM voxel.

3.3 Single Breath-Hold ^1H -MRS Cardiac Lipid Assessment

In this section a single breath-hold cardiac-gated ^1H -MRS acquisition is proposed as a time-efficient approach to measure myocardial lipid levels at 3 T. Breath-holding is commonly used for respiratory motion compensation in thoracic MR imaging, with comfortable end-expiration breath-hold periods limited to 25 seconds for healthy volunteers and nine seconds for patients (29,30). Hence, the aim was to investigate whether cardiac lipid levels can be measured accurately within a single breath-hold using ^1H -MRS at 3 T, and to evaluate the applicability and reproducibility of this method as a routine clinical research tool.

3.3.1 Methods

3.3.1.1 Subjects

In 15 healthy volunteers (11 men, four women; mean age $\pm$ standard deviation, 34 ± 10 years; age range, 22-58 years; body mass index (BMI), 19-30 kg/m²), myocardial lipid normalized to myocardial water content (myocardial lipid levels) were evaluated using ¹H-MRS. The volunteers were asked to fast overnight. None of the volunteers had a history of cardiovascular disease, diabetes or any other chronic disease. The Research Ethics Committee at our institution approved the study protocol, and all participants gave informed consent.

3.3.1.2 ¹H-MRS Technique

All studies were performed on a 3 T MR scanner (Tim Trio, Siemens Healthcare, Germany). The ¹H-MRS sequence was based on the STEAM sequence described in Chapter 1 that was modified to achieve a short TE of 10 ms. The water signal was suppressed using a WET module (31). In addition, a calibration pulse sequence was implemented to evaluate the optimal water suppression pulse scaling factor. Cardiac gated spectra were acquired using different water correction scaling factors in a 17 second breath-hold and the one yielding the most effective water-suppression was chosen and applied in the following experiments.

During the single breath-hold acquisition, the modified STEAM sequence was repeated four times: a spectrum without water suppression was obtained first, with the frequency centred at the water frequency followed by, three consecutive water-suppressed measurements with the frequency set to 3 ppm (Figure 3.5). The effective repetition time (TR) was cardiac gated and controlled by the pulse program to be at least 2 seconds and the total spectroscopy acquisition

time was less than 10 seconds. In order to determine whether or not the single breath-hold technique provided sufficient SNR for an accurate myocardial lipid assessment, it was compared to the multiple breath-hold method. This consisted of a total of six breath-holds of about 16 seconds each: five breath-holds, which allowed for the acquisition of 35 non-averaged water-suppressed spectra and a separate breath-hold setting the water suppression RF pulse power to zero (4 non-averaged water spectra were acquired). The total acquisition time was 5-7 minutes, including time for the volunteers to recover between breath-holds.

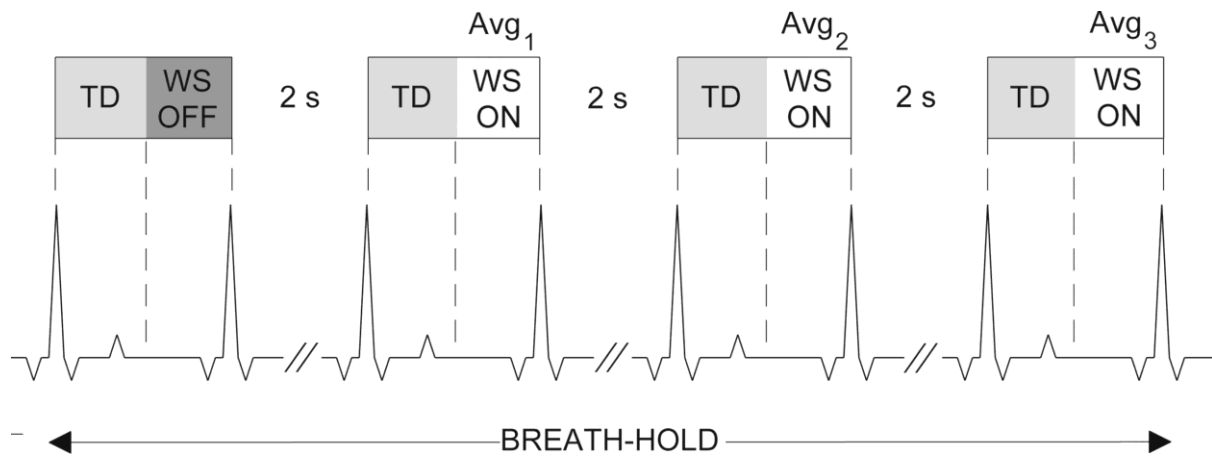


Figure 3.5 Single breath-hold acquisition scheme: a water-unsuppressed scan (WS OFF) was followed by 3 water-suppressed scans (WS ON), with a 2-second delay in between acquisitions. A trigger delay (TD) was used to position all acquisitions in mid-diastole.

3.3.1.3 Validation of Single Breath-Hold Lipid Content Measurement

For the validation of the method, the volunteers were positioned supine, the body coil was used for transmission and the anterior and posterior phased-array body coils for receiving, resulting in 15 channels of data. Cardiac gated MR cine imaging was performed to acquire the standardized four-chamber and short axis views for appropriate voxel placement in the septum and inspection of mid-diastole (Figure 3.6b and Figure 3.6c). Shim adjustment was performed on a 3D field map obtained from a gradient double echo acquisition within a

single breath-hold (18-22 s). Myocardial ^1H -MRS data were obtained at end-expiration from a $22 \times 12 \times 19 \times 32 \times 36$ mm (8-15 ml) voxel positioned in the interventricular septum far from epicardial fat (Figure 3.6). All acquisitions were ECG-triggered to mid-diastole. Spectroscopy parameters for the custom STEAM sequence included a TE of 10 ms, a mixing time of 7 ms, 1024 points were acquired at a bandwidth of 2000 Hz. Effective repetition times of at least 4 and 2 s were chosen to approach complete relaxation of the water and lipid signals, respectively. Furthermore, the scan frequency was set at 4.7 ppm during water-unsuppressed acquisitions and at 3 ppm during water-suppressed acquisitions, to minimize the effects of the large chemical shift displacement (≈ 420 Hz corresponding to 4.5 mm in the foot to head direction) between the lipid and water peaks at this field strength.

To investigate the reproducibility of the single breath-hold method, this technique was repeated five times in each subject without repositioning the voxel. To assess the accuracy of the single breath-hold method, the multiple breath-hold method was applied at an identical voxel location. Total session time comprising positioning of the patient, septum localization, shimming, water suppression factor adjustment and acquisition of a single breath-hold spectrum was on average 15 minutes. This time was increased by a further 5-7 minutes for the multiple breath-hold spectra acquisition.

3.3.1.4 Spectral Postprocessing

The algorithms for signal combination from individual coil elements (32) and averaging from different acquisitions were written in Matlab. The combination of the individual signals was performed using the amplitude and phase of the time-domain signal without water suppression to represent the weighting and phase correction factors required for weighted signal summation. The multiple ^1H -MRS acquisitions (i.e. three during single breath-hold

measurements and 35 during multiple breath-holds measurements) were phase-corrected with the zero-order phase of the dominant peak in the spectra (typically the residual water peak or the lipid peak) before averaging (33). Furthermore, individual signals acquired within different breath-holds were frequency aligned prior to the summation.

All spectral quantification was performed in the time domain, using the AMARES algorithm included in the jMRUI package (34). A total of six peaks were specified to fit the metabolite contributions in the water-suppressed spectra by using a Lorentzian line shape, representing total creatine with methylene group CH_2 at 3.9 ppm, trimethylammonium compounds at 3.2 ppm (TMA), total creatine with methyl group CH_3 at 3.0 ppm (Cr), lipids $\text{CH}_2\text{CH}_2\text{CO}$ at 2.2 ppm, lipids ($-\text{CH}_2$) at 1.3 ppm and lipids with methyl group CH_3 at 0.9 ppm. Prior knowledge including soft constraints was provided for all resonances. The linewidth was limited to 40 Hz and only the global zero-order phase was fitted, while the first-order term was kept constant (1.1 ms, three data points were acquired before the maximum of the stimulated echo to prevent possible baseline distortions due to incorrect amplitudes of the beginning of the FID after turning on the receiver). The amplitude of the lipid resonance at 1.3 ppm was selected for myocardial lipid quantification. The water peak amplitude from the water-unsuppressed scans was used as internal reference. The lipid content was calculated as a percentage relative to water as the amplitude of the lipid peak divided by the amplitude of the water peak, and multiplied by 100. The SNR of the lipid peak was estimated by taking the ratio of the fitted lipid peak amplitude to the noise SD extracted from the final 100 points of the real part signal in the time domain. Also, an estimate of the SNR was obtained by dividing the Cramer-Rao standard deviation (CRSD) of the lipid peak, which is an indicator of the accuracy of the spectral quantification provided by the AMARES fitting algorithm, by the lipid peak amplitude and converted to a percentage (rCRSD%) (35).

3.3.1.5 Statistical Analysis

Statistical analyses were performed using SPSS (version 16.0; Chicago, IL). Results were expressed as mean $\pm$ standard deviation (SD). To determine the reproducibility of the technique, coefficients of variation (CV) of the five repeated myocardial lipid content measurements in a single breath-hold were calculated as $CV = (\text{within-subject standard deviation}) / (\text{within-subject mean of five myocardial lipid \% measurements}) \times 100$. The Pearson correlation coefficient (r) and linear regression analysis were used to examine the relationship between myocardial lipid content measured in a single breath-hold and multiple breath-holds and between myocardial lipid content and BMI. Bland-Altman analysis was performed to evaluate the agreement between myocardial lipid levels by single and multiple breath-holds measurements. A paired t-test was used to compare myocardial lipid content, CRSD% and linewidth values between the two acquisition approaches. A $p < 0.05$ was considered statistically significant.

3.3.2 Results

Representative spectra from the septum of the healthy volunteers using both, single and multiple breath-hold methods are shown in Figure 3.6a. The spectra showed a well-defined resonance corresponding to myocardial lipids at 1.3 ppm, relative to the residual water at 4.7 ppm. Other resonances, such as TMA at 3.2 ppm, creatine at 3 ppm and other lipids at 0.9-2.5 ppm were also visible in the multiple breath-hold spectra.

Constructive averaging of the 35 STEAM spectra acquired using the multiple breath-hold method resulted in the expected increase in SNR and lower lipid rCRSD% compared to the single breath-hold method (SNR: 24 ± 14 vs 6.8 ± 4.2 , $p < 0.05$; rCRSD%: 4.4 ± 3.4 vs 9.1 ± 5.9 , $p < 0.05$; – multi vs single breath-hold). The mean phase variation for all subjects was 26

$\pm 19^\circ$ (mean $\pm$ SD, $n=15$). The water full-width-at-half-maximum (FWHM) in the unsuppressed water spectra was 14.3 ± 3.2 Hz and 13.3 ± 2.4 Hz ($p = 0.38$) when using the single breath-hold method (one average) and the multiple breath-hold method (four averages), respectively.

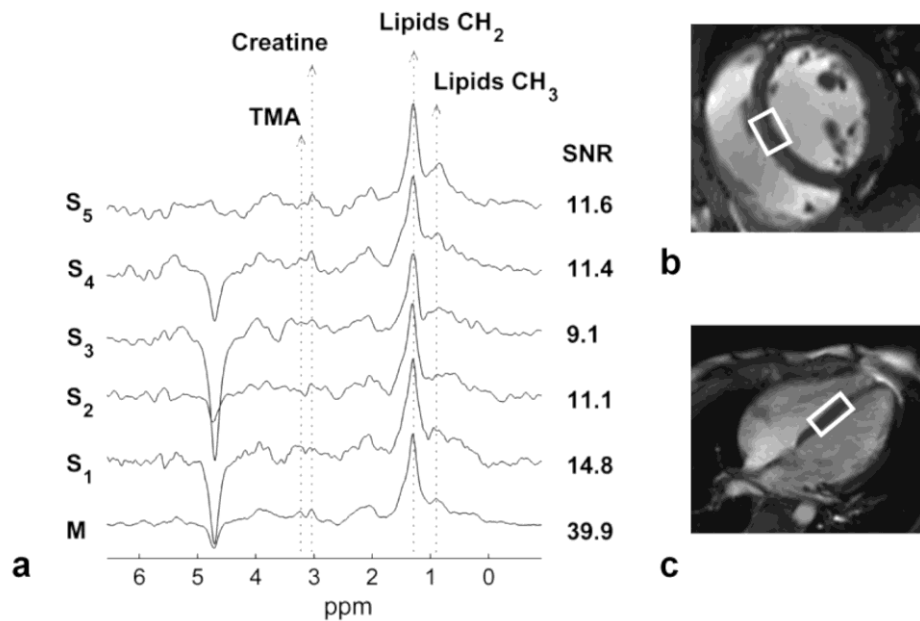


Figure 3.6 (a) Illustration of reproducibility of single breath-hold ^1H -MRS measurements compare to multiple breath-hold measurements. Within subject reproducibility was demonstrated by five repeated spectra (S_1 - S_5) acquired in the same session from a $22 \times 12 \times 32$ mm voxel positioned in the interventricular septum of a healthy volunteer as shown in (b) and (c). Depiction of the myocardial lipid resonance ($-\text{CH}_2$)_n at 1.3 ppm obtained in a single breath-hold (3 averages) showed sufficient SNR as confirmed by almost the same spectrum (M) acquired using multiple breath-hold ^1H -MRS (35 averages). The spectra were scaled to the respective water amplitude.

The mean myocardial lipid content in healthy volunteers with both single and multiple breath-hold ^1H -MRS was $(0.46 \pm 0.19)\%$ and $(0.45 \pm 0.20)\%$, respectively ($p = 0.94$). Within-subject reproducibility of myocardial lipid levels over repeated ^1H -MRS measurements in a single breath-hold showed a coefficient of variation of 19%. Moreover, a strong correlation with a slope close to 1 was confirmed between myocardial lipid levels obtained in a single breath-hold and multiple breath-holds ($r = 0.95$, $p < 0.05$, Figure 3.7).

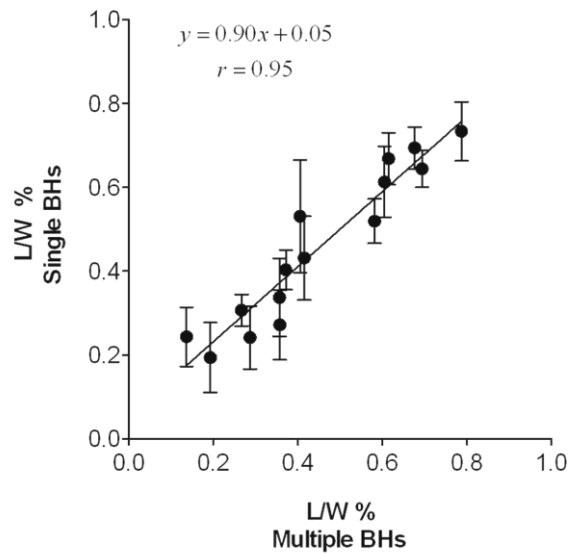


Figure 3.7 Linear regression analysis showing the strong correlation between myocardial lipid content measured relative to water in one breath-hold and multiple breath-holds ($p < 0.05$). The error bars represent the standard deviation of the 5 repeated scans using the single breath-hold method.

Bland-Altman analysis (Figure 3.8) showed an excellent agreement of the lipid levels measured in a single breath-hold and in multiple-breath-holds, with a mean difference between both methods of $(-0.02 \pm 0.06)\%$. All the differences between the two measurement methods were contained within the 95% limits of agreement (from -0.12% to 0.11%).

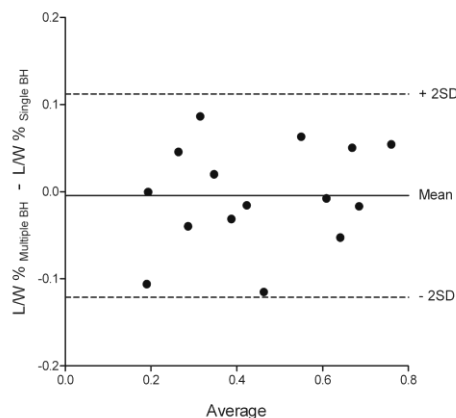


Figure 3.8 Bland-Altman plot for myocardial lipid levels acquired using single and multiple breath-holds methods. The solid line represents the mean of differences between levels obtained with different methods and the dashed lines indicate the confidence intervals $\pm 2SD$.

For the BMI range represented in this study, the myocardial lipid levels increased linearly with BMI (Figure 3.9a, single breath-hold: $r = 0.71$, $p < 0.05$; Figure 3.9b, multiple breath-holds: $r = 0.68$, $p < 0.05$). Moreover, the difference between the slopes of regression lines for both methods was not different ($p = 0.98$). The 15 volunteers were then divided into two groups: normal (BMI $< 25 \text{ kg/m}^2$, 4 male, 2 female) and overweight (BMI $\geq 25 \text{ kg/m}^2$, 7 male, 2 female). Myocardial lipid levels were significantly different between the two groups and independent of the method of measurement used (Figure 3.9c, single breath-hold: lipid levels lean group = $(0.32 \pm 0.12)\%$, lipid levels overweight group = $(0.54 \pm 0.17)\%$, $p = 0.02$; multiple breath-hold: lipid levels lean group = $(0.32 \pm 0.16)\%$, lipid levels overweight group = $(0.54 \pm 0.18)\%$, $p = 0.03$).

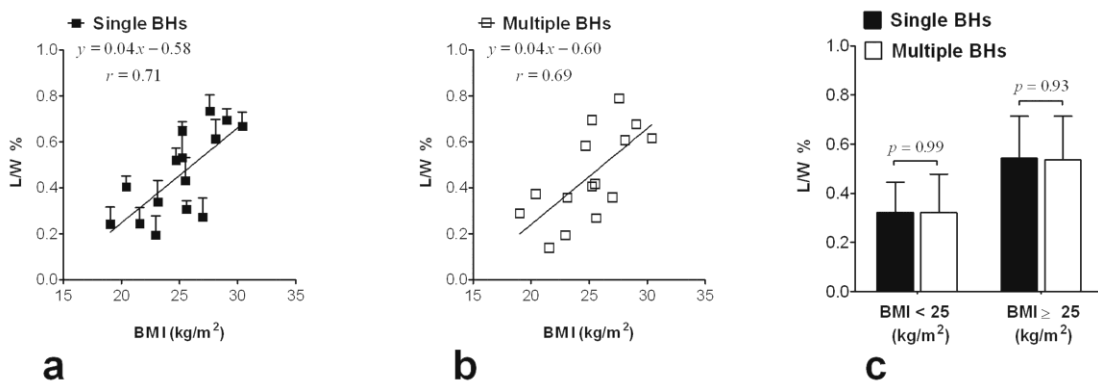


Figure 3.9 Myocardial lipid levels correlated positively with BMI for both single (a) and multiple (b) breath-holds acquisitions. The error bars in panel (a) represent the standard deviation of the 5 repeated scans using the single breath-hold method. Volunteers with BMI $\geq 25 \text{ kg/m}^2$ showed myocardial lipid levels significantly higher than volunteers with BMI $< 25 \text{ kg/m}^2$ ($p < 0.05$). No significant difference was found between methods for both BMI groups.

3.3.3 Discussion

This study has shown that myocardial lipid levels can be quantified during a short single breath-hold using cardiac triggered proton spectroscopy at 3 T. The mean lipid levels obtained using single and multiple breath-hold methods agreed well. Water was chosen as the internal reference as it is assumed that the water content remains constant in normal and pathological conditions as shown by Bottomley et al in the dog model of myocardial infarction (9). Other metabolites, such as creatine not only suffer from low SNR, but may also vary in pathologies.

The spectroscopic quality, characterized by the linewidth of the unsuppressed water signal (14.3 ± 3.2 Hz), was good using the single breath-hold method and similar to values published at 1.5 T. Van der Meer et al reported a myocardial water signal linewidth of 10.3 Hz using navigator gating and volume tracking for respiratory motion correction and 11.4 Hz without navigation (36). Other study obtained a 12 Hz myocardial water linewidth using a sequence gated to the respiratory cycle (37). Since linewidth is dependent on the T_2 -relaxation time and field homogeneity, the linewidth would be expected to increase as the field strength goes from 1.5T to 3 T. The similar values were attributed to the good local shimming achieved at 3 T in this study that used a cardiac gated 3D field map shimming method that controlled both the 1st and 2nd order shim coils. Furthermore, the rCRSD was on average 9.1% of the myocardial lipids demonstrating that single breath-hold acquisitions showed sufficient spectral quality (38) over the wide range of myocardial lipid levels observed (0.14-0.73%). This finding is also supported by the fact that an almost identical myocardial lipid range (0.14-0.79%) was observed using multiple breath-holds acquisition, with intrinsically lower rCRSD (4.4%).

The reproducibility of the single breath-hold method was validated by repeating single breath-hold lipid measurements at the same myocardial location for each subject. Although the volunteers were not repositioned in our reproducibility assessment, all the reference scans (i.e. scouting, frequency adjustment, RF-reference voltage measurement, shim current and water suppression calibration) were invalidated and forced to be re-calibrated in between measurements. In our experience, the calibration scans rather than the patient positioning represent the main source of variation for this kind of measurements. A CV = 19% was obtained during the same day. Szczepaniak et al (39) showed similar coefficient of variation (17%) for spectroscopy measurements of myocardial lipid levels using a pressure belt for respiratory gating. Felblinger reported a coefficient of variation of 13% using double triggering based on the ECG signal (40). Also, findings in this thesis compared well with more recent results obtained at 1.5 T using navigator gating and volume tracking (36).

Volunteers were divided into two groups according to their BMI. The threshold of 25 kg/m² was based on the National Institutes of Health classification of overweight by BMI (41). Importantly, single breath-hold ¹H-MRS showed sufficient SNR for the assessment of cardiac lipid levels, even for low, i.e. normal BMI volunteers. Moreover, a statistically significant positive correlation between BMI and myocardial lipid levels was found, which was also reported in previous studies where other methods were used for spectroscopy acquisition (39,42,43).

This study demonstrated the time efficiency obtainable from single breath-hold MR acquisitions. In a 10 second breath-hold, both unsuppressed and suppressed water signals were acquired with sufficient resolution for myocardial lipid levels quantification in healthy volunteers. Compared to multiple breath-hold averaging, single breath-hold ¹H-MRS

acquisition time decreased from 16 to 10 seconds, which allowed for a comfortable breath-hold and more importantly can be applied to patients, who can generally hold their breath only for shorter times compared to volunteers (30).

No T_1 corrections were considered in the calculation of the metabolite ratios. The T_1 of lipids was assumed to be between 300-400 ms (estimated based on values reported for 1.5T (44)). Hence, the TR of 2s was adequate to ensure full relaxation. The water T_1 -value for healthy myocardium was measured to be 1.2s (45). Although the water signal was not fully relaxed at a TR of 4s, the correction factor of 3% is within the accuracy of the method. Importantly, water was fully relaxed for the single-breath-hold method. Furthermore, the lipid level values obtained in this study were in concordance with previous published data (36,46-48).

The spectroscopy voxel was larger (8-15 ml) compared to previous studies (2-9 ml) (9,22,36,39,43,48). However, contamination from ventricular blood can be excluded due to the dark blood properties of the STEAM sequence (9,22,28), which we confirmed in preliminary work by placing the voxel entirely in the blood pool (section 3.2.2). Moreover, the spectroscopic volume was carefully positioned in the interventricular septal wall, avoiding areas of pericardial fat, confirmed by a single spectral peak at 1.3 ppm (39). The water-suppressed localized volume was based on the creatine resonance frequency (3 ppm) for possible assessment of the entire myocardial metabolic range, including creatine. For these experimental settings (≈ 3.4 kHz STEAM sinc refocusing pulses, length = 2.6 ms and voxel volume = 8-15 ml), the creatine and lipid resonances had $\approx 82\%$ of their corresponding voxels in common. A creatine peak was clearly visible in 12 of the 15 multiple breath-hold spectra acquired. However, creatine was not quantified in these spectra as the aim of this study was to establish and validate the single breath-hold technique. Furthermore, single

breath-hold cardiac spectra did not have sufficient SNR for reliable creatine quantification. Moreover, future single breath-hold experiments should be acquired at the lipid resonance.

Although only 15 volunteers were included in this study to investigate feasibility and to validate single breath-hold cardiac spectroscopy, the sample size was sufficient to achieve statistical significance by single breath-hold and multiple breath-hold lipid levels correlating with a slope of close to unity. However, ^1H -MRS was only performed in healthy volunteers with a BMI range 19-30 kg/m². Hence, the performance of the technique specifically in obese patients requires further investigation. Notably, a 10 second breath-hold is well within the comfortable range for healthy volunteers (29) and also for patients (30). A breath-hold period of similar duration is routinely used for functional cardiac MR imaging in our institution. Importantly, the minimal time requirement of the proposed protocol combined with the use of standard cardiac receive coils allows this method to be added to any routine cardiac MR exam without substantial increase of scan time.

3.4 Identification of Two Myocardial Lipid Pools

The use of high magnetic field strengths (3 T) has facilitated a more detailed quantification of various fatty acid components in hepatic tissue and skeletal muscle (49,50). The separate observation of intra- (IMCL) and extramyocellular lipids (EMCL) has also become an important application of ^1H -MRS in skeletal muscle (51-53). The physical basis for the observed 0.2-ppm shift in lipid resonances between the IMCL and EMCL storage pools is the distribution of magnetic susceptibility in tissue (54,55). IMCL is contained within spherical droplets and represents a metabolically highly active pool that is optimized for rapid turnover and supply of lipid substrates for cellular oxidation; whereas EMCL is located in an annular compartment oriented along muscle fibres and connective tissue and turns over slowly. In

human myocardium, the additional EMCL resonance has been attributed to epicardial fat when the voxel was located on the anterior left-ventricular wall (39). However, autopsy studies of myocardial lipids performed on obese individuals have shown fat infiltration in areas between myocardial fibres (56-58). While fat located throughout skeletal muscle is frequently seen in normal tissue, fatty infiltration of the myocardium seems to be a pathological pattern observed in morbid obesity (57).

The aim of this work was to assess the feasibility of ^1H -MRS at 3 T to differentiate EMCL and IMCL in a voxel of inter-ventricular myocardial tissue in patients with suspected high lipid myocardial content.

3.4.1 Methods

Patients with confirmed dystrophin mutations (Becker or Duchenne muscular dystrophies) were chosen for this study as these conditions are associated with elevated lipid levels in skeletal muscle (59) and also a high rate of cardiomyopathy (60). Five muscular dystrophy patients (age= 41 ± 13 yrs; BMI= 24.5 ± 2.7 kg/m²) with impaired septal contractility (peak circumferential strain $-11 \pm 2\%$; normal $-18 \pm 2\%$; $p < 0.001$) were scanned on a 3 T Siemens Tim Trio using the whole body coil in transmit mode and the 6-channel anterior and 24-channel posterior phased-array coils for signal receiving. Results from ^1H -MR spectra were compared to data obtained from five healthy volunteers and five healthy obese volunteers (Table 3.1)

Table 3.1 Subject characteristics. Values are mean $\pm$ standard deviation; BMI, body mass index

	Normal	Muscular Dystrophy	Obese
Age (years)	33 ± 14	41 ± 13	40 ± 7
BMI (kg/m ²)	21.4 ± 1.8	24.5 ± 2.7	45.0 ± 5.1
Sex (Male/Female)	3/2	5/0	2/3

Mid-ventricular long and short-axis cine series were acquired to determine the trigger delay for mid-diastole. A 22x18x32 mm (12.7 ml) STEAM voxel was planned on the corresponding cine frame (Figure 3.10a,b).

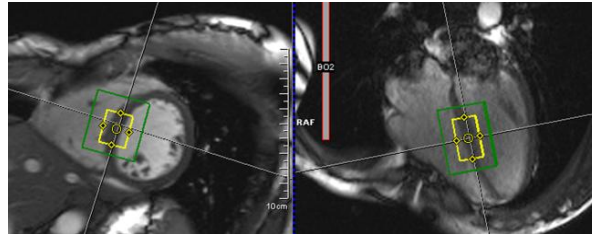


Figure 3.10 Cine short-axis (a) and long-axis (b) frames at mid-diastole used for positioning the STEAM voxel (yellow box) in the septum.

Shimming was performed based on a GRE dual echo 3D imaging data set. A cardiac-gated water spectrum (3 averages; TR of at least 4 s) was acquired in a single breath-hold to use as internal reference. Next, five to six WET water-suppressed scans (5 averages each; TR of at least 2 s) were acquired at mid-diastole in a series of single breath-holds. The acquisition parameters were: TE = 10 ms, TM = 7 ms, BW = 2000 Hz, 1024 data points. Individual coil signals were combined as described section 1.4. Following frequency correction, the 25 or 30 water-suppressed scans were constructively averaged (33). Spectra were quantified using the AMARES algorithm (34). Five peaks (TMA, creatine, EMCL, IMCL and CH₃ lipids at 3.2, 3.0, 1.5, 1.3 and 0.9 ppm respectively) were analyzed and prior knowledge was used for all peak locations by using soft constraints (Table 3.2). All peaks were fitted using Lorentzian lineshapes.

Table 3.2 Soft constrains used for spectral fitting

Metabolite	Chemical Shift Starting Values (ppm)	Chemical Shift Constrains (ppm)	Linewidth Constraints (Hz)
Residual Water	4.7	4.6-4.8	2-40
TMA	3.2	3.1-3.4	2-40
Creatine	3.0	2.9-3.1	2-40
EMCL	1.5	1.4-1.6	2-40
IMCL	1.2	1.1-1.4	2-40
CH ₃ Lipids	0.9	0.8-1.1	2-40

3.4.2 Results

Figure 3.11A shows a spectrum from a 12.7 ml volume in the septum of a muscular dystrophy patient. The two peaks corresponding to the EMCL and IMCL could be distinguished and were quantified in all MD patients and obese subjects. Conversely, only IMCL resonances were reliably quantifiable in the myocardial spectra of normal subjects (Figure 3.11B).

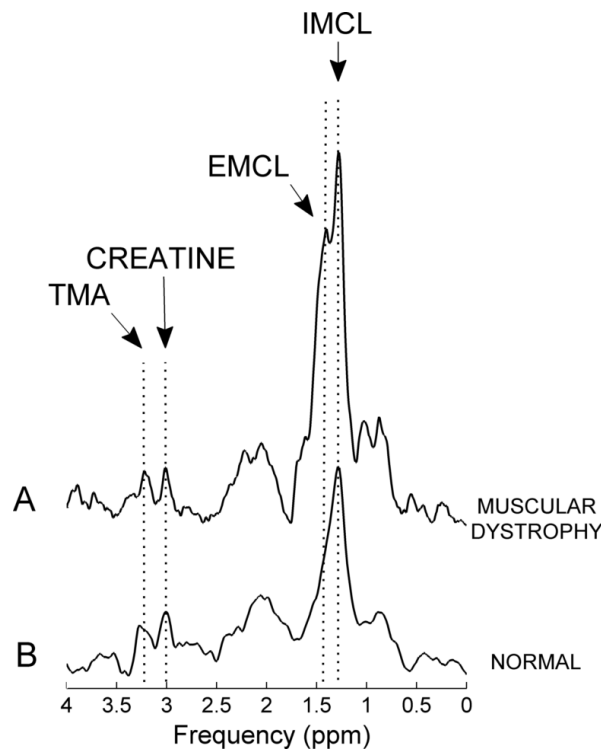


Figure 3.11 Proton spectra from septal myocardium of a muscular dystrophy (A) and a normal (B) subject. Both spectra are scaled to water content.

Figure 3.12 demonstrates the analysis of the real spectra using two model functions for IMCL and EMCL (left column) and only one model function (right column) for the five MD patients. IMCL and EMCL could be clearly resolved in spectrum MD2. EMCL signal was dominant in examples MD3 and MD5, whereas strongly overlapping IMCL and EMCL signals were observed in MD1 and MD4. The residuum (difference between acquired data and fit) was smaller when two model functions were used to fit the spectra $((2.9 \pm 0.7) \times 10^{-4})$

Figure 3.12 ^1H -MR spectra (black trace) of myocardial tissue obtained in five MD patients (MD1-5) and fitted data using two model functions (blue trace) for EMCL and IMCL and only one for the methylene protons (red trace). The residuals of fitting each spectrum are plotted underneath.

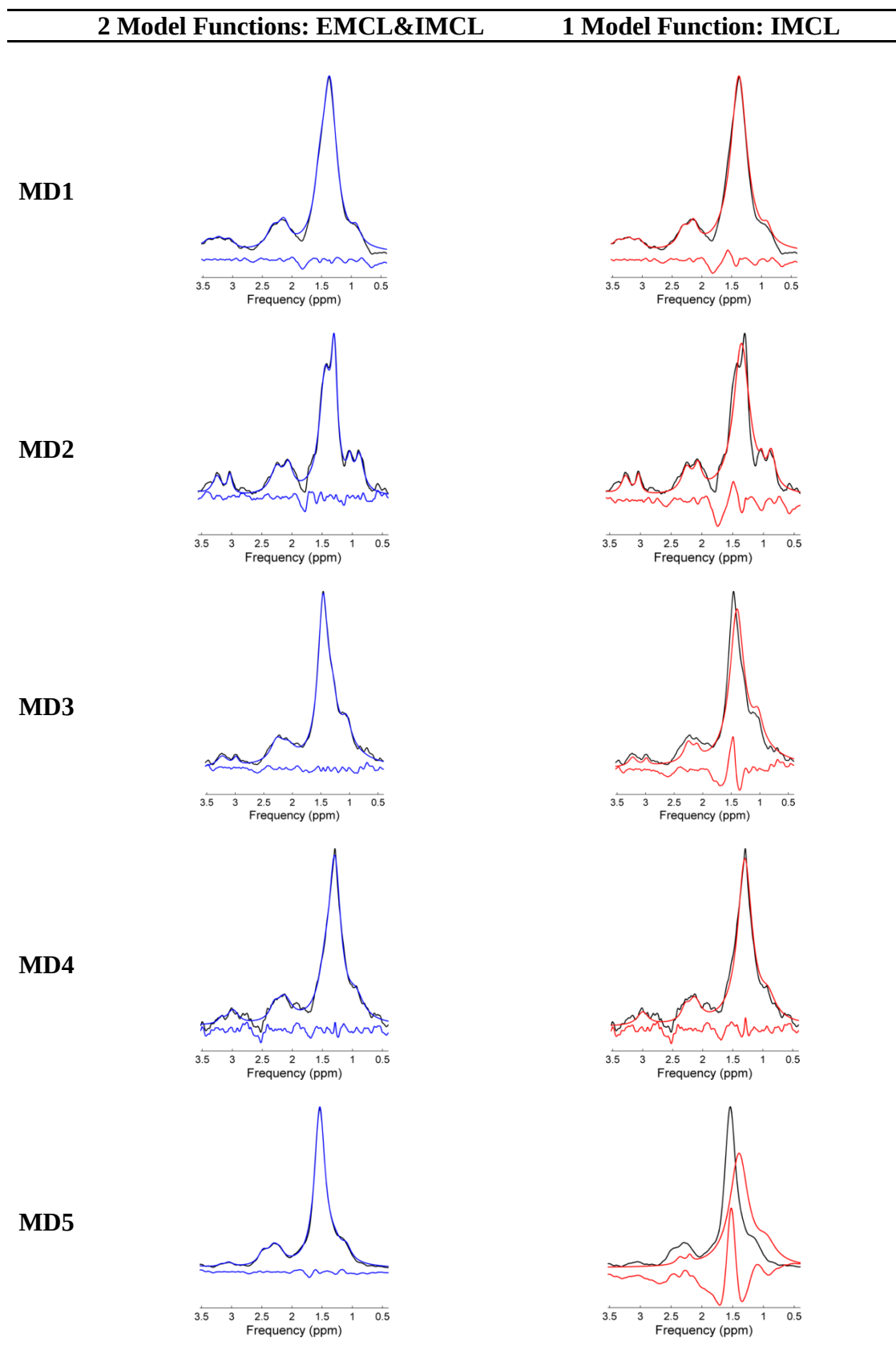
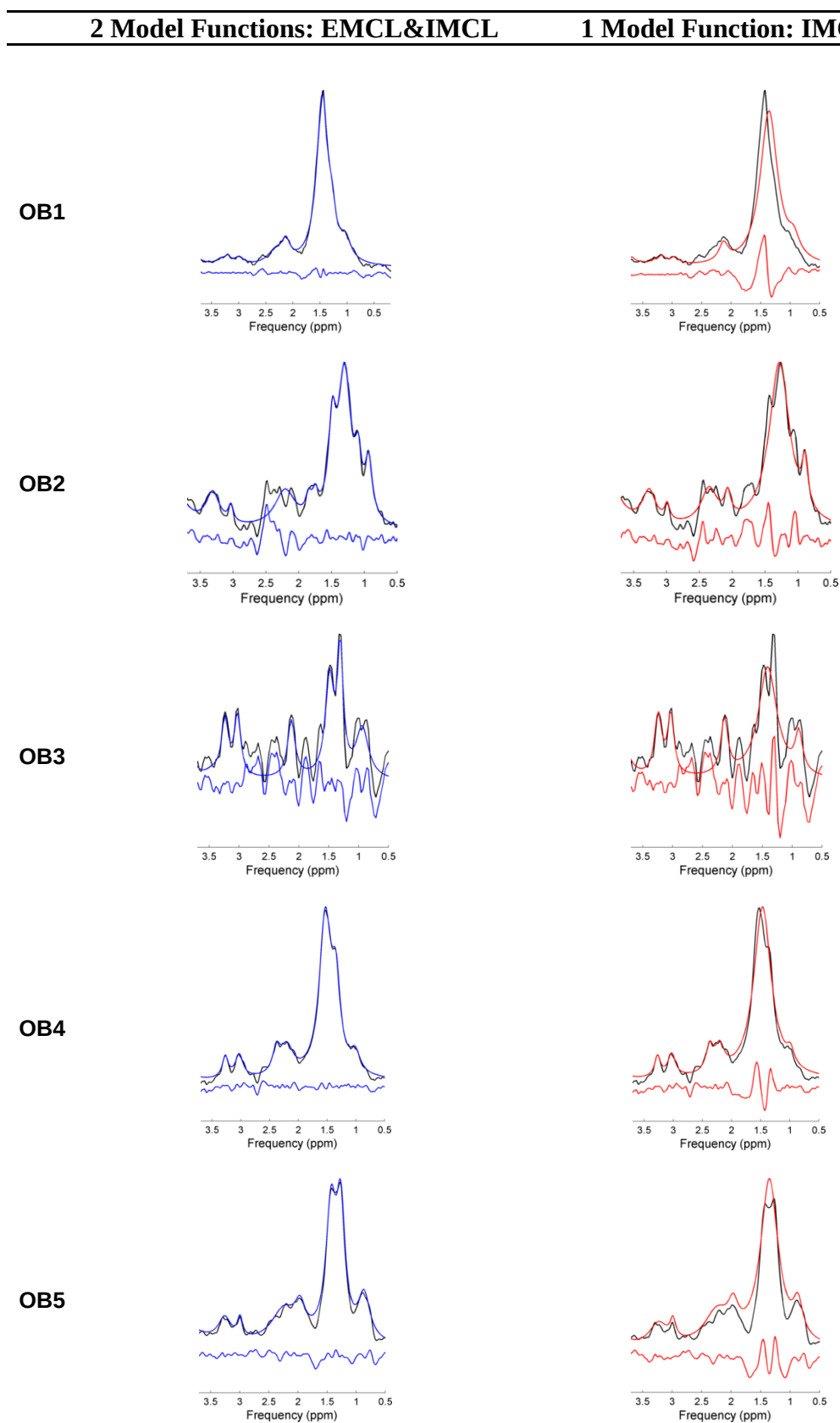


Figure 3.13 ^1H -MR spectra (black trace) of myocardial tissue obtained in five obese subjects (OB1-5) and fitted data using two model functions (blue trace) for EMCL and IMCL and only one for the methylene protons (red trace). The residuals of fitting each spectrum are plotted underneath.



au) than when only one model function was applied ($(7.4 \pm 9.0) \times 10^{-4}$ au). Figure 3.13 shows similar results obtained in obese subjects.

Water linewidths were 13.6 ± 3.0 Hz in normal volunteers, 15.0 ± 6.1 Hz in obese subjects and 15.5 ± 4.3 Hz in patients ($p = 0.4$). No significant difference was found between EMCL, IMCL and water linewidths when two model functions were used to fit the water-suppressed spectra in obese and muscular dystrophy subjects (Table 3.3). However, if only one model function is used to fit the spectra, obese and muscular dystrophy IMCL linewidths were significantly higher than IMCL and EMCL linewidths using two model functions ($p < 0.001$ and $p < 0.05$ respectively).

	LW _{Water} Hz	2 Model Functions		1 Model Function
		LW _{IMCL} Hz	LW _{EMCL} Hz	LW _{IMCL} Hz
Normal	13.6±3.0	-	-	20±7
Obese	15.0±6.1	15±6	19±7	39±1
M. Dystrophy	15.5±4.3	23±7	23±2	35±3

Table 3.3 Spectral full width at half maximum values of myocardial water, IMCL and EMCL obtained by using 2 model functions to fit the spectra and IMCL using only 1 model function. Mean $\pm$ SD; n=5 in each group

The mean chemical shift difference between EMCL and IMCL was 0.16 ± 0.01 ppm and 0.18 ± 0.01 ppm in the MD patients and in the obese subjects, respectively. Additionally, IMCL normalized to water content was significantly higher in patients than in normal volunteers, $(0.92 \pm 0.59)\%$ vs. $(0.27 \pm 0.10)\%$, $p < 0.05$. No significant difference in IMCL was found between obese subjects, $(0.34 \pm 0.12)\%$, and MD patients or normal volunteers. MD patients and obese subjects showed EMCL ranging from 0.26% to 4.25% and 0.16% to 2.12%, respectively, while a small (0.09%) EMCL content was found in only one volunteer (age: 29 years, BMI = 20.4, female (Figure 3.14).

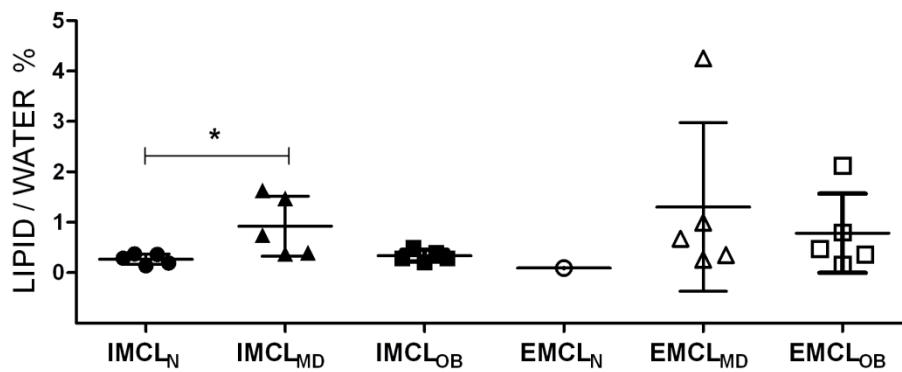


Figure 3.14 IMCL content was increased in MD patients, but was only significantly different to normal volunteers. A wide range of EMCL values was found in MD patients and obese subjects, whereas an EMCL peak was detected and fitted in only one normal volunteer. N: normal, MD: muscular dystrophy, OB: obese. * $p < 0.05$.

3.4.3 Discussion

To our knowledge, this is the first study demonstrating the feasibility of proton ^1H -MRS at 3 T to differentiate between IMCL and EMCL resonances in the inter-ventricular septum of subjects with suspected high myocardial lipid content. Boesch et al (52) introduced the terms IMCL and EMCL when investigating the use of ^1H -MRS to assess human muscle metabolism. Since the great potential of ^1H -MRS to observe non-invasively IMCL was shown, the number of exercise physiology and diabetology studies on IMCL increased considerably (61-65). Prior investigations reported that skeletal muscle spectra were apparently better resolved at higher magnetic field (50,66,67), as expected. Here, the use of a 3 T scanner and phased array coils provided higher SNR and sufficient spectral resolution to observe the same methylene chemical shift in cardiac muscle.

The crucial point for the quantification the two methylene resonances by means of ^1H -MRS is the separation of EMCL and IMCL signals. As previously reported, the incorporation of prior knowledge improves the accuracy of the fitting (34,68,69). Prior knowledge for processing

IMCL and EMCL was based on skeletal muscle studies (66,68,70), since values on cardiac tissue have not been published yet. Although Boesch et al (34,68,69) suggested Gaussian lineshapes to fit IMCL and EMCL resonances owing to the different chemical species in lipids resonances, Lorentzian lineshapes were used in this study. Gaussian lineshapes were also investigated, but no significant improvement was found in the residue and as well as for the other resonances in the cardiac spectra, Lorentzian lineshapes were taken. Moreover, AMARES algorithm does not contemplate Lorentzian-Gaussian lineshapes (Voigt lineshape) (71). Also the orientation of the myocardial volume in respect to the magnetic field will influence the fit quality. The best separation of the two resonances is achieved when the septum is roughly parallel to the static magnetic field. Unlike fibres in the leg that run in a single direction, myocardium fibres do not show such natural orientation.

Particularly, in the group of muscular dystrophy patients investigated here, the identification of fat infiltration to areas between the muscle fibres (EMCL) and also high fat content within the cytoplasm of cardiac myocytes suggests impaired fat utilization and suggests lipotoxicity as a potential disease mechanism. Thus, cardiac proton MR spectroscopy may help to determine whether or not myocardial lipid overstorage is important in this disease process.

3.5 Creatine: Baseline Values in Normal Hearts

Abnormalities in creatine metabolism of the dysfunctional heart have been demonstrated by biochemical methods (9,15,72-74), ^{31}P -MRS (75,76) and ^1H -MRS at 1.5 T (9,22,44). In this section the total myocardial creatine pool in healthy volunteers is observed in water suppressed ^1H -MR spectra via its resonance at 3 ppm (9,22). The purpose is to investigate the spectral resolution of ^1H -MRS performed during exhalation breath-holds and using phased arrays to quantify myocardial creatine in the human heart at 3 T.

3.5.1 Methods

In 15 healthy volunteers (11 men, four women; mean age $\pm$ standard deviation, 34 ± 10 years; age range, 22-58 years; body mass index (BMI), 19-30 kg/m²), creatine levels normalized to myocardial water content were evaluated using ¹H-MRS. None of the volunteers had a history of cardiovascular disease, diabetes or any other chronic disease. All participants gave informed consent.

¹H-MRS was performed using the multiple breath-hold STEAM method described in section 3.3.1.2. At acquisition, a data set of four signals was collected without water suppression in a single breath-hold and then a data set of 35 signals with water suppression was collected over five breath-holds for the creatine resonance.

The amplitude of the creatine peak was measured using AMARES with the prior knowledge previously described in section 3.3.1.4. Quantification of creatine spectra in the myocardium was performed using water as internal reference (9,22) and calculated using Eq 1.4. To compare the creatine values to the ones reported in previous ¹H-MRS studies (9,22,44) the creatine concentration was calculated according Eq 1.5-1.7 (77), with $M = CR$. The concentration of water in tissue $[W]$ was taken as 55 mol/kg times 72.7% (% myocardial water content by weight (78,79)); the ratio $N_W/N_{CR}=2/3$ protons on water divided by protons on the N-methyl group of the total creatine; $F_{CR/W}$ is the spin-lattice (T_1) correction factor; and $E_{CR/W}$ is the spin-spin correction (T_2) factor. Since myocardial metabolite relaxation times at a field strength of 3 T have not yet been published, creatine and water relaxations times were estimated from values reported at 1.5 T. T_1 increases approximately with the third root of the field strength (80), and was therefore estimated to $T_{1W}= 1524$ ms and $T_{1CR}= 1865$ ms (based on values used by Nakae et al (44)). On the other hand, increased field strength

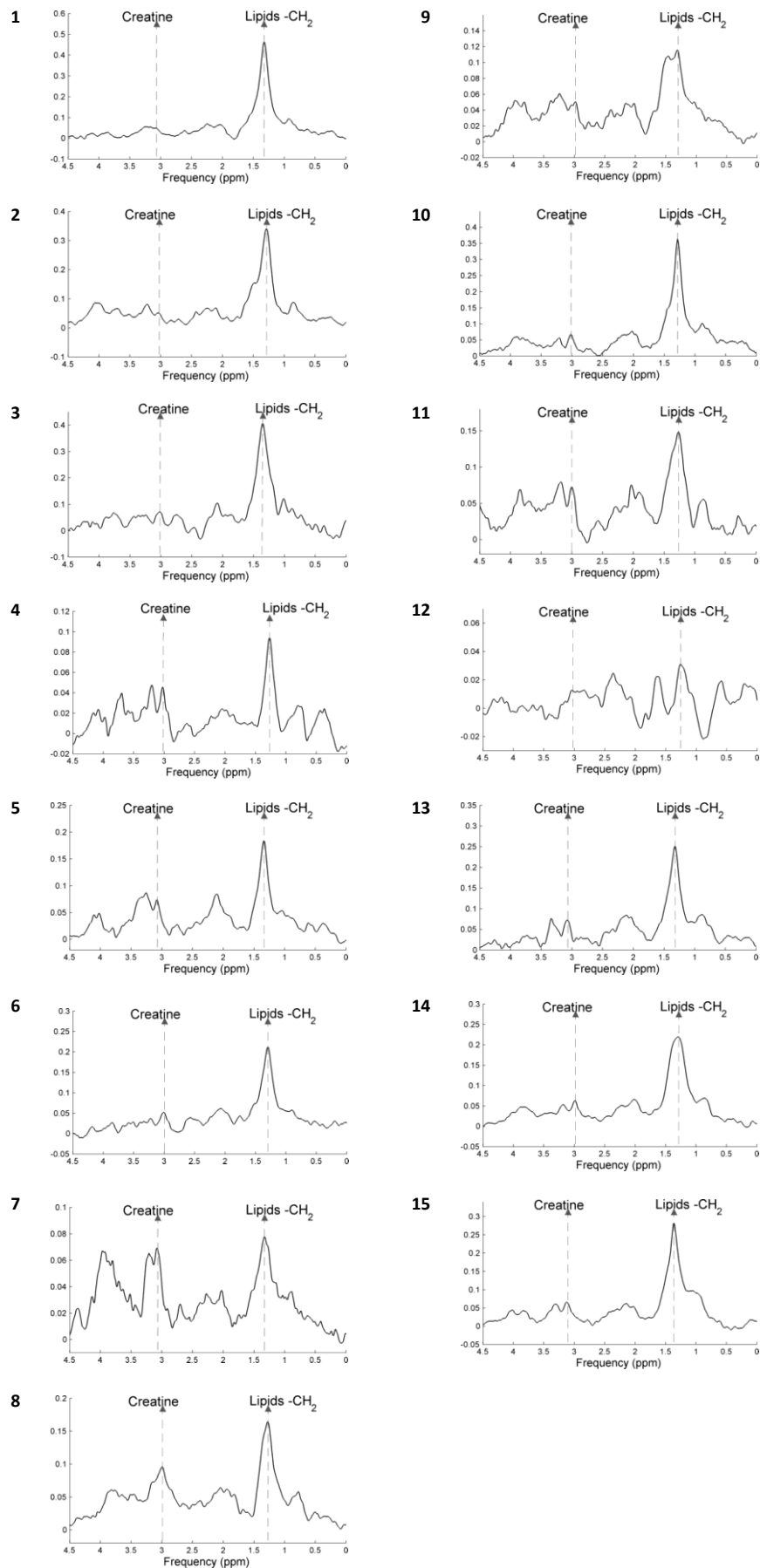
leads to a reduction in T_2 (81,82), accordingly estimate values of $T_{2W}= 24$ ms and $T_{2CR}= 96$ ms (based on 1.5 T values (44)) were used for T_2 correction.

3.5.2 Results

Figure 3.15 shows myocardial spectra from the septum of all volunteers. The spectra were collected in less than seven minutes from a voxel size of 8 to 15 ml and showed the detection of total creatine. ^1H -MRS provided quantifiable metabolic information in fourteen volunteers, while one spectrum did not have sufficient spectral resolution to quantify the creatine resonance reliably (Figure 3.15, example 12). The mean creatine and water signal linewidths were 9.8 ± 2.6 Hz and 15.0 ± 3.9 Hz respectively. The mean creatine to water ratio was 0.00052 ± 0.00023 , whereas the mean total creatine concentration without relaxation correction was 13.99 ± 6.19 $\mu\text{mol/g}$, 19.72 ± 8.72 $\mu\text{mol/g}$ after T_1 correction and 14.43 ± 6.38 $\mu\text{mol/g}$ after both T_1 and T_2 corrections (using Eq 1.4-1.7) (Table 3.4)

	LW _{CR} (Hz)	rCRSD _{CR} %	CR/Water 10 ⁻³			[CR] (μmol/g)
			Correction			
			None	T ₁	T ₁ &T ₂	
1	8.9	26.5	0.25	0.36	0.26	7.0
2	7.0	18.0	0.26	0.37	0.27	7.3
3	9.8	12.7	0.61	0.86	0.63	16.9
4	6.9	7.9	0.36	0.51	0.37	10.0
5	6.3	9.8	0.40	0.56	0.41	11.1
6	9.2	10.7	0.44	0.62	0.45	12.2
7	12.8	12.6	0.69	0.97	0.71	19.2
8	15.0	8.6	1.12	1.58	1.16	31.2
9	12.9	10.1	0.42	0.59	0.43	11.6
10	8.5	13.7	0.43	0.60	0.44	11.8
11	7.8	8.7	0.44	0.62	0.45	12.1
13	11.1	8.0	0.75	1.05	0.77	20.7
14	9.0	8.8	0.45	0.64	0.47	12.5
15	12.3	9.0	0.68	0.96	0.70	18.9

Table 3.4 Fitting parameters and creatine (CR) concentrations in 14 healthy volunteers. LW_{CR} creatine linewidth

Figure 3.15 Cardiac ^1H -MR spectra of 15 healthy volunteers showing creatine and lipid resonances

3.5.3 Discussion

This is the first report to describe the feasibility of cardiac-gated ^1H -MRS to detect and quantify creatine levels in the healthy human heart at 3 T using multiple phased-array coils and breath-holding for respiratory motion compensation. Formerly, it had been shown that creatine could be observed by ^1H -MRS at this field strength (33,48,83,84), but only studies performed at 1.5 T quantified creatine content in healthy and diseased human myocardium (9,22,23,44,85-87).

Bottomley et al were first to obtain creatine values ($28.0 \pm 6.0 \mu\text{mol/g}$, $n=10$) in healthy human hearts by ^1H -MRS using STEAM localization (9). A similar value ($27.6 \pm 4.1 \mu\text{mol/g}$, $n = 22$) was obtained by Nakae et al using PRESS localization (44). The mean water linewidth at 3 T ($15.0 \pm 3.9 \text{ Hz}$) was almost identical to that obtained at 1.5T ($19 \pm 8 \text{ Hz}$) (9). However, in spite of obtaining spectra with similar or superior resolution to that shown by these two groups, a significantly lower creatine concentration was obtained after T_1 and T_2 corrections ($14.4 \pm 6.4 \mu\text{mol/g}$, $n = 14$). The mean total creatine concentration obtained in this study corresponded to values found in the infarcted myocardium of patients ($9.8 \pm 8.6 \mu\text{mol/g}$, $n = 10$) (9), in patients with dilated cardiomyopathy ($14.80 \pm 4.80 \mu\text{mol/g}$, $n = 12$), with hypertrophic cardiomyopathy ($20.40 \pm 8.40 \mu\text{mol/g}$, $n = 8$) and ischemic cardiomyopathy ($19.40 \pm 6.30 \mu\text{mol/g}$, $n = 10$) (44). A reason for the diverging results may be the use of different measuring and data processing methods. Here the use of a shorter echo time of 10 ms may avoid losses in signal intensities due to relatively short T_2 times. However, Bottomley and Nakae applied 15 ms and 25 ms respectively. Moreover, these studies did not provide information about the respiratory correction method used, while it is known that PRESS and STEAM spectra acquired from the abdomen using respiratory gating of breath-holding have improved metabolite SNR (83,88). Another systematic variation between

studies was the difference in data treatment. The other studies fitted each peak to a Gaussian line (9) or to an 80% Gaussian and a 20% Lorentzian line (44). Linewidths can strongly influence the fitting errors, independently of the lineshape (38). In this study, both water and creatine peaks were fitted to a Lorentzian lineshape using AMARES and linewidths did not influence the CRSD of the peak amplitudes (increased linewidths did not lead to increased rCRSD, Table 3.4). The other studies did not report CRSD values (a good estimate of the reliability of the data). Although systematic over or underestimation of low concentration signals can be found in spectra with low SNR, poor resolution and complicated baselines (89), considering the good spectral resolution of some data sets (Figure 3.15, examples 4,14 and 15), the linewidth influence in spectral fitting can hardly explain the large discrepancy between this and previous studies.

Another explanation for the low creatine concentration found in this study maybe a magnetization transfer effect when applying a water suppression scheme (90-94). It was previously demonstrated in rat brain and skeletal muscle that water served as an intermediate in the transfer of magnetization between immobile and mobile protons in the creatine. Schneider et al reported a 30% reduction of the myocardial creatine in the ^1H -MRS study of perfused rat hearts at 11.7 T using three CHESS pulse for water suppression (7). In this thesis, spectroscopy data could not be compared to biochemical assays of myocardial tissue to prove this hypothesis.

However, to validate the method for the measurement of human myocardial creatine, Bottomley et al (9) also studied dogs using the same protocol and healthy tissue samples were subsequently analysed by fluorometric assays, showing a good agreement between both measuring methods ($n=6$, $26\pm 8\ \mu\text{mol/g}$ and $22\pm 3\ \mu\text{mol/g}$, respectively). Apparently, the CHESS method used for water suppression had no effect on the creatine resonance. Another

study found a slightly lower creatine concentration ($n=10$, $17 \mu\text{mol/g}$) by fluorometric assay of canine myocardial tissue (95). Even lower values were obtained by high-performance liquid chromatography (HPLC) in myocardium specimens obtained from unused donor hearts ($n=4$, $10.5 \pm 3.5 \mu\text{mol/g}$) (74) and from autopsy ($n=3$, $8.4 \pm 2.3 \mu\text{mol/g}$) (72).

Cardiac ^1H -MRS was performed during breath-holding for respiratory motion correction. A direct comparison to other respiratory motion correction methods, like double-triggered scans, would have been desirable. Unfortunately, respiratory gated-navigator based single-voxel sequences were not available on the platform. However, using this method to investigate cardiac lipid metabolism, as part of this thesis, the findings compared well with other results obtained at 1.5 T using navigator gating and volume tracking (36). Hence, breath-holding showed good spectral resolution and reproducibility to assess myocardial lipid levels. However, myocardial creatine suffers from low SNR compare to myocardial lipid levels and breath-holding may affect to a greater extent creatine measurements; since a potential problem with breath-holds is that many subjects have difficulties to have the breathing arrests in the same position. For this reason, additional measures, such as frequency and phase correction between multiple breath-holds, were to be taken in order to eliminate potential systematic errors. The feasibility of a similar acquisition approach than that used for lipid quantification is proposed for future creatine metabolism investigations, where water-unsuppressed and -suppressed spectra are measured in a single breath-hold and repeated several times to obtain sufficient SNR for creatine quantification. Since creatine longitudinal relaxation time is in the order of 2 s at 3T, as previously explained, TR is to be increased at least to 4 seconds, to avoid excessive saturation of the creatine signal. The acquisition of one spectrum without water suppression and three spectra with water suppression will result in a comfortable breath-hold of about 16 seconds (depending on the heart rate of the subject).

Cardiac creatine spectra acquired with 32 averages could be obtained over 11 breath-holds in approximately 5 minutes.

3.6 Conclusions

In conclusion, the study presents a rapid proton magnetic resonance spectroscopy method to evaluate human myocardial lipid levels in a single breath-hold at 3 T using a commercial whole-body system. During a 10 second breath-hold, water unsuppressed and suppressed spectra were acquired by two phased array coils using a short-echo time STEAM sequence ECG-triggered to mid-diastole. Thus, single breath-hold proton spectroscopy allows reliable and quick quantification of myocardial lipids at 3 T. In addition, this is a quick tool to better characterize the influence of cardiac lipid accumulation on cardiac function or to evaluate the effects of pharmacological or dietary interventions.

Furthermore, ^1H -MRS at 3 T allowed for differentiation of IMCL and EMCL resonances in the inter-ventricular septum of patients with suspected high myocardial lipid content. Hence, cardiac ^1H -MRS becomes a feasible method for the investigation of long term energy storage of the normal human heart and pathologies. Particularly, the identification of fat infiltration to areas between the muscle fibres (EMCL) and also high fat content within the cytoplasm of cardiac myocytes in muscular dystrophy patients suggests impaired fat utilization and suggests lipotoxicity as a potential disease mechanism.

Finally, this report has described for first time the feasibility of the detection and quantification of creatine content by ^1H -MRS in healthy myocardium at 3 T. The low creatine values obtained in healthy myocardium require further investigation. Future work needs to be done to fully understand the effect of water suppression on the creatine signal. On the other

hand, results obtained during breath-holding should be compared to spectra acquired using other respiratory motion correction methods, like navigator gating.

3.7 References

1. Neubauer S. Cardiac magnetic resonance spectroscopy: potential clinical applications. *Herz* 2000;25(4):452-460.
2. Neubauer S. Cardiac magnetic resonance spectroscopy. *Curr Cardiol Rep* 2003;5(1):75-82.
3. Burtscher IM, Holtas S. Proton MR spectroscopy in clinical routine. *J Magn Reson Imaging* 2001;13(4):560-567.
4. Di Costanzo A, Trojsi F, Tosetti M, Giannatempo GM, Nemore F, Piccirillo M, Bonavita S, Tedeschi G, Scarabino T. High-field proton MRS of human brain. *Eur J Radiol* 2003;48(2):146-153.
5. Howe FA, Opstad KS. ¹H MR spectroscopy of brain tumours and masses. *NMR Biomed* 2003;16(3):123-131.
6. Ugurbil K, Petein M, Maidan R, Michurski S, Cohn JN, From AH. High resolution proton NMR studies of perfused rat hearts. *FEBS Lett* 1984;167(1):73-78.
7. Schneider J, Fekete E, Weisser A, Neubauer S, von Kienlin M. Reduced (1)H-NMR visibility of creatine in isolated rat hearts. *Magn Reson Med* 2000;43(4):497-502.
8. Schneider JE, Tyler DJ, ten Hove M, Sang AE, Cassidy PJ, Fischer A, Wallis J, Sebag-Montefiore LM, Watkins H, Isbrandt D, Clarke K, Neubauer S. In vivo cardiac ¹H-MRS in the mouse. *Magn Reson Med* 2004;52(5):1029-1035.
9. Bottomley PA, Weiss RG. Non-invasive magnetic-resonance detection of creatine depletion in non-viable infarcted myocardium. *Lancet* 1998;351(9104):714-718.
10. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001;414(6865):782-787.
11. Abel ED, Litwin SE, Sweeney G. Cardiac remodeling in obesity. *Physiol Rev* 2008;88(2):389-419.
12. Sharma S, Adroque JV, Golfman L, Uray I, Lemm J, Youker K, Noon GP, Frazier OH, Taegtmeyer H. Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *Faseb J* 2004;18(14):1692-1700.
13. Witteles RM, Fowler MB. Insulin-resistant cardiomyopathy clinical evidence, mechanisms, and treatment options. *J Am Coll Cardiol* 2008;51(2):93-102.
14. Brindley DN, Kok BP, Kienesberger PC, Lehner R, Dyck JR. Shedding light on the enigma of myocardial lipotoxicity: the involvement of known and putative regulators of fatty acid storage and mobilization. *American journal of physiology* 2010;298(5):E897-908.
15. Ingwall JS, Kramer MF, Fifer MA, Lorell BH, Shemin R, Grossman W, Allen PD. The Creatine Kinase System in Normal and Diseased Human Myocardium. *New England Journal of Medicine* 1985;313(17):1050-1054.
16. Neubauer S, Horn M, Naumann A, Tian R, Hu K, Laser M, Friedrich J, Gaudron P, Schnackerz K, Ingwall JS, et al. Impairment of energy metabolism in intact residual myocardium of rat hearts with chronic myocardial infarction. *J Clin Invest* 1995;95(3):1092-1100.
17. Ten Hove M, Chan S, Lygate C, Monfared M, Boehm E, Hulbert K, Watkins H, Clarke K, Neubauer S. Mechanisms of creatine depletion in chronically failing rat heart. *J Mol Cell Cardiol* 2005;38(2):309-313.

18. Liao R, Nascimben L, Friedrich J, Gwathmey JK, Ingwall JS. Decreased energy reserve in an animal model of dilated cardiomyopathy. Relationship to contractile performance. *Circ Res* 1996;78(5):893-902.
19. Spindler M, Engelhardt S, Niebler R, Wagner H, Hein L, Lohse MJ, Neubauer S. Alterations in the myocardial creatine kinase system precede the development of contractile dysfunction in beta(1)-adrenergic receptor transgenic mice. *J Mol Cell Cardiol* 2003;35(4):389-397.
20. Jung WI, Sieverding L, Breuer J, Hoess T, Widmaier S, Schmidt O, Bunse M, van Erckelens F, Apitz J, Lutz O, Dietze GJ. 31P NMR spectroscopy detects metabolic abnormalities in asymptomatic patients with hypertrophic cardiomyopathy. *Circulation* 1998;97(25):2536-2542.
21. Crilley JG, Boehm EA, Blair E, Rajagopalan B, Blamire AM, Styles P, McKenna WJ, Ostman-Smith I, Clarke K, Watkins H. Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy. *J Am Coll Cardiol* 2003;41(10):1776-1782.
22. Nakae I, Mitsunami K, Omura T, Yabe T, Tsutamoto T, Matsuo S, Takahashi M, Morikawa S, Inubushi T, Nakamura Y, Kinoshita M, Horie M. Proton magnetic resonance spectroscopy can detect creatine depletion associated with the progression of heart failure in cardiomyopathy. *J Am Coll Cardiol* 2003;42(9):1587-1593.
23. Nakae I, Mitsunami K, Matsuo S, Horie M. Creatine depletion and altered fatty acid metabolism in diseased human hearts: clinical investigation using 1H magnetic resonance spectroscopy and 123I BMIPP myocardial scintigraphy. *Acta Radiol* 2007;48(4):436-443.
24. Young ME, Guthrie PH, Razeghi P, Leighton B, Abbasi S, Patil S, Youker KA, Taegtmeier H. Impaired long-chain fatty acid oxidation and contractile dysfunction in the obese Zucker rat heart. *Diabetes* 2002;51(8):2587-2595.
25. Chung CS, Karamanoglu M, Kovacs SJ. Duration of diastole and its phases as a function of heart rate during supine bicycle exercise. *Am J Physiol Heart Circ Physiol* 2004;287(5):H2003-2008.
26. Frahm JM, D. Hanicke, W. Haase, A. Stimulated Echo Imaging. *Journal of Magnetic Resonance* 1985;64:81-93.
27. Fahmy AS, Pan L, Stuber M, Osman NF. Correction of through-plane deformation artifacts in stimulated echo acquisition mode cardiac imaging. *Magn Reson Med* 2006;55(2):404-412.
28. Bottomley PA, Weiss RG. Noninvasive localized MR quantification of creatine kinase metabolites in normal and infarcted canine myocardium. *Radiology* 2001;219(2):411-418.
29. Jahnke C, Paetsch I, Achenbach S, Schnackenburg B, Gebker R, Fleck E, Nagel E. Coronary MR imaging: breath-hold capability and patterns, coronary artery rest periods, and beta-blocker use. *Radiology* 2006;239(1):71-78.
30. Marks B, Mitchell DG, Simelaro JP. Breath-holding in healthy and pulmonary-compromised populations: effects of hyperventilation and oxygen inspiration. *J Magn Reson Imaging* 1997;7(3):595-597.
31. Ogg RJ, Kingsley PB, Taylor JS. WET, a T1- and B1-insensitive water-suppression method for in vivo localized 1H NMR spectroscopy. *J Magn Reson B* 1994;104(1):1-10.
32. Roemer PB, Edelstein WA, Hayes CE, Souza SP, Mueller OM. The NMR phased array. *Magn Reson Med* 1990;16(2):192-225.

33. Gabr RE, Sathyanarayana S, Schar M, Weiss RG, Bottomley PA. On restoring motion-induced signal loss in single-voxel magnetic resonance spectra. *Magn Reson Med* 2006;56(4):754-760.
34. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997;129(1):35-43.
35. Lamb HJ, Doornbos J, den Hollander JA, Luyten PR, Beyerbach HP, van der Wall EE, de Roos A. Reproducibility of human cardiac ³¹P-NMR spectroscopy. *NMR Biomed* 1996;9(5):217-227.
36. van der Meer RW, Doornbos J, Kozerke S, Schar M, Bax JJ, Hammer S, Smit JW, Romijn JA, Diamant M, Rijzewijk LJ, de Roos A, Lamb HJ. Metabolic imaging of myocardial triglyceride content: reproducibility of ¹H MR spectroscopy with respiratory navigator gating in volunteers. *Radiology* 2007;245(1):251-257.
37. den Hollander JA, Evanochko WT, Pohost GM. Observation of cardiac lipids in humans by localized ¹H magnetic resonance spectroscopic imaging. *Magn Reson Med* 1994;32(2):175-180.
38. Kreis R. Issues of spectral quality in clinical ¹H-magnetic resonance spectroscopy and a gallery of artifacts. *NMR Biomed* 2004;17(6):361-381.
39. Szczepaniak LS, Dobbins RL, Metzger GJ, Sartoni-D'Ambrosia G, Arbique D, Vongpatanasin W, Unger R, Victor RG. Myocardial triglycerides and systolic function in humans: in vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magn Reson Med* 2003;49(3):417-423.
40. Felblinger J, Jung B, Slotboom J, Boesch C, Kreis R. Methods and reproducibility of cardiac/respiratory double-triggered (¹H)-MR spectroscopy of the human heart. *Magn Reson Med* 1999;42(5):903-910.
41. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. *Obesity research* 1998;6 Suppl 2.
42. Reingold JS, McGavock JM, Kaka S, Tillery T, Victor RG, Szczepaniak LS. Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method. *American journal of physiology* 2005;289(5):E935-939.
43. Kankaanpää M, Lehto HR, Parkka JP, Komu M, Viljanen A, Ferrannini E, Knuuti J, Nuutila P, Parkkola R, Iozzo P. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006;91(11):4689-4695.
44. Nakae I, Mitsunami K, Yoshino T, Omura T, Tsutamoto T, Matsumoto T, Morikawa S, Inubushi T, Horie M. Clinical features of myocardial triglyceride in different types of cardiomyopathy assessed by proton magnetic resonance spectroscopy: comparison with myocardial creatine. *J Card Fail* 2010;16(10):812-822.
45. Piechnik S, Ferreira V, Dall'Armellina E, Cochlin L, Greiser A, Neubauer S, Robson M. Shortened Modified Look-Locker Inversion recovery (ShMOLLI) for clinical myocardial T1-mapping at 1.5 and 3 T within a 9 heartbeat breathhold. *Journal of Cardiovascular Magnetic Resonance* 2010;12(1):69.
46. Rijzewijk LJ, van der Meer RW, Smit JW, Diamant M, Bax JJ, Hammer S, Romijn JA, de Roos A, Lamb HJ. Myocardial steatosis is an independent predictor of diastolic dysfunction in type 2 diabetes mellitus. *J Am Coll Cardiol* 2008;52(22):1793-1799.
47. Hammer S, van der Meer RW, Lamb HJ, Schar M, de Roos A, Smit JW, Romijn JA. Progressive caloric restriction induces dose-dependent changes in myocardial

- triglyceride content and diastolic function in healthy men. *J Clin Endocrinol Metab* 2008;93(2):497-503.
48. Liu CY, Redheuil A, Ouwerkerk R, Lima JA, Bluemke DA. Myocardial fat quantification in humans: Evaluation by two-point water-fat imaging and localized proton spectroscopy. *Magn Reson Med* 2010;63(4):892-901.
49. van Werven JR, Schreuder TC, Nederveen AJ, Lavini C, Jansen PL, Stoker J. Hepatic unsaturated fatty acids in patients with non-alcoholic fatty liver disease assessed by 3.0T MR spectroscopy. *Eur J Radiol* 2010;75(2):e102-107.
50. Velan SS, Durst C, Lemieux SK, Raylman RR, Sridhar R, Spencer RG, Hobbs GR, Thomas MA. Investigation of muscle lipid metabolism by localized one- and two-dimensional MRS techniques using a clinical 3T MRI/MRS scanner. *J Magn Reson Imaging* 2007;25(1):192-199.
51. Schick F, Eismann B, Jung WI, Bongers H, Bunse M, Lutz O. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn Reson Med* 1993;29(2):158-167.
52. Boesch C, Slotboom J, Hoppeler H, Kreis R. In vivo determination of intramyocellular lipids in human muscle by means of localized ¹H-MR-spectroscopy. *Magn Reson Med* 1997;37(4):484-493.
53. Boesch C, Decombaz J, Slotboom J, Kreis R. Observation of intramyocellular lipids by means of ¹H magnetic resonance spectroscopy. *Proc Nutr Soc* 1999;58(4):841-850.
54. Chu SC, Xu Y, Balschi JA, Springer CS, Jr. Bulk magnetic susceptibility shifts in NMR studies of compartmentalized samples: use of paramagnetic reagents. *Magn Reson Med* 1990;13(2):239-262.
55. Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, McGarry JD, Stein DT. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. *Am J Physiol* 1999;276(5 Pt 1):E977-989.
56. Alpert MA, Hashimi MW. Obesity and the heart. *Am J Med Sci* 1993;306(2):117-123.
57. Fornes P, Ratel S, Lecomte D. Pathology of arrhythmogenic right ventricular cardiomyopathy/dysplasia--an autopsy study of 20 forensic cases. *J Forensic Sci* 1998;43(4):777-783.
58. Pantanowitz L. Fat infiltration in the heart. *Heart* 2001;85(3):253.
59. Wren TAL, Bluml S, Tseng-Ong L, Gilsanz V. Three-Point Technique of Fat Quantification of Muscle Tissue as a Marker of Disease Progression in Duchenne Muscular Dystrophy: Preliminary Study. *Am J Roentgenol* 2008;190(1):W8-12.
60. Finsterer J, Stollberger C. The heart in human dystrophinopathies. *Cardiology* 2003;99(1):1-19.
61. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 1999;48(5):1113-1119.
62. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Ma YZ, Savoye M, Rothman DL, Shulman GI, Caprio S. Assessment of skeletal muscle triglyceride content by (¹)H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. *Diabetes* 2002;51(4):1022-1027.
63. van Loon LJ. Use of intramuscular triacylglycerol as a substrate source during exercise in humans. *J Appl Physiol* 2004;97(4):1170-1187.

64. Kiens B. Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiol Rev* 2006;86(1):205-243.
65. Stevenson EJ, Thelwall PE, Thomas K, Smith F, Brand-Miller J, Trenell MI. Dietary glycemic index influences lipid oxidation but not muscle or liver glycogen oxidation during exercise. *American journal of physiology* 2009;296(5):E1140-1147.
66. Krssak M, Mlynarik V, Meyerspeer M, Moser E, Roden M. ¹H NMR relaxation times of skeletal muscle metabolites at 3 T. *Magma (New York, NY)* 2004;16(4):155-159.
67. Wang L, Salibi N, Wu Y, Schweitzer ME, Regatte RR. Relaxation times of skeletal muscle metabolites at 7T. *J Magn Reson Imaging* 2009;29(6):1457-1464.
68. Weis J, Johansson L, Ortiz-Nieto F, Ahlstrom H. Assessment of lipids in skeletal muscle by LCModel and AMARES. *J Magn Reson Imaging* 2009;30(5):1124-1129.
69. de Graaf AA, Bovee WM. Improved quantification of in vivo ¹H NMR spectra by optimization of signal acquisition and processing and by incorporation of prior knowledge into the spectral fitting. *Magn Reson Med* 1990;15(2):305-319.
70. Szczepaniak LS, Dobbins RL, Stein DT, McGarry JD. Bulk magnetic susceptibility effects on the assessment of intra- and extramyocellular lipids in vivo. *Magnetic Resonance in Medicine* 2002;47(3):607-610.
71. Marshall I, Higinbotham J, Bruce S, Freise A. Use of Voigt lineshape for quantification of in vivo ¹H spectra. *Magn Reson Med* 1997;37(5):651-657.
72. Delanghe J, De Buyzere M, De Scheerder I, Vogelaers D, Vandenbogaerde J, Van den Abeele AM, Gheeraert P, Wieme R. Creatine determinations as an early marker for the diagnosis of acute myocardial infarction. *Ann Clin Biochem* 1988;25 (Pt 4):383-388.
73. Nascimben L, Ingwall JS, Pauletto P, Friedrich J, Gwathmey JK, Saks V, Pessina AC, Allen PD. Creatine kinase system in failing and nonfailing human myocardium. *Circulation* 1996;94(8):1894-1901.
74. Kalsi KK, Smolenski RT, Pritchard RD, Khaghani A, Seymour AM, Yacoub MH. Energetics and function of the failing human heart with dilated or hypertrophic cardiomyopathy. *Eur J Clin Invest* 1999;29(6):469-477.
75. Conway MA, Allis J, Ouwerkerk R, Niioka T, Rajagopalan B, Radda GK. Detection of low phosphocreatine to ATP ratio in failing hypertrophied human myocardium by ³¹P magnetic resonance spectroscopy. *Lancet* 1991;338(8773):973-976.
76. Neubauer S, Krahe T, Schindler R, Horn M, Hillenbrand H, Entzeroth C, Mader H, Kromer EP, Riegger GA, Lackner K, et al. ³¹P magnetic resonance spectroscopy in dilated cardiomyopathy and coronary artery disease. Altered cardiac high-energy phosphate metabolism in heart failure. *Circulation* 1992;86(6):1810-1818.
77. Bottomley PA, Lee Y, Weiss RG. Total creatine in muscle: imaging and quantification with proton MR spectroscopy. *Radiology* 1997;204(2):403-410.
78. Snyder WS, Cook MJ, Nasset ES, Karhausen LR, Howells GP, Tipton IH. Report of the Task Group on Reference Man. International Commission on Radiological Protection. Volume 23: Pergamon Press, Oxford; 1984. p pp. 280-285, p. 234, p. 116.
79. Bottomley PA, Atalar E, Weiss RG. Human cardiac high-energy phosphate metabolite concentrations by 1D-resolved NMR spectroscopy. *Magn Reson Med* 1996;35(5):664-670.
80. Bottomley PA, Hardy CJ, Argersinger RE, Allen-Moore G. A review of ¹H nuclear magnetic resonance relaxation in pathology: are T1 and T2 diagnostic? *Med Phys* 1987;14(1):1-37.
81. Duewell SH, Ceckler TL, Ong K, Wen H, Jaffer FA, Chesnick SA, Balaban RS. Musculoskeletal MR imaging at 4 T and at 1.5 T: comparison of relaxation times and image contrast. *Radiology* 1995;196(2):551-555.

82. Schär M, Kozerke S, Fischer SE, Boesiger P. Cardiac SSFP imaging at 3 Tesla. 2004;51:799-806.
83. Schar M, Kozerke S, Boesiger P. Navigator gating and volume tracking for double-triggered cardiac proton spectroscopy at 3 Tesla. *Magn Reson Med* 2004;51(6):1091-1095.
84. Krssak M, Winhofer Y, Gobl C, Bischof M, Reiter G, Kautzky-Willer A, Luger A, Krebs M, Anderwald C. Myocardial lipids and myocardial function in insulin resistant population. 2010; Proceedings of ISMRM Stockholm. p 174.
85. Walecki J, Michalak MJ, Michalak E, Bilinska ZT, Ruzyllo W. Usefulness of ¹H MR spectroscopy in the evaluation of myocardial metabolism in patients with dilated idiopathic cardiomyopathy: pilot study1. *Academic Radiology* 2003;10(10):1187-1192.
86. Nakae I, Mitsunami K, Yabe T, Inubushi T, Morikawa S, Matsuo S, Koh T, Horie M. Detection of metabolic abnormality in asynergic regions of ischemic human myocardium using ³¹P and ¹H magnetic resonance spectroscopy. *J Cardiovasc Magn Reson* 2004;6(3):685-696.
87. Nakae I, Mitsunami K, Matsuo S, Inubushi T, Morikawa S, Tsutamoto T, Koh T, Horie M. Myocardial creatine concentration in various nonischemic heart diseases assessed by ¹H magnetic resonance spectroscopy. *Circ J* 2005;69(6):711-716.
88. Katz-Brull R, Rofsky NM, Lenkinski RE. Breathhold abdominal and thoracic proton MR spectroscopy at 3T. *Magnetic Resonance in Medicine* 2003;50(3):461-467.
89. Kreis R, Boesch C. Bad spectra can be better than good spectra. 2003; Proceedings of ISMRM Toronto.
90. Dreher W, Norris DG, Leibfritz D. Magnetization transfer affects the proton creatine/phosphocreatine signal intensity: in vivo demonstration in the rat brain. *Magn Reson Med* 1994;31(1):81-84.
91. de Graaf RA, van Kranenburg A, Nicolay K. Off-resonance metabolite magnetization transfer measurements on rat brain in situ. *Magn Reson Med* 1999;41(6):1136-1144.
92. Kruiskamp M, Nicolay K. Unraveling the magnetization transfer effect on the ¹H-signal of creatine in rat skeletal muscle. 1999; Proceedings of ISMRM Philadelphia
93. Kruiskamp MJ, Nicolay K. On the importance of exchangeable NH protons in creatine for the magnetic coupling of creatine methyl protons in skeletal muscle. *J Magn Reson* 2001;149(1):8-12.
94. Helms G, Frahm J. Magnetization transfer attenuation of creatine resonances in localized proton MRS of human brain in vivo. *NMR Biomed* 1999;12(8):490-494.
95. Shen W, Spindler M, Higgins MA, Jin N, Gill RM, Bloem LJ, Ryan TP, Ingwall JS. The fall in creatine levels and creatine kinase isozyme changes in the failing heart are reversible: complex post-transcriptional regulation of the components of the CK system. *J Mol Cell Cardiol* 2005;39(3):537-544.

4

CONCLUSIONS

The primary aim of this thesis was to develop the techniques necessary to make in vivo cardiac ^1H -MRS measurements at 3 T as consistent, robust, and clinically practical as possible. In this chapter, a summary of the thesis achievements is presented, followed by some suggestions for future research.

In Chapter 1, the technical challenges for ^1H -MRS at 3 T were addressed in phantom and in vivo experiments. For this purpose, a STEAM sequence was optimized for single voxel localization. The pulse sequence was modified and the echo time was shortened to 10 ms to minimize the losses due to T_2 relaxation. To the best of our knowledge, this is the shortest echo time used for cardiac ^1H -MRS in humans. Furthermore, STEAM localization was combined with a WET module for water suppression. The ^1H -MR spectra obtained in creatine solution phantoms demonstrated the effectiveness of the water suppression scheme that did not impact on the creatine or lipid resonances (relevant metabolites for the

assessment of myocardial metabolism) but reduced the water resonance without affecting the spectral baseline. To mitigate the limited SNR of clinical MRS applications, an optimized method for the combination of individual coil signals from phased-array coils was developed and resulted in improved SNR compared to the previous available routine. Finally, the need for respiratory motion correction using breath-holding was described. The effects of breath-holding in the data were evaluated by analyzing the frequency and phase variation within and between breath-holds of the myocardial water resonance acquired by ECG triggered ^1H -MRS. An algorithm to average data acquired in consecutive breath-holds was described. Another contribution of this thesis is the modification of the STEAM sequence to accurately scale the RF pulses used for water suppression in the voxel of interest in a single breath-hold cardiac gated ^1H -MRS acquisition.

Chapter 2 provides a robust protocol to assess liver metabolism using the ^1H -MRS method described in the previous chapter. Liver spectroscopy was the previous developmental step towards cardiac ^1H -MRS. A reproducibility study of liver metabolites in healthy volunteers allowed the optimization of data acquisition protocols, evaluation of breath-holding and improvement of data post-processing algorithms. This thesis shows for the first time the possibility of using single voxel localization ^1H -MRS in the liver to investigate glycogen content in healthy volunteers. Also, measurements performed on the same day of lipids, choline containing compounds and glycogen showed a high degree of reproducibility. Moreover, between weeks reproducibility, which is clinically very relevant, of lipids (CH_2 group), choline components and glycogen showed a variation of 2%, 5% and 19%, respectively. Knowing these values of reproducibility is particularly useful when designing future clinical studies. The results of this study were published in Proceedings of the 26th Scientific Meeting European Society of Magnetic Resonance in

Medicine and Biology (2009). Chapter 2 also demonstrated the need for water-suppressed ^1H -MRS in order to obtain more complete metabolic information in the liver. Moreover, preliminary results from a collaboration study with the Oxford Centre for Diabetes, Endocrinology & Metabolism, investigating the effect of menopausal condition on liver lipid content measured by ^1H -MRS were presented in Chapter 2. Although final conclusions have not yet been drawn, since not all participants have been investigated, the utility and practicality of the developed ^1H -MRS method to address clinical research needs was illustrated and a paper is planned to be submitted to a clinical metabolic research journal.

Chapter 3 demonstrated the feasibility of ^1H -MRS to quantify myocardial lipid and creatine levels in healthy volunteers for the first time at 3 T. It was found that myocardial lipid levels in healthy volunteers can be accurately measured in a single breath-hold (10-12 s) with a coefficient of variation of 19% for repeated measurements on the same day. The same mean myocardial lipid levels in healthy volunteer were obtained than that reported in other studies at 1.5 T, but with the benefit of greatly reduced spectroscopic acquisition time (from 5-10 min to 10-12 s). This work was presented in the 19th Scientific Meeting of the International Society for Magnetic Resonance in Medicine (2010) and a communication has been submitted to Magnetic Resonance in Medicine. Furthermore, chapter 3 demonstrated that ^1H -MRS can resolve two different pools of myocardial lipids in patients with muscular dystrophy. The ^1H -MRS detection of IMCL and EMCL had been previously shown in skeletal muscle, but had not yet been observed in myocardial tissue. These results are planned to be submitted for publication. The clinical aspects of this work form part of the thesis by Dr Joseph J Suttie. This thesis also showed preliminary myocardial creatine content in healthy volunteers for the first time at 3 T. The

concentrations of creatine that were found are low when compared to that published from biochemical assays and previous ^1H -MRS studies. This suggests that further investigation is required to evaluate potential systematic errors.

This thesis not only provides a rapid breath-hold ^1H -MR technique that can easily be included in clinical research studies to evaluate hepatic and myocardial metabolism, but also the tools for the post-processing of spectra acquired.

This thesis set out to develop and validate a method for measuring the ^1H -MRS signals from fat and metabolites in the human heart using ^1H -MRS at 3 T. This method has been developed and published including both acquisition and post-processing techniques. Two clinical protocols have been developed for liver and cardiac ^1H -MRS and these have been used in clinical studies on population groups of scientific interest. Each of these protocols has been added to the armamentarium of our clinical research partners and will continue to be used within our clinical research facility for future investigative studies.