MRI of structural & functional changes associated with Western diet consumption

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Abstract

The Western diet (WD) is a high-fat, high-sugar diet increasingly common in the Western world and is associated with adverse effects in many organs, though the mechanisms behind these changes are unclear. Magnetic resonance imaging (MRI) techniques that provide structural and functional information non-invasively were used to investigate the effect of the WD on the liver and placenta in a guinea pig model.

The WD leads to a manifestation of the metabolic syndrome in the liver known as non-alcoholic fatty liver disease (NAFLD). Fat-fraction MRI was used to confirm the onset of NAFLD in a guinea pig model fed a lifelong WD, and hyperpolarized [1-13C]pyruvate magnetic resonance spectroscopy (MRS) was used to observe abnormal pyruvate metabolism in the liver. Importantly, this study included \textit{ex vivo} enzyme measurements that correlated with the MRS results, further validating its use in identifying potential biomarkers of metabolic disease.

While hyperpolarized MRS techniques were successfully applied in the liver, investigation of the placenta requires imaging, especially in the guinea pig where multiple fetoplacental units are common. A technical improvement to the hyperpolarized MRI acquisition technique involving an optimized flip angle scheme was shown to improve the signal-to-noise ratio, enabling it to be used more effectively for \textit{in vivo} evaluation of pyruvate metabolism in pregnancy research.

Continuing to investigate the effect of the WD, diffusion-weighted MRI and T2* maps were used to measure blood perfusion and oxygenation of the placenta in a guinea pig model. Lifelong maternal WD consumption led to decreased oxygen saturation in the placenta in conjunction with increased blood perfusion at mid-gestation, which eventually improved blood oxygenation in the placenta near term. Similar placental adaptations have previously been reported in other models, but this is the first study to propose a connection with maternal diet.

In conclusion, this dissertation contains applications of advanced MRI techniques to study the effect of the WD in multiple organs and provides an improved acquisition strategy for hyperpolarized MRI relevant to the study of metabolic disease. The findings presented here
validate and further motivate the use of hyperpolarized and diffusion-weighted MRI in studies of metabolic disease.

**Keywords:** Western diet, magnetic resonance imaging, hyperpolarized MRI, pyruvate, metabolism, intravoxel incoherent motion, oxygen saturation, proton density fat fraction, non-alcoholic fatty liver disease, placenta
Summary for Lay Audience

A high-fat and high-sugar diet, known as the Western diet (WD), is common in Western society and may lead to poor health outcomes. In this thesis, the effect of diet on the liver and placenta is investigated using magnetic resonance imaging (MRI) techniques.

The effect of diet on liver function was chosen as a focus because it is known that diets high in fat lead to the accumulation of fat in the liver and poor liver function. A specialized MRI technique was used in an animal model to investigate how diet affects the liver’s ability to break down simple sugars to generate energy. It was found that guinea pigs fed a WD had fatty livers and displayed a metabolism pattern that has previously been associated with liver damage.

The MRI technique used to gain information on metabolism in the body is an emerging method that suffers from poor image quality and difficulty in image acquisition. Part of this thesis aimed to improve image quality using a modified version of this technique. The new method was shown to produce higher quality images in our guinea pig experiments.

Poor diet during pregnancy has been shown to lead to poorer outcomes for the infant. One way this happens is through placental damage that limits the placenta’s ability to function properly. To measure the effect of diet on placental function, advanced MRI techniques were used to measure oxygenation and blood flow in a guinea pig model. Mothers fed a WD had less oxygen in the placenta but, interestingly, more blood flow than mothers fed a control diet. This data may suggest that the placenta recognizes the low oxygen levels associated with poor diet and reacts by increasing blood flow through the placenta in an attempt to provide the fetus with sufficient oxygen.

The research in this thesis is important for demonstrating advanced MRI techniques to study changes associated with poor diet. These results promote more investigation using these techniques that may eventually be incorporated into clinical examinations.
Co-Authorship Statement

Chapters 2, 3, and 4 of this thesis contain original research studies completed in collaboration with other authors. Acknowledgement of the contributions from myself and others to this work is described below for each project.

Chapter 2 was adapted from an original research manuscript accepted for publication in Journal of Magnetic Resonance Imaging in 2021 entitled “In vivo magnetic resonance spectroscopy of hyperpolarized [1-13C]pyruvate and proton density fat fraction in a guinea pig model of non-alcoholic fatty liver disease development after lifelong Western diet consumption”, DOI: 10.1002/jmri.27677. It was authored by Lauren M. Smith, Conrad B. Pitts, Lanette J. Friesen-Waldner, Neetin H. Prabhu, Katherine E. Mathers, Kevin J. Sinclair, Trevor P. Wade, Timothy R.H. Regnault, and Charles A. McKenzie. As first author, I was involved in imaging experiments, data analysis, interpretation, and preparation and submission of the manuscript. Lanette Friesen-Waldner was involved in imaging data collection and animal care. Conrad Pitts, Neetin Prabhu, and Katherine Mathers were involved in ex vivo tissue collection and analysis. Kevin Sinclair was involved in imaging data collection. Trevor Wade assisted with software involved in analysis of spectroscopy data. Timothy Regnault and Charles McKenzie were responsible for study conception and design and provided guidance on data interpretation and revisions of the manuscript.

Chapter 3 was adapted from an original technical development manuscript published in Magnetic Resonance in Medicine, Volume 83, Issue 3, pages 1510-1517, DOI:10.1002/mrm.28194. This work, entitled “Optimizing SNR for multi-metabolite hyperpolarized carbon-13 MRI using a hybrid flip-angle scheme”, was published in 2020 and authored by Lauren M. Smith, Trevor P. Wade, Lanette J. Friesen-Waldner, and Charles A. McKenzie. As first author, I was involved in study design, implementation, data collection, data analysis, interpretation, and preparation and submission of the manuscript. Trevor Wade was involved in the implementation of pulse sequence changes and data collection. Lanette Friesen-Waldner was involved in data collection and animal care. Charles McKenzie was responsible for study conception and provided guidance on revisions of the manuscript.
Chapter 4 was adapted from an original research manuscript prepared for submission to the *Journal of Magnetic Resonance Imaging* entitled “Perfusion and oxygenation of the mid-pregnancy and near-term placenta measured by magnetic resonance imaging in a guinea pig model of pregnancy following lifelong Western diet consumption” and authored by Lauren M. Smith, Lanette J. Friesen-Waldner, Conrad P. Rockel, Flavien Delhaes, Timothy R.H. Regnault, and Charles A. McKenzie. As first author, I was involved in study design, data collection, data analysis, interpretation, and preparation of the manuscript. Lanette Friesen-Waldner was involved in data collection and animal care. Conrad Rockel was involved in data collection and initial implementation of the diffusion-weighted imaging experiment. Flavien Delhaes was involved in study conception and design. Timothy Regnault and Charles McKenzie were responsible for study conception and design and provided guidance on data interpretation and revisions of the manuscript.
Dedicated to my mother and father.
Acknowledgements

It feels surreal to be at the end of my PhD journey after five years at Western in the McKenzie lab. I have so much to be thankful for, including the opportunity to work with wonderful people over the years and all the skills and lessons, both professional and personal, I’ve learned along the way.

I’d like to begin by thanking my supervisor, Dr. Charles McKenzie, for providing me with the opportunity to pursue a graduate degree in his lab. I feel truly lucky to have benefited from your dedicated mentorship that has been invaluable to my growth as a scientist. Thank you for sending me around the world, from Hawaii to Paris to Israel, to present my work and expand my research horizons. Thank you for encouraging me, believing in me, and for always making sure there were board games to play during Friday “meetings”.

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To Finnegan, I know you are a dog and cannot read this, but you helped me get through the end of this PhD. Thank you for taking me on daily walks and for giving me all the puppy kisses.

Of course, I would not have made it here without the love and encouragement of my parents, to whom I dedicate this thesis. Thank you for your unwavering support in all of my endeavours and for putting up with me when I said I wanted to go to school forever (this time I’m done for real)!

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<th>Description</th>
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</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>Flip angle (°)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Gyromagnetic ratio (rad·s$^{-1}$·T$^{-1}$)</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Initial angle of the net magnetization in the transverse plane</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>Larmor frequency (Hz)</td>
</tr>
<tr>
<td>$^1$H</td>
<td>Hydrogen-1</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>Carbon-13</td>
</tr>
<tr>
<td>$^{15}$N</td>
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<tr>
<td>$^{19}$F</td>
<td>Flourine-19</td>
</tr>
<tr>
<td>$^{29}$Si</td>
<td>Silicon-29</td>
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<tr>
<td>$^{31}$P</td>
<td>Phosphorus-31</td>
</tr>
<tr>
<td>$^{129}$Xe</td>
<td>Xenon-129</td>
</tr>
<tr>
<td>2D</td>
<td>Two dimensional</td>
</tr>
<tr>
<td>3D</td>
<td>Three dimensional</td>
</tr>
<tr>
<td>Acetyl-coA</td>
<td>Acetyl coenzyme A</td>
</tr>
<tr>
<td>ADC</td>
<td>Apparent diffusion coefficient</td>
</tr>
<tr>
<td>ALB</td>
<td>Albumin</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>$b$</td>
<td>$b$-value (s/mm$^2$)</td>
</tr>
<tr>
<td>$B_0$</td>
<td>Main magnetic field strength (T)</td>
</tr>
<tr>
<td>$B_1$</td>
<td>RF pulse magnetic field strength (T)</td>
</tr>
<tr>
<td>BA</td>
<td>Blood ammonia</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BOLD</td>
<td>Blood oxygen level dependent</td>
</tr>
<tr>
<td>BUN</td>
<td>Blood urea nitrogen</td>
</tr>
<tr>
<td>BW</td>
<td>Bandwidth</td>
</tr>
<tr>
<td>CD</td>
<td>Control diet</td>
</tr>
<tr>
<td>CFA</td>
<td>Constant flip angle</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>CHOL</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>CS</td>
<td>Citrate synthase</td>
</tr>
<tr>
<td>CSE-MRI</td>
<td>Chemical shift-encoded MRI</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>D</td>
<td>Diffusion coefficient (mm²/s)</td>
</tr>
<tr>
<td>D*</td>
<td>Perfusion coefficient (mm²/s)</td>
</tr>
<tr>
<td>d-DNP</td>
<td>Dissolution dynamic nuclear polarization</td>
</tr>
<tr>
<td>DNL</td>
<td>De novo lipogenesis</td>
</tr>
<tr>
<td>DOHaD</td>
<td>Developmental origins of health and disease</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion weighted imaging</td>
</tr>
<tr>
<td>EPI</td>
<td>Echo planar imaging</td>
</tr>
<tr>
<td>ETL</td>
<td>Echo train length</td>
</tr>
<tr>
<td>f</td>
<td>Perfusion fraction</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>FGR</td>
<td>Fetal growth restriction</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional MRI</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>GE</td>
<td>Gradient echo</td>
</tr>
<tr>
<td>GFE</td>
<td>Frequency encoding gradient</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma glutamyl-transferase</td>
</tr>
<tr>
<td>GPE</td>
<td>Phase encoding gradient</td>
</tr>
<tr>
<td>Gr</td>
<td>Gradient applied along the r direction (mT/m)</td>
</tr>
<tr>
<td>GSS</td>
<td>Slice selection gradient</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HP 13C MRI</td>
<td>Hyperpolarized carbon-13 MRI</td>
</tr>
<tr>
<td>HP MRI</td>
<td>Hyperpolarized MRI</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
</tr>
<tr>
<td>IDEAL</td>
<td>Iterative decomposition of water and fat with echo asymmetry and least-squares estimation</td>
</tr>
<tr>
<td>IVIM</td>
<td>Intravoxel incoherent motion</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LGA</td>
<td>Large for gestational age</td>
</tr>
<tr>
<td>$M_0$</td>
<td>Net magnetization</td>
</tr>
<tr>
<td>MAFLD</td>
<td>Metabolic dysfunction-associated fatty liver disease</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic syndrome</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>msVFA</td>
<td>Multi-spectral variable flip angle</td>
</tr>
<tr>
<td>MT</td>
<td>Mid-term</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acids</td>
</tr>
<tr>
<td>$M_{xy}$</td>
<td>Transverse magnetization</td>
</tr>
<tr>
<td>$M_z$</td>
<td>Longitudinal magnetization</td>
</tr>
<tr>
<td>NAD+</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide + hydrogen</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td>NASH</td>
<td>Non-alcoholic steatohepatitis</td>
</tr>
<tr>
<td>NEX</td>
<td>Number of excitations</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NT</td>
<td>Near-term</td>
</tr>
<tr>
<td>PD</td>
<td>Proton density</td>
</tr>
<tr>
<td>PDFF</td>
<td>Proton density fat fraction</td>
</tr>
<tr>
<td>PDH</td>
<td>Pyruvate dehydrogenase</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>pH</td>
<td>Power of hydrogen</td>
</tr>
<tr>
<td>PHIP</td>
<td>Parahydrogen induced polarization</td>
</tr>
<tr>
<td>PN</td>
<td>Postnatal</td>
</tr>
<tr>
<td>pPDH</td>
<td>Phosphorylated pyruvate dehydrogenase</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PRESS</td>
<td>Point resolved spectroscopy</td>
</tr>
<tr>
<td>PUFA</td>
<td>Poly-unsaturated fatty acid</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SABRE</td>
<td>Signal amplification by reversible exchange</td>
</tr>
<tr>
<td>S</td>
<td>MRI signal</td>
</tr>
<tr>
<td>S₀</td>
<td>Original MRI signal</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
</tr>
<tr>
<td>SE</td>
<td>Spin echo</td>
</tr>
<tr>
<td>SEOP</td>
<td>Spin exchange optical pumping</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated fatty acid</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>T</td>
<td>Tesla</td>
</tr>
<tr>
<td>T₁</td>
<td>Longitudinal relaxation time</td>
</tr>
<tr>
<td>T₂</td>
<td>Transverse relaxation time</td>
</tr>
<tr>
<td>T₂*</td>
<td>Apparent transverse relaxation time</td>
</tr>
<tr>
<td>TBIL</td>
<td>Bilirubin</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricarboxylic acid</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TG</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>TTP</td>
<td>Time-to-peak</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral adipose tissue</td>
</tr>
<tr>
<td>VFA</td>
<td>Variable flip angle</td>
</tr>
<tr>
<td>WD</td>
<td>Western diet</td>
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Chapter 1

1 Introduction

This introductory chapter provides information necessary to understand topics related to the following chapters of the thesis. A definition of the Western diet (WD) is provided with insight into the impact of the WD on the liver and placenta, two systems studied in detail in Chapters 2 and 4. Magnetic resonance imaging (MRI) techniques used in this thesis are explained in adequate detail for the reader to appreciate the technical details, advantages, and limitations of these imaging strategies.

1.1 Western Diet

The increasing trend of obesity and metabolic syndrome in North American society is due in part to the high-fat and high-sugar diets common to the Western world, referred to as the “Western diet” (WD) in this thesis. Hallmarks of the WD include high glycemic load, fatty acid composition, macronutrient composition, micronutrient density, acid-base balance, fibre content, and sodium-potassium ratio. There is a theory that humans were not able to genetically adapt quickly enough to the changes in diet and lifestyle that began with the rise of agricultural society, and these changes over time have led to an increased incidence of chronic metabolic diseases observed in modern societies. This concept is related to the hypothesis of a “thrifty phenotype” where the human body evolved to preserve glucose via insulin resistance in times of food deprivation and develop a robust immune system to defend against infectious disease. In the absence of our ancestors’ survival pressures, physiological stress may cause these adaptations to instead lead to the development of Western metabolic diseases, due in part to establishing a state of chronic metabolic inflammation. This chronic inflammation may be related to structural and behavioural changes to the microbiome brought on by WD consumption, highlighting the complex and multifaceted nature of the body’s response to the WD. The WD may also impact sex steroids which regulate both reproductive function and energy metabolism, potentially contributing to reproductive conditions such as hypogonadism and sex hormone imbalance. Many chronic non-communicable diseases in Western
societies can be partially attributed to diet, including diabetes, hypertension, obesity, cardiovascular disease (CVD), osteoporosis, and cancer. This post-agricultural diet primarily consists of foods humans have not evolved to consume, including dairy products, cereal grains, refined sugars, refined vegetable oils, excess salt, and alcohol. The WD is energy-dense, emphasizing saturated fats and simple sugars as a primary energy source with low nutritional quality. Studies have shown that diet composition is more important than caloric intake in the risk of developing metabolic syndrome.

Western diets, high in processed food, red meat, and sugar, have been associated with an increased risk of metabolic syndrome, even when controlled for other factors such as age, sex, education, physical activity, and body mass index (BMI).

In this thesis, I focus on the fatty acid composition, glycemic load, macronutrient composition, and micronutrient density aspects of the WD, using a diet particularly high in fat and sugar. The main aspects of the WD presented here that affect physiology are increased saturated fatty acids and significant fructose content, as seen in Figure 1.1.

**Figure 1.1: Components of the control and Western diet used in Chapters 2 and 4 of this thesis.**

Dietary fat can primarily be categorized as triglycerides (TG), which are three fatty acid molecules bound to a single glycerol molecule. Fatty acids fall into three categories: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Current evidence suggests that the risk of chronic disease is more impacted by the type of fat than the absolute amount of dietary fat, as MUFAs and some PUFAs are more beneficial than SFAs and trans fatty acids, which are detrimental in excessive quantities. Trans fatty acids, particularly industrial trans fatty acids found in fast foods and baked goods, increase cardiovascular disease risk through proinflammatory properties. The WD contains excessive SFA and trans fatty acids,
which increase the risk of CVD by elevating blood concentration of total cholesterol and low-density lipoprotein (LDL) cholesterol. High cholesterol has been associated with arterial lesions and may contribute to the risk of CVD. High-fat diets, particularly those high in SFAs, can induce weight gain, insulin resistance, and hyperlipidemia.

The overconsumption of sugar is associated with obesity, non-alcoholic fatty liver disease (NAFLD), insulin resistance, and CVD. Glycemic load is the potential of a food to increase blood glucose levels based on the quality and quantity of dietary carbohydrate. Carbohydrates with high glycemic loads elevate TGs and small-dense LDLs while reducing high-density lipoprotein (HDL) cholesterol. High glycemic load diets also promote C-reactive protein, which is an inflammatory marker and may be a strong predictor of CVD. Refined sugar products have high glycemic loads that lead to elevations in blood glucose and insulin concentrations after repeated exposure. In the long term, these changes may result in adverse metabolic effects that promote insulin resistance through hormonal and physiological changes. Though glycemic load is a helpful measure related to insulin resistance, it is not always an indication of “healthy” foods. For example, fructose has a low glycemic load but is known to induce insulin resistance. This may be due to its ability to cause a shift in balance from oxidation to the esterification of free fatty acids, which other sugars cannot do.

The liver takes up fructose rapidly, where it is phosphorylated and may be used to produce glucose, glycogen, lactate, and pyruvate. Fructose also plays a role in increasing hepatic gluconeogenesis and de novo lipogenesis (DNL), leading to increased plasma glucose, TG levels, and blood pressure. Compared to glucose, fructose consumption results in a larger production of lactate, which is related to liver damage and other conditions. Long-term exposure to a high fructose diet can cause muscle tissue to increase fatty acid oxidization and lead to decreased uptake and oxidation of glucose. Fructose lowers insulin and leptin blood concentrations, which increases the likelihood of weight gain and associated metabolic consequences. Insulin resistance, impaired glucose tolerance, adiposity, and hypertension result from high fructose diets in animal models. All this considered, the full metabolic effects of dietary fructose in humans remain unclear, though existing data suggests fructose may be detrimental in
terms of body weight, adiposity, and metabolic disease\textsuperscript{10}. The use of non-invasive techniques such as magnetic resonance spectroscopy (MRS) and MRI will allow us to learn more about the metabolic influence of high fructose dietary consumption\textsuperscript{15}.

The macronutrient composition of a diet also affects physiology and is a hallmark of the Western diet\textsuperscript{3}. The three major macronutrient groups are carbohydrates, fat, and protein. To maintain a healthy diet, the World Health Organization recommends that fat should not exceed 30\% of total energy intake, with protein maintained at 15\%\textsuperscript{16}. Although it is difficult to determine the historical macronutrient composition of our hunter-gatherer ancestors’ diets, it is likely humans evolved eating significantly elevated portions of protein (19-35\%) with fewer carbohydrates (22-40\%) compared to typical diets of today\textsuperscript{3}. There is evidence that high-protein diets are beneficial for a variety of reasons. One of these benefits is the improvement of blood lipid profiles, associated with lowering CVD risk. High-protein diets also decrease LDL cholesterol while increasing HDL cholesterol. Dietary protein has been linked to improvements in glucose and insulin metabolism in individuals with diabetes and lower blood pressure. Lastly, protein has a higher satiety value than fat or carbohydrates, so a high-protein diet may reduce caloric intake and be effective as a weight-loss strategy for individuals at risk due to being overweight or obese\textsuperscript{3}.

This section has highlighted aspects of the WD relevant to the studies presented in this thesis; however, there are additional aspects to the WD. These include low micronutrient density, where refined sugars and foods are void of essential vitamins and minerals. The replacement of nutrient-dense foods (fruit, vegetables, lean meats, seafood) with less nutrient-dense foods (refined sugars, grains, vegetable oils, dairy products) in the modern WD exacerbates the failure to meet the daily micronutrient requirements. The acid-base balance of the diet also impacts health as a typical WD has net acidity, leading to chronic, low-grade metabolic acidosis over time. In contrast, pre-agricultural diets tended to be base yielding. Another factor of the WD is the sodium-potassium ratio. Modern WDs typically have a higher sodium content than potassium content due to the addition of manufactured salt to foods and the displacement of potassium-rich foods with refined oils and sugars. These factors cause the WD to be high in sodium and low in potassium, the
inverse of our prehistoric ancestors’ diets. Lastly, the fibre content in the WD is significantly lower than recommended values (15.1g/d vs 25-30g/d). Soluble fibres, found in fruits and vegetables, have been found to reduce total and LDL cholesterol.

### 1.1.1 Metabolic Syndrome

Metabolic syndrome (MetS), also known as syndrome X or the insulin resistance syndrome, is a collection of metabolic abnormalities that include insulin resistance, visceral adiposity, atherogenic dyslipidemia, and endothelial dysfunction. Elevated levels of TGs and small-dense LDL and decreased levels of HDL cholesterol define atherogenic dyslipidemia. The small-dense LDL are susceptible to oxidation and promote atherosclerotic plaque formation, related to increased CVD risk. The features of MetS are interrelated, and the presence of more than one metabolic abnormality is necessary for the diagnosis of metabolic syndrome. MetS is related to the shared pathophysiological mechanisms of these abnormalities that may not be present in cases of isolated symptoms. Globally, it is estimated that 20-25% of the adult population has metabolic syndrome, which increases the lifetime likelihood of diseases such as stroke, heart attack, and type II diabetes. These conditions cause shortened lifespans in otherwise healthy individuals and contribute to socioeconomic pressure on regions with ageing populations. Diet-related chronic diseases are the largest cause of morbidity and mortality in most Western countries. Though metabolic syndrome has a broad definition and is difficult to diagnose, screening variables can be used to identify individuals at risk, including waist circumference, BMI, the concentration of TGs and HDL cholesterol in the blood, fasting glycemia, and blood pressure. Waist circumference is a more accurate variable to use instead of BMI to highlight the specific impact of visceral adipose tissue (VAT), which results in a combination of factors that are not always present in all individuals that are overweight or obese. While MetS is associated with poor diet, there has not been a single aspect of the diet identified as a sole risk factor for MetS. Instead, MetS is a result of the complex interactions of multiple nutritional factors characteristic of the WD.
While obesity is closely related to metabolic syndrome, there are also incidences of lean (BMI < 25 kg/m²) individuals who are not metabolically healthy⁹. It is important to consider that obesity, measured by BMI, is not a necessary criterion for metabolic syndrome, even though it is commonly used as part of the description of metabolic syndrome⁹. A study investigating ~800 young adults and the link between diet quality and MetS found that almost a third of the metabolically healthy group was overweight or obese while almost a third of individuals with MetS had a normal weight as defined by BMI⁹. A similar prevalence of lean individuals with MetS has been previously reported and is likely to rise with the increasing consumption of the WD in Western society²¹.

Individuals who are lean but have MetS are at a higher risk of developing diabetes, coronary heart disease, and stroke than individuals who are obese but metabolically healthy⁹. The cause of this lean MetS phenotype may be related to high volumes of VAT that are not easily measured using traditional anthropometric methods such as BMI, which does not provide information about fat distribution in the body⁹. Thus, common clinical assessments of obesity do not fully identify the risk associated with VAT in diagnoses. High volumes of VAT are associated with increased metabolic risk and may represent dysfunctional adipose tissue that cannot store energy excess appropriately²⁰,²².

Although both VAT and subcutaneous adipose tissue (SAT) are associated with metabolic syndrome, VAT is more strongly related to an adverse metabolic risk profile regardless of BMI or waist circumference (Figure 1.2)²². The metabolically active nature of VAT may explain this result. VAT secretes vasoactive substances such as inflammatory markers, adipocytokines, and growth factors that can increase cardiometabolic disease risk and drain directly into the liver²²,²³. Visceral obesity may also be a marker of defective fat being distributed between the adipose tissue, skeletal muscle, liver, and heart²⁰. VAT is a unique pathogenic fat deposit, and measuring its volume with imaging can provide additional information about the risk of metabolic disease compared to solely BMI and waist circumference measurements²². To accomplish this, imaging modalities including ultrasound, x-ray, CT, MRI, and PET can be used to quantify regional fat deposits with varying degrees of accuracy²⁴.
The importance of VAT in the context of metabolic disease highlights the need to recognize biomarkers of metabolic health that are independent of obesity. Related to this, diet quality, rather than caloric intake, is possibly a more important factor in the development of metabolic syndrome\textsuperscript{22}. Poor diet quality, evident in the WD, is associated with increased risk of metabolic syndrome\textsuperscript{25,26} and may be used as a predictor of metabolic health independent of body size and fat content measured by BMI\textsuperscript{9}. People with metabolic syndrome are more likely to consume a WD and have poor diet quality than metabolically healthy people across all BMI categories\textsuperscript{9}. It has been suggested that the excessive consumption of sugar and saturated fatty acids can trigger pathogenic mechanisms while not affecting adiposity, leading to a lean metabolically unhealthy phenotype in individuals who can maintain a healthy energy balance while still suffering from increased risk of metabolic and cardiovascular disease\textsuperscript{9}.

![Figure 1.2: Subcutaneous vs visceral fat storage](image)

As stated above, metabolic syndrome is a multifaceted collection of diseases with diverse effects on physiology and is strongly linked to modern WD trends in the developed world. Below, I will discuss the effects of the WD on the body systems focused on in this thesis.

1.1.2 Effects of the Western Diet on the Liver

The liver is a vital organ that regulates TG and cholesterol metabolism, among other functions\textsuperscript{27}. Perhaps the most evident structural change in the liver caused by poor diet quality is fat accumulation, otherwise known as steatosis\textsuperscript{28}. Steatosis gives rise to the NAFLD spectrum, discussed in section 1.1.2.1. The WD, inherently high-fat and calorie-
dense, typically results in weight gain, which generally drives hepatic fat accumulation, though this trend is not present in the proportion of metabolically healthy obese individuals. Hepatic fat accumulation is a result of an imbalance between lipid deposition and removal in the liver. Lipid intake, TG secretion, DNL, and mitochondrial fatty acid oxidation are the main factors that influence hepatic lipid accumulation and are themselves impacted by external factors including diet. The amount of dietary fat consumed has a positive correlation with liver fat content. Synthesis of TG in the liver is a mechanism used to store cytotoxic free fatty acids (FFAs); however, excess TG forms lipid droplets in hepatocytes that contribute to metabolic abnormalities. Vessels of fat accumulate in the liver within hepatocyte cells, displacing the cytoplasm and eventually the nucleus to the edge of the cell. FFAs are introduced into the liver from the diet, lipolysis of visceral fat, and DNL – the process by which excess carbohydrates are converted into fatty acids. Excessive FFAs in the liver may result from insulin resistance, which is associated with obesity, type 2 diabetes, and the WD. If the liver’s capacity to handle carbohydrates and fatty acids is overwhelmed, toxic lipid species can accumulate in the liver and induce hepatocellular stress, injury, and death. Fibrogenesis and predisposition to cirrhosis and hepatocellular carcinoma are long-term consequences of this devastated state.

The high-sugar WD contains glucose and fructose that both synthesize fatty acids via DNL. Fructose, in particular, has a large impact on hepatic fat accumulation as fructose is shunted to the liver and undergoes unregulated DNL, contributing to increased liver fat and cellular stress. Like fructose, alcohol and carbohydrates tend to be converted into fat in the liver. Diets rich in MUFA and PUFA tend to reduce liver fat, whereas the WD is high in SFAs and leads to increased liver fat. Exposure to the WD has been shown to induce pronounced steatosis and fibrosis in the liver of mice. In a study using a rat model of pre-steatosis, animals exposed to the WD showed a larger number of lipid droplets with larger diameters than those fed a control diet, though there were no macroscopic histological differences observed at this stage. In this pre-steatosis model of NAFLD, the WD was shown to increase cholesterol and the concentration of enzymes involved in the synthesis of TG, fatty acids, and cholesterol. This study provided evidence that DNL, TG, LDL cholesterol, and body weight are increased early in
NAFLD disease progression following exposure to the WD\textsuperscript{34}. In the same study, the WD was found to increase hepatic cells involved in fibrosis, as well as aspartate aminotransferase and bilirubin levels, both indicators of liver disease\textsuperscript{34}. Fibrosis, a symptom of advanced NAFLD, is the accumulation of extracellular matrix in the liver and may lead to cirrhosis, portal hypertension, and liver failure\textsuperscript{28}. Fibrogenesis is driven by signalling from stressed or injured hepatocytes and leads to the rapid production of matrix proteins that cannot be degraded as rapidly, leading to their accumulation in the liver\textsuperscript{28}.

Aside from altering liver architecture, several functional changes to the liver are also associated with the WD. In a mouse model, the WD was shown to increase inflammatory cell infiltration and proinflammatory gene expression in the liver\textsuperscript{33}. The presence of steatosis is strongly associated with chronic inflammation in the liver, and steatosis induced by a high-fat diet has been shown to elevate the hepatic expression of inflammatory cytokines\textsuperscript{35}. A fructose-rich WD has been shown to induce hepatic inflammation and significantly increase markers of fibrogenesis in a mouse model\textsuperscript{36}. Excessive fructose intake causes a gut microbiota imbalance, triggering macrophages in the liver\textsuperscript{27}. A high-fat diet is associated with mitochondrial dysfunction, which results in increased production of reactive oxygen species, leading to issues including oxidative stress and cell damage\textsuperscript{37}. Chronic exposure to the high-fat, high-fructose WD has shown to result in an imbalance of antioxidants, causing oxidative stress and contributing to liver damage in a mouse model\textsuperscript{38}. WDs lead to an increase in endoplasmic reticulum stress in the liver, which causes hepatocyte apoptosis and contributes to the development of hepatic steatosis\textsuperscript{39–41}. Oxidative stress contributes to inflammation and fibrogenesis in the liver, which ultimately results in the development of non-alcoholic steatohepatitis (NASH)\textsuperscript{42}. These changes also likely play a key role in the inhibition of fatty acid oxidation\textsuperscript{37}. Fructose causes immediate increases in pyruvate and lactate production in the liver, as it is rapidly taken up and metabolized in the liver\textsuperscript{9}. Additionally, long-term exposure to a high fructose diet results in a decreased ability to metabolize glucose in the liver\textsuperscript{15}. 
It is important to note that a diet high in fat, not necessarily a WD, is known to cause simple steatosis, insulin resistance, and expansion of adipose tissue in the liver but is unlikely to induce hepatic inflammation and fibrosis\textsuperscript{27}. On the other hand, diets high in fat and high in fructose, components of a typical WD, induce a more serious pathology of NAFLD, including extensive inflammation, fibrosis, and liver injury in addition to steatosis\textsuperscript{27,39}. Dietary cholesterol exacerbates the accumulation of free cholesterol in the liver and is an independent risk factor for the development of NASH and hepatocellular carcinoma\textsuperscript{27}. It is hypothesized that in addition to steatosis, multiple “hits” to liver health, including oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction, insulin resistance, inflammation, and gut microbiota imbalance, all potentially impacted by the WD, drive the progression of NAFLD into NASH and carcinoma\textsuperscript{27}. Most of the evidence for the pathogenesis of diet-induced NAFLD comes from animal studies with limitations in representing the full spectrum of disease\textsuperscript{43}. More accurate models and further research is warranted to elucidate details surrounding the mechanisms of these architectural and functional changes\textsuperscript{43}.

1.1.2.1 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease is defined by fat accumulation in the liver exceeding 5-10\% of the total liver weight\textsuperscript{31}. NAFLD differs from alcoholic-related fatty liver disease as individuals with NAFLD accumulate fat in the liver in the absence of alcohol abuse, though the distinction between these definitions is controversial as there is no universally accepted threshold for alcohol consumption\textsuperscript{31}. The prevalence of NAFLD is increasing globally with an estimated worldwide prevalence of \textasciitilde25\%\textsuperscript{28} and is the most common cause of chronic liver disease in the Western world\textsuperscript{31,44}.

NAFLD exists on a spectrum of liver disease and may progress to NASH and eventually liver fibrosis, cirrhosis, failure, or hepatocellular carcinoma, as seen in Figure 1.3\textsuperscript{31}. Hepatic steatosis is the primary indicator of NAFLD and involves vesicles of fat, primarily TGs, accumulating within hepatocytes without causing scarring, inflammation, or cell death\textsuperscript{31,45}. Steatosis may be either macrovesicular (related to an imbalance of hepatic synthesis and export of lipids) or microvesicular (related to defects in
mitochondrial function). NAFLD is generally considered benign, though patients with NAFLD have greater overall mortality than healthy populations. NAFLD may exacerbate previous liver damage caused by alcohol, industrial toxins, or hepatotropic viruses. Fatty livers are the most common symptom of the NAFLD spectrum, and only a percentage of patients experience progression of disease to advanced fibrosis (~20%) and cirrhosis (~3%)\(^1\), though methods of identifying this progression remain challenging. NASH, a more advanced stage of NAFLD, is defined by inflammation (hepatitis) and hepatocyte damage in addition to steatosis\(^2\) and has an increased liver-related mortality rate\(^4\) and risk of disease progression to cirrhosis (~21-16%)\(^4\).

Hepatocellular ballooning is a key feature of NASH associated with cell swelling and rounding of the cytoplasm. At the most extreme end of the spectrum, progressive fibrosis and cirrhosis in the liver cause irreversible damage and significantly increase the risk of developing hepatocellular carcinoma. Unlike other liver diseases, a large percentage of NASH patients develop hepatocellular carcinomas before cirrhosis and routine cancer screening. Features of NASH and advanced fibrosis are associated with a worse prognosis compared to individuals with just steatosis\(^1\). At this point, there is no longer fat accumulating in the liver, and the steatosis may disappear. NAFLD can be further described by grade (severity) and stage (degree of fibrosis) to clearly express the progression of disease.

Figure 1.3: The progression of NAFLD

The majority of NAFLD patients are asymptomatic, and when symptoms do occur, they are usually non-specific. Many NAFLD patients display normal lab profiles and may
progress to liver cirrhosis undiagnosed\textsuperscript{28}. Liver enzyme levels of aspartate aminotransferase and alanine aminotransferase (ALT), measured via blood test, are abnormal in patients with NAFLD, but the degree and patterns of these changes are nonspecific and cannot reliably be used to distinguish between NAFLD and NASH\textsuperscript{28}. Additionally, liver enzymes can be intermittently normal during any NAFLD stage and do not correlate with the degree of steatosis or fibrosis\textsuperscript{28}. Cytokeratin-18 fragment level is another serum biomarker related to hepatocyte apoptosis that has been externally validated but displays only modest accuracy in identifying NASH\textsuperscript{46,47}. Non-invasive imaging using ultrasound (US), computed tomography (CT), or MRI may be used to detect fat in the liver with limited sensitivity\textsuperscript{31}. US and CT can reliably detect liver fat of >20\% liver mass which may not be sensitive enough for detection of NALFD in patients with a lesser degree of steatosis\textsuperscript{28,31}. MRI is a more sensitive technique, able to detect fat as little as 5\% liver mass and is considered the gold standard to quantify hepatic steatosis\textsuperscript{28,47}. Liver stiffness measurements using US (Fibroscan) or MRI (elastography) show promise in estimating the presence of fibrosis, with MR elastography being the most accurate but limited by cost and availability\textsuperscript{46,47}. No imaging technique can reliably distinguish simple steatosis from NASH or distinguish between NASH cases with and without fibrosis, limiting the use of imaging for diagnosing the severity of disease\textsuperscript{31}. Liver biopsy is the most reliable diagnostic technique for NAFLD and can identify steatohepatitis and fibrosis, making it the only accurate technique for diagnosing NASH\textsuperscript{45}. While biopsy remains the gold standard for NASH diagnosis, it is limited by cost, sampling error, and biopsy-related morbidity and mortality\textsuperscript{45}. Considering these limitations, the diagnostic value of liver biopsy in routine clinical practice is debated since most patients with NAFLD have a good prognosis, and there is a lack of established, effective therapy for the NAFLD-spectrum of disease\textsuperscript{31}. Research and clinical trials currently focused on identifying novel serum biomarkers and developing imaging techniques to diagnose and monitor NASH may ultimately negate the need for liver biopsy in the future\textsuperscript{28}.

There are multiple causes of NAFLD, ranging from genetic risk factors to health-related behaviour\textsuperscript{31}. Further, the heterogeneity in the clinical presentation of NAFLD is likely influenced by many factors, including age, sex, ethnicity, diet, alcohol use, environmental
pollutants, microbiota, metabolic health, and epigenetics\textsuperscript{28,48}. Chapter 2 is focused on diet-related NAFLD and uses a high-fat, high-sugar Western diet to promote fatty livers in a guinea pig model. Metabolic dysfunction-associated fatty liver disease (MAFLD) has recently been proposed as a more accurate term to describe NAFLD associated with metabolic dysfunction\textsuperscript{48}. This proposed MAFLD nomenclature should be used to describe disease in patients with evidence of hepatic steatosis in addition to the presence of overweight/obesity, type 2 diabetes mellitus, or metabolic dysregulation\textsuperscript{49}. This nomenclature allows for a more comprehensive definition of the disease, recognizes a greater number of patients at risk of disease progression, and encourages holistic treatment that focuses on the overall metabolic health of the patient instead of solely liver function\textsuperscript{50}. While we recognize the purpose of the MAFLD definition and its relevance to work in this thesis, we will continue to use the term NAFLD to define the disease as it is more generally recognized and we have not confirmed evidence of metabolic dysregulation, as defined by Eslam et al., in our guinea pig model.

Diets high in fructose, sucrose, and saturated fats, combined with sedentary lifestyles, have been associated with NAFLD\textsuperscript{28}. One particular aspect of the WD that may contribute to NAFLD development is fructose consumption, as fructose has been shown to promote DNL in the liver, where DNL is the enzymatic pathway that converts dietary carbohydrates into fatty acids and eventually TGs\textsuperscript{51}. Dietary factors not related to the Western diet, including protein-calorie malnutrition, starvation, and rapid weight loss, have also been reported as secondary causes of steatosis\textsuperscript{31}.

NAFLD is a manifestation of the metabolic syndrome and is frequently associated with obesity (particularly central abdominal obesity), dyslipidemia, and insulin resistance\textsuperscript{31,44}. The rising incidence of NAFLD mirrors the rising prevalence of obesity and type 2 diabetes worldwide\textsuperscript{44}, and these diseases were found to be independent predictors of fibrosis and cirrhosis in NAFLD patients\textsuperscript{31}. The presence of MetS is a strong predictor of steatohepatitis in NAFLD patients and could be used to identify patients who would benefit from a liver biopsy\textsuperscript{45}. The relationship between MetS and the NAFLD spectrum is possibly bi-directional as NAFLD has been found to enhance features of MetS such as hypertension\textsuperscript{28}. The NAFLD spectrum has a substantial impact on cardiovascular disease,
and surprisingly the most common cause of death in these patients is CVD-related\textsuperscript{45}, followed by malignancy and then liver disease\textsuperscript{28}.

Due to the relationship between NAFLD and obesity and the lack of sensitive non-invasive diagnostic tests, NAFLD is often diagnosed on a presumptive basis when liver enzyme abnormalities are noticed in overweight or obese individuals\textsuperscript{31}. It should be noted that liver enzyme abnormalities are not consistent in all patients with NAFLD and often present differently in lean patients, limiting the reliability of this diagnostic measurement\textsuperscript{28}. Because of this, NAFLD patients in the normal BMI range (18.5-25 kg/m\(^2\)) represent a lean phenotype of NAFLD that is underrecognized\textsuperscript{52}, despite making up a significant percentage of patients with NAFLD (~15-20\%)\textsuperscript{48,53}, NASH (~60\%) and fibrosis (55\%)\textsuperscript{54}. This underdiagnosis of lean NAFLD may contribute to increased mortality reported in these patients compared to their overweight or obese counterparts\textsuperscript{55}. Lean NAFLD patients do not display the same risk factors of NAFLD, with lower insulin resistance levels and ALT but increased lobular inflammation compared to overweight/obese individuals, making the lean phenotype more difficult to diagnose\textsuperscript{52}. In Chapter 2 of this thesis, the effect of the WD on the liver is investigated in a lean phenotype of NAFLD using MRI to contribute to the identification of biomarkers associated with this disease in lean individuals.

Simple steatosis in early-stage NAFLD is reversible with diet intervention and gradual weight loss\textsuperscript{31}, though rapid weight loss can lead to liver damage and should be avoided\textsuperscript{56}. Although there is currently no proven effective therapy for NASH, research into pharmacological treatments is advancing rapidly, and numerous clinical trials are underway\textsuperscript{28,31}. Treatment targets include hepatic metabolism, cell stress and apoptosis, immune response, fibrosis, the microbiome, and combination therapies\textsuperscript{28}. Antioxidants, insulin-sensitizing drugs, lipid-lowering drugs, and ursodeoxycholic acid are examples of pharmaceutical treatments being investigated for use in NAFLD, though their efficacy and mechanisms of action require further study\textsuperscript{31}. A challenge of this research is assessing the survival benefit in patients with pre-cirrhotic disease as they are not usually at risk for short-term adverse outcomes\textsuperscript{28}. The interconnected relationship of NAFLD and
MetS suggests therapeutic interventions may alleviate symptoms and features of both diseases\[^{28}\].

### 1.1.3 Effects of the Western Diet in Pregnancy

As of 2018, 22.7\% of child-bearing-aged women (18-34) were obese according to the clinically relevant BMI definition\[^{15}\]. A significant contributor to obesity is the regular consumption of a high-fat and high-sugar Western diet\[^{57}\]. Diets high in saturated fats, refined carbohydrates, and low nutrient-dense foods were found to be commonly consumed by pregnant women in America\[^{58}\]. This finding is especially relevant in Western society as an increasing number of people are obese, accounting for 16\% of pregnant mothers in Canada between 2009-2011\[^{59}\]. Mothers who are obese experience a higher rate of stillbirth\[^{60,61}\] and are at higher risk for maternal and fetal complications than mothers with a normal BMI\[^{62,63}\]. WD consumption also contributes to the risk of folate deficiency\[^{64}\], abnormal fetal brain development\[^{65}\], and fetal predisposition to type II diabetes\[^{66}\].

It has been hypothesized that WD consumption, which leads to obesity and MetS, harms placental function during pregnancy\[^{67}\]. Mothers with MetS exhibit a higher risk of impaired fetal growth and intrauterine fetal death\[^{67}\]. Animal studies have shown that consumption of high-fat diets during pregnancy can lead to many issues for offspring, including but not limited to cardiovascular dysfunction, mitochondrial abnormalities\[^{68}\], and the development of type II diabetes\[^{69}\]. Metabolic syndrome has also been shown to increase the risk of placental dysfunction and pre-eclampsia, with individuals exhibiting a higher number of MetS characteristics having a higher risk of placental dysfunction\[^{67}\]. The placenta is responsible for transferring nutrients from the maternal blood to fetal blood and excretion from the fetus back to the mother\[^{70}\]. Any disruption to these functions may lead to adverse outcomes for the fetus.

Placental dysfunction is the leading cause of stillbirth, with rates of approximately 8/1000 pregnancies in Canada\[^{71}\]. Clinical studies have found that placental dysfunction can lead to major morbidities and is a primary cause of fetal growth restriction (FGR)\[^{72,73}\]. Further details of the fetal consequences of FGR are explained in Section 1.1.3.1. Chronic
hypoxia and hypoglycemia are also outcomes of placental insufficiency, with hypoxia being related to failed oxygen transfer between the placenta and fetus\textsuperscript{73,74}. Finally, metabolic dysfunction in the placenta could lead to a buildup of excess lactic or carbonic acid relative to homeostasis, leading to abnormal changes in placental pH and eventually fetal acidosis\textsuperscript{74}.

1.1.3.1 Developmental Origins of Health and Disease (DOHaD)

The developmental origins of health and disease (DOHaD), also known as the fetal origins hypothesis, suggests that the environment a fetus is exposed to \textit{in utero} impacts the lifelong health of the individual\textsuperscript{75}. Specifically, the theory states that adverse influences in early development may result in permanent changes in individuals’ physiology and metabolism that predispose them to non-communicable diseases later in life (Figure 1.4)\textsuperscript{76}. This approach evolved from the Barker hypothesis, first proposed by Dr. David Barker in the 1980s after studying correlations between low birth weight and incidence of coronary heart disease\textsuperscript{77}. The theory relies on assumptions that fetal adaptations persist into adulthood, are latent for long periods, and are caused by genetic programming due to the \textit{in utero} environment\textsuperscript{75}. Undernutrition is one of the main maternal factors involved in the fetal origins hypothesis and is believed to lead to fetal programming that increases the future risk of developing metabolic disease in adulthood\textsuperscript{78}. Birth weight, an early focus of DOHaD research, has been shown to have a direct correlation with adult BMI that has been demonstrated in more than 20 studies\textsuperscript{79}. Though details on the mechanisms underlying fetal programming are unclear and warrant further research, the “thrifty phenotype hypothesis” (introduced in Section 1.1) is believed to contribute to this increased risk of MetS with age\textsuperscript{80}. It is proposed that genetic adaptations which promote \textit{in utero} survival in instances of maternal undernutrition limit fetal growth and impair glucose uptake, promoting the adverse risk of metabolic diseases after birth when the individual has access to sufficient nutrition\textsuperscript{80}. Other possible mechanisms behind DOHaD include altered phenotypes from epigenetic changes in specific genes, altered microbiota, and the effect of an intrauterine stress response on neuroendocrine pathways that regulate metabolism and vasculature\textsuperscript{76,81}. It should be noted that adverse events during pregnancy may affect two generations, as genetic
information for reproduction is already present in the developing fetus and may also be affected by fetal programming\textsuperscript{76}.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{The DOHad Hypothesis}
\end{figure}

Since its initial inception, DOHaD has been expanded to investigate a variety of \textit{in utero} environmental pressures that influence the risk of developing a multitude of diseases, including type II diabetes\textsuperscript{76}, dyslipidemia (abnormal lipid concentration in the blood), obesity, cardiovascular disease\textsuperscript{81}, asthma\textsuperscript{82}, and cancer\textsuperscript{83}. Although maternal diet and metabolic health are still a focus of DOHaD, other stressors such as exposure to endocrine-disrupting chemicals that can harm the hormonal system have also been investigated as root causes of fetal programming\textsuperscript{76,84}. The fetus may be exposed to harmful chemicals via certain types of plastics (i.e. bisphenol-A), maternal smoking, or environmental pollution\textsuperscript{76}.

In Chapter 4, I focus on the impact of maternal diet on the placenta, and subsequently, the placenta’s impact on fetal growth. Birth size is a simple and easily obtained measurement
related to the *in utero* environment and thus was used as a staple metric in early
discussions of DOHaD, especially as a tool to look at historical epidemiological data.\(^85\).

Neonates may be classified as small for gestational age (SGA), which can be a
consequence of intrauterine growth restriction, also known as FGR. SGA is defined by a
fetal or neonatal body size more than two standard deviations below the mean or third
percentile for that gestational age.\(^86\) It is important to note that not all SGA fetuses result
from FGR, as some fetuses will naturally be small based on genetic or physiological
factors but were not growth restricted *in utero*.\(^73,87\) FGR neonates are known to have
health problems as they age, including but not limited to increased risk of MetS, type II
diabetes, CVD, obesity, neurodevelopmental issues, and cerebral palsy.\(^73,86–88\) One of the
mechanisms proposed to explain these fetal consequences is growth-triggered stress
induced by the undernourished individual’s rapid postnatal catch-up growth. This
postnatal stress has been shown to exacerbate metabolic defects.\(^81,87\).

On the other hand, lack of postnatal catch-up growth in FGR neonates is associated with
insulin resistance\(^86\) and risk of long-term growth issues.\(^87\) FGR neonates may be
classified as symmetric (20-30%) if they are proportionately small or asymmetric (70-
80%) if they disproportionally lag in abdominal growth.\(^89\) It is believed that asymmetric
FGR is caused by extrinsic influences such as placental insufficiency, and symmetric
FGR is caused by intrinsic influences such as intrauterine infections or genetic
abnormalities.\(^89\) These fetal growth patterns are complex and more research is needed to
confirm these causal associations.\(^89\) FGR symmetry may also be dependent on the onset
of FGR during gestation.\(^90\) Asymmetric FGR is thought to occur due to “brain sparing,”
where it is hypothesized that oxygen and nutrients are shunted to vital organs, including
the brain and heart, leaving other organs like the liver underdeveloped.\(^91\) There is no
definitive agreement on whether symmetric or asymmetric FGR is predictive of more
severe complications for the fetus.\(^89\).

On the other extreme of the size spectrum, infants born with high birth weight,
categorized as macrosomia, are also associated with adverse outcomes for both the
mother and child.\(^92\) Macrosomia is defined as infants with a birth weight of more than
4000 g, though the upper limit may be set between 3720-4500 g and is not universally
agreed upon\textsuperscript{93,94}. Another related definition is large-for-gestational-age (LGA), which is used for fetuses with a weight $>90^{\text{th}}$ percentile or above two standard deviations for that gestational age\textsuperscript{93}. Fetal macrosomia may be caused by genetic factors, including epigenetic regulation related to the \textit{in utero} environment, and maternal factors such as maternal age, BMI, body composition, diabetes status, gestational weight gain, and nutritional intake\textsuperscript{93,94}. LGA fetuses are at higher risk of intrauterine death, birth trauma, congenital anomalies, shoulder dystocia, and developing diabetes, MetS, obesity, asthma, and cancer later in life compared to fetuses that fall within the average range of size for gestational age\textsuperscript{70,93}.

Both SGA and LGA definitions that rely on absolute measurements or arbitrarily chosen percentiles are flawed as they regularly misclassify naturally small or large fetuses as abnormally sized\textsuperscript{87}. These definitions may also misclassify SGA fetuses as averaged sized if they lie within acceptable fetal size measurements but, in reality, have not met their growth potential\textsuperscript{87}. In other words, using statistical definitions does not reliably allow us to distinguish fetuses that grew normally throughout gestation from those that experienced flawed growth \textit{in utero}\textsuperscript{92}. A solution to this is customized fetal growth charts that consider genetic and physiological factors such as maternal age, sex, height, and parity to gauge the fetus’ growth respective to its genetically predetermined growth potential\textsuperscript{87}. Another solution would be to look at fetal body composition instead of just fetal size, which may be achieved using imaging. US and Doppler techniques are currently used to diagnose FGR using fetal body measurements and umbilical artery flow\textsuperscript{91}. MRI boasts superior soft-tissue contrast and though not commonly used in pregnancy, may provide high accuracy in fetoplacental measures of structure and function\textsuperscript{95}. Increased fetal brain:liver weight and volume ratios have been established as indicators of asymmetric FGR and predictors of fetal outcome\textsuperscript{96,97}. Measurements of placental volume and fetus:placenta volume ratio, also known as placental efficiency\textsuperscript{98}, can help identify at-risk fetuses, where very small or very large placentae relative to birth weight are associated with placental insufficiency and fetal death\textsuperscript{99,100}. It is important to note that the range of normal volume ratios change throughout gestation\textsuperscript{97}. Before birth, these volume ratios can be estimated using 3D ultrasound or MRI, where historically they were identified using post-mortem samples or by weighing the fetus and placenta after
Being able to measure volumes in utero allows for the classification of early-onset (<32 weeks) and late-onset (≥32 weeks) FGR, which have different causes and consequences. Additionally, the risk of perinatal mortality differs according to the gestational age during the onset of FGR.

Critiques of the DOHaD hypothesis question whether the correlations between birth weight and adulthood disease are due to random error, focus too heavily on potentially unreliable historical data, or fail to account for confounding factors. Some studies recognize the validity of further research to investigate early life mechanisms of the origin of chronic disease but argue that current work related to DOHaD is too premature to be used as a basis for clinical interventions. There have been examples of inconsistencies across DOHaD studies using observational data, which may not be suitable to confirm causal relationships. Another criticism of DOHaD is the overemphasis on early life factors while neglecting the importance of lifestyle factors accumulated throughout life that contribute to the risk of metabolic disease.

Nevertheless, there are still many questions to be answered concerning DOHaD, particularly in areas concerning maternal nutrient status, and experimental species may be used as a more reliable framework in place of historical data.

1.1.3.2 Effects of the Western Diet on the Placenta

The placenta is a vital temporary organ responsible for the exchange of nutrients between the mother and fetus and the production of growth factors and hormones during pregnancy. Knowledge of the effect of maternal diet on placental function is limited; however, there is evidence that maternal diet impacts placental structure and growth. Moreover, most placental studies related to maternal diet have focused on maternal undernutrition, where maternal overnutrition, obesity, and metabolic disease have been less of a focus. The literature is very limited concerning the effect of the WD on the placenta; however, multiple studies are focused on the placental response to the high-fat diet and maternal obesity, both closely related to the WD. Pre-pregnancy nutrition status and maternal diet during pregnancy are important factors that have the potential to influence placental function and consequently fetal growth. Placental size is impacted
by diet, and both placental weight and the birth weight:placental weight ratio (placental efficiency) can provide estimations of offspring health\textsuperscript{103}. At both extreme ends of the placental size spectrum, there is an increased risk of coronary heart disease, blood pressure, hypertension, stroke, and type II diabetes in the offspring’s adult life\textsuperscript{103}. Obesity is strongly correlated with the WD, and obesity during pregnancy is associated with increased placental weight and decreased placental efficiency\textsuperscript{104,106}. Other studies have shown decreased placental weight in rats fed a high-fat diet\textsuperscript{107} and in humans with a high maternal energy intake early in pregnancy\textsuperscript{105}. Obese mothers produce placentae with increased thickness compared to placentae from normal BMI mothers\textsuperscript{106}. A study in rats also found increased placental thickness and changes in placental layer distribution in mothers fed a high-fat diet compared to a control diet\textsuperscript{107}. Increased placental thickness is associated with reduced placental permeability, limiting the amount of oxygen that can successfully be delivered to the fetus\textsuperscript{106}. Placental nutrient transfer capacity is influenced by factors including placental surface area and thickness, which may impact fetal growth and development\textsuperscript{104}. Other structural changes of the placenta observed in animal models of a maternal high-fat diet include increased infarction, villous calcification, and accumulation of lipid droplets in the placenta\textsuperscript{104}.

Overweight and obese women demonstrate localized placental inflammation at higher rates than normal-weight mothers\textsuperscript{104–106}. This inflammation has also been demonstrated in non-human primates fed a chronic high-fat diet\textsuperscript{104}. Placental inflammatory cytokine expression and nitrative stress are elevated in obese women and impact placental nutrient transport\textsuperscript{105}. Increases in inflammatory cytokines are associated with FGR, angiogenesis, and decreases in placental growth factor gene expression\textsuperscript{105}. A proinflammatory state in the placenta is also a potential contributing factor to modified nutrient transport\textsuperscript{105}. A study in mice has shown that inflammation in the placenta at late gestation in obese mothers exhibited a sex-specific effect, with placentae of male fetuses showing greater inflammation and macrophage activation\textsuperscript{104}. Many human and animal studies of placental function and response to maternal diet demonstrate sex dimorphism\textsuperscript{104}. The placenta has the same genetic sex information as the fetus since the placenta has a fetal origin and generally demonstrates a greater ability of female placentae to adapt to perturbations of the \textit{in utero} environment, including a maternal Western or high-fat diet\textsuperscript{104}. 
Placental vasculature dictates the ability of the placenta to deliver oxygen and nutrients from the mother to the fetus. Lifelong exposure to the high-fat diet and/or maternal obesity has been associated with abnormal placental vasculature and altered trophoblast invasion. Specifically, chronic high-fat diet consumption has been shown to reduce uterine blood flow and blood flow volume on the fetal side of the placenta in primates. Overnutrition, a consequence of the WD, has led to impaired vascular development in the sheep placenta at early and mid-gestation. High-fat diet-induced obesity in the mother has caused blood vessel immaturity and hypoxia in the placentae of mice. A possible mechanism behind poor vascularization in the placenta is disturbances to the fetoplacental renin-angiotensin system – a hormone system that regulates blood pressure, vascular resistance, and fluid balance – which has previously been linked to maternal high-fat diet exposure. Poor placental vascular function in humans is associated with placental oxidative stress and pre-eclampsia.

Though it is clear that the placenta makes adjustments related to cell growth and nutrient transport based on maternal factors like nutritional status, it is unclear how the placenta “senses” these external factors. A proposed explanation of a placental nutrient sensor is the placental mammalian target of the rapamycin signalling pathway, which controls amino acid transport and is influenced by diet-induced obesity. Another system that may act as a nutrient sensor is composed of the various epigenetic mechanisms that control placental gene expression. In rodents, a maternal high-fat diet increased placental transport of glucose and amino acids via upregulation of their transporters, resulting in larger fetal size and potentially contributing to LGA or macrosomia in offspring. Obesity and high concentrations of cholesterol and TG in maternal blood have been linked to an increase in placental fatty acid transporter proteins, contributing to both fetal overgrowth and increased fetal adiposity. Mouse studies have shown that placental epigenetic alterations triggered by the maternal high-fat diet were sex-specific, particularly for genes involved in controlling cellular, metabolic, and physiological functions important for adaptation. Protein markers of poor placental function, such as the plasminogen activator inhibitor, are elevated in obese women, providing evidence of genetic changes associated with maternal diet and body composition. A high-fat diet and maternal obesity have been linked to mitochondrial fragmentation in the human
placenta, potentially caused by a decrease in a protein related to mitochondrial fusion and cell metabolism\(^ {112}\). Down-regulation of this protein was observed in women with unexplained miscarriages and contributed to mitochondrial alterations in the placenta\(^ {112}\). Although this data supports the hypothesis that maternal diet and obesity influence placental gene expression and nutrient transport, global epigenetic changes in the placenta are still poorly understood\(^ {113}\).

High fructose content is one aspect of the WD that generally harms physiology, though its impact on the placenta is understudied\(^ {3,114}\). In mice, maternal fructose consumption has led to placental inefficiency, increased lipids, and altered gene expression of systems that control oxidative stress in the placenta\(^ {115}\). In maternal fructose intake studies, sex-specific changes in placental size and fetal outcome have been reported, with female placentae and fetuses displaying worse outcomes\(^ {114}\).

Understanding how maternal diet affects placental structure and function is essential because the placenta is a programming agent of the adult health of the fetus\(^ {104}\). Adaptations made by the placenta in utero alter fetal nutrient supply, which in turn motivates epigenetic changes in the fetus that may contribute to DOHaD\(^ {104}\). Data supporting evidence of these placental trends associated with maternal diet come from various studies in both human and animal models using a diversity of species exposed to aspects of the WD for different durations. It should be emphasized that results may depend on the differences between studies, highlighting the need for further research on the impact of maternal diet on placental structure and function, especially in humans\(^ {104}\).

1.2 Guinea Pig Model

In the following chapters of this thesis, a guinea pig model is used in WD studies focusing on liver disease and pregnancy. Guinea pigs are often used in metabolism studies because they have a similar lipoprotein profile to humans where the majority of circulating cholesterol is transported as LDL, which is not observed in other rodents\(^ {116}\). Other similarities include comparable plasma lipoprotein metabolism enzymes, a requirement for dietary vitamin C, gender plasma lipoprotein differences, and similar
responses to exercise and dietary interventions that include lowered plasma TG and increased plasma HDL\textsuperscript{116,117}. Like humans, guinea pigs develop atherosclerosis and display a related inflammatory response, which may be induced with high cholesterol diets\textsuperscript{116}. Generally, the guinea pig is an ideal model for evaluating the mechanisms behind how diet interventions and drug treatments alter plasma lipids and lipoprotein metabolism\textsuperscript{116}. Guinea pigs may be used as a NAFLD model as they develop enlarged, fatty livers when exposed to diets high in fat and cholesterol and demonstrate markers of liver damage, including altered ALT activity\textsuperscript{117}. Importantly, unlike mice and rats, guinea pigs have shown to be reliable models for hepatic injury, steatosis, and cirrhosis without the need for genetic manipulations\textsuperscript{118}. The storage, synthesis, and catabolism of cholesterol in the liver is similar between humans and guinea pigs, which may be relevant in NAFLD as it has been suggested that cholesterol plays a central role in the transition of NAFLD to NASH\textsuperscript{118}. Certain risk factors for NAFLD are common in both humans and guinea pigs, including atherogenic hyperlipidemia, systemic inflammation, male gender, lack of exercise, and high-fat diet\textsuperscript{117}. These risk factors are also relevant for CVD, closely related to NAFLD in humans\textsuperscript{118}. Guinea pigs may be used as a model to evaluate therapeutic strategies for NAFLD as they display responses to interventions such as carbohydrate restriction, fatty acid saturation, dietary fibre, antioxidants, and pharmaceuticals that are similar to human responses\textsuperscript{117,118}.

The guinea pig is also an ideal model for pregnancy-related studies, as it has a relatively long gestation (~69 days) compared to other rodents but shorter than that of sheep or primates\textsuperscript{119}. The guinea pig is easily bred, thanks to modest husbandry requirements and a docile temperament, and is an ideal species for assessing multi-generational effects of in utero perturbations in studies of DOHaD\textsuperscript{120}. Because of this long pregnancy, guinea pigs deliver precocial, or relatively mature and mobile, pups with many developmental events occurring in utero similar to the human fetus\textsuperscript{119}. Examples of this are the similar timelines for muscle development and accumulation of adipose tissue in guinea pig and human fetal development\textsuperscript{120}. The relatively long guinea pig gestation allows for identifying critical periods during development that are vulnerable to in utero insults and displays adverse outcomes similar to those observed in humans\textsuperscript{120}. Unlike humans, guinea pigs usually produce more than one offspring (1-5); however, their litter sizes are
substantially lower than those typically seen in rats and mice \(^{(8-12)}\). Endocrine regulation is similar in the guinea pig and human pregnancy, with both species maintaining high and rising progesterone levels throughout birth\(^{(121)}\). The guinea pig placenta is hemomonochorial, having a similar structure to the hemochorial human placenta, and displays a deep trophoblast invasion similar to the human placenta\(^{(120)}\). Due to similarities with human placentation and the fetal/maternal transport barrier, the guinea pig is a well-established model to study placental transfer and FGR and is one of the few species known to develop pre-eclampsia\(^{(119,121,122)}\). A correlation between placental blood flow and fetal size has been shown in both guinea pigs and humans, highlighting the potential importance of this model for studying conditions that affect placental vascular function\(^{(120)}\). There are also metabolic similarities in human and guinea pig pregnancies, including the transfer of glucose and amino acids across the placenta. Today, the guinea pig is one of the most important animal models for placental studies in obstetric research\(^{(122)}\).

1.3 Magnetic Resonance Imaging

1.3.1 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is a phenomenon that occurs when magnetically sensitive nuclei are exposed to a strong magnetic field and perturbed by a weaker oscillating magnetic field\(^{(123)}\). Nuclei possess an intrinsic quantized angular momentum known as spin, which is largely determined by the number of unpaired nucleons\(^{(123)}\). “Spin-half” nuclei with half-integer quantum numbers occupy one of two discrete energy levels within an external magnetic field\(^{(124,125)}\). These spin-half nuclei experience a magnetic moment in an external magnetic field and, therefore, can be detected using NMR techniques\(^{(124)}\). Nuclei with integer or half-integer quantum numbers > \(\frac{1}{2}\) exhibit electric quadrupoles instead of dipoles, meaning they are NMR sensitive but are not suitable for high-resolution NMR\(^{(125)}\). Generally, the nucleus of interest in NMR is hydrogen \((^1\text{H})\), as it has a high natural abundance of 99.98% and is present in most organic compounds\(^{(125)}\). In NMR, \(^1\text{H}\) is often referred to as the proton itself since the electrons do not contribute to the magnetic resonance phenomenon\(^{(126)}\). The nuclei
involved in NMR are also colloquially referred to as spins, not to be confused with the nuclear spin introduced above\textsuperscript{126}.

A strong applied magnetic field interacts with the magnetic moment of a nucleus, produced by its angular momentum, and nearly aligns with the main magnetic field in either a parallel (low energy) or antiparallel (high energy) orientation\textsuperscript{69}. At thermal equilibrium, the energy levels of non-interacting, identical nuclei in a magnetic field are described by the Boltzmann distribution\textsuperscript{123}. There is an almost equal number of spins existing at the two energy levels in this state, with only a tiny excess in the lower energy orientation\textsuperscript{123}. The relative population difference in energy levels is defined by the polarization, which, in the high temperature approximation (kT >> 1, k \sim 8.6 \times 10^{-5} \text{ eV/K}), is inversely proportional to temperature and directly proportional to the magnetic field strength and an intrinsic property of the nucleus known as the gyromagnetic ratio (\gamma)\textsuperscript{123}. For hydrogen nuclei at body temperature in a 1.5 T field, only \sim 1/100,000 spins contribute to the NMR signal\textsuperscript{127}. This small polarization across the enormous number of spins in a sample produces a mean magnetic moment that aligns with the direction of the external magnetic field, referred to as the net magnetization (M\textsubscript{0})\textsuperscript{126}, as shown in Figure 1.5.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{magnetization.png}
\caption{Representation of the net magnetization vector M\textsubscript{0} in an external magnetic field B\textsubscript{0}.}
\end{figure}
Quantum mechanics governs the behaviour of each nucleus; however, when referring to a large number of protons, such as in the human body, classical mechanics may be used to describe the mean magnetic moment of the nuclei. \( M_0 \) precesses about the main magnetic field due to the spins creating a torque when interacting with the magnetic field. The angular frequency, referred to as the Larmor frequency (\( \omega_0 \)), of this magnetic moment vector is directly related to the main magnetic field (\( B_0 \)) and the gyromagnetic ratio (\( \gamma \)) of the nuclei, as expressed in Equation 1-1.

\[
\omega_0 = \gamma B_0
\]

To make a measurement, the net magnetization must be manipulated in a direction away from the main field since \( M_0 \) is much weaker than \( B_0 \) and would not be detectable along the same axis as \( B_0 \). To accomplish this detection of \( M_0 \), a radiofrequency (RF) pulse oscillating at the Larmor frequency is applied for a short time perpendicular to the main magnetic field, producing a weak magnetic field perpendicular to \( B_0 \) in the rotating field of reference, referred to as \( B_1 \). The excitation pulse is called an RF pulse because Larmor frequencies are typically in the MHz range, which corresponds to the radiofrequency range of the electromagnetic spectrum. The net absorption of energy from the RF pulse promotes spins to transition from the low energy state to the high energy state. The RF pulse also causes the net magnetization \( M_0 \) to experience nutation about the \( B_1 \) field as it is tipped into the transverse plane perpendicular to the \( B_0 \) field while the RF pulse is applied. The amplitude and duration of the RF pulse will determine the flip (or tip) angle (\( \alpha \)), describing the amount of nutation away from the axis of the \( B_0 \) field.

In simple experiments, \( \alpha \) is chosen to be 90° to achieve the maximum value of the transverse component of \( M_0 \) upon excitation. A 180° RF pulse would result in a net magnetization in the negative direction on the \( B_0 \) axis. In a Cartesian coordinate system, the magnetization component in the direction of the \( B_0 \) axis is called the longitudinal magnetization and is denoted as a vector in the z-direction, \( M_z \). The component of \( M_0 \) in the transverse plane or x-y plane is called the transverse
magnetization and is represented as a rotating phasor, $M_{xy}$\textsuperscript{125}. These components of the net magnetization can be described by Equations 1-2 and 1-3, where $\phi$ represents an arbitrary initial angle position, or “phase shift”, of $M_0$ in the transverse plane\textsuperscript{125}.

\[
M_z = M_0 \cos \alpha  \quad 1-2
\]
\[
M_{xy} = M_0 \sin \alpha \, e^{i(\omega_0 t + \phi)}  \quad 1-3
\]

According to Faraday’s law of induction, any changing magnetic field produces an electromotive force\textsuperscript{129}. Therefore, when an RF pulse is applied and $M_0$ experiences nutation, the spins radiate electromagnetic energy that is detected as an induced voltage by receive coils\textsuperscript{125}. This voltage is amplified and used to form the NMR signal measurement\textsuperscript{125}. The receive coil is only sensitive to magnetization perpendicular to $B_0$, and the voltage produced in the coil is proportional to the NMR signal and the square of the magnetic field strength of $B_0$\textsuperscript{126}.

After the magnetic moments of the nuclei are tipped into the transverse plane, they immediately start to lose phase coherence, or dephase, with each other, causing the $M_{xy}$ signal to decay exponentially and bringing the detected signal amplitude to 0 within a few milliseconds\textsuperscript{128}. The rate of this signal decay in an ideal homogeneous magnetic field is characterized by the transverse, or spin-spin, relaxation constant $T_2$\textsuperscript{130}. $T_2$ is a result of the intrinsic relaxation process in tissue and is related to the microscopic magnetic fields caused by adjacent protons and random molecular and atomic interactions\textsuperscript{131}. The decay of $M_{xy}$ over time due to $T_2$ is known as the free induction decay (FID) and can be expressed by Equation 1-4\textsuperscript{125}.

\[
M_{xy} = M_0 \sin \alpha \, e^{i(\omega_0 t + \phi)} \, e^{-t/T_2}  \quad 1-4
\]

Meanwhile, the longitudinal magnetization $M_z$ is recovered to $M_0$ in the absence of the RF pulse\textsuperscript{125}. This relaxation is completely independent of the transverse relaxation and is related to the transitions of excited spins back to their thermal equilibrium\textsuperscript{125}. The molecules in the sample undergo random translational, vibrational, and rotational motion, or “tumble”, in the external field\textsuperscript{125}. This tumbling motion within the $B_0$ field causes the dipole strength of an individual spin to fluctuate at the Larmor frequency and is further
affected by interactions with other fluctuating dipoles, known as intramolecular or dipole-dipole interactions$^{125}$. These interactions, determined by the random motion and spatial distance between nuclei, cause spin-state transitions between the low and high energy states$^{125}$. By this process, the extra energy absorbed from the RF pulse is released to the surrounding environment or “lattice”. This relaxation depends on the proximity of spins to other molecules, which varies according to tissue composition and pathophysiological state$^{125,126}$. This $M_z$ relaxation is characterized by the longitudinal, or spin-lattice, relaxation time $T_1$$^{125}$. The mathematical expression of the $M_z$ at a given time ($t$) after an RF pulse of flip angle $\alpha$ is given by Equation 1-5.

\[
M_z(t) = M_0 (1 - e^{-t/T_1}) + M_0 \cos \alpha e^{-t/T_1}
\]

$T_1$ is much slower than $T_2$, as seen in Figure 1.6, and the difference between these rate constants must be considered in all NMR spectroscopy or imaging$^{126}$. The unique $T_1$ and $T_2$ characteristics associated with different tissues in the body generate contrasts useful for imaging anatomy in vivo using MRI$^{126}$.

![Figure 1.6: Graphical representation of the longitudinal ($T_1$) and transverse ($T_2$) relaxation times.](image)

1.3.2 Magnetic Resonance Spectroscopy

In a basic sense, spectroscopy is the study of the interaction between matter and electromagnetic radiation$^{132}$. In magnetic resonance spectroscopy (MRS), the interaction of interest is between an applied external magnetic field and the resultant electromagnetic signals produced by nuclei within a sample of interest$^{133}$. NMR spectroscopy has been
used since the early 1950s in physical chemistry. When this technology is applied in a biomedical context it is generally referred to as MRS\textsuperscript{133}. The word “nuclear” was eliminated from the phrase to dispel the false implication that MRS is associated with nuclear radioactivity\textsuperscript{133}.

MRS provides chemical and structural information about molecules within the sample by observing differences in NMR due to the local magnetic fields around nuclei, which are slightly but measurably affected by their environment and placement within a molecule\textsuperscript{134}. Specifically, the magnetic field a nucleus experiences is influenced by the proximity and motion of nearby electrons and atoms, either having a shielding or an enhancing effect\textsuperscript{126,134}. The $B_0$ field causes electrons in an atom to circulate within their orbitals, and this induced motion generates a small local magnetic field at the site of the nucleus that is proportional to $B_0$\textsuperscript{123}. This shielding becomes more complex when considering a sample containing molecules instead of just atoms, with the resonant frequency of a nucleus characteristic of the electronic structures surrounding it\textsuperscript{123}. The phenomenon of slight differences in resonant frequency between nuclei in different local chemical environments is known as the “chemical shift”, and is a function of the chemical structure of the molecule\textsuperscript{134}. While the electron shielding factor itself is a constant, the chemical shift increases linearly with field strength, meaning that the spectral resolution is higher at large $B_0$ strengths\textsuperscript{125}.

A spin-spin coupling may occur within a molecule if multiple nuclei exist within different chemical environments and interact with each other\textsuperscript{134}. This phenomenon results in split peaks in the NMR spectrum, where spins occupying each energy level are slightly shifted away from each other, providing additional information about the arrangement of atoms in that molecule\textsuperscript{123}. Obtaining a very precise measurement of the spectroscopic peaks in a sample enables us to quantify the relative number of protons in each position on a molecule\textsuperscript{126}. MRS may be used to identify and quantify the concentration of metabolites \textit{in vitro} or \textit{in vivo} and provides a means of monitoring dynamic metabolic processes over time\textsuperscript{133}. 
Before the signal is acquired, the magnetic field is shimmed to correct for $B_0$ inhomogeneities that may affect the precision of the measurement\textsuperscript{134}. This shimming is done using shim coils placed around the sample that generate small local fields to provide the most homogenous $B_0$ field possible near the sample\textsuperscript{134}. In order to measure a signal, an RF pulse (as described in section 1.3.1) is applied either in short, intense increments at a specific frequency (pulsed method) or applied constantly and swept over varying frequencies (continuous wave method)\textsuperscript{134,135}. Although both methods have been used since the development of MRS, pulsed methods are more widely used today\textsuperscript{125}. The decay is measured as the previously introduced FID and consists of a superimposition of all resonances from the different nuclei in the sample\textsuperscript{134}. To get from the FID to the frequency spectra typically associated with MRS, the Fourier transform (FT) must be applied\textsuperscript{125}. The general idea behind the FT is the Fourier series, which is a way of representing any complex signal as a series of sinusoidal waves described by different frequencies, phases, and amplitudes\textsuperscript{126,136}. The FT enables the mathematical transformation of information from the FID in the time domain into the frequency domain, where distinct spectral peaks, or resonances, corresponding to each chemical shift are visible (Figure 1.7)\textsuperscript{134}. In other words, the FT is a mathematical tool that decomposes a complicated signal into the frequencies and relative amplitudes of its simple component waves expressed as a complex-valued function\textsuperscript{136}. The resultant complex signal consists of real (absorption spectrum) and imaginary (dispersion spectrum) components that contain information about the phase shifts corresponding to each frequency, though typically only the absorption spectrum is used in clinical MRS\textsuperscript{135}. If data is known in either the time or frequency domains, the FT or inverse FT can be used to compute data in the other domain\textsuperscript{136}. 
Each spectrum acts as a unique fingerprint of the compound in question, allowing for identification of organic compounds using MRS\textsuperscript{135}. The multiple peaks in the MR spectrum represent the relative contributions of individual nuclei that make up metabolites in the region sampled, existing at distinct chemical shifts from each other\textsuperscript{136}. In the MR frequency domain, the area under each peak corresponds to the concentration of that chemical species and, though not common, direct quantification of metabolites is possible using advanced acquisition protocols, calibrations, and relaxation time measurements\textsuperscript{125,134}. The chemical shift of each molecule is usually expressed in dimensionless units (parts per million, ppm) using a reference material such as water\textsuperscript{134}.

\textit{In vivo} MRS is performed to detect small molecules within cells or extra-cellar spaces and monitor metabolic changes in response to disease or treatment\textsuperscript{137}. $^1\text{H}$ and $^{31}\text{P}$ are the most commonly used nuclei for \textit{in vivo} MRS as they are naturally abundant and have a high gyromagnetic ratio, though $^{13}\text{C}$, $^{23}\text{Na}$, and $^{19}\text{F}$ are also targets of clinical MRS\textsuperscript{134}. Water suppression is usually used in $^1\text{H}$ MRS as the spectrum would otherwise be dominated by the water peak, and metabolite peaks would be difficult to detect above the background noise\textsuperscript{125}. Though the chemical shift is mostly affected by electron shielding, other factors, including temperature and pH, may have measurable effects on the chemical shifts of specific compounds\textsuperscript{135}. \textit{In vivo} MRS is most commonly done using single-voxel spectroscopy, where a single voxel of interest within an organ is selectively targeted using gradients (expanded upon in section 1.3.3) and excited to produce a spectrum from that voxel\textsuperscript{126,134}. Smaller voxels require a higher number of signal
averages, resulting in longer scan durations\textsuperscript{134}. For greater anatomical coverage, chemical shift imaging uses a matrix of voxels to form the spectra, typically in a 2D single slice\textsuperscript{134}. Advantages of \textit{in vivo} MRS include its capacity for direct measurement of distinct metabolites, robustness, reproducibility, rapid sampling, a minimal requirement for sample preparation, and the ability to sample small volumes\textsuperscript{134}. A disadvantage of this technique is its failure to detect metabolites in low abundance or metabolites co-resonant with higher concentration metabolites that are therefore hidden in the spectra\textsuperscript{134}. Interpretation of data from \textit{in vivo} experiments must consider sources of potential error related to hardware, water suppression efficiency, localization of voxel, choice of analysis technique, and tissue characteristics\textsuperscript{134}.

The most common application of \textit{in vivo} \textsuperscript{1}H MRS is in the brain, as many important cerebral metabolites are present in the proton spectrum, including N-acetyl aspartate, choline, creatine, myo-inositol, glutamine and glutamate, and lactate\textsuperscript{134}. Pattern changes in the MR spectrum over time in the brain may indicate disease and aid in diagnosing conditions such as white matter demyelination, Alzheimer’s disease, or ischemia\textsuperscript{137}. Conditions that affect brain metabolism are also a focus of \textit{in vivo} MRS research, including Parkinson’s disease, epilepsy, cancer\textsuperscript{137}, and traumatic brain injury\textsuperscript{138}. Lipid signals in the brain are generally associated with necrosis observed in high-grade brain tumours and have also been observed in MR spectra of multiple sclerosis patients\textsuperscript{137}. \textsuperscript{1}H MRS has also been used as a research tool in the context of liver disease to provide insight into mechanisms involved in NAFLD, cirrhosis, and hepatocellular carcinoma\textsuperscript{134}. \textsuperscript{31}P MRS can provide measurements related to intracellular pH and adenosine triphosphate (ATP) synthesis and has been used to assess energy metabolism in the skeletal muscle, brain, heart, and liver\textsuperscript{139}.

\subsection{Magnetic Resonance Imaging}

While both MRS and MRI are based on NMR theory, they differ in application\textsuperscript{133}. MRS is used to detect signals from chemical compounds to evaluate \textit{in vivo} biochemistry, whereas MRI detects signals produced by water in tissue to acquire images of the macroscopic anatomy\textsuperscript{133}. 

In MRI sequences, the FID is manipulated to create an echo, which is the refocusing of the spin magnetization used to measure the NMR signal\textsuperscript{126}. Two types of echo can be generated: the spin echo (SE) or gradient echo (GE)\textsuperscript{126}. In both cases, an RF pulse is applied to flip the magnetization into the xy-plane, similar to how the FID would be generated\textsuperscript{126}. Typically, a 90° RF pulse may be used to flip all of the magnetization into the xy-plane but smaller flip angles (< 90°) may also be used to generate echoes. To create a spin echo, the transverse magnetization is left to dephase for some time after the RF pulse is applied. After this delay, a 180° RF pulse is applied to flip all the spins about the x-axis in the transverse plane\textsuperscript{126}. This rotation reverses the phase angles of the spins, causing them to rephase and produce an echo, as seen in Figure 1.8\textsuperscript{126}.

![Spin Echo Diagram](image)

**Figure 1.8: Formation of a spin echo.** (A) Magnetization is in the $M_z$ direction (B) A 90° RF pulse tips the magnetization into the xy-plane (C) Spins begin to de-phase with each other (D) A 180° RF pulse is applied to flip the spins in the xy-plane (E) Spins move back into phase with each other (F) An echo is formed.

The gradient echo is generated instead using a single 90° RF pulse followed by a gradient – a magnetic field that changes in magnitude across a specific direction and distance – that refocuses the phase of the nuclei spins\textsuperscript{126}. A negative gradient lobe is applied immediately after the RF pulse to rapidly dephase the magnetization in the transverse
plane, followed by a positive but equal in magnitude gradient lobe that reverses the magnetic field gradient, causing the spins to rephase and come back into phase\textsuperscript{126}. Like the FID, these echoes are detected by a receive coil, where they can be recorded as NMR signals. The signal produced by an echo depends on the concentration of spins, intrinsic relaxation properties ($T_1$, $T_2$) in a particular tissue, and the echo time (TE) – the time between applying the RF pulse and creating the echo\textsuperscript{126}. The time period between the application of successive RF pulses is the repetition time (TR)\textsuperscript{126}.

Images generally display a contrast, or weighting, primarily dependent on the $T_1$, $T_2$, or proton density (PD) of the sample\textsuperscript{126}. PD-weighted images display high signal intensities in areas of high proton density, $T_1$-weighted images display high intensity in areas with short $T_1$, and $T_2$-weighted images display high signal intensity in areas with long $T_2$\textsuperscript{126}. The dominant weighting of the image can be selected by manipulating the TE, TR, and $\alpha$ of the sequence, as detailed in Table 1.1\textsuperscript{126}.

| Table 1.1: Image weighting via manipulation of TE, TR, and $\alpha$\textsuperscript{126} |
|---------------------------------|-----------------|-----------------|
| **Spin Echo Sequences**         | Short TE (< 40 ms) | Long TE (> 75 ms) |
| Short TR (< 750 ms)              | $T_1$-weighted   | Not useful       |
| Long TR (> 1500 ms)              | PD-weighted      | $T_2$-weighted   |
| **Gradient Echo Sequences**     | Short TE (< 15 ms) | Long TE (> 30 ms) |
| Small $\alpha$ (< 40°)           | PD-weighted      | $T_2$-weighted   |
| Large $\alpha$ (> 50°)           | $T_1$-weighted   | Not useful       |

The information is spatially encoded in MRI, producing a matrix of information that is transformed into a 2D image or 3D volume\textsuperscript{125}. Magnetic field gradients enable the spatial localization and encoding of information in three dimensions in a Cartesian coordinate system\textsuperscript{125}. The ground-breaking work surrounding magnetic field gradients that eventually resulted in the first MRI of a human was pioneered by Paul Lauterbur and further developed by Peter Mansfield; these joint efforts won them the Nobel Prize in Physiology or Medicine in 2003\textsuperscript{125}. Magnetic field gradients are linearly varying
magnetic fields applied along an axis that cause the spin’s location to be encoded in its Larmor frequency\textsuperscript{125,127}. Since the magnetic field of the gradient changes linearly with position along the applied axis, spins along that axis experience slightly different magnetic fields based on their position in space\textsuperscript{125}. The gradient strength is \( \sim 1\% \) of the \( B_0 \) field strength, meaning that the gradients result in very small perturbations to the magnetic field\textsuperscript{127}. The addition of the gradient field to \( B_0 \) causes spins along the gradient axis to precess faster or slower than the Larmor frequency, depending on their position. Note that the center of the gradient will only experience the main field \( B_0 \)\textsuperscript{126}. Therefore, as shown in Equation 1-6, the angular frequency of any spin (where \( r \) is a location described by \( x, y, z \)) can be written as a function of the gradient strength, where \( G_r \) is the strength of the gradient applied along the \( r \) direction\textsuperscript{125}.

\[
\omega_0(r) = -\frac{\gamma}{2\pi} (B_0 + G_r \cdot r)
\]  

1-6

The spatial localization of the MR signal requires three orthogonal linear gradients that are typically applied along the \( z \) (parallel to the MRI bore), \( x \) (left-right), and \( y \) (top-bottom) axes\textsuperscript{126}. Since the gradients have both magnitude and direction, they can be considered vectors and can be used to generate magnetic field gradients applied along any direction if applied in combination\textsuperscript{127}. These gradient fields are produced by gradient coils inside the bore of the MRI scanner\textsuperscript{126}. The gradient strength is expressed as mT/m, and larger gradient strengths allow for images to be acquired with a smaller field of view and thinner slices\textsuperscript{126}. Gradients both allow for slice selection, the selective excitation of particular anatomical planes of interest, and spatial encoding of the positions of resonating spins\textsuperscript{125}.

Combining a band-limited RF pulse with a gradient restricts the MR interactions to a 2D plane, or slice, of a prescribed location and thickness\textsuperscript{126,127}. The slice selection gradient causes spins along that axis to resonate at progressively increasing frequencies, and an RF pulse is transmitted at a bandwidth of frequencies corresponding to spins within the slice of interest (Figure 1.9)\textsuperscript{125}. Slice selection can be performed in any direction, producing transverse, sagittal, coronal, or oblique slices\textsuperscript{126}. After the RF pulse is applied and gradient removed, only the spins in that slice experience transverse magnetization.
and contribute to the MR signal, while all other spins remain aligned with the $B_0$ field\textsuperscript{127}. The width and location of the slice can be controlled via the gradient strength and range of frequencies in the RF pulse bandwidth\textsuperscript{127}. After the RF pulse is applied, a refocusing gradient with equal and opposite amplitude to the slice selection gradient is used to bring the spins in that slice back into phase with each other to avoid signal loss\textsuperscript{125}.

![Schematic of slice selection using an RF pulse with a bandwidth from $f_1$ to $f_2$ applied in combination with a gradient in the z-direction. Only spins within the bandwidth are selectively excited by the RF pulse.](image)

**Figure 1.9:** Schematic of slice selection using an RF pulse with a bandwidth from $f_1$ to $f_2$ applied in combination with a gradient in the z-direction. Only spins within the bandwidth are selectively excited by the RF pulse.

After a slice is selectively excited, the next step is spatially encoding the spins within the slice in the two directions orthogonal to the slice selection gradient\textsuperscript{125}. These are known as the frequency and phase encoding gradients (Figure 1.10). By convention, slice selection is performed along the z-direction, frequency encoding along the x-direction, and phase encoding along the y-direction\textsuperscript{125}.
The frequency encoding gradient, also known as the readout gradient, is applied along the x-axis continuously while the MR signal is being sampled, resulting in spins with linearly varied resonant frequencies along the x-axis\textsuperscript{125,126}. Since the gradient is present during the MR signal measurement, the signal’s frequency will depend on the position of the material within the gradient field, so the signal is “frequency encoded” in the x-direction\textsuperscript{126}. The signal will consist of a mixture of many frequency components, which, as explained in section 1.3.2, can be decomposed using the Fourier transform into a spectrum that represents a one-dimensional projection of the sample\textsuperscript{126}. Therefore, for a single RF excitation, all the spatial frequencies in the x-direction can be collected in real time\textsuperscript{126}.

Phase encoding is done in a similar way to frequency encoding, with the difference being that the phase encoding gradient is not continuously on during acquisition; rather, it is turned on for a short amount of time to cause the spins to experience a phase change\textsuperscript{126}. Although it would be time-efficient to have two continuous orthogonal gradients frequency encoding simultaneously, the frequency value is scalar, and therefore it would not be possible to discern which gradient a particular spin’s frequency originated from using this strategy\textsuperscript{126}. Instead, one of the gradients manipulates the phase of the spins so that each spin has a unique combination of phase and frequency that corresponds to its position in 2D space. In practice, the phase encoding gradient is applied before the frequency encoding gradient so that the spins experience different frequencies during sampling while still retaining their previous phase shifts\textsuperscript{126}. When the gradient is on, the precession of the nuclei increases or decreases depending on their position along the y-axis. When the gradient is turned off, the nuclei will revert to the Larmor frequency but will retain the different phase angles acquired during the time the gradient was applied; in other words, they are “phase-encoded”\textsuperscript{125}. The relative phase difference between these spins persists until another gradient is applied or the signal decays due to T\textsubscript{2} relaxation\textsuperscript{125}. Unfortunately, there is no way to distinguish signals along the phase-encode direction using a single acquisition since only the sum of all phase shifts for each frequency is measured\textsuperscript{126}. By repeating the excitation and measurement at many different phase-encoding steps, or phase changes, multiple data points are acquired that demonstrate different strengths of the MR signal over a range of gradient moments\textsuperscript{126}. With this
information, we can disentangle the individual phase contributions using the inverse Fourier transform, where each phase component corresponds to a different spatial position along the y axis\textsuperscript{126}. The MR sequence must be repeated with a different phase-encoding gradient until all possible spatial frequencies have been sampled, corresponding to the number of rows of pixels in the image\textsuperscript{126}. The total scan time of an MR sequence is equal to the number of signal averages multiplied by the number of phase encoding steps multiplied by the TR\textsuperscript{126}. Signal averaging is achieved by repeating measurements under similar conditions to increase SNR by suppressing effects from random noise\textsuperscript{126}.

![Gradient Echo Pulse Sequence Diagram](image)

**Figure 1.10:** An example of a basic gradient echo pulse sequence diagram showing the slice selection (G\textsubscript{SS}), phase encoding (G\textsubscript{PE}), and frequency encoding (G\textsubscript{FE}) gradients at work to encode the spatial location of the echo.

A 2D inverse Fourier transform is used to convert the spatially encoded data in one slice from a raw data matrix to a 2D image\textsuperscript{126}. Unique to MRI, compared to other imaging modalities, the raw data is complex and contains both a real and imaginary component\textsuperscript{125}. These components can be used to construct magnitude and phase images, where the magnitude image is used for diagnostic purposes, and the phase image may be used to identify regions of B\textsubscript{0} inhomogeneity\textsuperscript{125}. The complex nature of this data means it contains inherent properties such as symmetry that may be exploited to enable more efficient image acquisition and reconstruction\textsuperscript{125}. 3D MRI is an alternative to acquiring 2D images at multiple slices and may be advantageous for certain applications that
require high axial resolution images\textsuperscript{126}. The 3D acquisition technique requires a second phase-encode axis, resulting in longer scan times\textsuperscript{126}.

### 1.3.4 Chemical Shift-Encoded MRI

As introduced in section 1.3.2, chemical shift is a property that allows nuclei to be distinguished from each other based on slight differences in their resonant frequencies due to their local chemical and molecular environments\textsuperscript{125}. Perhaps the most common example of chemical shift in MRI is the shift of 3.5 ppm between water and fat protons\textsuperscript{125,140}. Water and fat are obvious initial targets of chemical-shift encoded MRI (CSE-MRI) as they have good SNR at clinical field strengths and are much more abundant than other endogenous metabolites\textsuperscript{141}. This chemical shift exists because the methyl group (CH\textsubscript{3}) on fatty acids causes spins to experience a different electron density distribution and magnetic shielding than spins on a water molecule\textsuperscript{125}. Chemical shift-encoded MRI is an imaging technique that uses multiple TEs to measure the phase variation between signals originating from different metabolites\textsuperscript{142}. CSE-MRI techniques employ modified pulse sequences to encode the chemical shift difference into the signal’s phase that can be used to achieve separation of fat and water during postprocessing\textsuperscript{140}.

While MRS techniques can detect and separate signals from fat and water, CSE-MRI has the advantage of faster acquisition times and providing spatial information\textsuperscript{142}. As long as the chemical shifts between the components are known, it is possible to calculate the TEs at which the two signals will be in-phase and out-of-phase with each other\textsuperscript{140}.

W. Thomas Dixon first introduced this method in 1984, using a modified SE sequence to acquire images using TEs when fat and water were in-phase and 180° out-of-phase (Figure 1.1)\textsuperscript{141}. The in-phase and out-of-phase images are combined to create fat-only and water-only images of anatomy\textsuperscript{126,141}. Using a conventional SE, the water and fat signals are in-phase at the echo time, but due to their different precession frequencies, they move out of phase over time\textsuperscript{141}. By shifting the readout gradient slightly during a second acquisition, it is possible to acquire the minimum signal that occurs when water and fat are 180° out-of-phase\textsuperscript{140,141}. This pioneering work is known as the two-point Dixon method and is the simplest iteration of the fat-water separation technique\textsuperscript{126}. Using
this method, the in-phase image contains the sum of signals from fat and water, while the out-of-phase image is the difference of the fat signal from water signal\textsuperscript{126}. The water-only image is useful for fat suppression in instances where the hyperintense fat signal may obscure pathology, and both images may be used to quantify the fat and water content in an area of interest\textsuperscript{140}. Since this method only requires the addition of a time delay to an otherwise conventional SE sequence, there is little to no sacrifice of spatial resolution, SNR, or imaging speed required to obtain the additional information\textsuperscript{141}. This technique is limited by $B_0$ inhomogeneity which appears as a phase error and affects the summation and subtraction approach to fat and water image construction\textsuperscript{140}.

![Diagram of MR signal in-phase and out-of-phase with TEs](image)

**Figure 1.11**: An example of the MR signal from fat and water coming in and out of phase with each other at different TEs. In a two-point Dixon acquisition, images would be acquired at the TEs that correspond to the purple boxes.

The attempt at correction for $B_0$ inhomogeneity led to the introduction of the three-point Dixon method, which acquires a third image with an in-phase TE\textsuperscript{126}. Using the three-point Dixon method, the two in-phase images are used to calculate and remove the phase acquired from $B_0$ inhomogeneities in each voxel, followed by the simple postprocessing
of the two-point method\textsuperscript{140}. Additions to the three-point method include the four-point Dixon method, which involves an additional acquisition at another TE to provide information that can be used to calculate the line width of the fat spectra\textsuperscript{143}. Another strategy involves obtaining a T$_2^*$ map, the accelerated T$_2$ decay that may be caused by microscopic magnetic susceptibilities (further discussed in section 1.1.3.6), in addition to fat, water, and B$_0$ images\textsuperscript{143}.

Modern magnet design and increased shimming capabilities led to renewed interest in the Dixon technique in the early 2000s, with the emergence of more complex algorithms including IDEAL (Iterative Decomposition of water/fat using Echo Asymmetry and Least-squares estimation)\textsuperscript{144}. The IDEAL method utilizes multiple asymmetrically acquired echoes and a more complex least-squares decomposition algorithm to separate fat and water\textsuperscript{144}. This method was introduced to minimize noise and eliminate the dependence of noise on the fat:water ratio in a voxel\textsuperscript{144}.

So far, the fat peak has been simplistically described by a single peak, but in reality, the fat signal consists of several resonant frequencies, including those originating from methylene (-CH$_2$-) and olefin (-CH=CH-) groups that, in addition to the methyl group, are present on TG molecules\textsuperscript{143}. This issue can be overcome with fat spectrum modelling, either using \textit{a priori} MRS measurements or estimating the fat spectrum from the measured CSE-MRI data\textsuperscript{145}. Not only does this modelling provide more accurate estimations of T$_2^*$ and fat/water content, but it may also be used to measure fatty acid chemical composition, which is relevant to studies of obesity, hepatic steatosis, and cancer\textsuperscript{146}. Additionally, IDEAL methods can identify and characterize brown adipose tissue and differentiate it from white adipose tissue \textit{in vivo} using the known difference in fat fraction between these compounds\textsuperscript{147}.

As more improvements were continuously made to CSE-MRI, algorithms have been introduced to address confounding factors of measurement, including relaxation parameters (T$_1$, T$_2^*$), the spectral complexity of fat, and noise biases\textsuperscript{148}. By correcting these inconsistencies, it is possible to estimate the proton density fat fraction (PDFF), defined as the ratio of the density of mobile protons from fat to the total density of mobile
protons in the sample\textsuperscript{149}. The PDFF is constructed by dividing the fat image by the sum of the fat and water images and provides information on the percentage of fat in the body\textsuperscript{148,149}. Today, PDFF is the most practical and meaningful MRI-based biomarker of tissue fat concentration based on its robustness, reproducibility, accuracy, and precision\textsuperscript{149}. The PDFF is highly correlated with \textit{ex vivo} measurements of TG concentration\textsuperscript{149}. A more complex IDEAL algorithm can be used to achieve simultaneous quantification of PDFF and T\textsubscript{2}\textsuperscript{*}\textsuperscript{150}, similar to the IDEAL technique used in Chapters 2 and 4 of this thesis to quantify these measurements. Modern developments have led to the use of CSE-MRI sequences in routine clinical settings\textsuperscript{140}.

CSE-MRI is often used for PDFF estimation in the liver, particularly in the study of NAFLD, and has been validated for use at clinical field strengths\textsuperscript{148,151}. Another popular application is its use in the assessment of T\textsubscript{2}\textsuperscript{*} and PDFF in osteoporosis\textsuperscript{152}. Aside from PDFF and fat suppression, CSE-MRI is used to produce B\textsubscript{0} maps, useful for field shimming and quantitative susceptibility mapping\textsuperscript{140,153}. T\textsubscript{2}\textsuperscript{*} mapping, which may be achieved with CSE-MRI, is used to assess the concentration of iron in tissue\textsuperscript{140}. CSE-MRI methods can be employed for non-proton imaging, including in \textsuperscript{19}F imaging\textsuperscript{154} and are demonstrated in Chapter 3 of this thesis to separate signal from hyperpolarized [\textsuperscript{1-13}C]pyruvate (see section 1.3.7.1) and its metabolites using a modified IDEAL technique\textsuperscript{155}.

### 1.3.5 Diffusion-Weighted Imaging

Diffusion-weighted imaging (DWI) is sensitive to the molecular diffusion of water molecules within the body, providing information on the microstructure of organs\textsuperscript{156}. In this context, diffusion refers to Brownian motion, or the random displacement of molecules due to movement and collisions driven by thermal energy\textsuperscript{157}. In a perfectly homogeneous medium, this displacement would be random, isotropic, and described by a Gaussian distribution dependent on the type of molecule, the temperature of the medium, and time allowed for diffusion\textsuperscript{158}. The spread, or variance, of the Gaussian distribution depends on the diffusion time interval and the diffusion coefficient (D), which represents
the magnitude of the molecule’s diffusion as a function of displacement and velocity\textsuperscript{157,158}. \(D\) is approximately equal to \(3 \times 10^{-9} \text{ m}^2/\text{s}\) for water at \(37^\circ \text{C}\textsuperscript{158}.

The complex structure of the human body does not allow for perfectly random diffusion. Instead, water molecules exist within cells and in extracellular compartments coming into contact with barriers of varying diffusivity\textsuperscript{158,159}. While extracellular water molecules experience relatively free diffusion, intracellular water molecules experience “restricted diffusion”\textsuperscript{159}. Restricted diffusion due to compartmental boundaries and molecular obstacles results in a decreased diffusion distance compared to particles experiencing unrestricted diffusion\textsuperscript{158}. Different tissues in the human body experience different diffusion properties based on characteristic cellular architecture and proportions of intra- and extracellular regions, which may be further altered by disease\textsuperscript{159}. Therefore, DWI provides a functional dimension to conventional anatomic MRI that may be used to obtain information regarding microscopic architecture and blood flow\textsuperscript{159}.

DWI uses magnetic field gradients, termed diffusion gradients, to identify the displacement of spins based on small differences in their Larmor frequencies\textsuperscript{156,159}. The most common approach involves a spin echo sequence with equal area gradient pulses applied before and after the 180\(^\circ\) refocusing pulse\textsuperscript{156}. A moving spin will accumulate phase from the diffusion gradient proportional to its displacement in the direction of the gradient\textsuperscript{156}. If there is no motion, the phase shifts accrued from the two equal diffusion gradients cancel out. If all the spins are moving coherently within the field, they acquire an identical phase. The case of diffusion is unlike these two scenarios. Instead, the spins experience a random displacement, and the phase shifts accumulated by individual spins differ from each other\textsuperscript{156}. Since the spins move between the application of the two gradients, each spin experiences a slightly different applied gradient strength each time and the spins do not successfully rephase, causing signal attenuation\textsuperscript{160}. Because of this, the resulting diffusion-weighted image displays low signal intensity in regions of high diffusion along the diffusion gradient\textsuperscript{158}. The amount of signal attenuation is dependent on how far the molecule travels between the application of the two gradients and can be described by Equation 1-7\textsuperscript{156}.
\[ M_{(b, TE)} = M_0 e^{-TE \times T_2} e^{-b \times D} \]

Where \( M_{(b, TE)} \) represents the magnetization at a specific b-value and echo time, \( M_0 \) represents the original magnetization, TE is the echo time, \( T_2 \) is the transverse relaxation constant, D is the apparent diffusion coefficient, and b represents the “b-value” used to describe the strength of diffusion gradients. The b-value is proportional to the squares of the duration and amplitude of the applied gradient and the time delay between the two diffusion gradients\(^{159}\). A fully accurate calculation of the b-value should include contributions from other gradients used in imaging, such as the readout gradient, but these effects are usually overshadowed by the diffusion-weighted gradients at sufficiently high b-values. The degree of diffusion weighting is dependent on the b-value, where a higher b-value results in a more pronounced diffusion-related signal attenuation\(^{159}\). Therefore, the signal attenuation displays a mono-exponential decay with respect to b-value that positively correlates with the diffusion coefficient\(^{157}\). Different b-values may enhance different properties of the tissue. For example, longer intervals between gradients will increase the distinction between diffusion occurring in different directions\(^{158}\).

If we assume the diffusion in the image can be described by a free diffusion model with a 3D isotropic Gaussian distribution, we can estimate the diffusion coefficient, referred to in this model as the apparent diffusion coefficient (ADC)\(^{158}\). The ADC can be calculated from at least two measurements using Equation 1-8\(^{158}\):

\[ ADC = -b \times \ln \left( \frac{DWI}{b_0} \right) \]

Here, DWI is the diffusion-weighted image intensity for a b-value, b, and \( b_0 \) represents signal from a reference image with no diffusion weighting\(^{158}\). The ADC is dependent on the direction of diffusion encoding. To overcome this directional dependence, three orthogonal measurements may be applied and averaged in the calculation of ADC\(^{158}\). Tissues with more restricted diffusion appear hypointense on an ADC map, allowing for
a more intuitive interpretation compared to DWI\textsuperscript{159}. The ADC provides limited information, especially in anisotropic anatomy like axon bundles in the brain\textsuperscript{158}. More advanced techniques that account for greater degrees of freedom, including diffusion tensor imaging, q-Ball imaging, and diffusion spectrum imaging, make use of longer acquisitions and more complex postprocessing to gain information about the orientation and anisotropy of diffusion\textsuperscript{158}.

Clinical use of DWI became possible in the 1990s with the availability of echo-planar imaging (EPI), which provided a fast sequence that negated issues associated with motion artifacts\textsuperscript{159}. Pathologic processes that alter the structural organization on a cellular level are targets of diagnosis via DWI technology\textsuperscript{156}. Water diffusion can be modulated by cell density, cell membrane orientation, or cell size; for example, cell swelling as observed in stroke\textsuperscript{161}. DWI is commonly used in brain imaging and has been used to improve knowledge of brain connectivity and research conditions including multiple sclerosis, dyslexia, schizophrenia, and trauma\textsuperscript{156,162}. DWI has also been implemented clinically for use in identifying ischemia \textit{in vivo} and has had a large clinical impact in the management of stroke\textsuperscript{156,162}. DWI is widely used to detect metastases in areas including the breast, prostate, and liver\textsuperscript{162}. While uncommon, recent studies have applied DWI to study the placenta in human and animal models to measure blood movement through the placenta to the fetus\textsuperscript{163}.

DWI is limited in its accuracy and image quality because the algorithms used in reconstruction make assumptions that do not hold in reality, including perfect field homogeneity, infinitely fast gradient changes, and perfectly shaped RF pulses\textsuperscript{159}. DWI is susceptible to artifacts such as ghosting, blurring, and distortions\textsuperscript{159}. T\textsubscript{2} shine-through may occur from tissues with long T\textsubscript{2} times that retain signal on high b-value images, resulting in artificially bright signals on ADC maps\textsuperscript{159}. Conversely, the T\textsubscript{2} blackout effect results in low signals on an ADC map in regions with very low T\textsubscript{2} signal, for example areas lacking water protons, that may mask the diffusion sensitivity\textsuperscript{159}. The reproducibility of ADC values is questionable, as DWI typically suffers from low SNR and artifacts common to single-shot EPI sequences\textsuperscript{159}. The implementation of non-EPI sequences for DWI has helped to overcome some of these limitations\textsuperscript{159}.
1.3.5.1 Intravoxel Incoherent Motion (IVIM)

It has been acknowledged that the ADC model describing a mono-exponential relationship between the DWI signal and b-value does not perfectly describe the behaviour of water in tissue\(^{164}\). A more appropriate and sophisticated model, referred to as intravoxel incoherent motion (IVIM), can separate the DWI signal into two mechanisms – diffusion and perfusion\(^{165}\). The concept behind IVIM was introduced in 1986 when it was discovered that the collective low velocity motion of blood in randomly oriented capillaries mimics the diffusion process\(^{165}\). IVIM enables the estimation of quantitative parameters that independently reflect tissue diffusivity and microcapillary perfusion without the need for novel DWI sequences or contrast agents\(^{157,164}\).

At low b-values (0-100 s/mm\(^2\)), the measured signal attenuation in DWI results from water diffusion in tissues and blood within the capillary network\(^{164}\). Since the blood perfusing these capillaries does not move in a coherent orientation, it can be thought of as “pseudo-diffusion” that depends on the velocity of the flowing blood and the vascular architecture\(^{164}\). The amount that the pseudo-diffusion effect contributes to the signal attenuation is dependent on the b-value and, as opposed to ADC estimation, a larger number of b-values are required to estimate the pseudo-diffusion coefficient (\(D^*\))\(^{164}\). Conveniently, \(D^*\) is close enough to D to be sensitive to DWI, resulting in a signal decay sensitive to tissue diffusion and blood microcirculation\(^{157}\). Due to the comparatively faster velocity and larger displacement of molecules in the microvasculature during gradient application, the signal attenuation from pseudodiffusion is an order of magnitude greater than that from tissue diffusion\(^{164}\). This means that at sufficiently high b-values (b >100 mm/s), the measured signal attenuation is mostly or completely a result of the tissue diffusion, and at these b-values, the ADC is approximately equal to D (Figure 1.12)\(^{164}\).
Therefore, the IVIM model uses a biexponential function to describe the DWI data, assuming that the measured signal attenuation is due to a combination of tissue diffusion and perfusion effects. Equation 1-9 describes the biexponential decay of the MRI signal \((S)\) from the original signal \((S_0)\) at a certain b-value \((b)\), where \(D\) represents the water diffusion coefficient in the tissue, \(D^*\) represents the pseudo-diffusion coefficient in the microvasculature, and \(f\) represents the perfusion fraction or fraction of signal attenuation resulting from perfusion in the microvasculature.

\[
\frac{S}{S_0} = f \times e^{-b(D*D)} + (1 - f) e^{-b(D)}
\]

The perfusion fraction may be thought of as the percent of a voxel volume occupied by capillaries and is used to describe the vascularity of the tissue. Fitting the MRI signal to this equation at multiple b-values allows for the estimation of \(D\), \(D^*\), and \(f\), which can each be used to construct parametric maps.

For this technique to be useful clinically, it must be used in regions of the body that display a bi-exponential response to DWI. IVIM may have a clinical application as an alternative to perfusion MRI in patients with contraindications to contrast agents. Perfusion imaging is important for evaluating microstructure heterogeneity and
The most studied and promising clinical application of IVIM is in oncology, as a collection of research has shown that IVIM measurements demonstrate distinct characteristics in tumours and may be used to monitor treatment efficacy. Tumours in the prostate, breast, kidneys, pancreas, liver, and head and neck regions have been identified as potential diagnostic targets of IVIM as they display distinct $D$, $D^*$, and/or $f$ values compared to healthy tissue in these areas. In the liver, IVIM has been used to identify fibrosis and cirrhosis, highlighting its potential use in identifying $D^*$ as a biomarker of NASH. IVIM is particularly useful in the kidneys as diffusion parameters can differentiate between the cortex and medulla and may also be used to predict renal function. IVIM is sensitive to flow other than blood and may be used to investigate tubular flow in the kidneys, though it may be difficult to disentangle this signal from the microvascular flow. Any type of active transport from glandular secretion, including breast ducts, salivary glands, and pancreas glands, may be a target of investigation using IVIM. Like DWI, an obvious application of IVIM is studying the brain, as regional cerebral blood flow is closely related to functional brain activity and may be useful in studies focusing on trauma or stroke. IVIM techniques may also be used in conjunction with functional MRI (fMRI) by removing blood flow effects to improve the spatial resolution of activation maps.

There are some limitations to IVIM, including reproducibility issues as there is no consensus on the number of b-values that should be used for measurement. The MRI platform must allow for customized b-values during acquisition, as IVIM requires multiple low b-values to avoid errors and SNR variations. IVIM has a variable sensitivity to vessel size, which should also be considered when selecting b-values for different regions in the body. More sophisticated data processing may help decrease uncertainty in parameter estimation and lead to shorter acquisition times. To provide more useful quantitative information, the relationship between IVIM parameters and blood volume and flow estimates from gold-standard approaches must be clarified. This quantification may not be straightforward as the perfusion fraction measured with IVIM is likely influenced by other physiologic processes such as glandular secretion and ductal flow that may be difficult to isolate from the microvasculature contribution.

Future work related to IVIM includes manipulating this technique for application in MR
elastography to measure tissue shear stiffness and angiography to detect laminar flow in larger vessels\textsuperscript{157}.

1.3.6 $T_2^*$ MRI

When imaging physiological tissue, local $B_0$ inhomogeneities and differences in magnetic susceptibilities within the tissue result in a shortened $T_2$ relaxation time, known as the apparent transverse relaxation time, $T_2^*$ (Figure 1.13)\textsuperscript{130}. This effect is caused by variations in the local magnetic field that randomly cause nuclei to experience phase shifts and local diffusion, leading to accelerated phase incoherence and transverse relaxation\textsuperscript{167}. While it may seem that correcting for $T_2^*$ in images would be beneficial to measure $T_2$, $T_2^*$ is useful in itself for measuring functional parameters such as blood oxygen saturation. Oxyhemoglobin is weakly diamagnetic and does not produce a magnetic moment, while deoxyhemoglobin is paramagnetic due to the presence of four unpaired electrons\textsuperscript{168}. As paramagnetic substances cause local field distortions, the $T_2^*$ is shortened by the presence of deoxygenated hemoglobin in the blood, which causes detectable susceptibility changes within vessels that have high concentrations of deoxyehmoglobin\textsuperscript{126}. Therefore, on a $T_2^*$-weighted image, areas with a concentration of deoxygenated blood appear dark\textsuperscript{131}. $T_2^*$ maps can also be produced where the $T_2^*$ value is quantitatively measured and mapped to each voxel in the image\textsuperscript{131}. Unlike spin echo sequences that eliminate effects of field inhomogeneities with the 180° refocusing pulse, gradient echo sequences do not correct for this effect and therefore depend on $T_2^*$\textsuperscript{131}. $T_2^*$ weighting may be emphasized by acquiring an image using a small flip angle, long TR, and long TE\textsuperscript{126,131}.

![Figure 1.13: Graph of $T_2$ and $T_2^*$ relaxation curves.](image)
It should be noted that blood oxygenation is also related to the inherent $T_2$, as the transverse relaxation rate ($1/T_2$) has a quadratic dependence on deoxyhemoglobin fraction$^{169}$. This quantitative relationship has been validated using *in vivo* animal studies over multiple oxygenation conditions$^{169}$. $T_2$ measured with a SE sequence is sensitive to spin diffusion caused by the field inhomogeneity, whereas $T_2^*$ measured using a GE sequence is sensitive to spin dephasing and spin diffusion$^{169}$. Because of this, measurable $T_2^*$ changes can be detected from smaller variations in blood oxygen saturation, making $T_2^*$ the more sensitive and preferred method of detection for many oxygenation studies$^{169}$, including the work presented in Chapter 4 of this thesis.

Although $T_2^*$ is primarily dependent on oxygenation, it is also affected by tissue characteristics, including villus density, inhomogeneous distribution of oxygenated blood, and the presence of other paramagnetic molecules$^{167}$. Furthermore, the $T_2^*$ signal is affected by macroscopic field inhomogeneities, such as magnet imperfections, poor shimming, and tissue-air interfaces that should be taken into account if possible$^{170}$. Therefore, $T_2^*$ alone cannot be used as a direct quantitative measure of blood oxygen saturation, but rather as an indirect measure of changes in blood oxygen saturation *in vivo*$^{169}$. $T_2^*$ is dependent on the blood oxygen saturation and partial pressure of oxygen in and around the blood vessels, both of which are useful quantitative measures related to oxygen availability *in vivo*$^{170}$. Quantification of these measurements is possible through animal and *ex vivo* studies that account for factors such as the hemoglobin concentration, blood flow, and blood volume fraction within each voxel$^{170–172}$.

There are many clinical applications of $T_2^*$-weighted MRI and $T_2^*$ mapping for detecting hemoglobin oxygenation levels throughout the body. One focus of this application is in myocardial imaging to detect damage due to infarction and differentiate between irreversible necrosis and reversible oedema that would benefit from restoring perfusion to the area$^{173}$. $T_2^*$ has been used in placental imaging to detect baseline differences in oxygenation between fetal and maternal areas, differences between normal and dysfunctional placentae$^{167}$, and to evaluate the placenta’s response to hyperoxygenation$^{172}$. Other than detecting blood oxygen saturation, $T_2^*$-weighted
imaging may be used to visualize hemorrhage, arteriovenous malformation, cavernoma, thrombosed aneurysm, calcification, and iron deposits in various tissues\(^{131}\).

The relationship between dynamic changes in T\(_2^*\) as a response to neural activity is termed the blood oxygenation level-dependent (BOLD) effect and was first observed in the 1990s\(^{126}\). BOLD contrast reflects changes in cerebral blood volume, blood flow, and oxygen consumption\(^{130}\). The BOLD effect is the backbone of fMRI work, the most common application of T\(_2^*\)-based imaging\(^{126}\). In fMRI studies, the relative local change in the raw T\(_2^*\)-weighted signal is measured over time to visualize the increased cerebral blood flow in response to neuronal activity in a region of interest in the brain\(^{131,167}\). Quantitative BOLD techniques utilize complex biophysical modelling to distinguish information from cerebral venous blood volume oxygen saturation\(^{174}\).

The long TEs required for T\(_2^*\)-weighted imaging pose technical difficulties arising from body motion and pulsatile blood flow\(^{169}\). Despite any challenges in applying this technique, T\(_2^*\) MRI provides an alternative imaging method to positron emission tomography (PET) for mapping blood oxygenation that does not require ionizing radiation and is more widely available\(^{174}\). T\(_2^*\) imaging also provides advantages over the standard procedure of pulse oximetry, which is limited to superficial capillary sampling and invasive blood sampling, which poses risks when used to gather information about deep vessels\(^{169}\).

### 1.3.7 Hyperpolarized MRI

Imaging metabolic biomarkers provides the opportunity to probe the state of a biological system. Although MRI is often used to detect signal from the \(^1\)H nuclei on water molecules, this technology can detect signals from other nuclei, potentially enabling imaging of metabolically important biomolecules. For many reasons, non-hydrogen MRI is challenging. Firstly, hydrogen has the highest gyromagnetic ratio of all magnetically sensitive, stable nuclei. This makes other NMR-sensitive nuclei challenging to use for MR imaging as, compared to hydrogen nuclei, they have smaller polarizations. While spectroscopy, especially at ultra-high field strengths, may be possible using lower \(\gamma\)
nuclei, imaging requires faster acquisitions at lower, clinical field strengths, making \textit{in vivo} non-proton imaging difficult\textsuperscript{137}.

Secondly, as human bodies are composed of approximately 75\% water, the concentration of hydrogen nuclei dominates the concentration of other nuclei in the body. While \textsuperscript{15}N is present in many biochemically interesting molecules, these metabolites exist in concentrations below thresholds necessary for detection using traditional MRI methods\textsuperscript{175}. One may assume carbon would be high enough in concentration due to the large number of hydrocarbons present in living systems; however, the natural abundance of the magnetically sensitive \textsuperscript{13}C isotope is only 1\%. In contrast, hydrogen has almost 100\% abundance of its magnetically sensitive isotope \textsuperscript{1}H. Combining the low abundance of \textsuperscript{13}C with its low $\gamma$, thermal NMR measurements of \textsuperscript{13}C can only achieve 0.0176\% of the sensitivity of \textsuperscript{1}H\textsuperscript{176}. Despite these limitations, non-proton MRI has long been desired as a method to detect specific enzyme-catalyzed biological reactions as altered metabolism is central to many human diseases, including cancer, cardiovascular disease, diabetes, and a variety of inflammatory conditions\textsuperscript{175,177}. Attempts have been made to increase NMR sensitivity for non-proton nuclei to make non-proton MRI a practical clinical imaging tool.

A successful method of boosting the MR signal of nuclei is external nuclear polarization, otherwise known as hyperpolarized MRI (HP MRI)\textsuperscript{124}. Hyperpolarization is a process in which nuclei are aligned to a polarization many orders of magnitude greater than what would normally be possible at thermal equilibrium \textit{in vivo}\textsuperscript{124}. If the hyperpolarized state can be maintained long enough for uptake and metabolism in cells at the site of interest, the hyperpolarized probe may be useful for qualitative and quantitative measurements of \textit{in vivo} metabolism in various applications. A few methods may be used to boost the signal of these nuclei temporarily. Some methods are unique to hyperpolarized gases and some are used for liquid solutions containing hyperpolarized nuclei. Hyperpolarization methods most commonly used in preclinical and clinical studies include spin-exchange optical pumping (SEOP), parahydrogen induced polarization (PHIP), signal amplification by reversible exchange (SABRE), and dissolution dynamic nuclear polarization (d-DNP)\textsuperscript{178}. 
SEOP methods are used to produce hyperpolarization in noble gases, most commonly in $^3$He and $^{129}$Xe. The SEOP method uses circularly polarized resonant light aimed at the vapour of an alkali metal in a glass cell to transfer the photons’ spin angular momentum absorbed by the metal vapour to the alkali metal atoms. This transfer produces spin polarisation in the valence electrons of these atoms via optical pumping. The setup also contains a large amount of the target noble gas to facilitate collisions between the alkali metal atoms and noble gas atoms that result in a transfer of the electron-spin polarization to the nuclei of the noble gas.

PHIP is a polarization method that relies on the pairwise incorporation of hyperpolarized parahydrogen ($^2$H with proton spins in opposing directions) into the molecule of interest, usually across unsaturated chemical bonds. PHIP does not require the use of radicals, and instead, proton hyperpolarization is transferred to carboxyl $^{13}$C using RF-based or field-cycling approaches. An advantage of PHIP is that it can be achieved quickly, with hyperpolarization transfer occurring in a matter of seconds, and it may be stored for months at room temperature. PHIP is limited in its application as it is confined to use in probes that are unsaturated and have appropriate symmetry to incorporate the parahydrogen into the molecule structure.

SABRE is related to PHIP but differs in technique as the parahydrogen does not chemically react with the target molecule. Instead, the parahydrogen and substrate are brought into temporary contact via a transition metal-based host. In this state, the high spin order of parahydrogen is transferred to net polarization in a matter of seconds when exposed to a sudden change in the external magnetic field. Though promising, the SABRE technique, introduced in 2009, is relatively new and d-DNP remains the most commercialized and utilized polarization technique for liquid-state probes.

The final polarization method, d-DNP, is the hyperpolarization technique used in Chapters 2 and 3 of this thesis. This technique involves the transfer of polarization from unpaired electrons to the target nuclei via low temperatures (near absolute zero) and strong static magnetic fields. To facilitate this, a stable free radical is mixed in a solution with the biomolecule probe to produce a glass matrix at a temperature ~1 K.
inside a magnetic field of ~3-5 T. A high-powered microwave then irradiates the glass matrix at the electron resonant frequency to enable the transfer of the spin polarization from electrons to the nuclei of interest in the solid state. Finally, the solid-state solution is rapidly dissolved and warmed, ready for injection into the subject and subsequent imaging. d-DNP methods have been shown to increase polarization by 10,000 in liquid state NMR.

HP MRI is limited in the types of probes that may be used for imaging. The nuclei must be stable and able to produce net magnetization in an external magnetic field. The target nuclei must also be isolated from other magnetic moments, such as unpaired electrons (i.e. O₂) or other magnetic nuclei on the molecule to prevent loss of alignment with the external magnetic field. Target nuclei found in vivo, such as ¹H, ¹³C, ¹⁵N, and ³¹P, are obvious choices for biological applications of HP MRI. If needed, these nuclei may be incorporated onto endogenous biomolecules via isotope enrichment. These hyperpolarized biomolecule probes may then be administered to the subject at near or supra-physiological concentrations without negative effects and provide information on native metabolic processes in vivo. Non-toxic molecules such as ¹⁹F and ²⁹Si may also have application for HP MRI, and inert gases ³He and ¹²⁹Xe are chosen as target nuclei for HP imaging of airways.

![Diagram](image)

Figure 1.14: Example of the enrichment of NMR-sensitive nuclei followed by hyperpolarization

The HP probe must be selected to be biologically relevant, have the capacity to attain high polarization levels, and have relatively long T₁ decay constants. T₁ decay is an important factor for probe selection as there is a lower limit to the time scale required for image acquisition, and the rate of decay impacts the choice of acquisition techniques,
suitability for 3D imaging, and image quality\textsuperscript{178}. Biologically relevant applications of the probes may vary and can include measurement of cellular uptake, metabolic pathway kinetics, redox state, ion concentrations, pH, drug efficacy, or oncologic signalling\textsuperscript{186}. Metabolically active probes such as $^{13}$C-enriched pyruvate may be used to acquire signals from both the injected molecule and its downstream metabolites, as the hyperpolarized nuclei are transferred between metabolites \textit{in vivo}\textsuperscript{185}. The known chemical shifts of each metabolite may be used to separate their acquired signals during image reconstruction using chemical shift imaging or CSE-MRI techniques\textsuperscript{185}.

Hyperpolarized MRI technology is often compared to nuclear medicine imaging modalities such as PET, as both are functional imaging methods used to probe biodistribution and cellular uptake of biologically relevant molecules \textit{in vivo}. Like PET, HP MRI requires anatomic reference images\textsuperscript{144} and conveniently, anatomic MRI acquisitions may be performed during the same exam and patient setup to allow for simple image registration. Using MRI for reference images also has the advantage of excellent soft-tissue contrast to localize metabolically active regions indicated by HP MRI. PET exhibits some benefits over HP MRI, including its sensitivity to nanomolar concentrations of tracer, allowing for administration of a very small amount of radiotracer that is unlikely to affect \textit{in vivo} metabolism\textsuperscript{187}. HP MRI has some advantages over PET, as it does not produce ionizing radiation, enabling safety for use in vulnerable populations, including children and pregnant women\textsuperscript{177}. Additionally, unlike HP MRI, PET techniques are limited to providing information on the uptake and perfusion of the injected biomolecule and cannot provide information on the downstream metabolites of the probe\textsuperscript{177}. Combining HP MRI and PET information may prove beneficial as the two methods provide complementary information, especially in cancer metabolism\textsuperscript{188}. At centers that possess the equipment necessary for HP MRI and PET, it is estimated that the cost of clinical administration of these exams would be similar, negating any financial advantages of either technology\textsuperscript{177}.

Although the concept behind d-DNP was first explored in 1953\textsuperscript{189}, technological advancements in the construction of compact, high strength magnets have only recently made commercially available polarizers a reality, including polarizers capable of
producing sterile injections suitable for clinical use\textsuperscript{190}. Though not yet a routine clinical imaging modality, HP MRI has been used in clinical studies since 2013\textsuperscript{191}. Even after almost a decade of clinical studies, HP technology is still in its infancy, and polarizer methods should continue to be optimized for cost-effective, reliable polarizations that can be achieved faster and at higher levels of polarization\textsuperscript{188}. Other improvements that should be prioritized are methods for prolonged storage of HP probes, enriched probes with longer T\textsubscript{1}s, and established routine protocols for quality assurance, calibration, and data analysis\textsuperscript{175}. Improvements in hardware, including commercially available dual-tuned MRI coils and higher performance gradients, would also be beneficial for advancing the field of HP MRI\textsuperscript{188}. Novel techniques to improve acquisition speed, spatial and temporal resolution, and motion are still being developed to improve HP MRI's feasibility in clinical settings\textsuperscript{124}. Additionally, kinetic modelling techniques are being explored and improved upon to obtain quantitative information related to metabolic rates and uptake; external validation of these measurements using \textit{ex vivo} samples is integral to highlighting the clinical use of quantitative HP MRI\textsuperscript{175}. Moving forward, it will be necessary to continue publishing literature concerning the biomedical value and specific human applications of HP MRI to encourage further support of translating this modality to eventual clinical use.

1.3.7.1 Hyperpolarized Pyruvate MRI

Projects described in Chapters 2 and 3 specifically utilize hyperpolarized MRI to gain information about pyruvate's biodistribution and metabolism \textit{in vivo}. Pyruvate is an end product of glycolysis, which involves the metabolism of glucose – a simple sugar and the most abundant monosaccharide carbohydrate\textsuperscript{14,192}. Glucose is the most important energy source in all organisms, and dietary glucose may be sourced from many fruits, vegetables, and grains\textsuperscript{192}. Pyruvate is less commonly generated via the oxidation of lactate, transamination of alanine, or conversion from malate\textsuperscript{14,193}. Pyruvate is a key molecule in cellular energy production and is critical for mitochondrial ATP production\textsuperscript{14}. Normally, pyruvate enters the mitochondrial matrix where it is converted into acetyl coenzyme A (acetyl-CoA) via pyruvate dehydrogenase (PDH) or oxaloacetate via pyruvate carboxylase, which may both enter the tricarboxylic acid (TCA) cycle,
otherwise known as the citric acid cycle or Krebs cycle, to generate ATP via oxidative phosphorylation\textsuperscript{193}. Under anaerobic conditions, pyruvate may be reduced to lactate in the cytosol via lactate dehydrogenase (LDH), which also produces NAD$^+$ from NADH\textsuperscript{194}. NAD$^+$ is necessary to maintain glycolysis, and as such, the conversion of pyruvate to lactate indirectly facilitates energy production via the promotion of anaerobic glycolysis, though this method of energy production is less efficient than the TCA cycle\textsuperscript{194}.

There are two carbons on the pyruvate molecule that may be enriched with a $^{13}$C nucleus and used for HP MRI. The choice of which carbon is targeted will determine which metabolites the enriched carbon is transferred to and subsequently which energy production pathways may be understood via imaging. Focusing on $^{13}$C enrichment of the C2 atom on pyruvate, we can probe information on the oxidative phosphorylation pathway and specifically the TCA cycle where [2-$^{13}$C]pyruvate and its downstream metabolites [2-$^{13}$C]lactate, [1-$^{13}$C]citrate, [1-$^{13}$C]acetyl-carnitine, and [5-$^{13}$C]glutamate contribute to the MRI signal\textsuperscript{124,195,196}. Acetyl-CoA retains the enriched C2 nuclei from pyruvate and may be metabolized into acetyl-carnitine, which does not enter the TCA cycle, or citrate, an intermediate of the TCA cycle that goes on to be metabolized into $\alpha$-ketoglutarate. $\alpha$-ketoglutarate is in metabolic flux with glutamate, which exits the TCA cycle\textsuperscript{197}. TCA cycle intermediates including acetyl-CoA and $\alpha$-ketoglutarate carry the enriched C2 nuclei but suffer from a low signal that is not typically sensitive enough for MRI detection. Both C1 and C2-enriched pyruvate may be used to detect signal from the amino acid alanine, which is synthesized from pyruvate via the ALT enzyme\textsuperscript{176,198}. Alanine is usually produced in lower quantities than lactate; however, pyruvate’s conversion to alanine is a useful biomarker for tissues in the liver and skeletal muscles heavily involved in alanine metabolism\textsuperscript{124}. A limited number of studies have focused on using HP [2-$^{13}$C]pyruvate MRI due to the relatively low oxidative phosphorylation activity in many tissues, resulting in low signal and difficulty imaging these TCA cycle intermediates\textsuperscript{124}.

Due to its longer $T_1$ and higher capacity for polarization, [1-$^{13}$C]pyruvate is the most common probe used in hyperpolarized MRI\textsuperscript{124}. Chapters 2 and 3 focus on [1-
\(^{13}\text{C}\)pyruvate HP MRI, allowing us to gain information on three main metabolic fates of the pyruvate molecule, as demonstrated in Figure 1.15. Similar to C2-enrichment, the C1-enriched nuclei provides signal from lactate and alanine. In this case, the signal is provided by the C1-enriched carbon on these molecules\(^{198}\). Since the C1 nucleus is not transferred to acetyl-CoA, it does not flow into the TCA cycle, preventing direct detection of downstream metabolites in the TCA cycle\(^{199}\). Instead of being carried to acetyl-CoA, the C1 from pyruvate is transferred to carbon dioxide during this reaction and is then rapidly converted to bicarbonate. \([1-^{13}\text{C}]\text{bicarbonate}\) can be measured with HP MRI, giving us an indirect measure of the proportion of pyruvate being used to drive the TCA cycle\(^{188}\).

![Figure 1.15: Schematic of pyruvate metabolism highlighting the fate of the \([1-^{13}\text{C}]\text{pyruvate}\) nuclei](image)

It should be noted that C1 may enter the TCA cycle through other means, specifically pyruvate's conversion into oxaloacetate, which may then be converted to aspartate and exit the TCA cycle or be converted to other TCA cycle intermediates – malate and fumarate\(^{185}\). Due to the short \(T_1\) and/or rapid metabolism of these biomolecules, their signal is not usually visible with HP MRI, and applications primarily focus on imaging pyruvate, lactate, alanine, and bicarbonate\(^{188}\). Signal from these metabolites may be used
to estimate metabolic kinetics, including modelling metabolic rate constants associated with the enzymatic activities of PDH, LDH, and ALT in vivo. Quantification of these constants from imaging data is complex, with many uncertainties including polarization levels, $B_1$ inhomogeneity, transmitter/receiver gain settings, and $T_1$ relaxation. There is currently no consensus on the best method of quantifying HP metabolic data, and this remains a future goal of HP [1-$^{13}$C]pyruvate MRI.

There are many HP [1-$^{13}$C]pyruvate MRI applications, with the majority of projects centred around tumour detection and characterization, as the Warburg effect causes altered and distinct glucose metabolism in cancer cells. Specifically, the Warburg effect is a hallmark of cancer, presenting as a shift from aerobic to anaerobic metabolism in tumour cells as tumours expand beyond the local blood supply’s perfusion and oxygenation capabilities and rely on anaerobic glycolysis for cellular energy production. The first human study involving hyperpolarized [1-$^{13}$C]pyruvate MRI was done in prostate cancer patients in 2013 and demonstrated successful translation of this technology to clinical research. This study confirmed increased lactate production as a characteristic of tumour cells while also demonstrating this technique’s safety in human patients. Preclinical studies using HP [1-$^{13}$C]pyruvate MRI have identified lactate and alanine as potential biomarkers for prostate cancer, and the lactate/pyruvate ratio has been correlated with tumour grade in the prostate. Other sites targeted for tumour detection and treatment response include the brain, kidneys, and breast.

Although tumour imaging is the most popular application of HP [1-$^{13}$C]pyruvate MRI, the probe has also been used to characterize metabolic patterns in models of acute injury, inflammation, and normal in vivo metabolism. HP [1-$^{13}$C]pyruvate MRI has been used to examine the metabolic profile of a healthy heart and changes to this profile in cardiac ischemia cases, resulting in increased lactate and decreased bicarbonate production. In addition to brain cancer, HP MRI has been used to characterize metabolic changes occurring in post-traumatic brain injuries, with findings of lowered bicarbonate production suggesting impaired mitochondrial pyruvate metabolism due to local decreases in aerobic respiration. The kidneys typically have a high $^{13}$C signal as they receive 20% of the cardiac output, making them a target for HP [1-$^{13}$C]pyruvate MRI to
study the metabolism of healthy kidneys and metabolic changes with respect to blood glucose levels in diabetes \textsuperscript{124}. The liver is another target for [1-\textsuperscript{13}C]pyruvate as it is the most metabolically active organ in the body, and pyruvate metabolism has been used to probe information about hepatic tumours and non-alcoholic fatty liver disease \textsuperscript{205}. HP inert gases are commonly used for imaging the airways, but HP [1-\textsuperscript{13}C]pyruvate has also been used in the lung as an angiography tool for pulmonary vasculature. Since HP [1-\textsuperscript{13}C]pyruvate MRI of the lung is challenging due to low tissue density, B\textsubscript{0} inhomogeneity, and motion, most studies are done on \textit{ex vivo} perfused lung models of ischemia and reperfusion \textsuperscript{124}. HP [1-\textsuperscript{13}C]pyruvate MRI has also been used in unique applications such as monitoring placental metabolism \textsuperscript{206}. Lastly, skeletal muscle metabolism may be probed using HP [1-\textsuperscript{13}C]pyruvate MRI and used for comparison with metabolic profiles in other organs or functional experiments of muscle activation \textsuperscript{124}.

1.4 Thesis Outline

This thesis is motivated by a need to understand the complex and often unknown effects of diet on the body, particularly in relation to liver and placental function. Advanced MRI techniques capable of providing structural and functional information about the areas of interest were employed in an animal model of Western diet-induced metabolic disease. The main objective of this thesis was to investigate the effects of the WD on anatomy \textit{in vivo}, using a variety of measurements. Technical improvements in cutting-edge hyperpolarized MRI were applied to improve the investigation of \textit{in vivo} metabolism in real time. The major themes of this thesis are represented in Figure 1.16.
Chapter 2 of this thesis presents work adapted from an original research manuscript entitled “In vivo magnetic resonance spectroscopy of hyperpolarized [1-13C]pyruvate and proton density fat fraction in a guinea pig model of non-alcoholic fatty liver disease development after lifelong Western diet consumption”\(^1\). The purpose of this project was to use hyperpolarized MRS and PDFF measurements to investigate the effects of a chronic, lifelong exposure to the WD in a guinea pig model resulting in non-alcoholic fatty liver disease. It was hypothesized that the WD would result in the development of NAFLD in association with altered pyruvate metabolism. Animals fed a lifelong WD had increased PDFF in the liver and demonstrated abnormal hepatic pyruvate metabolism indicated by an increased rate of lactate production. PDFF and HP MRS results were significantly correlated with ex vivo measurements of TG concentration and enzyme activity.

Chapter 3 is adapted from an original research article entitled “Optimizing SNR for multi-metabolite hyperpolarized carbon-13 MRI using a hybrid flip-angle scheme”\(^2\). This project aimed to improve the SNR of hyperpolarized \(^{13}\text{C}\) MRI of [1-13C]pyruvate. This was achieved using a multi-spectral variable flip angle (msVFA) scheme where the spectral profile and flip angle varied dynamically with time to be optimized for each
metabolite. The novel msVFA scheme was compared to a constant flip angle (CFA) scheme through simulations and \textit{in vivo} experiments in pregnant guinea pigs where regions of interest were placed in the placenta, maternal liver, and maternal kidneys. \textit{In vivo} experiments showed significant increases in SNR using the msVFA technique relative to CFA for all metabolites and regions of interest.

Chapter 4 of this thesis describes an original research project prepared as a manuscript for submission to a peer-reviewed scientific journal titled “Perfusion and oxygen saturation of the mid-pregnancy and near-term placenta measured by magnetic resonance imaging in a guinea pig model of pregnancy following lifelong Western diet consumption”. The purpose of this work was to investigate the effect of a lifelong maternal WD exposure on placental structure and function at mid- and near term in a guinea pig model of pregnancy. T$_2^*$ mapping and diffusion-weighted imaging techniques were applied to gain information on placental oxygen saturation, diffusion, and perfusion \textit{in vivo}. Sows fed a lifelong WD displayed higher placental perfusion, lower placental oxygen saturation, and decreased placental efficiency compared to sows on a control diet. These results suggest that hypoxic conditions induced by the WD led to placental vascular adaptations resulting in increased blood perfusion at mid-gestation and improved oxygen saturation at late gestation.

Chapter 5 concludes this thesis with a summary of the work presented within and suggestions for future work related to this thesis.
Chapter 2

2 In vivo magnetic resonance spectroscopy of hyperpolarized \[1-^{13}C\]pyruvate and proton density fat fraction in a guinea pig model of non-alcoholic fatty liver disease development after lifelong Western diet consumption

2.1 Introduction

With increasing consumption of the 'Western diet' (WD) that is high in fat and sugar, there has been a corresponding increased incidence of non-alcoholic fatty liver disease (NAFLD) and comorbidities in Western society in both lean and overweight/obese populations\(^4\). NAFLD has become the leading cause of chronic liver disease in developed nations, imposing significant burdens on healthcare systems and decreasing overall life expectancy\(^4\). The ability to non-invasively assess liver metabolism may provide a means of monitoring NAFLD severity as it has been reported that altered pyruvate metabolism, specifically increased lactate production, is an indicator of liver disease\(^14\). The increased production of lactate from pyruvate is an indication of the shift from oxidative metabolism to anaerobic glycolysis and is associated with liver damage\(^207\). Elevated lactate in the liver, induced by a high-fat diet, has been found in obese mice\(^205\) and may be linked to a disturbance in hepatic lipid synthesis\(^208\). The ability to measure lactate production \textit{in vivo} would prove useful for observing NAFLD's effects on liver damage, stress, and lipid accumulation.

MRI is used to evaluate structure, function, and composition of organs, making it an ideal method for characterizing liver disease\(^209\). Chemical shift-encoded imaging is an MRI technique that can be used to separate signal from fat and water within the body, enabling \textit{in vivo} measurements of fat fractions in the liver\(^209\). Additionally, MRI can detect signal from nuclei besides hydrogen, allowing for the selective imaging of certain biomolecules of interest\(^210\). Carbon-13 \((^{13}C)\) magnetic resonance spectroscopy (MRS) has historically been used to investigate glycolysis but is limited by inherently low sensitivity and long
acquisition times\textsuperscript{210}. To address these limitations, hyperpolarized $^{13}$C MRS is used to temporarily boost the signal-to-noise ratio, allowing for rapid acquisition of the spectroscopy signal from $^{13}$C-enriched substrates\textsuperscript{155}. By enriching pyruvate with $^{13}$C and using hyperpolarized technology to significantly enhance the MR signal, it is possible to inject and subsequently image the distribution of [1-\textsuperscript{13}C]pyruvate \textit{in vivo} in real-time\textsuperscript{155}. Additionally, this technique allows us to simultaneously acquire and subsequently differentiate signals from the pyruvate molecule and its downstream metabolites that retain the $^{13}$C nuclei over the acquisition duration\textsuperscript{155}. Concentrations and time curves of each metabolite can be quantified, allowing for examination of metabolic processes. Time-to-peak (TTP) is a quantitative indirect measurement of enzyme concentration as the temporal dynamics of the metabolic reactions are directly related to its concentration\textsuperscript{201}. Animal studies are crucial in understanding the fundamental biochemical properties of disease and in validating emerging technologies such as hyperpolarized MRI by correlating $^{13}$C exchange rates with \textit{ex vivo} measurements\textsuperscript{188}. Hyperpolarized [1-$^{13}$C]pyruvate MRS has previously been used in a NAFLD rat model where both [1-$^{13}$C]alanine and [1-$^{13}$C]lactate were identified as potentially useful non-invasive markers of the progression of NAFLD\textsuperscript{205}.

To study a model of NAFLD, guinea pigs were fed a WD previously shown to induce NAFLD without causing the accumulation of subcutaneous fat in this species\textsuperscript{8}. Thus, this study aimed to validate MRI techniques used to investigate the effect of long-term WD consumption on liver metabolism in a pre-clinical guinea pig model of lean NAFLD and to investigate the hypothesis that exposure to the WD will result in NAFLD in association with altered pyruvate metabolism.

**2.2 Methods**

**2.2.1 Ethical Approval**

Animal care, maintenance, and procedures were performed following the national council's standards and guidelines on animal care. All procedures were reviewed, approved, and monitored by the institutional animal care and ethics committee.
2.2.2 Animal Model and Welfare

Guinea pigs were used in this study as they differ from other rodents as a model for NAFLD in that their lipoprotein metabolism and hepatic enzyme activity closely mimics human physiology. Male Dunkin-Hartley guinea pig pups were born in-house to mothers fed a standard diet throughout gestation and lactation in a 12/12 hour light-dark schedule in individual cages. At approximately fifteen days postnatal (PN), pups were weaned onto their respective diets, feeding ad libitum in individual cages. Male guinea pig pups (matched for litter) were randomly weaned onto either a control diet (CD: 21.6% protein, 18.4% fat, 60% carbohydrates, n = 14) or WD (21.4% protein, 45.3% fat, 33.3% carbohydrates, n = 14). Percentages indicate the calorie contribution from each macronutrient to the total dietary calories. The fat content (CD: 3% SFA, 4% MUFA, 11% PUFA; WD: 32% SFA, 12% MUFA, 2% PUFA) and carbohydrate content (CD: 10% sucrose, 40% corn starch; WD: 19% sucrose, 6.5% fructose, 9% corn starch; % by weight) of the diets differed in terms of their compositions. The WD had a higher caloric density (4.2 vs 3.8 kcal/g) and included 0.25% cholesterol. Daily food consumption (g/day/kg body weight) and animal weights were recorded for the 10 days before MRI scanning and during the period between MRI and euthanisation. At 144 days PN, animals underwent scanning (details below), and at approximately 150 days PN, animals were euthanised by CO₂ inhalation in a sealed chamber. Blood samples were immediately collected from the descending vena cava and analyzed using VetScan VS2 Chemistry Analyzer (VetScan® Mammalian Liver Profile reagent, Abaxis, Union City, CA) to quantify levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma glutamyl-transferase (GGT), blood ammonia (BA), bilirubin (TBIL), albumin (ALB), blood urea nitrogen (BUN), and cholesterol (CHOL). Livers were then harvested, weighed, snap-frozen in liquid nitrogen, and stored at -80°C until later biochemical determinations. Kidneys, brains, and hearts were harvested and weighed.
2.2.3 In Vivo Proton MRI Determination of Fat Content and In Vivo Measurements of Hepatic Metabolism with Hyperpolarized $^{13}$C MRS

At 144 +/- 4 days PN (equivalent to ~ 18-22 human years$^{213}$), animals were imaged using a 3T MRI scanner (GE Discovery MR750; GE Healthcare, Waukesha, WI, USA) under anesthetic$^{206}$. Animals were anesthetized using 4.5% isoflurane with 2L/min O$_2$ and maintained between 1.5-2.5% isoflurane with 2L/min O$_2$. A catheter was inserted into the hind foot saphenous vein for intravenous administration of the hyperpolarized $^{13}$C pyruvate during the MRI exam. Vital signs were monitored throughout the experiment, and body temperature was maintained at 37°C. To standardize metabolic state at the start of the experiment, all animals underwent MRI at roughly the same time of day. All animals were fasted for 2 hours before imaging and a subcutaneous injection of glycopyrrolate (0.01mg/Kg body weight) was administered half an hour before administration of anesthetic to reduce saliva production and risk of aspiration$^{214}$.

Anatomical T$_1$-weighted gradient echo (repetition time/echo time [TR/TE] = 5.1/2.4 ms, flip angle = 15°, number of averages = 4, slice thickness = 0.9 mm, total scan time ~ 7 min) and T$_2$-weighted spin echo (TR/TE = 2000/120 ms, number of averages = 2, slice thickness = 0.9 mm, total scan time ~ 7min) images with 0.875 x 0.875 mm$^2$ in-plane resolution were obtained using a 32-element cardiac coil (In Vivo Corp., Gainesville, FL). Water-fat images were acquired using a modified IDEAL acquisition (TR/ΔTE = 9.4/0.974 ms, echoes = 6, flip angle = 4°, number of averages = 4, slice thickness = 0.9 mm, total scan time ~ 13 min) with a 0.933 x 0.933 mm$^2$ in-plane resolution and reconstructed into PDFF images. CSE-MRI (IDEAL-IQ) used parallel MRI to accelerate the acquisition by a factor of 1.75 in the phase and slice directions. Regions of interest were drawn around the whole liver, hind limb tissue, whole body, subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) by L.S. (5 years’ experience), who was blinded to the diet group. These segmentations were done manually with digitizing monitors using 3D Slicer (version 4.10.0)$^{215}$. For this study, the VAT was defined as the adipose tissue visible on IDEAL fat images below the diaphragm and above the pelvis.
external to the abdominal cavity organs. SAT and VAT were reported as absolute values and as a percentage of the total body volume, calculated by dividing the adipose tissue volume by the whole body volume. These segmentations were used to measure the total volume and median PDFF of each region when overlaid on IDEAL fat fraction images. Areas of fat-water swaps were excluded from volumes that were used to measure PDFF.

Anatomical images were used as a reference to select a slab through the liver for 1D localized MRS. PRESS chemical shift MRS (TR/TE = 1082/35 ms, echoes = 1, slice thickness = 20.4 mm) was used to acquire hyperpolarized $^{13}$C spectra over 90 s with a 1s time resolution using a custom $^{13}$C birdcage coil (Morris Instruments, Ottawa, Canada). $[1^{-13}$C]pyruvate (Cambridge Isotope Labs, Massachusetts, USA) with 15mM Ox063 (Oxford Instruments, Oxford, UK) and 1.5mM Dotarem (Guebert, Villepinte, France) was hyperpolarized (Hypersense, Oxford Instruments), and a 3.5 mL bolus of the 80 mM solution (pH balanced, 37 °C) was injected over approximately 12 s into a vein in the hind leg. Spectra were analyzed using SAGE software (Spectroscopy Analysis by General Electric, GE Medical Systems, Chicago, IL, USA), and the TTP was measured as the time between the pyruvate peak and metabolite peak to mitigate effects due to slight differences in injection times. TTP is a model-free analysis metric that roughly displays an inverse correlation with enzyme concentration. The animals were monitored, warmed, and kept on 100% O₂ until they began to wake up. They were then placed under a heating lamp and monitored until they were fully awake and mobile, at which time they were returned to their cages.

2.2.4 Ex Vivo Hepatic Determinations

2.2.4.1 Triglyceride Content

The left liver lobe was ground into a frozen powder over liquid nitrogen and analyzed for liver triglyceride levels using a colorimetric assay (Cayman Chemicals, Ann Arbor, MI, USA) following the manufacturer's instructions. Briefly, approximately 200 mg tissue was homogenized in NP-40 buffer containing leupeptin using an electric homogenizer. Samples were centrifuged at 10 000 g, and the supernatant was harvested. Samples from
CD-fed animals were not diluted, whereas samples from WD-fed animals were diluted 1:4 in NP-40 before assaying. After incubating the samples in the enzyme mixture for 15 minutes in the dark, the plate was read at 530 nm, 540 nm, and 550 nm. The absorbance at the three wavelengths was averaged and used to calculate triglyceride concentration based on the standard curve. Triglyceride concentration was normalized to protein concentration by Pierce BCA assay (ThermoFisher, Waltham, MA, USA).

2.2.4.2 Liver Cholesterol Content

Total lipids were extracted from approximately 150 mg of frozen liver tissue following the Folch method. Total cholesterol, free cholesterol, and cholesteryl ester levels in lipid extracts were determined by enzymatic, colorimetric assays (Wako Diagnostics, Richmond, VA, USA) performed through the Metabolic Phenotyping Laboratory in Robarts Research Institute (London, Ontario, Canada). Cholesterol levels were normalized to the mass of the extracted tissue.

2.2.4.3 Western Blot

Protein was isolated from approximately 100 mg of frozen, ground liver tissue using RIPA buffer containing Aprotinin, Leupeptin, PMSF, NaF, and Sodium Orthovanadate (New England BioLabs, Ipswich, MA, USA). Tissue was homogenized using an electric homogenizer, sonicated at 30% amplitude for 5 second processing time, and centrifuged at 12 000 g for 30 mins at 4°C. The supernatant was harvested and stored at -80°C until use. Protein samples were prepared in laemmli buffer containing b-mercaptoethanol at a final concentration of 5%. Twenty mg of protein was run through 10% polyacrylamide tris-glycine gels and transferred onto PVDF membrane. Membranes were probed for PDH, phosphorylated PDH (pPDH), and LDH (Table 1) overnight at 4°C. Anti-rabbit secondary antibodies (Table 1), conjugated to HRP, were used to detect primary antibodies by incubating for one hour at room temperature. Proteins were detected using Amersham ECL reagent (GE Healthcare, Chicago, IL, USA) and ChemiDoc imager (BioRad, Hercules, CA, USA) with ImageLab software. Protein expression was normalized to total protein by amido black staining.
Table 2.1: Antibodies used in Western blot analysis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Host Animal</th>
<th>Company</th>
<th>Catalog #</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate Dehydrogenase</td>
<td>Rabbit mAb</td>
<td>Cell Signaling</td>
<td>3205</td>
<td>1:1000 in 5% BSA</td>
</tr>
<tr>
<td>Phosphorylated Pyruvate Dehydrogenase-E1α (pSer^{232})</td>
<td>Rabbit pAb</td>
<td>EMD Millipore</td>
<td>AP1063</td>
<td>2:2000 in 5% milk</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>Rabbit pAb</td>
<td>Cell Signaling</td>
<td>2012</td>
<td>1:1000 in 5% BSA</td>
</tr>
</tbody>
</table>

2.2.4.4 Enzyme Activity Assays

Six samples were randomly selected from each diet group for enzyme activity assays. Liver tissue was homogenized in 9 volumes of homogenization buffer (25 mM HEPES, 2 mM EDTA, 0.1% (v/v) Triton X-100, 10 mM sodium fluoride, 1 mM sodium orthovanadate, pH 7.8) using a microtube plastic pestle. Following incubation at 4°C for 20 min, homogenates were centrifuged at 10000 g for 10 min at 4 °C. Floating lipid was removed by aspiration, and pellets were resuspended within the supernatant for each sample. Samples were subjected to three rounds of freeze-thaw in liquid nitrogen, then assayed immediately for enzyme activity. Enzyme assays were performed at 37°C using a Spectramax plate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) in a 96-well plate. Enzyme activities were expressed relative to total protein concentration for each homogenate (determined by bicinchoninic acid (BCA) assay).

Lactate dehydrogenase (LDH) activity was measured following the addition of 5ml liver homogenate (diluted 1:20 in homogenization buffer) to 295ml assay mixture containing 50 mM HEPES (pH 7.4), 0.2 mM NADH, 1.0 mM pyruvate. Absorbance values (340 nm) were collected for 3-5 mins, and LDH activity was calculated using an extinction coefficient of 6.22 L mol^{-1} cm^{-1}.

Pyruvate dehydrogenase (PDH) activity was measured following the reduction of iodonitrotetrazolium chloride (INT) at 500 nm. Background rates were collected following the addition of 5 ml liver homogenate to 290 ml assay mixture containing 50
mM Tris (pH 7.8), 0.5 mM EDTA, 2.5 mM NAD+, 0.2 mM Coenzyme A, 0.1 mM sodium oxalate, 0.4 mM thiamine pyrophosphate, 1 mg/ml bovine serum albumin, 0.1% (v/v) Triton X-100, and 1U/ml diaphorase. The PDH reaction was then initiated by the addition of 5 ml 0.6 M sodium pyruvate. PDH activity was calculated from the difference between the rates with and without pyruvate, using an extinction coefficient of 15.4 L mol⁻¹ cm⁻¹.

Citrate synthase (CS) activity was measured following the addition of 10 ml liver homogenate (diluted 1:20 in homogenization buffer) to 287 ml assay mixture containing 50 mM Tris (pH 8.0), 0.1 mM 5,5-dithiobis(2-nitro-benzoic acid) DTNB), and 1.15 mM acetyl CoA. Parallel reactions were run with and without the addition of 0.5 mM oxaloacetate. Absorbance values (412 nm) were collected for 5 min, with CS activity calculated from the difference between the rates with and without oxaloacetate, using an extinction coefficient of 13.6 L mol⁻¹ cm⁻¹.

2.2.5 Statistical Analysis

Unpaired two-tailed Student's t-tests were used to determine differences in all measurements between animals in the two diet groups. A Shapiro-Wilk normality test was used to confirm the normal distribution of the data, and subsequently, the Pearson correlation coefficient was calculated for correlations between MRI and ex vivo data using a two-tailed P-value and a 95% confidence interval. Results are shown as mean ± SEM, and statistical significance was set at p < 0.05. Data analysis was performed using GraphPad Prism 6 (San Diego, CA, USA).

2.3 Results

2.3.1 Animal Body Weights and Food Intake

Average daily food consumption and daily calorie consumption did not significantly differ between the diet groups in the 10 days before MRI (CD 45.27 ± 2.64 g/day/kg body weight vs WD 45.56 ± 4.67 g/day/kg body weight, p = 0.958; CD 121.1 ± 9.48 kcal/day vs WD 130.6 ± 25.87 kcal/day, p = 0.578) or during the time between MRI and
euthanasia (CD 51.11 ± 2.84 g/day/kg body weight vs WD 54.43 ± 7.58 g/day/kg body weight, p = 0.667; CD 132.1 ± 11.42 kcal/day vs WD 150.0 ± 33.54 kcal/day, p = 0.416). On average, guinea pigs in the WD group weighed significantly less than animals in the CD group based on weight recording in the 10 days before (CD 770.6 ± 17.97 g vs WD 693.6 ± 18.52 g, p <0.05) and the period after the MRI examination (CD 774.6 ± 21.63 g vs WD 708.3 ± 19.01 g, p <0.05).

2.3.2 Blood Profiles Show Elevated Indicators of Liver Damage in Western Diet Animals

ALT levels were significantly elevated in WD-fed animals compared to CD-fed animals (p <0.05, Table 2). Blood cholesterol levels were also significantly greater in WD-fed animals compared to CD-fed animals (p < 0.05, Table 2). No significant differences were observed in levels of ALP (p = 0.1301), GGT (p = 0.7814), BA (p = 0.5457), TBIL (p = 0.2914), or BUN (p = 0.7276) between the two diet groups, though there was a non-significant trend towards elevated ALB in WD-fed animals (p = 0.0508, 95% CI = [-0.001085, 0.5189]; Table 2).

Table 2.2: Liver function and tissue profiles

<table>
<thead>
<tr>
<th>Liver Function Profile</th>
<th>CD</th>
<th>WD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphate (ALP) [u/L]</td>
<td>62.43 ± 11.06</td>
<td>43.14 ± 5.65</td>
<td>0.1301</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT) [u/L]</td>
<td>48.14 ± 2.80</td>
<td>82.00 ± 9.58</td>
<td>0.0071</td>
</tr>
<tr>
<td>Gamma glutamyl-transferase (GGT) [u/L]</td>
<td>6.14 ± 0.74</td>
<td>6.63 ± 1.53</td>
<td>0.7814</td>
</tr>
<tr>
<td>Blood ammonia (BA) [µmol/L]</td>
<td>52.57 ± 13.65</td>
<td>65.38 ± 15.14</td>
<td>0.5457</td>
</tr>
<tr>
<td>Bilirubin (TBIL) [mg/dl]</td>
<td>0.20 ± 0</td>
<td>0.24 ± 0.02</td>
<td>0.2914</td>
</tr>
<tr>
<td>Albumin (ALB) [g/dl]</td>
<td>4.03 ± 0.08</td>
<td>4.29 ± 0.09</td>
<td>0.0508</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN) [mg/dl]</td>
<td>22.75 ± 1.87</td>
<td>23.50 ± 0.98</td>
<td>0.7276</td>
</tr>
<tr>
<td>Cholesterol (CHOL) [mg/dl]</td>
<td>71.57 ± 14.37</td>
<td>440.4 ± 29.84</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liver Tissue Component</th>
<th>CD</th>
<th>WD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (TG) [mg/mg protein]</td>
<td>0.02 ± 0.00</td>
<td>0.11 ± 0.01</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total cholesterol [mg/mg tissue]</td>
<td>2.44 ± 0.12</td>
<td>19.34 ± 1.91</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Free cholesterol [mg/mg tissue] 1.85 ± 0.11 4.72 ± 0.28 < 0.0001
Cholesterol ester [mg/mg tissue] 0.59 ± 0.07 14.62 ± 1.66 < 0.0001

2.3.3  Impact of Lifelong Western Diet on Body and Organ Fat Content

Using MRI, at 144 ± 4 days PN, WD-fed animals had a significantly lower body volume compared to CD-fed animals (CD 684800 ± 20000 mm³ vs WD 631200 ± 16100 mm³, p <0.05; Figure 2.1A). Additionally, following lifelong WD feeding, guinea pigs had significantly elevated liver volume (CD 28300 ± 1000 mm³ vs WD 41400 ± 3200 mm³, p < 0.05; Figure 2.1B) compared to CD-fed animals, with no significant differences in hind limb volume (p = 0.6667, Figure 2.1C) between the two groups. WD-fed animals showed a significantly elevated liver PDFF (CD 6.20 ± 0.34 % vs WD 10.64 ± 0.87 %, p < 0.05; Figure 2.1D) and significantly decreased hind limb PDFF (CD 5.47 ± 0.58 % vs WD 3.86 ± 0.25 %, p <0.05; Figure 2.1E) compared to CD-fed animals. The SAT (CD 34897 ± 2339 mm³ vs WD 28196 ± 2173 mm³, p < 0.05) and VAT (CD 29374 ± 2316 mm³ vs WD 22299 ± 2075 mm³, p < 0.05) volumes were significantly larger in CD-fed animals compared to WD-fed animals. When considering the SAT and VAT as a percentage of total body volume, there were no differences between animals in the two diet groups (Figure 2.2A, B); however, there was a reduction in total adipose tissue (VAT + SAT) as
a percentage of total body volume in WD-fed animals vs CD-fed animals (CD 9.32 ± 0.43 % vs WD 8.01 ± 0.44 %, p <0.05; Figure 2.2C).

Figure 2.1: Total volumes estimated from MRI for the whole body (A), liver (B), and hind limbs (C). WD-fed animals displayed a significantly decreased whole-body volume and increased liver volume compared to the CD-fed animals. Median PDFF percentages of the liver (D) and hind limbs (E) show a significantly elevated PDFF in the livers and lower PDFF in the hind limbs of WD-fed animals compared to CD. * indicates p < 0.05, **** indicates p < 0.0001. (F) Examples of proton-density fat fraction image slices from an animal in the CD (left) and WD (right) groups. The livers and hind limbs are outlined in red and yellow, respectively, in both images. The PDFF is visibly elevated in the WD-exposed liver, as indicated by a lighter colour, and visibly reduced in the WD hind limb. In the box-and-whisker plots, the boxes extend from the 25th to 75th percentiles, the middle line is the median, and the whiskers extend from the smallest to the largest value in the data set.
2.3.4 Western Diet Feeding Results in Accelerated Hepatic Lactate Production Rate

Of the 28 animals scanned in MRS experiments, 26 spectra produced viable data (CD n = 13, WD n = 13; Figure 2.3A). Lactate TTP in WD-fed animals was significantly lower than in CD-fed animals (CD 14.92 ± 1.14 sec vs WD 11.15 ± 1.06 sec, p <0.05; Figure 2.3B). The TTP related to the rate of metabolism for pyruvate to alanine did not significantly differ with respect to diet conditions (p = 0.2422, Figure 2.3C).
Figure 2.3: (A) Examples of hyperpolarized [1-13C]pyruvate magnetic resonance spectra (left) and stack plots (right) from one CD (top) and one WD (bottom) fed guinea pig liver. Frequency is relative to the center of the pyruvate peak. Stack plots display spectra from the first 60 seconds of acquisition with a 1 second time resolution. Mean time to peak (TTP) measured from the time of the pyruvate peak for lactate (B) and alanine (C) in both diet groups. WD (n = 13) animals show a significant decrease in lactate TTP compared to CD (n = 13) animals.* indicates p < 0.05.

2.3.5 Lifelong Western Diet Alters Body and Liver Weights

At 150 ± 6 days PN, tissue collection samples showed WD-fed guinea pigs were significantly lighter than CD-fed guinea pigs (CD 787.6 ± 21 g, n = 14 vs WD 718.8 ± 17.9 g, n = 14; p <0.05), despite WD-exposed livers being significantly heavier than CD-exposed livers both in absolute weight (CD 26.70 ± 1.18 g vs WD 42.33 ± 2.47 g; p < 0.05) and as a fraction of total body weight (CD 0.034 ± 0.001 vs WD 0.059 ± 0.003; p < 0.05). Kidneys from WD-fed animals were found to be lighter than those from CD-fed animals as an absolute weight (CD 5.45 ± 0.16 g vs WD 5.00 ± 0.12; p <0.05) but no differences were found in kidney weight as a fraction of total body weight (CD 0.0070 ± 0.0002 vs WD 0.0070 ± 0.0002; p = 0.883). Brain and heart weights were not significantly different between the two diet groups in absolute weight (CD 3.96 ± 0.31 g vs WD 3.91 ± 0.24 g, p = 0.55; CD 3.28 ± 0.56 g vs WD 2.84 ± 0.58 g, p = 0.15) or as a fraction of total body weight (CD 0.0055 ± 0.0001 vs WD 0.0051 ± 0.0002, p = 0.072, 95% CI = [-3.812*10^-5, 8.296*10^-4]; CD 0.0042 ± 0.0005 vs WD 0.0039 ± 0.0005, p = 0.323).
2.3.6 Triglyceride and Cholesterol Levels are Elevated in WD-exposed Livers

WD-fed animals had significantly elevated hepatic TGs compared to CD-fed animals (p < 0.05, Table 2). The hepatic triglyceride concentration displayed a moderate correlation to the PDFF in the livers of animals in both diet groups combined (r = 0.692, Figure 2.4). The correlation between TG and PDFF becomes weak and non-significant when only considering animals from the WD group (r = 0.311, p = 0.301, n = 14). Total cholesterol (p < 0.05; Table 2), free cholesterol (p < 0.05; Table 2), and cholesteryl ester (p < 0.05; Table 2) were significantly increased in the WD-exposed liver tissues.

![Figure 2.4: Liver triglyceride concentrations plotted against PDFF in the liver. There is a moderate positive correlation (r = 0.6917, p = 0.0001) between TG and PDFF for all animals. The linear fit line is shown with 95% confidence intervals. There is a non-significant weak correlation between TG and PDFF when only considering animals from the WD group (r = 0.311, p = 0.301).](image)

2.3.7 Altered PDH and LDH Activity in WD-exposed Livers

PDH protein levels were significantly increased in WD-fed animals compared to CD-fed animals (CD 0.28 ± 0.02, n = 13 vs WD 0.42 ± 0.04, n = 14; p <0.05, Figure 2.5A), with no difference observed in phosphorylated PDH (p = 0.5694, Figure 2.5B), although PDH activity was significantly decreased in WD-fed animals (CD 1.56 ± 0.14 μmol/min*mg
protein, n = 6 vs WD 0.50 ± 0.24 μmol/min*mg protein, n = 5; p < 0.05, Figure 2.5D). Note that one of the samples from an animal in the CD group was not abundant enough to be used for protein level analysis and thus was not included in this data. Despite no difference in protein levels of LDH (p = 0.1477, Figure 2.5C), LDH activity was significantly elevated in WD-exposed livers (CD 388.7 ± 35.3 μmol/min*mg protein, n = 6 vs WD 638.6 ± 73.1 μmol/min*mg protein, n = 6; p < 0.05, Figure 2.5E). No significant difference with respect to diet was observed in CS activity (p = 0.1031, Figure 2.5F).

Figure 2.5: Liver enzyme protein levels for pyruvate dehydrogenase (PDH; A), lactate dehydrogenase (LDH; B), and phosphorylated PDH (pPDH; C). Enzyme activity measured from liver tissue for PDH (D), LDH (E), and citrate synthase (CS; F). * indicates p < 0.05, ** indicates p < 0.01.
2.3.8 Correlations

All data passed the Shapiro-Wilk normality test. LDH activity displayed a significantly strong positive correlation with PDFF in the liver ($r = 0.829$, $p < 0.05$; Figure 2.6A) and PDH activity displayed a significantly strong negative correlation with liver PDFF using data from both diet groups ($r = -0.835$, $p < 0.05$; Figure 2.6B). LDH activity was also shown to have a moderate negative correlation with the lactate TTP measurement across all animals in both diet groups ($r = -0.600$, $p = 0.051$; Figure 2.6C).

Figure 2.6: (A) Liver PDFF plotted against LDH activity displays a strong positive correlation ($r = 0.8289$, $p = 0.0016$). (B) Liver PDFF plotted against PDH activity displays a very strong negative correlation ($r = -0.8350$, $p = 0.0026$). (C) LDH enzyme activity plotted against lactate TTP displays a moderate negative correlation ($r = -0.6004$, $p = 0.0508$). Linear fit lines are shown with 95% confidence intervals.

2.4 Discussion

2.4.1 PDFF Findings Consistent with Lean NAFLD Phenotype

The increase in liver PDFF with a decline in hind limb PDFF is evidence that this model of lifelong WD consumption promotes NAFLD; differential fat deposition occurs, displaying a lean NAFLD phenotype. High-fat and high-fat/high-sugar diets have typically been found to induce obesity in animal models\textsuperscript{218}, although lean phenotypes of NAFLD are also reported\textsuperscript{211}. PDFF values of guinea pig\textsuperscript{8} and rat\textsuperscript{219} livers previously reported in the literature are similar to PDFFs reported in this study for both WD and CD groups. Also of note, the liver PDFF values observed in WD-fed animals were within the range of PDFF values (10-20\%) previously reported in human patients with grade 1 and 2 steatosis\textsuperscript{151}. This pre-clinical assessment of lean NAFLD using PDFF MRI techniques
similar to those that have been established in human studies\textsuperscript{209} provides motivation for further research focused on lean NAFLD in humans, which is often overlooked clinically and in the literature\textsuperscript{220}. The increased volume and weight of livers in WD-fed animals reflect fat accumulation in the liver and are likely indications of enlarged hepatocytes\textsuperscript{221}. WD-fed animals had significantly elevated hepatic TG and cholesterol levels, with all liver TG concentrations being above the lower limit for hepatic steatosis defined in humans\textsuperscript{222}. Elevated liver TG and cholesterol levels found in WD-fed animals indicate that lifelong exposure to the WD may result in lipid overload and dysfunction in the liver\textsuperscript{223}. Related to this, elevated serum ALT was observed in WD-fed animals, indicating liver damage\textsuperscript{224}. ALT is a reliable marker of hepatocellular injury or necrosis, suggesting that the WD group experienced liver cell damage due to diet\textsuperscript{224}. Although not statistically significant, a trend towards elevated serum ALB in WD-fed animals was observed and is another indication of liver damage\textsuperscript{225}.

Animals in the WD group exhibited a smaller body volume, weight, and proportion of adipose tissue compared to animals in the CD group. Specifically, the decreased percentage of adipose tissue may be explained by the WD's high fructose content, as fructose has been shown to decrease lipogenesis in rat adipose tissue\textsuperscript{226}. The overload of dietary fructose in rats has also been found to increase the release of free fatty acids from adipose tissue into the bloodstream, where the fatty acids may eventually be taken up by hepatocytes and stored as TGs in the liver\textsuperscript{227}. The loss of muscle mass has been reported in chronic liver diseases such as NAFLD\textsuperscript{228}, and it is speculated that this may also explain the overall decreased body weight and volume of WD-fed animals in this study. While other studies have found acute high fructose diets to increase visceral adiposity\textsuperscript{13,228}, it is speculated here that the lifelong exposure to a high fructose diet during critical development stages in this study results in a reduced capacity of extrahepatic adipose tissues to synthesize and store fatty acids. Hexokinase, a hepatic enzyme responsible for fructose metabolism in adipose tissue, is adaptive to diet and may be responsible for the reduction of adipose tissue as a response to high fructose exposure\textsuperscript{226}. Similar changes of reduced body size and adiposity have been noted after exposure to high fructose diets in neonates\textsuperscript{229} and in utero\textsuperscript{230}, suggesting that adaptations to an overload of dietary fructose may be programmed at a very young age or even before birth. Previous studies in guinea
pigs have reported a lean phenotype with decreased adipose tissue after lifelong WD exposure with the same diet composition as the WD used in this study\(^8\). The mechanisms of this adaptation to a high fructose diet in the adipose tissue during early development is an important topic that warrants further research.

2.4.2 Altered Pyruvate Metabolism and Evidence of Damaged Livers Found in WD-fed Animals

The decreased \textit{in vivo} lactate TTP, indicating an increased rate of lactate production, and the increased \textit{ex vivo} LDH activity in the WD group highlight, in two techniques, a shift towards lactate production via anaerobic glycolysis in the fatty livers\(^{205}\). In the current study, PDFF in the liver showed a strong correlation to LDH enzyme activity, supporting the relationship between fatty livers and the promotion of anaerobic glycolysis. Increased lactate concentration may be linked to a disturbance in liver lipid synthesis\(^{211}\) and has been found in obese mice, indicating that lactate may act as a metabolic biomarker for a diet-induced fatty liver\(^{231}\). WD-fed animals showed a significant increase in PDH protein levels but a significant decrease in PDH activity, suggesting inhibition of PDH in the livers of WD-fed animals. Reports of decreased PDH activity in liver disease are associated with increased lactate as PDH normally regulates lactate production in healthy livers\(^{232}\). A previously published study by Lee et al. used a rat model to investigate the effects of a lifelong exposure to a high-fat diet and found no change in lactate production, but rather an increase in aspartate and malate production in the livers of these animals\(^{210}\). The diet used in their study was not high in sugar, unlike the diet used in the current study, and these diet differences may explain the disparity in lactate production findings in the liver. Another relevant study used a rat model with induced obesity to investigate the effects of an acute exposure to a high-fat diet\(^{205}\). This study observed increased lactate in the liver after six weeks of exposure to the high-fat diet, in conjunction with NAFLD, which would be consistent with our findings of increased lactate as a result of NAFLD\(^{205}\). A major difference in the acute study was the finding of increased alanine production in the liver, which was not observed in the chronic WD-induced NAFLD model presented here. It may be hypothesized that the mechanisms resulting in increased liver alanine may be associated with the onset of NAFLD and become more subtle over time in a chronic
model of NAFLD like the one presented in this study. In support of this hypothesis, a previous study has found no evidence of elevated ALT in NAFLD patients with portal chronic inflammation, which is a marker of advanced disease state\textsuperscript{233}. Considering data from these multiple studies allows us to investigate liver function differences between acute and chronic exposure to high-fat diets. Demonstrating the sensitivity of HP [1-\textsuperscript{13}C]pyruvate MRS in measuring liver metabolism in different species and experimental conditions is an essential step in its external validation for use as an \textit{in vivo} biomarker of liver dysfunction. This technology is already being implemented in clinical studies to measure pyruvate metabolism in tumours, and studies providing motivation for its use in NAFLD patients may help to accelerate its clinical translation for this application\textsuperscript{188}.

An approach to consider for future work may include a longitudinal study that repeats these imaging experiments in both sexes, at different time points in the guinea pigs' development to understand the impact of sex upon these changes and also when liver metabolic changes occur in the animal's lifespan. Additionally, these imaging methods could be implemented in a study investigating whether diet reversal, exercise, or therapeutic interventions can modulate the metabolic effects of the WD. Previous studies have demonstrated the benefits of dietary intervention and exercise in decreasing liver volume and fat accumulation but have not specifically looked at the impact of these interventions on hepatic pyruvate metabolism \textit{in vivo}\textsuperscript{234}.

2.4.3 Limitations

First, due to the low signal-to-noise ratio of bicarbonate in MRS experiments, there was insufficient data to measure the bicarbonate TTP. Information on the rate of bicarbonate production \textit{in vivo} would have been valuable to correlate to PDH activity and further confirm the value of hyperpolarized \textsuperscript{13}C MRS as a tool to measure hepatic [1-\textsuperscript{13}C]pyruvate labelled metabolism. Second, labelling the first carbon on the pyruvate molecule limits which downstream metabolites can be measured via MRS. Other experiments may label the second carbon on pyruvate instead to measure the signal from metabolites involved in the metabolic pathway following pyruvate’s conversion into acetyl-CoA. Third, our TTP measurement precision is limited by the 1 s temporal
resolution of our MRS experiments. Finally, the animals' metabolic environment may be affected by the anesthetic during MRI experiments, limiting our ability to observe homeostasis in vivo\textsuperscript{235}. To combat this, the animals were returned to their cages to recover in the days between the MRI examination and tissue collection.

2.4.4 Conclusion

PDFF imaging showed increased fat fractions, corresponding to increased TG levels in the livers of male guinea pigs fed a lifelong WD. Further, the application of hyperpolarized $^{13}$C MRS demonstrated utility in probing metabolic events in the liver that correlated with ex vivo liver enzyme activities. Hyperpolarized $^{13}$C spectroscopy results, in conjunction with altered LDH enzyme activity, highlight a shift from oxidative metabolism of pyruvate to anaerobic glycolysis and increased lactate production in the fatty livers of animals fed a WD. These results highlight lactate production as an indication of changes brought on by NAFLD development after chronic exposure to a WD in a guinea pig model of lean NAFLD. Hyperpolarized MRS techniques provide a non-invasive method of examining liver metabolism that correlates well with ex vivo findings and may eventually be used to help diagnose and determine the efficacy of treatment for NAFLD.
Chapter 3

3 Optimizing Signal-to-noise Ratio for Multi-metabolite Hyperpolarized Carbon-13 Magnetic Resonance Imaging using a Hybrid Flip Angle Scheme

3.1 Introduction

The placenta produces a variety of biomolecules during pregnancy that are important for both maternal and fetal metabolism\(^{236}\). In vivo assessment of placental metabolism would allow us to characterize healthy metabolism at all stages of pregnancy and investigate potential placental metabolic abnormalities in conditions such as intra-uterine growth restriction\(^{237}\). Imaging assessment of the placenta during pregnancy is generally limited to the non-ionizing imaging modalities of ultrasound and MRI. These imaging techniques can contribute structural information and limited functional data about the placenta\(^{237,238}\).

Hyperpolarized carbon-13 magnetic resonance imaging (HP\(^{13}\)C MRI) is an ideal imaging modality for real-time in vivo monitoring of metabolism. Using d-DNP methods, it is possible to temporarily increase signal up to 10,000 fold and acquire images of the distribution of molecules enriched with the hyperpolarized substrate\(^{239}\). This technique has a variety of applications in research, including tumour monitoring, inflammation, cardiac disease, and fetoplacental development\(^{177,206}\).

One of the more common HP\(^{13}\)C MRI substrates is [1-\(^13\)C]pyruvate, which following an intravenous injection allows us to resolve signal from its metabolites: lactate, alanine, and bicarbonate. Pyruvate metabolism is a key step in glycolysis and indicative of normal and abnormal cell function. The metabolic by-products of pyruvate follow different metabolic pathways, some aerobic and some anaerobic, and the metabolic fates of these biomolecules provide insight into the biological state of the region or organ of interest. The metabolic conversion rate constant from pyruvate to a metabolite (e.g. \(K_{PL}\) is the conversion rate of pyruvate \(\rightarrow\) lactate) can be calculated from the time-resolved
measurements of the different metabolites and provides a quantitative measure of metabolism related to enzyme concentrations \textit{in vivo}\textsuperscript{240}. Measuring the entire concentration-time curve of each metabolite is necessary for a robust sampling of data in order to calculate metabolic rates. As such, a sufficiently high signal-to-noise ratio (SNR) must be maintained throughout the entire acquisition.

The metabolites of pyruvate we wish to image exist at distinct chemical shifts (between -9.7 to 12.6 ppm relative to pyruvate). Since metabolism of the injected pyruvate occurs rapidly, the relative abundance of each metabolite changes over the course of the experiment. The metabolites are typically found in quantities > 6-fold lower than pyruvate. In past experiments we have taken advantage of this by utilizing a spectrally varying RF pulse such that pyruvate is exposed to a smaller flip angle than its metabolites that are lower in abundance\textsuperscript{206}. This flip angle scheme is spectrally varying but constant in time and will be referred to as the constant flip angle (CFA) scheme.

The rapid T\textsubscript{1} decay of the hyperpolarized state provides challenges to maximizing SNR. Previous HP \textsuperscript{13}C MRI studies\textsuperscript{241–246} have addressed this by proposing variable flip angle (VFA) schemes that increase the flip angle over time for one metabolite. These studies typically use a spectral-spatial RF pulse shape and vary the pulse amplitude at each excitation, continuously increasing to 90\textdegree to maintain a constant signal from a single spectral peak of a hyperpolarized \textsuperscript{13}C molecule despite the rapid decay of polarization.

The aim is to expand upon the CFA scheme to optimize SNR in HP \textsuperscript{13}C MRI experiments by introducing a novel approach that allows us to deliver optimized time-varying flip angles to each metabolite by taking advantage of the spectral variance of CFA. This will be referred to as the multi-spectral variable flip angle (msVFA) scheme. The individual trajectories for each metabolite using msVFA are calculated using previously published VFA methods and applied to each metabolite simultaneously by progressively varying both the shape and amplitude of the spectrally varying pulse. This allows us to produce a distinct VFA trajectory for each metabolite and apply the SNR benefits associated with VFA to all metabolites simultaneously\textsuperscript{247}. 
In this study, the novel msVFA is compared to the CFA scheme. The comparison CFA to msVFA was chosen as it allows for comparison of the SNR for all four metabolites of interest simultaneously. Additionally, this comparison highlights an alternative to the spectral-spatial RF method as an improvement of the current IDEAL technique. Therefore, the focus of this work is to investigate the benefit of adding time-varying flip angles to the CFA method to provide optimal SNR for multiple metabolites simultaneously.

It is hypothesized that the msVFA scheme will provide SNR equivalent to VFA for each metabolite; therefore, it will significantly increase SNR throughout the experiment for each metabolite compared to the CFA scheme. If successful, the msVFA has potential to improve HP $^{13}$C MRI image quality in a variety of research applications including, but not limited to, fetoplacental research.

3.2 Methods

3.2.1 Simulations

Flip angles for each metabolite were calculated using the method described by Xing et al.\textsuperscript{245}, which takes into account both the T\textsubscript{1} decay and metabolic conversion of each metabolite. This method was used to calculate the flip angles that would optimize SNR for each metabolite throughout the experiment. The calculated flip angles for the msVFA scheme were compared to a CFA scheme previously described by Friesen-Waldner et al.\textsuperscript{206}. Figure 3.1 shows the flip angle trajectories applied in this work for both of these methods.
Figure 3.1: The desired flip angle trajectories for each metabolite are shown for CFA (top) and msVFA (bottom) strategies. These flip angles were achieved using a double Gaussian RF pulse. Dotted vertical lines indicate the start time of each acquisition.

Bloch equations were used to simulate the SNR of different metabolites using Matlab (MATLAB 2018a, Mathworks, 2018). Equation 3-1 was used to estimate SNR at each n\textsuperscript{th} time point for every metabolite (met) where the concentration of each metabolite was calculated using estimated T\textsubscript{1} decay and metabolic conversion rates found in the literature\textsuperscript{248,249}. The predicted SNR resulting from using the implemented flip angle in msVFA was compared to the predicted SNR using the exact VFA for each metabolite.

\[
SNR_{met}(n) = [Met(n)]e^{\frac{(n-1)TR}{T1_{met}}}(\sin \alpha)\cos \alpha^n
\]
3.2.2 msVFA Implementation and Verification

In the msVFA experiments, a unique RF waveform is applied for each image acquisition\(^{247}\). Additional improvement would likely be possible by applying a unique RF waveform for each excitation of each image. This may be feasible for single-shot pulse-and-acquire type sequences but becomes impractical to implement for the thousands of custom RF pulse shapes required for the sequence used here.

The double Gaussian pulse allows us to implement unique flip angles for each metabolite; however, the double Gaussian pulse shape limits our accuracy in delivering the exact calculated flip angles to every metabolite. If there was interest in imaging only two or three metabolites, the double Gaussian pulse could be used to deliver the exact calculated flip angles; however, with four metabolites, some approximations must be made due to the different spectral positions. This is why lactate and alanine are delivered the same flip angle in msVFA experiments.

For \(^{13}\text{C}\) acquisitions, a quadrature volume transmit and receive coil was used (12 cm diameter, 19 cm long)\(^{155}\). The desired flip angles were applied to each metabolite using double Gaussian RF spectral profiles designed for each acquisition. The RF profiles for each acquisition are shown in Figure 3.2.
Figure 3.2: The double Gaussian RF spectral profile used in each msVFA acquisition is shown here, centered on the pyruvate peak. The resonant frequency of each metabolite is indicated by vertical dashed lines. The magnitude and shape of the RF profile progressively changes with each acquisition to follow the msVFA trajectory.

These RF pulses were implemented in a version of our previously described chemical shift encoded [1-13C]pyruvate imaging pulse sequence\textsuperscript{155,206,247}. The modified pulse sequence was tested on a phantom containing 7 mol/L thermal [1-13C]sodium acetate phantom doped with a gadolinium-based contrast agent\textsuperscript{250} to ensure that RF pulses were scaling appropriately to prescribed flip angles over time. Frequency sweeps of the RF pulses were used to determine RF amplitude at various chemical shifts relevant to [1-13C]pyruvate imaging. The pulse sequence was run at a longer TR (1.5 s) than hyperpolarized experiments to permit for the T\textsubscript{1} recovery of the thermal phantom.

3.2.3 Animal Experiments

Six pregnant adult guinea pigs (gestational age = 48.2 ± 11.7 days, number of pups = 3.4 ± 0.9) were imaged under the approval of the institution’s Animal Care Committee. Due to a technical failure of the RF coil in one experiment, one animal was excluded from analysis, leaving a total of 5 guinea pigs and 17 placentae. Animals were anesthetized...
using 4.5% isoflurane with 2 L/min O\textsubscript{2} and maintained between 1.5-2.5% isoflurane with 2 L/min O\textsubscript{2}. Animal vital signs (breathing, heart rate, temperature, blood oxygenation) were monitored throughout the experiment. Experiments were done at roughly the same time of day, and all animals were fasted for 2 hours before the experiment tostandardize their metabolic state at the start of the experiment. All animals underwent an ultrasound examination three days prior to the MRI experiment to confirm pregnancy.

Each animal received two pyruvate injections during an experiment, one imaged with CFA and the other with msVFA. The order of the msVFA and CFA acquisitions were randomized between experiments. The second injection occurred approximately one hour after the first to allow time for hyperpolarization of the second dose of pyruvate.

For each injection, [1-\textsuperscript{13}C]pyruvic acid (Cambridge Isotope Laboratories, Tewksbury, MA) containing 15 mM OX63 trityl radical (Oxford Instruments, Abingdon, UK) and 1 mM ProHance (Bracco, Milan, Italy) was hyperpolarized using the Hypersense DNP polarizer (Oxford Instruments). 75 mg/kg of the hyperpolarized 250mM [1-\textsuperscript{13}C]pyruvate solution was delivered as a bolus injection via intravenous catheter into the saphenous vein over \~12 seconds, approximately 15 seconds after the dissolution had been released from the polarizer. Imaging was initiated 7.5 seconds after the start of pyruvate injection. An image was acquired every 7.5 seconds following the first acquisition, resulting in a total of seven images acquired at different time points. Hyperpolarized imaging was done using a 3D multiphase broadband fast gradient recalled multiecho pulse sequence with the following parameters: FOV 20 x 0.6 cm, slice 8.5 mm, BW 8.93 kHz, echoes 8, NEX = 1, ETL 4, first TE 4.2 ms, echo spacing 1.1 ms, acquisition time = 7.5 s.

\textit{T\textsubscript{1}}-weighted gradient echo (repetition time/echo time [TR/TE] = 5.1/2.4 ms, flip angle = 15°, number of averages = 4, slice thickness = 0.9 mm, total imaging time \~7 min) and \textit{T\textsubscript{2}}-weighted spin echo (TR/TE = 2000/120 ms, number of averages = 2, slice thickness = 0.9 mm, total imaging time \~7 min) images with 0.875 \times 0.875 mm\textsuperscript{2} in-plane resolutions were obtained as anatomical references for the \textsuperscript{13}C images. All experiments were done on a 3T MRI system (Discovery MR750, GE Healthcare, Waukesha, WI). \textsuperscript{1}H images were acquired using a 32-element human cardiac coil array (In Vivo Corp., Gainesville, FL),
and $^{13}$C images were acquired using a custom-built $^{13}$C birdcage coil (Morris Instruments, Ottawa, Canada). Polarization levels were measured with an MQC spectrometer shortly after the dispensation of the sample for each injection.

### 3.2.4 Signal-to-Noise Ratios

The SNR of the *in vivo* images was calculated as the mean signal in a region of interest (ROI) divided by the standard deviation of signal in a signal-free ROI placed outside the animal. ROIs were drawn on the $T_1$ images for each placenta, maternal liver, and maternal kidney and then transferred to the HP $^{13}$C images. SNR was calculated for each metabolite and acquisition. Paired t-tests were done to compare SNR for each metabolite in each ROI. Significance was defined at 0.05 for this test.

### 3.3 Results

Using the msVFA double Gaussian pulse, it is possible to achieve flip angles very similar to those of the optimal VFA calculated for each metabolite. Bloch simulations found that the predicted SNR using the flip angles implemented with msVFA was very similar to the predicted SNR using the exact VFA calculated for each metabolite. The SNR ratio summed over time for each metabolite using msVFA compared to the true VFA are as follows: 97% for pyruvate, 89% for lactate, 97% for alanine, and 95% for bicarbonate.

An increase in SNR in the placentae was observed for all metabolites using msVFA at time points up to 37.5 s, as displayed in Figure 3.3A. Statistically significant ($p < 0.00005$) increases in SNR were observed for pyruvate, lactate, and bicarbonate signal in the placentae using the msVFA acquisition compared to the CFA acquisition. An exception of this SNR increase in the placentae is the alanine signal; however, placental alanine SNR is very low, suggesting very little alanine was being produced in the placentae, and as such, it is not possible to increase the SNR when negligible signal is present.

Significant increases of pyruvate and lactate SNR were observed in the maternal liver ($p = 0.005$ and $p = 0.006$ respectively). There is no significant difference in the SNR of
alanine and bicarbonate in the maternal liver, but low SNR indicates that very little alanine and bicarbonate were produced in the maternal liver. A significant increase in pyruvate SNR was observed using msVFA in the maternal kidneys ($p = 0.015$) and trends of increased mean SNR were found using msVFA for all metabolites (see Figure 3.3C).

Typically, there is no increase in SNR using msVFA at the last time point (52.5 s) since there is not much hyperpolarized signal left to acquire at this time point. In some cases, SNR is increased in the CFA images at 52.5 s, and this may be due to the fact that CFA is less efficient at using all the signal by the end of the scan, leaving “wasted” signal. SNR was not corrected for polarization level in this analysis because all polarization measurements were between 5-7% and polarization level was not significantly different between msVFA and CFA, as determined by a paired t-test ($p = 0.15$).
Figure 3.3: The mean SNR for each metabolite averaged over all (A) placenta, (B) maternal liver, and (C) maternal kidney ROIs are plotted here. The mean SNR at each time point may be compared between msVFA (red squares) and CFA (blue circles) acquisitions. A: The increased SNR provided by the msVFA acquisition is apparent for all metabolites, except alanine. This is likely due to limited production of alanine in the placenta providing very little metabolite signal that could be increased by the use of msVFA. B: The increased SNR provided by the msVFA acquisition is apparent for pyruvate and lactate, while there is limited improvement for alanine and bicarbonate. C: There appears to be increased mean SNR using msVFA for all metabolites in maternal kidney ROIs; however, these trends were not found to be significant. Note for all ROIs, the y-axis SNR scale is larger for pyruvate due to larger amount of signal from pyruvate compared to other metabolites.

Qualitatively, it can be appreciated that the increased SNR in images collected using the msVFA acquisition allows for a more easily discernible signal in less perfused and/or metabolically active areas than the lower SNR images acquired using the CFA method. This allows for a clearer distinction of low metabolism values from noise and detect small changes in metabolism. It was observed that signal in the fetal livers was distinguishable more often in images acquired using msVFA compared to CFA. An example of this is displayed in Figure 3.4, where there is more signal in placentae and fetal livers in the image acquired using the msVFA technique.
3.4 Discussion and Conclusions

In this study, it was demonstrated that a spectrally varying dynamic flip angle scheme individually optimized for each metabolite increases the SNR for all metabolites in time-resolved metabolic imaging. Up to 250% increases in SNR in pyruvate, lactate, and bicarbonate images of the placentae were observed; and similar SNR increases for metabolites in the maternal liver was demonstrated. The lack of alanine signal in the
maternal liver may be related to the known decrease of alanine aminotransferase enzyme activity enzymes in the liver that occurs during pregnancy, though this has only been reported on in human studies\textsuperscript{251,252}. The increased incidence of signal seen in fetal livers using msVFA compared to CFA leads us to believe that msVFA may be useful for metabolic analysis of fetal organs, where otherwise signal may not be distinguishable from noise.

The msVFA approach combats the rapid decay of hyperpolarized signal while accounting for the variation in metabolite concentrations and T\textsubscript{1} rates in hyperpolarized [1-\textsuperscript{13}C]pyruvate MRI. The delivery of flip angles optimized for different metabolites in HP\textsuperscript{13}C MRI builds on studies that have shown the advantage of VFA techniques to counter-act the rapid hyperpolarized decay\textsuperscript{244,245,253–255}. In these previous studies, VFA was optimized for one metabolite, usually either pyruvate or lactate, leading to sub-optimal flip angles for other metabolites.

Alternative approaches to optimize SNR for multiple metabolites have used spectrally-selective RF pulses which apply a unique flip angle for each metabolite but only excite one metabolite per RF pulse\textsuperscript{243,245,246,255}. These approaches to date have only been used to optimize up to two metabolites, usually pyruvate and lactate, and are usually implemented for imaging 2D slices. In our application of fetoplacental imaging, 3D imaging has the advantage of ensuring we can image multiple placentae and fetuses, which would be difficult if limited to a single 2D slice. The msVFA technique uses a spectrally varying flip angle which delivers unique flip angles for each metabolite during every RF excitation. The msVFA method does not limit the number of metabolites for which we may use optimized flip angles, and scaling up msVFA for more metabolites would be possible with a change in the RF excitation pulse shape. The current implementation of msVFA does not use the exact VFA flip angles for each metabolite due to limitations of the double Gaussian RF pulse. Delivering the exact desired flip angles may be possible as one could imagine using a more sophisticated RF pulse design; however, more complex pulses may be associated with additional complications such as higher SAR and likely longer pulses, which should be avoided in hyperpolarized experiments. While both methods of achieving multi-metabolite VFA are valid, unique
advantages and weaknesses exist for either method. This step-wise msVFA is considered to be a step toward a fully optimized VFA for 3D volumes.

The msVFA technique may be generalized for any set of metabolites in hyperpolarized imaging, assuming a rough estimate of the $T_1$ and metabolic rate constant is known. This would be valuable for hyperpolarized imaging of any metabolite, particularly if the end goal is quantitative analysis. The $in$ $vivo$ $T_1$ rates of these metabolites are difficult to measure and not well characterized; however, the msVFA optimization is dependent on the ratio of $TR/T_1$ and hyperpolarized imaging sequences typically use $TR << T_1$ due to the limited lifetime of the hyperpolarized signal. Therefore, the msVFA optimization calculation is relatively insensitive to errors of $in$ $vivo$ $T_1$ estimation. Similarly, flip angle optimization calculations from Xing et al.\textsuperscript{245} used assumed metabolic rate constant values in the estimation of the effective $T_1$, which is inversely related to the rate constant. These metabolic rate constants are not well known $in$ $vivo$, but as above, errors in rate constant estimation are not expected to affect SNR since $TR << T_{1eff}$ in this sequence.

The metabolic rate constants measured $in$ $vivo$ are typically on the order of $10^{-2}$ or $10^{-3} \, s^{-1}$ and precision is important when quantifying such small values. It is predicted that the increased SNR produced in images acquired with msVFA will improve the precision of metabolic rate fitting, enhancing the ability to detect small changes in these values. A future application of this work would be to quantify and compare metabolic rate constant values in diseased and healthy placentae using the msVFA acquisition.

In this study, there is a large degree of biological variation between our animals, including different maternal ages, gestational ages, and number of fetoplacental units. A diverse population was chosen for $in$ $vivo$ experiments to demonstrate that the advantages of using msVFA are applicable to different populations, meaning that the msVFA would not bias results when comparing two groups of animals.

For these $^{13}$C acquisitions, the birdcage coil was used to limit the guinea pig volume we wished to image. To translate this technique to application in large animal or human studies, the RF pulse would need to be redesigned to include spatial selectivity. This adaptation is an important topic for future work.
In summary, it has been demonstrated that msVFA results in improved overall SNR for all metabolites in *in vivo* hyperpolarized [1-$^{13}$C]pyruvate MRI. This was achieved using a spectrally varying RF pulse that increases in amplitude over the duration of the scanning protocol, preserving hyperpolarized $^{13}$C signal more effectively than a flip angle scheme that is constant over time. Although this study has focused on reporting the SNR for ROIs in the placentae, maternal liver, and maternal kidneys, this technique could improve SNR for any organ where metabolism is occurring. Additionally, this technique may be generalized for metabolic imaging of any hyperpolarized substrate. The msVFA technique improves hyperpolarized $^{13}$C images to allow better quantitative analysis of metabolic rates, and this is an important step in translation of hyperpolarized $^{13}$C MRI to clinical applications.
Chapter 4

4 Perfusion and oxygen saturation of the mid-pregnancy and near-term placenta measured by magnetic resonance imaging in a guinea pig model of pregnancy following lifelong Western diet consumption

4.1 Introduction

Consumption of a high-fat and high-sugar diet common to the Western world, known as the Western diet (WD), is linked to several metabolic diseases, including type II diabetes and cardiovascular disease. Animal studies have shown that consuming high-fat diets in pregnancy can lead to postnatal negative health outcomes for offspring, including, but not limited to, cardiovascular dysfunction, obesity, and type II diabetes. Maternal obesity and metabolic syndrome, often induced by WD consumption, are associated with placental dysfunction. Placental dysfunction is a leading cause of stillbirth and major morbidities, including fetal growth restriction (FGR), with the severity of these morbidities dependent on when they develop during gestation. It is hypothesized that the WD negatively impacts placental function during pregnancy which, in turn, predisposes the fetus to increased risk of later life disease, though the mechanisms behind this association are unclear.

Historically, birth weights of the fetus and placenta have been used as a measure of placental efficiency; however, with modern imaging techniques, it is possible to obtain a clearer picture of fetoplacental physiology in utero. Ultrasound (US) is the most commonly used imaging modality for assessing fetoplacental growth but is limited in its ability to quantify placental function. Umbilical artery Doppler US is used to provide an indirect measure of fetoplacental circulation but even with this technology, it remains difficult to detect microscopic changes to the placental microcirculation that are commonly associated with placental dysfunction. US wave reflection is a technique...
that can isolate US signal from the placental vasculature and has potential for detecting placental vascular pathology but is still in early pre-clinical stages of application\textsuperscript{258}. To better understand physiological changes associated with diet during pregnancy, we investigate placental function using specialized MRI techniques. MRI has advantages over US, including superior soft-tissue contrast and the ability to make quantitative measurements related to tissue microstructure and function\textsuperscript{171}. X-ray CT is often used to examine vascular function\textsuperscript{259}, but, unlike MRI, it is not generally considered safe for use in pregnancy as it produces ionizing radiation\textsuperscript{260}.

In this study, T\(_2^*\) mapping was used as an indirect measurement of blood oxygen saturation in the placenta, fetal brain, and fetal liver as T\(_2^*\) decay is sensitive to deoxygenated hemoglobin\textsuperscript{171}. Diffusion-weighted imaging (DWI), specifically intravoxel incoherent motion (IVIM) techniques, were used to quantify diffusion and perfusion in the placenta, providing information on placental microstructure\textsuperscript{261}. IVIM provides estimates of the diffusion coefficient (D) related to cellular density and regional fibrosis, the pseudodiffusion coefficient (D\textsuperscript{*}) related to the perfusion of blood in the microcirculation, and the perfusion fraction (f) related to the density of blood vessels in a voxel\textsuperscript{165}.

The purpose of this study was to investigate the impact of lifelong maternal WD consumption on placental structure and function \textit{in utero} in a guinea pig model at two points in pregnancy to observe any changes over gestation. T\(_2^*\) and IVIM measurements were used to provide placental oxygen saturation and microvascular information. Measurements of the fetus:placenta and fetal brain:liver volume ratios were used as well-defined proxy measurements of placental efficiency and fetal growth symmetry\textsuperscript{97,98}. Based on previous studies focusing on the impact of poor maternal nutrition\textsuperscript{108,262,263} on placental structure and function, it was postulated that lifelong maternal exposure to the WD would result in placental oxygen saturation and blood perfusion decreasing over gestation compared to placentae in sows consuming a control diet (CD).
4.2 Methods

4.2.1 Animals

All animals were housed at the Animalium of Western University, London, Canada. They were allowed feed and water ad libitum throughout the experiment and were examined regularly, and all efforts were made to minimize any distress as per the approved protocol and with veterinary oversight. This study was carried out in strict accordance with the recommendations of the Canadian Council of Animal Care (CCAC). The protocol was approved by the Animal Care Committee of Western University (AUP# 2019-116), and day-to-day operation was overseen by the Institution’s post-approval monitoring program. Thirty female guinea pigs were weaned from birth randomly onto either a control diet (CD: 21.6% protein, 18.4% fat, 60% carbohydrates, N=15) or a Western diet (WD: 21.4% protein, 45.3% fat, 33.3% carbohydrates, N=15)\textsuperscript{211}. The percentages listed indicate the calorie contribution from each macronutrient to the total calories of that diet. The two diets differ in terms of fat content (CD: 3% SFA, 4% MUFA, 11% PUFA; WD: 32% SFA, 12% MUFA, 2% PUFA) and carbohydrate content (CD: 10% sucrose, 40% corn starch; WD: 19% sucrose, 6.5% fructose, 9% corn starch; % by weight)\textsuperscript{211}. Additionally, the WD had a higher caloric density of 4.2 kcal/g than the CD at 3.4 kcal/g\textsuperscript{211}. The guinea pigs used in this study were mated in-house, and all had experienced one pregnancy prior to this study. The animals underwent MRI at either 40 ± 1 days (N = 13) or 63 ± 1 days (N = 17) gestation (term ~68 days)\textsuperscript{122}. These gestational ages correspond to the mid-term (MT) and near-term (NT) groups, respectively. Each guinea pig was carrying between 1-5 pups, with the CD group containing 41 fetuses and the WD group containing 44 fetuses.

4.2.2 Imaging and Analysis

Animals were transported from the Animalium to the MRI suite and anesthetized using 4.5% isoflurane with 2L/min O\textsubscript{2}. Once stable, the sow was maintained between 1.5-2.5% isoflurane with 2L/min O\textsubscript{2} and imaged at 3T (Discovery MR750, GE Healthcare, Waukesha, WI) using a 32-element cardiac coil (In Vivo Corp., Gainesville, FL).
Anatomical $T_1$-weighted gradient echo ($\text{TR/TE} = 5.1$ ms/2.4 ms, flip angle = 15°, number of averages = 4, slice thickness = 0.9 mm, total scan time ~ 7 min) and $T_2$-weighted spin echo ($\text{TR/TE} = 2000$ ms/120 ms, number of averages = 2, slice thickness = 0.9 mm, total scan time ~ 7 min) images with $0.875 \times 0.875$ mm$^2$ in-plane resolutions were acquired. Water-fat images were acquired using a modified IDEAL acquisition ($\text{TR/ATE} = 9.4$ ms/0.974 ms, echoes = 6, flip angle = 4°, number of averages = 4, slice thickness = 0.9 mm, total scan time ~ 13 min) with a $0.933 \times 0.933$ mm$^2$ in-plane resolution and reconstructed into R2* maps and PDFF images. DWI were acquired using a diffusion-weighted echo-planar imaging acquisition ($\text{TR/TE} = 3940$ ms/61 ms, number of averages = 2, in-plane resolution = $0.6 \times 0.6$ mm$^2$, slice thickness = 1 mm, total scan time ~ 8 min, with the following b-values: 10, 20, 35, 50, 65, 80, 95, 110, 125, 160, 200, 300, 400, 500, 600, 700, 750, 800, 900, and 1000 s/mm$^2$ acquired in 3 directions.

Regions of interest (ROIs) were manually segmented by L.S. (5 years of experience) using a digitizing monitor in 3D Slicer (version 4.10.0) around the placental, fetal, fetal brain, and fetal liver volumes. Placenta, fetal liver, and fetal brain ROIs were then overlaid on R2* maps to calculate the mean $T_2^*$ in each volume. A Matlab (The MathWorks, Inc., 2008 version R2020a) IVIM fitting tool was used to produce voxel-wise maps of $D$, $D^*$, and $f$ within the placenta ROIs and the mean values were recorded. All measurements between the two diet groups were compared using a linear mixed model two-way ANOVA with a post hoc Tukey test to account for intra-litter effects, computed in R (R Foundation for Statistical Computing, Vienna, Austria). The mean and standard error of measurements are reported with significance defined at $p < 0.05$.

4.3 Results

The results are presented in a different sub-section for each type of measurement: volumes, oxygen saturation, and IVIM estimates. Within each sub-section, the results will be grouped by independent variables: gestational age disregarding maternal diet,
maternal diet group disregarding gestational age, and the interaction of gestational age and maternal diet.

4.3.1 Volumes and Volume Ratios

Without considering maternal diet, the fetal, placental, fetal liver, and fetal brain volumes significantly increased with gestational age (p < 0.001; Table 4.1). Normalizing fetal organs to fetal body volume, the fetal liver:body and fetal brain:body ratios decreased with gestational age (p < 0.001; Figure 4.1A, Table 4.1). The fetal brain:liver volume ratio also decreased with gestational age (p < 0.001; Figure 4.1C, Table 4.1) and the fetus:placenta volume ratio increased with gestational age (p < 0.001; Figure 4.1D, Table 4.1).

Disregarding gestational age, placental volume was increased in the WD group (p < 0.05; Table 4.1). There were no significant differences in absolute fetal, fetal liver, or fetal brain volumes with respect to diet (Table 4.1). The fetal liver:body and fetal brain:body ratios were significantly decreased in the WD group (p < 0.01; Figure 4.1A, B, Table 4.1) compared to the CD group. The fetal brain:liver volume ratio did not show any significant differences with respect to maternal diet (Figure 4.1C, 4.1A, Table 4.1). The fetus:placenta volume ratio showed a non-significant trend towards being decreased in the WD group (p = 0.061; Figure 4.1D, Table 4.1) compared to the CD group.

When considering the interaction of maternal diet and gestational age, the absolute fetal, placental, fetal liver, and fetal brain volumes were increased with gestational age within both the WD and CD groups (p < 0.001). The placental volume was increased in the WD group at NT (p < 0.05). There was a non-significant trend towards (p = 0.061) a decreased fetal volume in WD-fed sows compared to CD-fed sows at NT. The fetal liver:body and fetal brain:body ratios were decreased with gestational age in the CD group (p < 0.001) and decreased in the WD group compared to the CD group at MT (p < 0.05; Figure 4.1A, B). The fetal brain:liver volume ratio decreased with gestational age in the CD group (Figure 4.1A, B). The fetus:placenta volume ratio increased with gestational age within both diet groups and was decreased in WD-fed mothers at NT compared to CD-fed mothers at NT (Figure 4.1 D).
Table 4.1: Fetal and placental volumes for each diet and gestational age group.

<table>
<thead>
<tr>
<th>Volume (mm$^3$)</th>
<th>CD</th>
<th>WD</th>
<th>p-value</th>
<th>MT</th>
<th>NT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetus</td>
<td>40322 ± 3299</td>
<td>33671 ± 3067</td>
<td>0.512</td>
<td>6234 ± 3459</td>
<td>67760 ± 2907</td>
<td>&gt; 0.001</td>
</tr>
<tr>
<td>Placenta</td>
<td>5828 ± 449</td>
<td>6367 ± 418</td>
<td>&gt; 0.05</td>
<td>2873 ± 471</td>
<td>9358 ± 396</td>
<td>&gt; 0.001</td>
</tr>
<tr>
<td>Fetal liver</td>
<td>2956 ± 311</td>
<td>2681 ± 287</td>
<td>0.902</td>
<td>709 ± 25</td>
<td>4928 ± 272</td>
<td>&gt; 0.001</td>
</tr>
<tr>
<td>Fetal brain</td>
<td>1890 ± 200</td>
<td>1642 ± 177</td>
<td>0.640</td>
<td>653 ± 209</td>
<td>2879 ± 168</td>
<td>&gt; 0.001</td>
</tr>
</tbody>
</table>

Figure 4.1: Fetal liver to fetal body (A), fetal brain to fetal body (B), fetal brain to fetal liver (C), and placenta to fetus (D) volume ratios measured for animals in each diet and gestational age group. The violin plots show the empirical distribution of the data, with a bold dashed line at the median and dotted lines at the first and third quartile. Horizontal lines indicate measurements that are different.
from each other, pronged horizontal lines indicate differences between gestational age disregarding maternal diet, and pronged vertical lines indicate differences between diet groups disregarding gestational age. Significance is denoted by: * p < 0.05, ** p < 0.01, and *** p < 0.001.

4.3.2 Oxygen Saturation

Mean placental T$_2$* measurements were significantly reduced with gestational age (p < 0.001; Figure 4.2A), not taking into account maternal diet. T$_2$* measurements in the fetal liver (p < 0.001; Figure 4.2B) and brain (p < 0.001; Figure 4.2C) also decreased with gestational age.

Disregarding gestational age, mean placental T$_2$* measurements were significantly decreased in WD-fed animals compared to CD-fed animals (p < 0.05; Figure 4.2A). T$_2$* in the fetal liver and brain was not affected by maternal diet.

T$_2$* significantly decreased from MT to NT in the CD-exposed placenta (Figure 4.2A). Decreased T$_2$* in the placentae of WD-fed sows compared to CD-fed sows was significant at MT (Figure 4.2A). Mean T$_2$* in the fetal liver and fetal brain were significantly decreased with gestational age in both the CD and WD groups (Figure 4.2B, C).

Figure 4.2: Mean T$_2$* measurement in placentae (A), fetal liver (B) and fetal brain (C) in each diet and gestational age group. Horizontal lines indicate measurements that are different from each other, pronged horizontal lines indicate differences between gestational age disregarding maternal diet, and pronged vertical lines indicate differences between diet groups disregarding gestational age. Significance is denoted by: * p < 0.05, ** p < 0.01, and *** p < 0.001.
4.3.3 Placental Microstructure

Without considering maternal diet, D decreased over gestational age (p < 0.01; Figure 4.3A) and D* increased with gestational age (p < 0.001; Figure 4.3B). The perfusion fraction did not change with gestational age.

Disregarding gestational age, D* was increased in the WD-fed group (p < 0.05; Figure 4.3B). D and f did not differ with respect to maternal diet.

The interaction of gestational age and maternal diet was significant for D* as it was significantly increased with gestational age in the CD-fed group. D showed a non-significant trend (p = 0.068) towards being decreased from MT to NT in the WD-fed animals.

![Figure 4.3: Mean diffusion parameters from all placentae: (A) apparent diffusion coefficient, (B) pseudodiffusion coefficient, and (C) perfusion fraction in each diet and gestational age group. Horizontal lines indicate measurements that are different from each other, pronged horizontal lines indicate differences between gestational age disregarding maternal diet, and pronged vertical lines indicate differences between diet groups disregarding gestational age. Significance is denoted by: * p < 0.05, ** p < 0.01, and *** p < 0.001.](image)

4.4 Discussion

This study aimed to investigate the effect of a high-fat, high-sugar WD on placental oxygen saturation and microvasculature at mid- and near-term. IVIM techniques were used to non-invasively assess possible microstructural changes in placentae and T2* maps were used to assess oxygen saturation in the placenta, fetal liver, and fetal brain. The most intriguing results were that WD-fed sows produced placentae with lower blood
oxygen saturation and increased blood perfusion compared to their CD-fed counterparts. By NT, the WD-exposed placentae did not have significantly different T2* than CD-exposed placentae. It is hypothesized that the increased perfusion in WD animals is an adaptation to the low oxygen environment and results in improved oxygen saturation at NT. Even with this proposed vascular adaptation, the WD-fed animals displayed a lower fetus:placenta volume ratio at late gestation compared to the CD group. The most intriguing results are summarized in Figure 4.4.

![Figure 4.4: Summary of interesting results (A) in the WD group compared to CD group at MT and NT and (B) from MT to NT in CD and WD animals. The red “down” arrows indicate a decrease in the measurement, green “up” arrows indicate an increase in the measurement, and blue equals signs indicate no change in the measurement. All changes presented here are significant.]

### 4.4.1 Volumes and Volume Ratios

Absolute measures of fetal body and placental volume over gestation were not impacted by maternal diet. Interestingly, when normalized to the fetal body volume, the fetal liver:body and fetal brain:body volume ratios were smaller in the WD group than the CD group at MT, indicating that fetal liver and brain growth are negatively impacted by the lifelong WD consumption of the sow. This may be related to the reduced placental T2* at MT in MD-fed sows, though the fetal liver and brain T2* measurements were not affected by maternal diet. Decreased fetal liver volumes have been previously documented in models of reduced placental blood flow. Previous work in the pregnant guinea pig using the same WD has shown decreased fetal brain:body volume ratios late in gestation, though here this relationship is only observed at MT.
The fetal brain to liver volume ratio was measured as an indicator of asymmetric FGR due to brain sparing as an adaptation to a poor *in utero* environment\(^7\). There was no indication that maternal diet is causing FGR in this study.

It is not surprising that the fetus:placenta volume ratio increased with gestational age as the placenta reaches its maximum size early in pregnancy, whereas the fetus grows rapidly and reaches its maximum size in late pregnancy\(^7\). The fetus:placenta volume ratio is often used as an indicator of placental efficiency, where a low placental efficiency is associated with fetal death\(^8\). The placental volume and placental efficiency were impacted by diet, with WD-fed sows having an increased placental volume and decreased fetus:placenta volume at NT. As pregnancy advances, the fetus:placenta ratio increases as a reflection of a more efficient placenta\(^7\). While this increase across gestation was observed in both diet groups, on average, the fetus:placental volume ratio increased by 382\% in the CD group and increased by only 311\% in the WD group. In human pregnancies, overweight and obese mothers have been shown to have heavier placentae\(^8\) and lower placental efficiencies\(^8\) compared to mothers with normal BMIs.

### 4.4.2 Oxygen Saturation

The mean T\(_2^*\) in the placenta, a measure related to blood oxygen saturation, significantly decreased from MT to NT in the CD group, while T\(_2^*\) was comparatively low in the WD group at both time points. The decrease in placental T\(_2^*\) decay with gestational age has been previously documented\(^7\) and is likely due to a combination of decreasing placental oxygen over gestation\(^7\) and maturation of placental architecture over gestation\(^7\). As the placenta ages, increased fibrin\(^7\) and calcification\(^7\) accelerate the T\(_2\) decay, which is closely related to the T\(_2^*\) measurement.

Placental T\(_2^*\) may be a sensitive marker of placental dysfunction as decreased T\(_2^*\) has been associated with placental insufficiency and FGR in human and animal studies\(^7\). Diet impacted placental oxygen saturation in this study as T\(_2^*\) was significantly reduced in WD-fed sows compared to CD-fed sows at MT. A study in rats found reduced placental tissue oxygen saturation in mothers fed a lifelong high-fat diet compared to
those on a control diet\textsuperscript{278}. Disruptions in oxygen transfer across the placenta may be caused by various factors, including maternal or fetal blood flow, the oxygen-carrying capacity of maternal or fetal blood, oxygen diffusion capacity, or fetoplacental oxygen consumption\textsuperscript{171}. Using T\textsubscript{2}* mapping MRI technology does not allow us to elucidate the mechanism behind this observation, but histological studies may help determine the relationship between the WD and decreased placental oxygen saturation. The oxygen saturation in the fetal organs did not differ with respect to maternal diet, indicating that the fetal oxygen supply was not affected by the decreased placental oxygen saturation in WD-fed animals at MT.

4.4.3 Placental Microstructure

The guinea pig is an excellent model for placental studies as it shares a number of important similarities with the human placenta, including a similar discoidal, haemomonochorial structure and fetal/maternal transport barrier\textsuperscript{122}. Even with these similarities, it is vital to consider differences in architecture between the human and guinea pig placenta when interpreting diffusion-weighted measurements, focusing on the labyrinthine structure of the GP placenta. Sections of the GP placenta perfused by both fetal and maternal blood vessels make up the labyrinth and are responsible for the majority of fetal and maternal exchange sites\textsuperscript{122}. The maternal and fetal microvasculature run counter-current to each other, in parallel but opposite orientations, facilitating effective diffusion for oxygen and nutrient exchange\textsuperscript{122}.

In contrast, the human placenta uses a less efficient immersion fixation for nutrient exchange where the fetal villous trees containing blood vessels are immersed in a slow-moving pool of maternal blood\textsuperscript{279}. This exchange occurs in the cavity between the basal and chorionic plates where villous trees containing fetal microcirculation are bathed in maternal blood released from openings in the basal plate, which percolates through the intervillous space and is eventually drained through the uterine veins\textsuperscript{279}. In human placental IVIM studies, the slow-moving maternal blood mimics free diffusion and contributes to signal loss described by D, while the fetal blood compartment flowing through convoluted villous trees contributes to signal loss described by D*\textsuperscript{280}. In the case
of the guinea pig placenta, maternal blood is also moving through vessels, and therefore it cannot be assumed that the D measurement includes a significant contribution from the maternal blood. Instead, we may assume that the D* measurement represents blood perfusion from both the maternal and fetal microvasculature within the labyrinth structure.

D decreased with gestational age, which has been reported previously. This result is likely due to changes in microstructure as the placenta matures\textsuperscript{271,281,282}. D* appears to increase with gestational age in the CD group. Interestingly, WD-exposed pregnancies displayed a trend of elevated D* at mid-term that was maintained through gestation and was not significantly different from D* in the CD group at NT. This observation is unexpected, as placental vascularity has previously been shown to be reduced due to maternal obesity\textsuperscript{263} and maternal high-fat diet exposure\textsuperscript{262} at both mid- and near-term in rodent models. Previous findings from maternal obesity studies have shown placental blood vessel immaturity related to hypoxia, increased inflammation, and cellular stress markers in the placenta\textsuperscript{108}. It should be noted that both of these studies utilized a high-fat diet and resulted in maternal obesity, whereas our study used a WD that included a high-sugar component in an animal model where WD did not induce maternal obesity.

It is speculated that increased perfusion measured in the placentae of WD-fed animals results from an increased blood volume flowing through the placental capillaries. While it may seem counter-intuitive that WD-fed placentae with lower T_{2}* values would have more vascular blood movement, we may be observing a placental adaptation to the low-oxygen environment in WD-exposed placentae at mid-term. Guinea pigs exposed to hypoxia have been shown to have increased fetal capillary volume density in the placental labyrinthine areas while still demonstrating reduced fetus:placenta weight ratios compared to guinea pigs in normoxic environments\textsuperscript{283}. Other studies have supported the claim that hypoxic conditions can result in adaptations to the fetal capillaries in the placenta, manifesting as a dilation of the capillary sinusoids and thinning of the villous membrane\textsuperscript{284}. Placentae with coiled, randomly branched, and dilated placental microvascular architecture have been reported in mice as a compensatory mechanism to facilitate increased oxygen supply to the developing fetus\textsuperscript{285}. 
In the current study, there were no changes in the perfusion fraction with respect to diet or gestational age, indicating that the high $D^*$ in WD-fed sows at MT is not a result of any change in placental vascular density or angiogenesis. Instead, this increased perfusion may be explained by the dilation of capillaries previously reported in the literature\textsuperscript{285} as an adaptation to the low placental oxygen saturation in the WD group at MT. It is hypothesized that chronic maternal WD exposure results in a hypoxic placenta which promotes placental vascular adaptations. Physiologically, this vascular dilation may be related to the placenta growth factor and vascular endothelial growth factor, which are vasodilators known to be upregulated in human and rodent endothelial cells exposed to hypoxia\textsuperscript{286–290}.

4.4.4 Limitations, Future Work, and Conclusions

This study's limitations include a lack of \textit{ex vivo} histology to verify MRI findings and potentially explore differences in the microvascular architecture between CD and WD-fed sows. The range of normal $T_2^*$\textsuperscript{171} and IVIM\textsuperscript{291} measurements has not yet been defined in the placenta, and particularly the guinea pig placenta, limiting our ability to categorize healthy and damaged placentae with quantitative MRI measurements.

Future work may include follow-up studies involving offspring of mothers in both diet groups to determine whether these placental changes resulted in later life metabolic disease. Measurements including body composition, glucose tolerance, liver fat percentage, and TG serum concentrations in the offspring may help determine any heightened risk of metabolic disease based on maternal diet. Diffusion tensor imaging (DTI) provides additional information on the trajectory of perfusion and has been performed in the human placenta to distinguish between normal and non-functional tissue in healthy and FGR pregnancies\textsuperscript{292}. Although it may be challenging to implement in the small guinea pig placenta, DTI may provide more information on the spatial distortion of functional placental tissue and potential changes in microvessel structure with respect to maternal diet\textsuperscript{163}. Another future aim of this work is to determine spatial patterns of $T_2^*$ and IVIM measurements as they appear to be heterogeneous across the placenta. It would be fascinating to compare oxygen saturation, diffusion, and perfusion in areas of the
placenta associated with maternal and fetal blood supply to further explore the mechanisms behind observed changes of T$_2^*$ and perfusion. Due to the complex architecture of the guinea pig placenta, methods used to segment maternal and fetal sections of the human placenta would likely not be translatable. Additionally, the small size of the guinea pig placenta makes it difficult to visualize placental substructure at a clinical MRI field strength.

In conclusion, it was found that CD-exposed placentae had increased placental blood perfusion from mid-to near-term while WD-exposed placentae displayed high perfusion at both gestational ages, possibly compensating for low oxygen saturation at mid-term. WD-exposed placentae had lower oxygen saturation and were associated with smaller fetal liver:body and fetal brain:body volume ratios at MT, but these measurements did not differ from the CD group at NT. Despite some improvements at NT, low placental efficiency was observed in the WD group, which may be a "trade-off" for the earlier placental adaptations. Further studies focusing on the WD's effect on placental function should explore the mechanisms behind the observed changes in placental T$_2^*$ and D* associated with diet. Insight into these WD-related mechanisms may help diagnose and evaluate therapeutic interventions for placental insufficiency in the future.
Chapter 5

5 Conclusions

This chapter will conclude the thesis with suggestions for future work and a summary of the research, highlighting the significance of the scientific contributions presented in this thesis.

5.1 Thesis Summary

The Western diet has a multifaceted impact on physiology, shown in this thesis to manifest as metabolic changes in the liver and vascular adaptations in the placenta. It was demonstrated that lifelong exposure to the WD results in abnormal pyruvate metabolism in the liver in conjunction with fat accumulation typical of NAFLD. The main finding of this work, described in Chapter 2, was the observation of increased lactate production observed in the livers of WD-fed animals and validated by ex vivo enzyme activity measurements. In Chapter 4, the effect of a lifelong maternal WD on placental oxygenation and blood perfusion was observed at mid- and near term. The findings from this study indicate that maternal WD exposure results in poor blood oxygen saturation in the placenta at mid-gestation, which was hypothesized to trigger a vascular response that increased placental blood perfusion. This adaptation resulted in increased oxygen saturation in the WD-exposed placentae late in gestation, though low placental efficiency was observed in these animals.

This thesis demonstrates the use of multiple MRI techniques to study metabolic disease, including hyperpolarized $^{13}$C MRS, hyperpolarized $^{13}$C MRI, chemical shift-encoded imaging, and diffusion-weighted imaging. The variety of information obtained using different MRI techniques and contrast testify to the versatility of MRI and its capability to provide structural and functional information relevant to in vivo processes. Although MRI has been in use for biomedical applications since the 1970s,$^{125}$ novel techniques such as hyperpolarized MRI are still evolving and being improved upon. This evolution is evidenced in Chapter 3, where a novel RF pulse trajectory was established to improve SNR in HP MRI. This technical improvement was shown to increase SNR in our in vivo
experiments and may allow for more precise measurements of metabolic kinetics in future work.

5.1.1 Significance and Impact

The work from Chapter 2 of this thesis demonstrated *in vivo* metabolic changes in the livers of animals with diet-induced NAFLD. These results were validated using *ex vivo* measurements and promote the use of HP [1-13C]pyruvate MRS in evaluating liver disease. The increased production of lactate in these livers indicates, alongside other animal studies, that [1-13C]lactate may be a useful biomarker of early-stage NAFLD and a non-invasive diagnostic alternative to liver biopsy.

Chapter 3 described a technical improvement in image acquisition for HP [1-13C]pyruvate MRI that may be adapted for any probe or application of HP MRI. Improvement in SNR from the msVFA contributes to the overall goal of overcoming difficulties associated with HP MRI and supporting HP MRI as a feasible imaging option in routine clinical use. This work represents a small step towards implementing HP MRI in human studies of pregnancy and metabolic disease.

The findings from Chapter 4 of this thesis demonstrate, for the first time, a placental vascular response to an adverse environment promoted by maternal diet. Although similar adaptations have been reported in cases of hypoxia, studying this response in the context of maternal diet may provide important information on the placenta’s role in fetal programming that may impact the later-life health of the offspring. Hopefully, this study will prompt further investigation into the underlying mechanisms connecting maternal diet, placental oxygen saturation, and placental perfusion.

In conclusion, the work in this thesis demonstrated and promoted the benefits of advanced MRI techniques to study metabolic disease, which is not a conventional application of these imaging methods. Further, this thesis has highlighted two examples of an adverse response to a lifelong WD and may inspire future work to investigate the underlying mechanisms of these changes and the efficacy of treatments for metabolic disease.
5.2 Future Directions

5.2.1 Longitudinal Studies

Longitudinal studies would be beneficial in future work related to the research presented in Chapters 2 and 4 of this thesis. In Chapter 2, metabolic changes were observed in conjunction with NAFLD in the guinea pig as a result of a lifelong WD exposure. One of the advantages of HP MRI is its ability to provide information about in vivo metabolism without invasive or harmful effects. Repeating this study with imaging experiments at multiple time points would allow us to understand better when these metabolic changes occur during the development of NAFLD and whether these changes remain stagnant over time as the disease progresses. These additional experiments would be especially important to observe in the progression of NAFLD to NASH to pinpoint early biomarkers of the transition from benign steatosis to more harmful hepatitis in the liver.

Related to pregnancy studies focused on maternal diet, the DOHaD hypothesis states that the lifelong health of an offspring is impacted by its in utero environment\(^8^1\). In Chapter 4, evidence that maternal WD consumption impacts placental function was presented, and it would be interesting to perform follow-up studies involving offspring from these mothers to determine if the placental changes resulted in any later life metabolic disease. Further, sex-specific abnormalities in the offspring should be investigated as there is evidence that maternal metabolic health has a sex-specific effect on the placenta\(^1^0^4\).

5.2.2 Diet Intervention and Exercise Studies

In this work, adverse effects of WD consumption in the liver and placenta were demonstrated. As with many metabolic diseases, primary treatment typically involves calorie reduction via diet intervention and suggestions for increased physical activity\(^2^6\). It is known that early-stage NAFLD is reversible with gradual weight loss\(^5^0\), leading to decreased liver volume and hepatic fat accumulation\(^2^3^4\). It is unclear whether the metabolic adaptation of increased lactate production observed in Chapter 2 would revert or persist with these diet and exercise interventions. An HP \(^{1^3}\)C imaging study designed
to induce NAFLD followed by a period of calorie restriction and/or increased exercise is proposed to investigate the impact of these interventions on in vivo hepatic metabolism.

Benefits of a healthy diet and exercise have been shown in the placenta to aid in placental growth\textsuperscript{293}, prevent placental hypoxia\textsuperscript{294}, prevent placental overgrowth, and reverse impaired placental vascularization\textsuperscript{263}. In Chapter 4, adverse effects in the placenta of WD-fed sows related to vascularization and oxygen saturation were observed. It would be interesting to conduct a study comparing placental oxygenation and blood perfusion between mothers fed a WD with and without diet or exercise intervention. This could also lead to a longitudinal study comparing the metabolic health of the offspring from each group later in life.

5.2.3 HP [2-$^{13}$C]pyruvate MRI/MRS

Chapters 2 and 3 of this thesis focused on MR spectroscopy and imaging of [1-$^{13}$C]pyruvate and its downstream metabolites to study metabolic disease. Abnormal pyruvate metabolism in the livers of WD-fed animals were observed in Chapter 2, indicated by an increased rate of lactate production. Based on related enzyme activity results, it was hypothesized that WD-fed animals also experienced decreased bicarbonate production, suggesting fewer pyruvate molecules undergoing oxidative phosphorylation via the TCA cycle compared to CD-fed animals. Using [1-$^{13}$C]pyruvate as the hyperpolarized probe limits our ability to visualize metabolism within the TCA cycle. A similar study using a hyperpolarized [2-$^{13}$C]pyruvate probe would be useful to determine the metabolic fate of pyruvate in the TCA cycle and determine whether diet influences aerobic metabolism in the liver. HP [2-$^{13}$C]pyruvate is less commonly used as it has a shorter T\textsubscript{1} and less polarization than [1-$^{13}$C]pyruvate\textsuperscript{196} but nevertheless is capable of providing information involving the integrity of the TCA cycle in different metabolic states.

Previous research in the McKenzie lab has observed no significant changes with respect to maternal diet and pyruvate placental metabolism using HP [1-$^{13}$C]pyruvate MRI; however, it may be beneficial to repeat this study using [2-$^{13}$C]pyruvate imaging as maternal diet may have an impact on the placental aerobic metabolism within the TCA
cycle which is not detectable using \([1^{-13}C]\)pyruvate\(^{295}\). The general RF pulse strategy presented in Chapter 3 may be tailored and implemented to increase SNR of \([2^{-13}C]\)pyruvate and its downstream metabolites for HP MRI in these applications.

5.2.4 HP \(^{31}\text{P}\) MRI

Metabolic dysfunction resulting in the buildup of excess lactate may lead to acidosis, resulting in abnormal changes to local pH in extreme cases\(^{74}\). Severe chronic hypoxia, which may be induced by altered metabolism and tissue remodelling in NAFLD, may also lead to pH changes in the liver\(^{296}\). Decreased oxygen saturation in the placenta, demonstrated in Chapter 4, can result in hypoxia over time and may be associated with fetoplacental acidosis, resulting in deviations from normal pH\(^{74}\). The ability to detect extracellular pH non-invasively would be beneficial for both of these applications.

Since pH is regulated by the bicarbonate buffer system, the ratio of bicarbonate and CO\(_2\) can be used to estimate pH\(^{297}\). While this is possible to measure with HP \(^{13}\text{C}\) MRI, images of these metabolites typically have low SNR and are difficult to acquire\(^{297}\). Other compounds such as inorganic phosphate experience chemical shifts at different pH\(^{298}\). Conveniently, \(^{31}\text{P}\) is NMR-sensitive and 100% abundant, motivating the use of inorganic phosphate’s pH-sensitive chemical shift to construct pH maps using MRI. As with most non-proton nuclei, it is difficult to acquire \(^{31}\text{P}\) MR images due to low concentration and low \(\gamma\), but it has recently been shown that inorganic phosphate can be hyperpolarized via d-DNP\(^{298}\). This technique has been used to evaluate pH in \textit{ex vivo} samples using MRS, with a hyperpolarized solution of inorganic phosphate with phosphocreatine as a reference\(^{298}\). It is theoretically possible to use this technique for \textit{in vivo} MRI, using chemical shift-encoded imaging to construct maps of extracellular pH. An adaptation to the optimized RF pulse trajectory presented in Chapter 3 can be applied to HP \(^{31}\text{P}\) MRI to increase image SNR further. This is an area of future research in the McKenzie lab and may be applied in studies of NAFLD and placental insufficiency.
5.2.5 Human studies

The imaging experiments in this thesis made use of a guinea pig model. Animal models provide many advantages, including control of the animals’ environment and diet, a short lifespan and gestation, and the ability to extract tissue components for *ex vivo* comparisons. The disadvantage of animal models is their physiological differences to humans and the need for anesthesia in imaging studies which may impact metabolism during imaging. While the guinea pig is an ideal model for studying metabolic disease and pregnancy due to its lipoprotein profile and placental structure being similar to humans, there are also many differences. A major difference between the guinea pig and human is the tendency for humans to become obese when exposed to the WD while the guinea pig remains lean, though still displaying metabolic consequences such as increased visceral adipose tissue. Regarding pregnancy, the guinea pig produces multiple offspring while humans typically birth one offspring per pregnancy, which may cause differences in resource allocation during gestation. As discussed in Chapter 4, despite the similarities in placental structure between the two animals, there remain differences regarding placental vasculature, with the guinea pig displaying a labyrinthine structure.

To overcome the limitations of an animal model, clinical studies provide more accurate information on human metabolism and disease. The eventual goal of these advanced MRI techniques is their application in clinical settings. All imaging experiments in this thesis were performed on a commercial clinical 3T MRI and are theoretically possible to replicate in human subjects.

HP $^{13}$C MRI and MRS have been implemented in clinical studies and have demonstrated safety and success in identifying biomarkers for oncologic applications. To date, only preclinical studies of HP $^{13}$C MRI/MRS for NAFLD have been published; however, previous work and work presented in Chapter 2 provide evidence of its use for identifying potential metabolic biomarkers of NAFLD and motivation for its use in human studies. Work presented in Chapter 3 that demonstrated improved SNR for HP $^{13}$C MRI may also be implemented in clinical studies that pose additional challenges of larger
field of view coverage and longer durations for delivery of the hyperpolarized injection into the subject.

Diffusion-weighted imaging and IVIM methods have been implemented in the human placenta\(^{302,303}\), demonstrating safety and adequate sensitivity. These imaging techniques have not yet been implemented to study the effects of maternal diet on placental function in humans, though the work presented in Chapter 4 motivates this investigation. In Chapter 4, an adaptation of the placental vasculature in response to poor oxygenation influenced by maternal diet was demonstrated in the guinea pig. It would be interesting to observe if this placental adaptation is common to the human placenta as well. The human placenta also provides an opportunity to compare changes in different placental compartments, as they are more easily discernable than in a small animal model\(^{280}\). Translation of these MRI techniques to clinical studies may enable us to learn more about physiological changes associated with diet in the human body and potentially lead to improvements in personalized patient care.
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Appendices

Appendix A: Ethics Approvals

**AUP Number:** 2010-229  
**PI Name:** Regnault, Timothy  
**AUP Title:** In Utero Origins Of Adult Insulin Resistance  
**Approval Date:** 09/08/2014

**Official Notice of Animal Use Subcommittee (AUS) Approval:** Your new Animal Use Protocol (AUP) entitled "In Utero Origins Of Adult Insulin Resistance" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal. 2010-229::5

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura  
on behalf of the Animal Use Subcommittee  
University Council on Animal Care

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**AUP Number:** 2015-063  
**PI Name:** Regnault, Timothy  
**AUP Title:** Hyperpolarized 13c Mri Of Placental Metabolic Abnormalities Resulting From The Western Diet  
**Approval Date:** 01/22/2016

**Official Notice of Animal Use Subcommittee (AUS) Approval:** Your new Animal Use Protocol (AUP) entitled "hyperpolarized 13c Mri Of Placental Metabolic Abnormalities Resulting From The Western Diet" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal. 2015-063::1

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura  
on behalf of the Animal Use Subcommittee  
University Council on Animal Care
AUP Number: 2019-116
AUP Title: Hyperpolarized 13C MRI of Placental Metabolic Abnormalities Resulting from the Western Diet
Yearly Renewal Date: 02/01/2022

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2019-116 has been approved by the Animal Care Committee (ACC), and will be approved through to the above review date.

Please at this time review your AUP with your research team to ensure full understanding by everyone listed within this AUP.

As per your declaration within this approved AUP, you are obligated to ensure that:

1) Animals used in this research project will be cared for in alignment with:
   a) Western’s Senate MAPPs 7.12, 7.10, and 7.15
      http://www.uwo.ca/univac/policies_procedures/research.html
   b) University Council on Animal Care Policies and related Animal Care Committee procedures
      http://uwo.ca/research/services/animalethics/animal_care_and_use_policies.html

2) As per UCAC’s Animal Use Protocols Policy,
   a) this AUP accurately represents intended animal use;
   b) external approvals associated with this AUP, including permits and scientific/departmental peer approvals, are complete and accurate;
   c) any divergence from this AUP will not be undertaken until the related Protocol Modification is approved by the ACC; and
   d) AUP form submissions - Annual Protocol Renewals and Full AUP Renewals - will be submitted
      and attended to within timeframes outlined by the ACC... http://uwo.ca/research/services/animalethics/animal_use_protocols.html

3) As per MAPP 7.10 all individuals listed within this AUP as having any hands-on animal contact will
   a) be made familiar with and have direct access to this AUP;
   b) complete all required CCAC mandatory training ([%20%20training@uwo.ca]: training@uwo.ca); and
   c) be overseen by me to ensure appropriate care and use of animals.

4) As per MAPP 7.25,
   a) Practice will align with approved AUP elements;
   b) Unrestricted access to all animal areas will be given to ACVS Veterinarians and ACC Leaders;
   c) UCAC policies and related ACC procedures will be followed, including but not limited to:
      i) Research Animal Procurement
      ii) Animal Care and Use Records
      iii) Sick Animal Response
   iv) Continuing Care Visits

5) As per institutional OH&S policies, all individuals listed within this AUP who will be using or potentially exposed to hazardous materials will have completed in advance the appropriate institutional OH&S training, facility-level training, and reviewed related (M)SDS Sheets,
   http://www.uwo.ca/hr/learning/required/index.html

Submitted by: Copeman, Laura
on behalf of the Animal Care Committee
University Council on Animal Care
Curriculum Vitae

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**Conference Proceedings:**


15. Fradin, C., Barkley, C., Smith, L., Nadkarni, R., Hashmi, M. Taking the Temperature of Rotating Magnetotactic Bacteria. Oral presentation at 5\textsuperscript{th} Annual International Magnetotactic Bacteria Meeting in Marseille, France on September 13, 2016.