THE ROLE OF LIPID METABOLISM IN ALTERED INSULIN SENSITIVITY IN ENDOCRINE DISEASE

A THESIS
SUBMITTED TO THE UNIVERSITY OF MANCHESTER
FOR THE DEGREE OF
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IN THE FACULTY OF MEDICAL AND HUMAN SCIENCES

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SCHOOL OF MEDICINE
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# Contents

Abstract 15

Declaration 16

Copyright 17

Acknowledgements 18

Contributions To This Work 20

Sources of Funding 22

1 Introduction 23

1.1 Introduction .............................................. 23
   1.1.1 Overview of Study Plan ............................ 23
   1.1.2 Body Composition ................................. 24
   1.1.3 Body Composition in acromegaly and Cushing’s disease ... 25

1.2 Insulin and its actions .................................. 25

1.3 Overview of Lipid Metabolism .......................... 26
   1.3.1 Regulation of Lipid Metabolism ................... 29
   1.3.2 Pharmacological Inhibition of Lipolysis .......... 30

1.4 Growth Hormone ........................................ 30
   1.4.1 Growth Hormone and the IGF-I axis .............. 31
   1.4.2 Maintaining Glucose Homeostasis ............... 32
   1.4.3 Acromegaly ........................................ 33
1.5 Cortisol ................................................................. 35
  1.5.1 Cushing’s syndrome ............................................. 35
1.6 The role of Lipids in Insulin Sensitivity .......................... 36
1.7 Summary .............................................................. 37

2 Background - Imaging Study ................................. 38
  2.1 Methods of Body Composition measurement ............... 38
    2.1.1 Macroscopic Body Composition ............................ 38
  2.2 1H Magnetic Resonance Spectroscopy ......................... 39
  2.3 Software .......................................................... 41
    2.3.1 MRicro ......................................................... 41
    2.3.2 jMRUI ......................................................... 41

3 Patients and Methods - Imaging Study ....................... 43
  3.1 Patients .......................................................... 43
    3.1.1 Patient Characteristics .................................... 44
  3.2 Study Design ..................................................... 45
  3.3 Whole Body MRI .................................................. 46
    3.3.1 Image Acquisition .......................................... 46
    3.3.2 Image analysis .............................................. 48
    3.3.3 Calculating Adiposity within Body compartments ....... 48
    3.3.4 Definitions of body compartments ....................... 49
  3.4 Magnetic Resonance Spectroscopy ............................. 49
    3.4.1 Liver Spectroscopy .......................................... 50
    3.4.2 Muscle Spectroscopy ....................................... 50
  3.5 Analysis of Spectra .............................................. 50
    3.5.1 Liver ........................................................ 50
    3.5.2 Muscle ..................................................... 51
  3.6 Calculations and Statistics ................................... 53

4 Results - Imaging Study ........................................ 54
  4.1 Introduction ..................................................... 54
  4.2 Body Composition by Bioimpedance ............................ 56
  4.3 Multi Slice Magnetic Resonance Imaging ....................... 59
  4.4 1H Magnetic Resonance Spectroscopy ........................ 63
    4.4.1 Liver ........................................................ 63
    4.4.2 Skeletal Muscle ........................................... 64
  4.5 Summary of Results ............................................. 67
### CONTENTS

#### 4.5.1 Bioimpedance ................................. 67
#### 4.5.2 Multi-Slice MRI ............................. 67
#### 4.5.3 Magnetic Resonance Spectroscopy .......... 67

#### 5 Background - Metabolic Study 68

- 5.1 Methods of Measuring Insulin sensitivity (and β cell function) .......... 68
- 5.2 Fasting Measures of Insulin Sensitivity ........................................ 68
- 5.3 Oral Glucose Tolerance Test ......................................................... 69
- 5.4 Insulin Clamps ............................................................................. 69
- 5.5 Frequently Sampled IVGTT ......................................................... 70
- 5.6 Stable Isotope Studies ................................................................. 70
- 5.7 Modelling Glucose Kinetics ......................................................... 70
  - 5.7.1 MINMOD ................................................................. 70

#### 6 Patients and Methods - Metabolic Study 72

- 6.1 Patients ......................................................................................... 72
- 6.2 Patient Characteristics ................................................................. 73
- 6.3 Study Design ............................................................................... 75
- 6.4 Study Protocol ............................................................................. 75
- 6.5 Overnight Study ........................................................................... 76
- 6.6 Intravenous Glucose Tolerance Test .............................................. 76
- 6.7 Handling of Blood samples ........................................................... 76
  - 6.8 Samples ....................................................................................... 77
    - 6.8.1 Glucose and glucose for isotope analysis ................................. 77
    - 6.8.2 Glycerol, Free Fatty Acid and BOH measurements .................. 77
    - 6.8.3 Insulin .................................................................................... 77
    - 6.8.4 Isotope Samples ..................................................................... 77
- 6.9 Glucose and Glycerol Isotope analysis .......................................... 78
  - 6.9.1 Calculating Isotopic $R_a$ and $R_d$ ............................................. 78
  - 6.9.2 Calculations and Statistics ....................................................... 79

#### 7 Results - Metabolic Study 80

- 7.1 Results .......................................................................................... 80
- 7.2 Overnight and Basal ....................................................................... 81
  - 7.2.1 Healthy Volunteers ................................................................. 81
  - 7.2.2 Acromegaly .............................................................................. 81
  - 7.2.3 Cushing’s ................................................................................ 88
- 7.3 Minimal Modelling Results from Intravenous Glucose Tolerance Test .. 93
## CONTENTS

7.3.1 Healthy Volunteers - Cold Model .......................... 94
7.3.2 Acromegaly - Cold Model ............................... 94
7.3.3 Acromegaly - Hot Model ................................. 98
7.3.4 Cushing’s - Cold Model ................................. 102
7.3.5 Cushing’s - Hot Model ................................. 102
7.4 Summary of Results ........................................ 103
  7.4.1 Overnight and Basal ..................................... 103
  7.4.2 IVGTT and MINMOD .................................... 104

### 8 Discussion 105

8.1 Summary of Findings .......................................... 105
  8.1.1 Acromegaly .............................................. 105
  8.1.2 Cushing’s ............................................... 106
8.2 Implications of these findings ................................ 106
  8.2.1 Acromegaly .............................................. 106
  8.2.2 Cushing’s ............................................... 108
8.3 Limitations of The Studies .................................... 109
  8.3.1 Lack Of Control Data ................................... 109
  8.3.2 Numbers of Patients Studied ............................ 109
  8.3.3 Sampling and Modelling ............................... 110
8.4 Further Work .................................................. 110

References 118

A First Principles of Minimal Modelling - Adapted from Caumo 119
  A.1 Minimal Modelling ......................................... 119

---

Final word count: 16982
List of Figures

1.1 The insulin receptor and phosphorylation cascade .......................... 26
1.2 Overview of protein, carbohydrate and lipid metabolism .................. 27
1.3 Overview of Non Esterified Fatty Acids (NEFA) and TriAcyl Glycerol (TAG) trafficking ........................................ 27
1.4 Enzymatic pathways involved in NEFA and TAG metabolism ............. 28
1.5 A summary of insulin action on lipid metabolism ........................... 29
1.6 Intracellular growth hormone signalling ...................................... 31
1.7 Neuroendocrine regulation of growth hormone (GH) secretion .......... 32
1.8 Randle’s Theory ........................................................................ 36

2.1 $^1$H spectra from gastrocnemius after water peak removed ............... 40
2.2 $^1$H spectra from gastrocnemius after peak identification ............... 40
2.3 Abdominal cross-sectional MRI slice from MRIcro viewer ................ 42

3.1 Magnetic Resonance representation of the slice and gap thickness ........ 47
3.2 Abdominal cross-sectional MRI slice from MRIcro viewer ................. 48
3.3 $^1$H MRS spectra from liver, created on jMRUI software ................ 50
3.4 $^1$H spectra from gastrocnemius after water peak removed ............... 51
3.5 $^1$H spectra from gastrocnemius after peak identification ............... 52

4.1 BMI of Healthy Volunteers, Acromegaly and Cushing’s Patients ........ 57
4.2 Total Adipose Tissue (TAT) by Bioimpedance ............................... 57
4.3 Total Body Water (TBW) by Bioimpedance .................................. 58
4.4 Fat Free Mass (FFM) by Bioimpedance ........................................ 58
4.5 Total Adipose Tissue (TAT) by MRI ........................................... 60
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6</td>
<td>Subcutaneous Adipose Tissue (SAT) by MRI</td>
<td>60</td>
</tr>
<tr>
<td>4.7</td>
<td>Visceral Adipose Tissue (VAT) by MRI</td>
<td>61</td>
</tr>
<tr>
<td>4.8</td>
<td>Intra-Muscular Adipose Tissue (IMAT) by MRI</td>
<td>61</td>
</tr>
<tr>
<td>4.9</td>
<td>Mean Total Adipose Tissue Bioimpedance vs. MRI</td>
<td>62</td>
</tr>
<tr>
<td>4.10</td>
<td>Hepatic Triglyceride :water ratio by $^1$H MRS</td>
<td>64</td>
</tr>
<tr>
<td>4.11</td>
<td>Triglyceride CH$_2$ :water ratio in Tibialis Anterior by $^1$H MRS</td>
<td>65</td>
</tr>
<tr>
<td>4.12</td>
<td>Triglyceride CH$_2$ :water ratio in Gastrocnemius by $^1$H MRS</td>
<td>65</td>
</tr>
<tr>
<td>4.13</td>
<td>Triglyceride CH$_3$ :water ratio in Tibialis Anterior by $^1$H MRS</td>
<td>66</td>
</tr>
<tr>
<td>4.14</td>
<td>Triglyceride CH$_3$ :water ratio in Gastrocnemius by $^1$H MRS</td>
<td>66</td>
</tr>
<tr>
<td>6.1</td>
<td>Timeline of study protocol</td>
<td>75</td>
</tr>
<tr>
<td>7.1</td>
<td>Mean Overnight Plasma Insulin in Healthy Volunteers and Acromegaly Patients</td>
<td>82</td>
</tr>
<tr>
<td>7.2</td>
<td>Mean AUC for Overnight Plasma Insulin in Healthy Volunteers and Acromegaly Patients</td>
<td>83</td>
</tr>
<tr>
<td>7.3</td>
<td>Mean Overnight Plasma Glucose in Healthy Volunteers and Acromegaly Patients</td>
<td>83</td>
</tr>
<tr>
<td>7.4</td>
<td>Mean AUC for Overnight Plasma Glucose in Healthy Volunteers and Acromegaly Patients</td>
<td>84</td>
</tr>
<tr>
<td>7.5</td>
<td>HOMA-IR in Healthy Volunteers and Acromegaly Patients</td>
<td>85</td>
</tr>
<tr>
<td>7.6</td>
<td>HOMA-$\beta%$ in Healthy Volunteers and Acromegaly Patients</td>
<td>85</td>
</tr>
<tr>
<td>7.7</td>
<td>Hepatic Insulin Sensitivity (HIS) in Acromegaly Patients</td>
<td>87</td>
</tr>
<tr>
<td>7.8</td>
<td>Mean Overnight Plasma Insulin in Healthy Volunteers and Cushing’s Patients</td>
<td>88</td>
</tr>
<tr>
<td>7.9</td>
<td>Mean AUC for Overnight Plasma Insulin in Healthy Volunteers and Cushing’s Patients</td>
<td>89</td>
</tr>
<tr>
<td>7.10</td>
<td>Mean Overnight Plasma Glucose values for Healthy Volunteers and Cushing’s Patients</td>
<td>89</td>
</tr>
<tr>
<td>7.11</td>
<td>Mean AUC for Overnight Plasma Glucose in Healthy Volunteers and Cushing’s Patients</td>
<td>90</td>
</tr>
<tr>
<td>7.12</td>
<td>HOMA-IR in Healthy Volunteers and Cushing’s Patients</td>
<td>91</td>
</tr>
<tr>
<td>7.13</td>
<td>HOMA-$\beta%$ in Healthy Volunteers and Cushing’s Patients</td>
<td>91</td>
</tr>
<tr>
<td>7.14</td>
<td>Hepatic Insulin Sensitivity (HIS) in Cushing’s Patients</td>
<td>92</td>
</tr>
<tr>
<td>7.15</td>
<td>Comparison of (a) Si (p&lt;10$^{-7}$) and (b) Comparison of DI (p&lt;10$^{-7}$)</td>
<td>93</td>
</tr>
<tr>
<td>7.16</td>
<td>Cold Model Insulin Sensitivity (Si) in Acromegaly Patients</td>
<td>95</td>
</tr>
<tr>
<td>7.17</td>
<td>Cold Model Glucose Effectiveness (Sg) in Acromegaly Patients</td>
<td>96</td>
</tr>
<tr>
<td>7.18</td>
<td>Acute Insulin Response to Glucose (AIRg) in Acromegaly Patients</td>
<td>97</td>
</tr>
</tbody>
</table>
7.19 Cold Disposition Index (DI) in Acromegaly Patients. ............... 98
7.20 Hot Model Insulin Sensitivity (Si) in Acromegaly Patients. .......... 99
7.21 Hot Model Glucose Effectiveness (Sg) in Acromegaly Patients. ...... 100
7.22 Hot Model Disposition Index (DI) in Acromegaly Patients. .......... 101

A.1 One-pool glucose and two-pool insulin model without isotope ....... 120
A.2 Glucose and insulin model with ‘hot’ glucose ($G^b$). ............... 120
### List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Healthy Volunteers in the imaging studies</td>
<td>44</td>
</tr>
<tr>
<td>3.2</td>
<td>Acromegaly Patients in the imaging studies</td>
<td>45</td>
</tr>
<tr>
<td>3.3</td>
<td>Cushing’s Patients in the imaging studies</td>
<td>46</td>
</tr>
<tr>
<td>4.1</td>
<td>Imaging details of Acromegaly Patients</td>
<td>54</td>
</tr>
<tr>
<td>4.2</td>
<td>Imaging details of Cushing’s Patients</td>
<td>55</td>
</tr>
<tr>
<td>4.3</td>
<td>Body Composition by Bioimpedance</td>
<td>56</td>
</tr>
<tr>
<td>4.4</td>
<td>Adipose tissue compartments by total body MRI</td>
<td>59</td>
</tr>
<tr>
<td>4.5</td>
<td>(^1)H Magnetic Resonance Spectroscopy results</td>
<td>63</td>
</tr>
<tr>
<td>6.1</td>
<td>Acromegaly Patients</td>
<td>73</td>
</tr>
<tr>
<td>6.2</td>
<td>Cushing’s Patients</td>
<td>74</td>
</tr>
<tr>
<td>6.3</td>
<td>Healthy Volunteers</td>
<td>74</td>
</tr>
<tr>
<td>7.1</td>
<td>Hepatic Insulin Sensitivity (HIS) in Acromegaly Patients</td>
<td>86</td>
</tr>
<tr>
<td>7.2</td>
<td>Hepatic Insulin Sensitivity (HIS) in Cushing’s Patients</td>
<td>92</td>
</tr>
<tr>
<td>7.3</td>
<td>Data for the 'cold’ glucose kinetics model for Healthy Volunteers</td>
<td>94</td>
</tr>
<tr>
<td>7.4</td>
<td>Data for the 'cold’ glucose kinetics model for Acromegaly Patients</td>
<td>94</td>
</tr>
<tr>
<td>7.5</td>
<td>Data for the 'hot’ glucose kinetics model for Acromegaly Patients</td>
<td>99</td>
</tr>
<tr>
<td>7.6</td>
<td>Data for the 'cold’ glucose kinetics model for Cushing’s Patients</td>
<td>102</td>
</tr>
<tr>
<td>7.7</td>
<td>Data for the 'hot’ glucose kinetics model for Cushing’s Patients</td>
<td>103</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>Active Acromegaly</td>
<td></td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
<td></td>
</tr>
<tr>
<td>AHT</td>
<td>Anti-Hypertensive Therapy</td>
<td></td>
</tr>
<tr>
<td>AIRg</td>
<td>Acute Insulin Response to Glucose</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Transaminase</td>
<td></td>
</tr>
<tr>
<td>AMARES</td>
<td>Advanced Method for Accurate Robust and Efficient Spectral fitting of MRS data</td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Transaminase</td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>Adipose Tissue</td>
<td></td>
</tr>
<tr>
<td>ATAGL</td>
<td>Adipose Triacylglycerol Lipase</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
<td></td>
</tr>
<tr>
<td>BOH</td>
<td>Beta Hydroxy Butyrate</td>
<td></td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin Releasing Hormone</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
<td></td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
<td></td>
</tr>
<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
<td></td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual Energy X-ray Absorptiometry</td>
<td></td>
</tr>
<tr>
<td>DI</td>
<td>Disposition Index</td>
<td></td>
</tr>
<tr>
<td>DICOM</td>
<td>Digital Imaging and Communications in Medicine</td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>Extra-Myocellular</td>
<td></td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
<td></td>
</tr>
<tr>
<td>fMRI</td>
<td>functional MRI</td>
<td></td>
</tr>
<tr>
<td>FSIVGTT</td>
<td>Frequently Sampled Intravenous Glucose Tolerance Test</td>
<td></td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas Chromatography - Mass Spectrometry</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>Growth Hormone</td>
<td></td>
</tr>
<tr>
<td>GHD</td>
<td>Growth Hormone Deficiency</td>
<td></td>
</tr>
<tr>
<td>GHR</td>
<td>Growth Hormone Receptor</td>
<td></td>
</tr>
<tr>
<td>GHRH</td>
<td>Growth Hormone Releasing Hormone</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>Gastro-Intestinal</td>
<td></td>
</tr>
<tr>
<td>GLUT4</td>
<td>Glucose Transporter Type 4</td>
<td></td>
</tr>
<tr>
<td>HIS</td>
<td>Hepatic Insulin Sensitivity</td>
<td></td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis Model Assessment</td>
<td></td>
</tr>
<tr>
<td>HRMAS-NMR</td>
<td>High Resolution Magic Angle Spinning Spectroscopy - Nuclear Magnetic Resonance</td>
<td></td>
</tr>
<tr>
<td>HSL</td>
<td>Hormone Sensitive Lipase</td>
<td></td>
</tr>
<tr>
<td>HV</td>
<td>Healthy Volunteers</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>IAAT</td>
<td>Intra-Abdominal Adipose Tissue</td>
<td></td>
</tr>
<tr>
<td>ICH GCP</td>
<td>International Conference on Harmonisation Good Clinical Guidance</td>
<td></td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin like Growth Factor</td>
<td></td>
</tr>
<tr>
<td>IGFBP</td>
<td>Insulin Growth Factor Binding Protein</td>
<td></td>
</tr>
<tr>
<td>IHTG</td>
<td>Intrahepatocellular Triglyceride</td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>Intra-Myocellular</td>
<td></td>
</tr>
<tr>
<td>IMAT</td>
<td>Intramuscular Adipose Tissue</td>
<td></td>
</tr>
<tr>
<td>IMTG</td>
<td>Intramyocellular Triglyceride</td>
<td></td>
</tr>
<tr>
<td>IPSS</td>
<td>Inferior Petrosal Sinus Sampling</td>
<td></td>
</tr>
<tr>
<td>IR</td>
<td>Insulin Resistance</td>
<td></td>
</tr>
<tr>
<td>IRS</td>
<td>Insulin Receptor Substrates</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
<td></td>
</tr>
<tr>
<td>IVGTT</td>
<td>Intravenous Glucose Tolerance Test</td>
<td></td>
</tr>
<tr>
<td>JAK/STAT</td>
<td>Janus Kinase and Signal Transducer and Activator of Transcription</td>
<td></td>
</tr>
<tr>
<td>KW</td>
<td>Kruskal Wallis</td>
<td></td>
</tr>
<tr>
<td>LDDST</td>
<td>Low Dose Dexamethasone Suppression Test</td>
<td></td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein Lipase</td>
<td></td>
</tr>
<tr>
<td>LREC</td>
<td>Local Research Ethics Committee</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
<td></td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic Resonance Spectroscopy</td>
<td></td>
</tr>
<tr>
<td>MWU</td>
<td>Mann Whitney U test</td>
<td></td>
</tr>
<tr>
<td>NEFA</td>
<td>Non Esterified Fatty Acid</td>
<td></td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral Glucose Tolerance Test</td>
<td></td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>OHA</td>
<td>Oral Hypoglycaemic Agents</td>
<td></td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-Kinase</td>
<td></td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome Proliferator-Activated Receptor</td>
<td></td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid Hormone</td>
<td></td>
</tr>
<tr>
<td>QUICKI</td>
<td>Quantitative Insulin-Sensitivity Check Index</td>
<td></td>
</tr>
<tr>
<td>Rₐ</td>
<td>Rate of Appearance</td>
<td></td>
</tr>
<tr>
<td>Rᵋ</td>
<td>Rate of Disappearance</td>
<td></td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
<td></td>
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<td>SAT</td>
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<td>SD</td>
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<tr>
<td>Sg</td>
<td>Glucose Effectiveness</td>
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<td>Si</td>
<td>Insulin Sensitivity</td>
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<td>SIM</td>
<td>Selected Ion Monitoring</td>
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<tr>
<td>SRIF</td>
<td>Somatotropin Release Inhibiting Factor</td>
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<td>T2DM</td>
<td>Type 2 Diabetes</td>
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<tr>
<td>TAT</td>
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<td>tert.ButyldiMethylSilyl</td>
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<td>TBW</td>
<td>Total Body Water</td>
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<td>Triglycerides</td>
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<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
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<tr>
<td>TSH</td>
<td>Thyroid-Stimulating Hormone</td>
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<td>Abbreviation</td>
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<tr>
<td>TSS</td>
<td>Trans-Sphenoidal Surgery</td>
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<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
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<tr>
<td>VAPRO</td>
<td>Variable Projection</td>
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<td>VAT</td>
<td>Visceral Adipose Tissue</td>
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<tr>
<td>VLDL</td>
<td>Very Low-Density Lipoprotein</td>
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<td>Voxel</td>
<td>Volumetric Picture Element</td>
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ACROMEGALY AND CUSHING’S DISEASE ARE RARE ENDOCRINE CONDITIONS. THE LEAN PHENOTYPE OF ACROMEGALY IS IN STARK CONTRAST TO THAT OF CUSHING’S, WHERE INCREASED FAT DEPOSITION IS SEEN. BOTH CONDITIONS ARE ASSOCIATED WITH ALTERED INSULIN SENSITIVITY AND LIPID METABOLISM.

USING MULTI-SLICE WHOLE BODY MRI AND 1H MAGNETIC RESONANCE SPECTROSCOPY, MACRO AND MICROSCOPIC FAT DISTRIBUTION WAS OBSERVED. USING STABLE ISOTOPES OF Glucose AND GLYCEROL, INSULIN SENSITIVITY AND RATES OF LIPOLYSIS WERE ASSESSED IN THE BASAL AND STIMULATED STATE.

USING ACIPIMOX (SYNTHETIC NICOTINIC ACID) TO BLOCK LIPOLYSIS, THE IMPACT OF FREE FATTY ACIDS (FFA) ON INSULIN SENSITIVITY IN THESE CONDITIONS WAS OBSERVED.

ACROMEGALY PATIENTS HAD REDUCED INTRAHEPATIC TRIGLYCERIDE, AS MEASURED BY 1H MRS. THIS IS A NOVEL FINDING. INSULIN SENSITIVITY WAS IMPROVED BY LIPOLYSIS INHIBITION IN BOTH THE BASAL AND STIMULATED STATE IN ACROMEGALY; IN ADDITION TO IMPROVED TISSUE SENSITIVITY, LIPOLYSIS BLOCKADE IMPROVED FIRST PHASE INSULIN SECRETION INDICATING A POSSIBLE ROLE OF FREE FATTY ACIDS IN β Cell toxicity.

CUSHING’S DISEASE WAS ASSOCIATED WITH HIGHER VISCERAL ADIPOSE TISSUE (VAT) AND HAD HIGHER INTRAHEPATIC TRIGLYCERIDE BY 1H MRS, ALTHOUGH THE LATTER WAS NOT STATISTICALLY SIGNIFICANT. BLOCKING LIPOLYSIS HAD A MORE MODEST EFFECT ON BASAL INSULIN SENSITIVITY IN CUSHING’S.

THESE RESULTS SUGGEST THAT LIPOLYSIS IS CRITICAL TO THE DEVELOPMENT OF INSULIN RESISTANCE IN ACROMEGALY, AND THE LOW HEPATIC TRIGLYCERIDE SUGGESTS THAT FFA FLUX THROUGH THE LIVER MAY BE RESPONSIBLE FOR THE HEPATIC INSULIN RESISTANCE SEEN. ANTI-LIPOLYSIS HAS A MORE MODEST EFFECT ON INSULIN RESISTANCE IN CUSHING’S, PERHAPS REFLECTING THE DIFFERING PATHOPHYSIOLOGY INVOLVED.
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I would like to express my gratitude to the following people. Their participation (unless stated here) is detailed in the following section, *Contributions To This Work*.

Firstly, to my research supervisors- Professor Peter Trainer at The Christie for his wisdom, support and guidance, and to Dr Tara Kearney at Salford Royal Hospital for her support and encouragement, and for allowing me this research opportunity.

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Mr David Ryder at The Christie and Sigrid Whiteside at University Hospital of South Manchester who provided statistical guidance.

Mrs Sara Wassell, Laboratory Assistant at HNR-MRC Cambridge for processing the isotope samples.

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My consultant colleagues at University Hospital of South Manchester who allowed me time to complete the writing of this thesis.

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To K for her love, sympathy and understanding.

This thesis is dedicated to the patient (and healthy) volunteers without whom this research would not have been possible.
Dr Claire Higham and Prof. Peter Trainer designed the imaging study protocols in collaboration with Steve Williams, Professor of Imaging Science at University of Manchester. The scanning was carried out in the Translational Imaging Unit at Salford Royal NHS Foundation Trust.

The original protocols for the metabolic aspects of this research were designed by Dr Claire Higham and Prof. Peter Trainer in Manchester, in collaboration with Prof. David Dunger, Dr Les Bluck and Dr Burak Salgin in Cambridge. Dr Bluck in particular provided expertise in the use of stable isotopes and glucose modelling using the Frequently Sampled Intra-Venous Glucose Tolerance Test.

Liquid Gas Chromatography-Mass Spectroscopy was carried out in The Human Nutrition Research Laboratory, Cambridge by Sara Wassell. Dr Les Bluck at HNR Cambridge produced glucose and isotopic modelling.

I recruited both patients and healthy volunteers for the imaging and metabolic studies. I was involved in the development of the $^1$H Magnetic Resonance Spectroscopy protocols in collaboration with Prof. Steve Williams, and derived data from the scans using MRIcro and jMRUI software. I worked with Prof. Williams in the discussion and analysis of these results. With the help of the Research Nurses from the Endocrine Unit, The Christie, I carried out the overnight studies and IVGTTs and was involved in the processing, spinning and storage of the blood samples. I helped develop the methods for the IVGTTs in an attempt to minimise haemolysis of the samples, and assisted Sara Wasell with some LGC-MS processing in Cambridge. I collaborated with Dr Bluck in the analysis of the glucose modelling results.
CONTRIBUTIONS TO THIS WORK

David Ryder at The Christie NHS Trust and Sigrid Whiteside at Wythenshawe Hospital UHSM Trust provided statistical guidance.
The metabolic studies were financed from The Christie Endocrine Research Fund.

Scanning time at the Transitional Imaging Unit, Salford Royal Hospital, was provided by University of Manchester.

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1.1 Introduction

1.1.1 Overview of Study Plan

Acromegaly and Cushing’s disease are conditions arising from pathological hypersecretion of Growth Hormone and cortisol respectively. Despite quite differing biochemical profiles and phenotypes, both these conditions are associated with insulin insensitivity and diabetes mellitus. It is hypothesised that there are several differing mechanisms for the associated insulin resistance.

While increased adiposity in general has been known to have a detrimental effect on insulin sensitivity, it has been postulated that the distribution of fat may have an influence on insulin signalling and action. It has also been suggested that microscopic i.e. intracellular concentrations of lipids in key organs on which insulin acts (liver and skeletal muscle) may also be of importance. The characteristic phenotypes of acromegaly and Cushing’s disease relate, at least in part, to an alteration in body composition. There is also evidence that lipid turnover is altered in these disease states.

The role of lipid metabolism in the alteration of insulin sensitivity in these conditions is to be studied, with a focus on two areas: firstly, the macro and microscopic distribution of fat observed in acromegaly and Cushing’s. This will be done using whole body multi slice magnetic resonance imaging (MRI) scan in conjunction with $^1$H magnetic resonance
spectroscopy (MRS), the latter measuring \( \text{CH}_2 \) and \( \text{CH}_3 \) moieties of triglyceride in muscle and liver to assess intracellular lipid content. Secondly, we hypothesise that lipolysis (resulting in increased Free Fatty Acid [FFA] production) is a modulator of insulin sensitivity in these conditions. Stable isotopes of glucose and glycerol will be used to assess insulin sensitivity and glycerol production in active Cushing’s and acromegaly, in both the basal (overnight) and stimulated state, intravenous glucose tolerance test (IVGTT). By studying patients on two occasions, both before and on Acipimox (a pharmacological inhibitor of lipolysis), the effect of lipolysis/FFA production in this insulin sensitivity will be studied.

The Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT) will permit, using a modified minimal model, calculations of insulin sensitivity (Si), glucose effectiveness (Sg), Acute Insulin Response to glucose (AIRg) and Disposition Index (DI). AIRg provides a measurement of \( \beta \) cell function and DI is a product of this and the stimulated insulin sensitivity. These measures will be calculated using MINMOD modelling software. This technique, and others used in measuring insulin sensitivity will be detailed in chapter 2.

### 1.1.2 Body Composition

Excess adipose tissue has long been associated with morbidity. It has been postulated that the distribution of this fat may have an influence on insulin signalling and action. Abdominal obesity, and more specifically visceral adipose tissue (VAT) appears to play a significant role in the development of the metabolic syndrome, namely decreased insulin sensitivity, type 2 diabetes (T2DM), hypertension and Cardiovascular Disease (CVD). The more female ‘pear’ vs. male ‘apple’ distribution of fat was, or is thought to explain, at least in part, the increased incidence of cardiovascular disease among men. The correlation between Subcutaneous Adipose Tissue (SAT) and metabolic risk factors are weaker, and favourable improvements in intra-abdominal adipose tissue (IAAT) via diet and exercise confer a lowering of risk not observed in similar reductions in SAT e.g. via liposuction.

There is also evidence that intracellular deposition of fat in key insulin target organs may be important; Intrahepatocellular triglyceride (IHTG) and intramyocellular triglyceride (IMTG) measured using \(^1\text{H} \) MRS have shown a correlation between increasing IHTG and IMTG in obesity, insulin resistance and T2DM. Whether this is cause or effect has not been elucidated.
1.1.3 Body Composition in acromegaly and Cushing’s disease

As previously stated, central adiposity, and more specifically visceral adipose tissue is correlated with impaired insulin sensitivity.

Endocrine disease often results in characteristic body composition changes. Paradoxically, Cushing’s disease and acromegaly both have strong associations with insulin resistance and type 2 diabetes, but with quite different distribution of adiposity; Cushing’s patients develop truncal obesity, with reduced limb muscle mass [1], whereas patients with acromegaly are known to have reduced fat mass, including reductions in visceral adiposity [2]. It has been postulated that this may be due to growth hormone (GH) causing increased β adrenergic receptor activation, in turn activating Hormone Sensitive Lipase (HSL) [3], and that visceral adipose tissue is more responsive to this stimulation than subcutaneous fat stores [4].

It is also observed that both GH deficiency (GHD) and acromegaly are associated with insulin resistance, but with differing body composition. GH deficiency causes increased fat mass and reduction in lean muscle mass [1] while pathological GH excess have opposite effects; In GHD total body mass does not change significantly with treatment, as the decrease in fat is accompanied by an increase in lean muscle. In acromegaly, this does not tend to result in a reduction in weight, as total body water has been shown to significantly increase [5].

In these studies we will use MRS to assess whole body fat content, and 1H MRS to assess intramyocellular and intrahepatocellular triglyceride content in active acromegaly, Cushing’s disease and normal volunteers. These techniques, and others used to study body composition, will be detailed in chapter 2.

1.2 Insulin and its actions

Insulin is a peptide hormone isolated in 1921 by a group led by Banting and Best [6]. It is secreted by β cells of the pancreas (as a pro-hormone) in response to a postprandial rise in plasma glucose and amino acid levels. Insulin acts via its cell surface receptor. This has 2 alpha and 2 beta subunits that are disulphide bonded into an a2b2 heterotetrameric complex. This, and the insulin-signalling pathway, are simplified in Figure 1.1.

At the liver, insulin decreases gluconeogenesis and glycogenolysis, which in turn limits hepatic glucose production. It facilitates glucose uptake in other areas (primarily skeletal muscle and adipose tissue) via translocated glucose transporter type 4 (GLUT4) receptors
1.3. OVERVIEW OF LIPID METABOLISM

that (in the basal state) are sequestered in cytoplasmic vesicles. The phosphorylation cascade which occurs in muscle and adipose tissue is summarised below in Figure 1.1 [2].

![Figure 1.1: The insulin receptor and phosphorylation cascade](image)

[2] Insulin Receptor activation phosphorylates IRS-1, which binds to and activates phosphoinositol 3 kinase (PI3K). PI3K catalyses the reaction PIP2 + ATP → PIP3. PIP3 activates protein kinase B (PKB). PKB phosphorylates glycogen synthase kinase (GSK) and thereby inactivates GSK. This then cannot phosphorylate Glycogen synthase, allowing glycogen production via GS. PKB/Akt also facilitates migration of GLUT4 to the cell surface.

Insulin promotes hepatic fatty acid synthesis, prevents breakdown of triglyceride to FFA within adipocytes, and by facilitating glucose transport into adipocytes, contributes to fat synthesis within these tissues.

In addition to these roles, insulin is a powerful anabolic hormone, necessary for tissue development and growth.

1.3 Overview of Lipid Metabolism

Carbohydrate, lipid and indeed protein metabolism have common pathways. All three can be broken down into Acetyl-CoA, a key fuel for cellular respiration. This is illustrated in figure 1.2.

Most dietary fat is ingested as triglycerides (known as TG). Free Fatty Acids, the product
1.3. OVERVIEW OF LIPID METABOLISM

The enzymatic pathways in lipid metabolism is described in more detail in figure 1.4 and the citric acid cycle is outlined in figure 1.8, Randle’s Theory.

of dietary fat digestion i.e TG, are a major source of fuel for oxidative metabolism, glucose being the other. For transport in the circulation, these are either bound to albumin or are re-esterified with glycerol to form TG and complexed in lipoprotein packages (chylomicrons from the gut, Very low-density lipoprotein (VLDL for export from the liver). Most are stored as TG in adipose tissue, but are taken up in smaller quantities by hepatic and muscle tissue. This is summarised in figure 1.3.

Figure 1.3: Overview of Non Esterified Fatty Acids (NEFA) and TriAcyl Glycerol (TAG) trafficking.


27
In order for the circulating TG to enter adipose tissue for storage, it must first be hydrolysed by lipoprotein lipase (LPL) present on the luminal surface of the capillary endothelium [8] before being esterified with glycerol to TG. At this point, TG can either be hydrolysed (under the influence of ATAGL, ATAGL and HSL) for use as fuel in the mitochondria via $\beta$ oxidation, or released back into the circulation. These enzymatic pathways are outlined in Figure 1.4 below.

Figure 1.4: Enzymatic pathways involved in NEFA and TAG metabolism. Adapted from [7]. Acc, acetyl-CoA carboxylase; Acsl, long chain acyl-CoA synthetase; Agpat, 1-acylglycerol-3-phosphate O-acyltransferase; ATGL, adipose triglyceride lipase; Cpt1, carnitine palmitoyl transferase; DAG, diacylglycerol; Dgat; DAG O acyltransferase; DNL, de novo lipogenesis; Fas, fatty acid synthase; Gpat, glycerol-3-phosphate O-acyltransferase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; mGpat, mitochondrial glycerol phosphate acyltransferase; PAP, phosphatidate phosphatase.
1.3. OVERVIEW OF LIPID METABOLISM

When attempting to measure adipose tissue lipolysis, glycerol is a more accurate marker than FFAs, as the latter can appear in the circulation without ever having been taken up into the adipocyte, whereas glycerol cannot be removed from the circulation as adipose tissue lacks glycerol kinase, the enzyme required for this transport.

1.3.1 Regulation of Lipid Metabolism

This is a tightly regulated process and can be considered in both the acute phase and long term; in the fed state, insulin secretion is increased, promoting LPL activity in adipose tissue (but down regulating LPL in muscle). Insulin also acts to suppress lipolysis in adipocytes (see figure 1.5). In the fasted state, insulin levels fall and GH secretion increases; while circulating adrenalin and noradrenalin acutely promote lipolysis, growth hormone has a delayed and less potent effect. LPL has been shown to increase adipose tissue lipolysis 2-3 hours after a pulse, [9] and has important regulatory effects in both exercise and fasting. The inter-relationship between insulin and GH is more complex still; increased GH secretion results in increased Insulin like Growth Factor-I (IGF-I) production, which (as its name suggests) has insulin-like effects on lipolysis, albeit with less potency. Furthermore, the main source of circulating IGF-I is the liver, and insulin governs the sensitivity of hepatic tissue to the effects of GH. In the fasted state, when insulin secretion is low and GH high, the liver becomes de-sensitised to GH effect, and IGF-I production decreases. In contrast, hyperinsulinaemia sensitises the liver to GH. IGF-I production is therefore attenuated by both insulin and GH, and in the healthy physiologic state, provides a modulating bridge in the control of fat storage, mobilisation and oxidation.

![Figure 1.5: A summary of insulin action on lipid metabolism](image)

Longer-term control of lipid metabolism is influenced by environmental factors- the availability of food, eating patterns and exercise. It is less well understood than the acute setting, but nuclear hormone receptor activation (including Peroxisome proliferator-activated receptors PPARs and gonadal steroid receptors) affects adipocyte differentiation among other actions. While thyroid-stimulating hormone (TSH) and parathyroid hormone (PTH)
are also known to stimulate lipolysis, Alpha agonists and adenosine (among others) have inhibitory effects.

1.3.2 Pharmacological Inhibition of Lipolysis

Both GH and cortisol modulate insulin sensitivity by direct and indirect mechanisms. A potentially important indirect mechanism is the respective impact of these hormones on lipolysis. Acipimox, a nicotinic acid derivative has been found to be a potent short-term inhibitor of lipolysis. In diabetic and insulin resistant subjects, insulin resistance and FFA production was reduced by short term administration of Acipimox [10], and when given to GHD adults, the decrease in insulin sensitivity resulting from GH administration was not observed [11]. An improvement in β cell function was observed with short term Acipimox administration to type 2 diabetics and obese patients [12]. This may imply that free fatty acids have a direct toxic effect on β cells.

1.4 Growth Hormone

Growth hormone is a 22 kDa single, linear chain of 191 amino acids within four alpha helical cores held by two disulphide bonds and with two distinct receptor binding sites. The Growth Hormone Receptor (GHR) exists as preformed dimers which, when bound to GH results in a conformational change and activation/phosphorylation of (JAK/STAT) pathway (see Figure 1.6).

Growth Hormone is perhaps poorly named; while it has an important function promoting linear growth in children, it is primarily an anabolic hormone, promoting cell multiplication, cartilage and bone deposition, protein synthesis, hepatic glucose production and lipolysis. It has both direct (via the GH receptor) and indirect effects via IGF-I and lipolysis.

The exact nature of the effects of GH on insulin sensitivity is not well understood. Some small studies (mainly under artificial physiological conditions) have suggested a role for lipolysis in GH modulation of insulin sensitivity. Direct effects of GH via receptor binding and subsequent intracellular signalling via the JAK/STAT pathway have pointed to a potential duality of both insulin sensitising and resisting effects, outwith its IGF-I modulated effects. GH binding to its receptor stimulates IGF-I production at the liver and other tissues that have insulin-like effects, and indeed GH itself stimulates phosphorylation of insulin receptor substrates (IRS) that (via Phosphoinositide 3-kinase, PI3K) transiently
stimulate glucose transport, lipogenesis and protein synthesis. This PI3K stimulation is not however a straightforward amplification of insulin signalling; PI3K exists as 2 dimeric subunits, p85 and p110, and the intracellular ratios of these determine whether they block or facilitate insulin signalling. The precise regulation of this process remains unknown. These have remained difficult to unravel due to the shared pathways of GH, IGF-I and insulin, and the complex nature of their interaction.

![Figure 1.6: Intracellular growth hormone signalling](image)

1.4.1 Growth Hormone and the IGF-I axis

GH and IGF-I secretion and metabolism are under complex physiological control and is mediated via the hypothalamus, anterior pituitary and the liver. GH (and as a consequence IGF-I) is a major component in the maintenance of glucose homeostasis under normal conditions. GH synthesis and release are chiefly governed by growth hormone releasing hormone (GHRH), ghrelin and somatostatin (see figure 1.7).

For clarity, Figure 1.7 shows only the most proximal modulators affecting pituitary somatotrophs. Central nervous system mechanisms integrate responses to stress, exercise, caloric intake, sleep, and circadian and ultradian rhythms, responding with changes in hypothalamic secretion of growth hormone releasing hormone, somatostatin (SRIF), and (possibly) ghrelin. Caloric balance, stress, disease, and other factors in turn affect synthesis of IGF-I (insulin-like growth factor–I) by the liver and other GH target organs. Ghrelin stimulates hunger and food intake, although the physiologic importance of ghrelin for normal GH regulation is not well defined.
IGF-I is a 7964 Da polypeptide of 70 amino acids. This, and IGF-2 are part of a family of insulin like growth factors which are closely homologous to (pro-) insulin. While IGF-I is secreted by a number of tissues, including muscle and bone, the majority of circulating IGF-I is hepatic in origin; its production in other tissues is not thought to reach the circulation, acting only locally in a paracrine manner. Six IGF binding proteins have been identified (IGFBP 1-6), but in healthy subjects over 80% are bound to IGFBP3 and an acid labile subunit as it circulates in the form of a 150 KDa ternary complex. Both insulin and IGF-I are able to bind to each other’s cognate receptors, albeit with lower affinities. They share common signalling pathways as demonstrated in Figure 1.1.

Under normal physiological conditions, the complex interaction outlined in figure 1.7 results in pulsatile release of GH. Feeding, largely a daytime phenomenon, supresses GH secretion, so these pulses occur mainly at night during slow wave sleep, with somatostatin inhibition the key influence on the GH peaks [13].

1.4.2 Maintaining Glucose Homeostasis

GH and IGF-I act to maintain glucose homeostasis, and supply or store energy as the need arises. In the fed state, when a rise in glucose causes increased insulin secretion,
GH release is correspondingly reduced. In a fasted state, GH rises and its gluconeogenic and lipolytic effects predominate, perhaps to provide alternate substrate for tissues, while a fall in IGF-I levels is indicative of a state of relative GH resistance.

Studies using knockout mouse models showed that hepatic IGF-I deficient mice had high GH, insulin and glucose levels, which, when bred with GH antagonistic transgenic mice had significantly improved glucose and insulin levels. This suggests that even modest levels of IGF-I (hepatic IGF-I knockout mice had 75% less circulating IGF-I) can improve insulin sensitivity in the absence of the effect of GH [14]. The fluctuations in insulin and GH levels with satiety and fasting contrast with the relatively stable IGF-I levels; in the fasted state, GH is abundant, the liver is relatively resistant to its effects resulting in lower IGF-I and decreased free IGF-I, while in the fed state (when GH is low) hyperinsulinaemia sensitises the liver to the effects of GH, raising IGF-I, synergising insulin effects. Therefore, in common with the modulation of lipolysis, IGF-I has a stabilising influence on the mobilisation, storage and oxidation of glucose.

In summary, the fluctuating production, yet complimentary actions of GH and insulin are responsible for normal physiological glucose homeostasis. IGF-I on the other hand, remains stable over a 24h period, and is more likely to provide a stable background modulation of this action.

### 1.4.3 Acromegaly

Acromegaly results from autonomous excessive GH secretion (typically from a somatotroph adenoma of the anterior pituitary), with both circulating GH and IGF-I levels being elevated. Patients have impaired hepatic and peripheral insulin sensitivity and diabetes mellitus [15], while developing a ‘lean’ phenotype [2], [5].

The underlying mechanisms causing these changes in insulin sensitivity in acromegaly are not fully understood. While glucose homeostasis is maintained by the *yin and yang* of GH and insulin under normal conditions, the insulin resistance and diabetes seen in acromegaly is less well described; the constant production of GH in this condition contrasts with its normal nocturnal pulsatility, and its effects, both direct and indirectly via increased lipolysis, and on change in body composition, may all play a part in this insulin resistant state. Furthermore, this resistance encourages insulin production, and in contrast to the complementary effects in of GH and insulin in normal physiology, the pancreas increases secretion secondary to GH induced insulin resistance.

GH increases lipolysis in adipocytes independently of IGF-I (there are limited IGF-I re-
ceptrons on adipocytes). A single pulse of GH in normal subjects significantly increases serum FFA levels up to 2x the baseline at approximately 2-3 hrs. post pulse [16]. This increase in FFA can be inhibited by Acipimox and is therefore lipolysis dependent [11]. The replacement of GH in GHD patients has been shown to increase serum levels of FFAs.

The assumption therefore is that GH excess in acromegaly stimulates lipolysis. During a hyperinsulinemic clamp however, FFA production in acromegalic patients is not as susceptible to suppression by low dose insulin as are normal controls [15]. The pattern of secretion of GH is probably important, with an increased baseline secretion causing increased lipolysis, and increased pulsatility having separate effects on insulin sensitivity [16].

Serum levels of FFA and glycerol reflect both rates of lipolysis and rates of substrate uptake. Glycerol production rate is a more accurate reflection of rate of lipolysis and can be measured effectively with stable glycerol isotopes but this has not yet been fully studied in acromegaly.

A study using Pegvisomant (GH receptor antagonist) to examine the effect of acromegaly on glucose and lipid metabolism showed improved central and peripheral insulin sensitivity, but without any alteration in FFA metabolism (measured by palmitate appearance) [17]. A later study by Higham et al (2009) used stable isotopes, overnight sampling and a hyperinsulinaemic euglycaemic clamp. This showed reductions in overnight FFA production with Pegvisomant, with corresponding improvements in insulin sensitivity [18]. They could not however reproduce Jorgensen’s reduction in hepatic glucose production (HGP), as the supraphysiological insulin levels in the clamp completely suppressed HGP.

In summary, the autonomous secretion of GH in acromegaly appears to disrupt the complex interplay among GH, insulin and IGF-I, with significant effects on carbohydrate and lipid metabolism. The precise mechanisms are poorly understood. Lipolysis is increased, and in a previous study does not appear to be suppressed by insulin, suggesting that of the actions of GH may be dominant. Blockade of GH action (using Pegvisomant) improves insulin sensitivity, but the observed effect on lipolysis was inconsistent. It is likely that the relationship between acromegaly, impaired insulin sensitivity and lipid metabolism is complex; by blocking lipolysis, the effect of FFA production on this could be studied. The use of a FSIVGTT allows observation of β cell function, and the effect of short term inhibition of lipolysis on insulin secretion. Secondly, measurement of intrahepatocellular lipid in acromegaly has not been studied, and this may suggest potential mechanisms for the hepatic insulin insensitivity.
1.5 Cortisol

Cortisol is the primary glucocorticoid in man and is synthesised from a cholesterol base in the adrenal cortex using 11 β hydroxysteroid dehydrogenase enzymes catalysing the inactive cortisone to active cortisol. It is secreted in response to adrenocorticotropic hormone (ACTH) stimulation from the pituitary that is itself stimulated by hypothalamic corticotropin-releasing hormone (CRH) in response to stress. This axis exhibits diurnal variation, with cortisol levels peaking in the early morning. While GH/IGF-I play a key role in glucose homeostasis during normal physiological conditions, cortisol provides an alternative mechanism for preventing hypoglycaemia at times of stress by stimulating hepatic gluconeogenesis and decreasing glucose uptake in skeletal muscle. The latter is thought at least in part due to inhibition of translocation of GLUT4 receptors to the muscle cell membrane [19].

1.5.1 Cushing’s syndrome

Cushing’s syndrome is the consequence of long-term inappropriate excessive cortisol secretion and is associated with decreased insulin sensitivity, and an increased incidence of diabetes mellitus. It has characteristic body composition changes -increased truncal obesity, increased visceral fat and wasting of predominantly proximal muscles.

Cortisol is known to affect enzymes controlling rates of lipolysis and lipogenesis, lipoprotein lipase and hormone sensitive lipase. Lipid metabolism in Cushing’s has been investigated with conflicting results. Studies in active Cushing’s syndrome revealed no difference in FFA and glycerol serum concentrations between Cushing’s and normal controls [20] although other studies have demonstrated significantly increased levels of FFA compared to controls [21], [22]. More recent investigations using isolated subcutaneous adipose tissue and microdialysis techniques revealed increased rates of lipolysis as evidenced by increased local glycerol production [23]. It is unclear whether these in vitro observations are reproduced in human subjects; hypercostisolaemia, in addition to effects on adipocyte differentiation and modulation of lipolysis, is a potent appetite stimulator, and this contributes to increased adiposity, providing an increased source of FFAs. VLDL production is up regulated in Cushing’s syndrome, giving rise to atherogenic rises in LDL, and intrahepatic TG synthesis is potentiated by up regulation of some of the enzymes involved. While overall rates of lipolysis were not increased in Cushing’s studies, the microdialysis studies may suggest selective up-regulation of lipolysis (as observed in subcutaneous tissue), but the study measuring regional fat depot lipolysis has not yet been
1.6 The role of Lipids in Insulin Sensitivity

Randle’s Theory over forty years ago, postulated that the ’alternative fuel source’ had a role in modulating insulin sensitivity [24]. This is illustrated in figure 1.8.

Figure 1.8: Randle’s Theory

[25] Glucose-fatty-acid cycle within the myocyte proposed by Randle and colleagues. CoA=coenzyme A. PDH=pyruvate dehydrogenase. PFK=phosphofructokinase. G6P=glucose-6-phosphate. HK=hexokinase. Red circle with minus sign represents inhibition. Black line with arrowhead represents increase or accumulation of substrate. Blue dotted line with arrowhead indicates a pathway that is inhibited.

Subsequent studies have shown that, rather than FFAs competitively inhibiting glucose oxidation, the defect arises in insulin signalling, leading to a decrease in glucose uptake.
(via GLUT 4 transport) in muscle, which in turn causes more substrate to arrive at the liver. De-novo lipogenesis can occur, causing both an accumulation of TG in the liver, and an increase in VLDL manufacture to carry FFA to adipose tissue for storage. The intermediate metabolites of TG synthesis (the prime suspect diacylglycerol, DAG) cause inhibition of intrahepatic insulin signalling which results in increased hepatic glucose production.

Plasma FFAs are also implicated in the modulation of $\beta$ cell function. In the short term, increased levels (within physiological limits) of FFAs were associated with an improvement in $\beta$ cell function, whereas inhibition of lipolysis (via Acipimox) in Type 2 diabetes resulted in augmented insulin secretion in response to a glucose load.

1.7 Summary

Studies to date have suggested possible mechanisms by which altered lipid metabolism can impair insulin sensitivity. Similarly, studies of body composition and intracellular lipid content have demonstrated that visceral and intrahepatocellular fat are associated with the metabolic syndrome, insulin resistance and diabetes.

Acromegaly and Cushing’s syndrome provide two models of insulin resistance. The exact mechanisms remain poorly understood; by detailed measurement of micro and macroscopic fat distribution in these conditions, and observing the effects of lipolysis on the attenuation of insulin sensitivity, our understanding of these mechanisms may be improved.
2.1 Methods of Body Composition measurement

Excess adipose tissue has long been associated with morbidity and mortality. It is an independent risk factor in the development of cardiovascular disease, hypertension, diabetes and cancer. The mechanisms conferring this increased risk, including the distribution of body fat, have been extensively studied over the past thirty years. The development of non-invasive techniques for studying fat content at an intracellular level within the tissues—particularly $^1$H Magnetic Resonance Spectroscopy (‘proton’ MRS)—has widened scope for study.

2.1.1 Macroscopic Body Composition

Several techniques have been validated to investigate body composition. For studies involving large number of patients, a quick, readily available and relatively inexpensive test is advantageous. 2-D DEXA (Dual Energy X-ray Absorptiometry) has been used to measure lean body mass and adiposity using relatively small doses of ionising radiation [26]. For 3-D examination, whole body CT provides more detailed information, making it more suitable for smaller studies where detection of subtle differences between study groups is required. The main disadvantages are long scanning times and exposure to high dose radiation, which makes it largely unsuitable for research purposes.
MRI scanning can offer similarly high detail imaging, whilst exposing the patient to no harmful radiation. Total body scanning provides images that allow accurate calculations of body fat distribution and mass [27], in addition to lean muscle mass. Total body scanning involves multiple (in our study 66) slices, and can prove uncomfortable for the subject. Whilst it is a highly accurate method, scan times of up to an hour make this a costly method, and coupled with analysis time and financial constraints often make this method suitable only for studies with a small number of recruits.

Using a single abdominal slice to estimate SAT and VAT has been performed [28], although recent reviews have questioned the reliability of this method [29]. The advantage of a single slice is that it can be performed and analysed rapidly. It is therefore useful in larger population studies.

The use of mobile pallet scanners, where the patient is transported through the scanner on a mechanised tray, is limited by the subject’s weight (a scanning table will typically allow a 120kg limit) and by body habitus; MRI scanners in particular have a relatively narrow aperture. As the main interest in studying body fat distribution is within obese study groups, body habitus can limit the suitability of this modality.

### 2.2 $^1$H Magnetic Resonance Spectroscopy

Until recently, the measurement of metabolites or compounds within tissues required tissue biopsy and subsequent biochemical assay techniques. While these provided highly detailed and reproducible data, they were limited by the invasive nature of the procedure. Magnetic Resonance Spectroscopy (MRS) allows non-invasive, real time quantification of metabolites.

$^1$H MRS produces strong signal to noise ratios from the sensitive proton nucleus. This allows quantification of both fat and water signals using similar techniques to clinical Magnetic Resonance Imaging. Unlike $^{31}$P and $^{13}$C imaging, it cannot be used for real-time assessment of metabolism.

While $^1$H MRS has been used predominantly in the study of the brain with functional MRI (fMRI), the proton nuclei in both CH$_2$ and CH$_3$ moieties of triglyceride (TG) can be detected by $^1$H MRS in both muscle [30] and liver [31]. Furthermore, intracellular and extracellular Triglyceride concentrations can be estimated separately. A spectrum from gastrocnemius is shown in Figure 2.2.

Intramyocellular triglyceride (IMTG) concentrations have been found to correlate with
2.2. $^1$H MAGNETIC RESONANCE SPECTROSCOPY

Figure 2.1: $^1$H spectra from gastrocnemius after water peak removed.
*The original trace shows choline and creatine peaks at 3.2 ppm and 3.02 ppm respectively, and the four TG peaks appear as a single deflection. Y-axis has no scale (arbitrary units).*

Figure 2.2: $^1$H spectra from gastrocnemius after peak identification.
*After peak identification using the AMARES function of jMRUI, four peaks are found: $A = ECH_2$, $B = ICH_2$, $C = ECH_3$, $D = ICH_3$. Y-axis has no scale (arbitrary units).*

decreased insulin sensitivity, in patients with Type 2 Diabetes Mellitus, in obesity [32], increasing age and high fat feeding [33]. Prolonged fasting (72 hours) also resulted in significantly increased IMTG [34]. No studies have previously been performed in active acromegaly or Cushing’s, although administration of Growth Hormone to healthy individuals did not result in significant alterations in IMTG [35]. Some studies have suggested that IMTG is significantly altered by exercise [30] and diet [33], and regimes for standardising this measurement have been proposed [36].

In a large study intrahepatic TG was found to be closely correlated with decreasing insulin sensitivity, and importantly, remained unaltered in the fed and 12 hour fasting states [37]. Hepatic (but not intramyocellular) TG was reduced after treatment with thiazolidinedione
[38], with subsequent improvement in insulin sensitivity in T2DM patients.

2.3 Software

MRIcro (http://www.mccauslandcenter.sc.edu/mricro), a radiology image viewer, was used to quantify adipose tissue distribution from multi-slice MRI scanning, and jMRUI (http://www.mrui.uab.es/mrui) to semi-quantify intracellular triglyceride content using $^1$H MRS spectra from liver and muscle. These are validated tools for body composition studies.

2.3.1 MRIcro

Rorden and Brett devised this primarily for imaging brain lesions [39], although it can be used as a general radiology image-viewing platform. It allows analysis of Volumetric Picture Elements (Voxels), which are in essence the 3D equivalents of pixels. If the voxel volume is known then the volume of a particular body composite e.g. fat, muscle etc. can be calculated on each slice. Multi-slice MRI images can be uploaded either through DICOM or Philips file managers. Pixel intensity can be set to highlight iso-dense areas within the image, and this can be applied to all slices in the imaging sequence. This facilitates rapid identifying of bone, brain, muscle and (in this study) adipose tissue. The resulting images can be ‘cleaned up’ manually - areas wrongly identified as fat can be deleted, and adipose tissue not identified automatically can be added. See Figure 2.3.

2.3.2 jMRUI

The Java based MRI User Interface (jMRUI) enables user-friendly time-domain analysis of magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) and High Resolution Magic Angle Spinning Spectroscopy - Nuclear Magnetic Resonance (HRMAS-NMR) signals [40]. It can be used in $^{13}$C, $^{31}$P and $^1$H MRS applications. In these studies, AMARES deconvolution and HSVLD (dominant peak suppression) applications were used.
Figure 2.3: Abdominal cross-sectional MRI slice from MRIcro viewer.

Subcutaneous fat is highlighted in red.
3.1 Patients

Eight patients with acromegaly (AA), seven with Cushing’s disease and five healthy volunteers were recruited for the study. Patient and healthy volunteer characteristics are described in tables 3.1, 3.2 and 3.3.

Inclusion Criteria

- All subjects (Healthy Volunteers, Acromegaly patients, Cushing’s patients): Age >18 years
- Acromegaly patients: active disease as evidenced by elevated Growth Hormone (GH) and IGF-I > upper limit of normal (ULN)
- Cushing’s patients: active disease as evidenced by raised 24 hour Urinary Free Cortisol (24h UFC) levels on two occasions
- Ability to give consent

Exclusion Criteria

- All subjects: Pregnancy; presence of cardiac pacemaker / implantable cardiac defibrillator / metallic heart valve / metal fragments in the eye / cochlear implants; other conditions that may result in abnormal GH /IGF-I/cortisol (e.g. severe hepatic disease / severe renal impairment / malnutrition / alcohol abuse / levodopa /
heroin abuse; inability to comply with study procedures; claustrophobia; ALT or AST >3 × ULN or clinically significant hepatic disease.

- Healthy volunteers: Prescribed regular medications and/or diagnosed with a chronic disease.

### 3.1.1 Patient Characteristics

Five healthy volunteers were recruited. Characteristics are presented in table 3.1.

<table>
<thead>
<tr>
<th>Healthy Volunteer</th>
<th>Sex</th>
<th>Age</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>39</td>
<td>28.7</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>32</td>
<td>26.4</td>
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<td>M</td>
<td>28</td>
<td>24.9</td>
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<td>F</td>
<td>32</td>
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</tr>
<tr>
<td><strong>Median</strong></td>
<td></td>
<td>30.5</td>
<td>28.1</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td></td>
<td>28-39</td>
<td>24.9-29.0</td>
</tr>
</tbody>
</table>

Table 3.1: Healthy Volunteers in the imaging studies

Eight patients with acromegaly, mean ages 39.5 (23-66) years were recruited, two were female. All were Caucasian. Mean IGF-I:ULN was 1.80 (1.22-4.44). Two had prior Trans-sphenoidal surgery (TSS) and one of these had radiotherapy and was on stable replacement therapy for TSH and Gonadotropin deficiency. Two other patients were treated medically. Patient characteristics for acromegaly are presented in table 3.2.

Seven patients with Cushing’s disease, median age 37, (range 19-53) were recruited, three were female. Patient characteristics are presented in table 3.3. All were Caucasian. One had adrenal Cushing’s and was taking Metyrapone therapy; she had Type 2 Diabetes and was taking oral hypoglycaemic agents (OHAs) and anti-hypertensive therapy (AHT). Three patients were diabetic and were taking OHAs. Three patients with Cushing’s disease had pituitary macroadenomas, one had documented cyclical disease without an identified source, and the other had ACTH driven hypercortisolaemia with a pituitary microadenoma and no other detectable source, although Inferior Petrosal Sinus Sampling (IPSS) had shown equivocal results.

The study was approved by Liverpool Local Research Ethics Committee (LREC) and sponsored by the R&D Dept. at The Christie NHS Foundation Trust. Patients were
3.2. STUDY DESIGN

<table>
<thead>
<tr>
<th>Acromegaly Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Other pituitary insufficiency</th>
<th>On treatment</th>
<th>IGF-I(ng/ml)(^1)</th>
<th>IGF-I:ULN</th>
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<tr>
<td>1</td>
<td>M</td>
<td>23</td>
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<td>LAG 120mg/4wk LAR 30mg/4wk</td>
<td>610</td>
<td>1.70</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>40</td>
<td>Nil</td>
<td></td>
<td>326</td>
<td>1.22</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>38</td>
<td>Nil</td>
<td>Sx + LAR 30mg/4wk + CBG 0.5mg/wk</td>
<td>833</td>
<td>2.93</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>51</td>
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<td></td>
<td>277</td>
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</tr>
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<td>5</td>
<td>M</td>
<td>39</td>
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<td></td>
<td>1244</td>
<td>4.44</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>56</td>
<td>TSH and GN</td>
<td>Sx + XRT</td>
<td>369</td>
<td>1.64</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
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<td></td>
<td>602</td>
<td>1.96</td>
</tr>
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<td>8</td>
<td>M</td>
<td>66</td>
<td>Nil</td>
<td></td>
<td>480</td>
<td>2.4</td>
</tr>
<tr>
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<td></td>
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<td></td>
<td></td>
<td>326-1244</td>
<td>1.22-4.44</td>
</tr>
</tbody>
</table>

Table 3.2: Acromegaly Patients in the imaging studies

Notes to table: CBG=Cabergoline, GN=Gonadotrophin, LAG=Lanreotide Autogel, LAR=Sandostatin LAR, Sx=Surgery, TSH=Thyroid Stimulating Hormone, XRT=External Beam Radiotherapy. 1) Serum IGF-I levels for imaging study patients were measured using an Immulite-2000 solid-phase enzyme-labeled chemiluminescent immunometric assay (Siemens, Los Angeles, CA). (Patients 5-8 also participated in the metabolic studies, in which IGF-I was measured using IDS-iSYS assay, hence the difference in results c.f. Table 6.1).

Table 3.2: Acromegaly Patients in the imaging studies

Notes to table: CBG=Cabergoline, GN=Gonadotrophin, LAG=Lanreotide Autogel, LAR=Sandostatin LAR, Sx=Surgery, TSH=Thyroid Stimulating Hormone, XRT=External Beam Radiotherapy. 1) Serum IGF-I levels for imaging study patients were measured using an Immulite-2000 solid-phase enzyme-labeled chemiluminescent immunometric assay (Siemens, Los Angeles, CA). (Patients 5-8 also participated in the metabolic studies, in which IGF-I was measured using IDS-iSYS assay, hence the difference in results c.f. Table 6.1).

3.2 Study Design

Following written informed consent, the subjects attended on two occasions. They were recruited via Manchester endocrine clinics, and the healthy volunteers via approved in-hospital advertising at The Christie NHS Foundation Trust, Manchester. Approved patient information sheets were provided in advance of obtaining consent and all study procedures were carried out in accordance with International Conference on Harmonisation Good Clinical Guidance (ICH GCP) principles.
### 3.3. WHOLE BODY MRI

#### 3.3.1 Image Acquisition

Scanning of whole-body adiposity was adapted from Thomas et al 1998 [27]. A rapid T1 weighted spin echo sequence with echo time 38 ms, flip angle 120°, 100% rectangular field of view, 256×256 matrix, phase-conjugate symmetry, and a slice thickness of 10 mm was used. Images in the legs were collected with two averages, those in the torso with one average. Subjects maintained a prone position with arms above the head (if tolerated). This position was chosen to minimize respiratory motion, a potential source of artefact. All images were acquired as single slices at the isocenter to avoid image distortion. Subjects were scanned from the toes to the shoulder-tops, acquiring 10 mm

---

<table>
<thead>
<tr>
<th>Cushing’s Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Other pituitary insufficiency</th>
<th>Prior therapy</th>
<th>Source</th>
<th>Diabetes</th>
<th>24hr UFC average (nmol/24hr)</th>
</tr>
</thead>
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<td>1</td>
<td>F</td>
<td>28</td>
<td>Nil</td>
<td>-</td>
<td>Unknown</td>
<td>No</td>
<td>1109</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>36</td>
<td>Nil</td>
<td>-</td>
<td>Macro</td>
<td>OHA¹</td>
<td>531</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>40</td>
<td>Nil</td>
<td>-</td>
<td>Pit</td>
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<td>471</td>
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<tr>
<td>4</td>
<td>F</td>
<td>37</td>
<td>Nil</td>
<td>-</td>
<td>Micro</td>
<td>No</td>
<td>262</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>53</td>
<td>Nil</td>
<td>Metyrapone 250mg/tds</td>
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<td>OHA²</td>
<td>359</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>19</td>
<td>Nil</td>
<td>-</td>
<td>Pit</td>
<td>No</td>
<td>257</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>43</td>
<td>Nil</td>
<td>-</td>
<td>Macro</td>
<td>No</td>
<td>316</td>
</tr>
</tbody>
</table>

Median 37
Range 19-53

Table 3.3: Cushing’s Patients in the imaging studies

*Notes to table: 1) Metformin + Gliclazide, 2) Metformin alone*

impedance data was recorded (*Tanita* bioimpedance analyser). On a second occasion, subjects attended the Translational Imaging Unit at Salford Royal NHS Foundation Trust, Manchester. Using an *Intrinsa* 3 Tesla MRI scanner with a movable platter (*Philips*). The protocols performed are described in the following section.
3.3. WHOLE BODY MRI

thick slices with 30 mm gaps in the legs, and 10 mm thick slices with 10 mm gaps in the trunk. This resulted in the lower 30 stacks being taken with a 30 mm gap, and the upper 36 with a 10 mm gap (Figure 3.1).

All patients and Healthy Volunteers had body composition measured by bioimpedance and multi-slice MRI, and liver and muscle triglyceride by $^1$H MRS unless otherwise stated. This 66-slice imaging sequence took approximately 40 minutes.

Figure 3.1: Magnetic Resonance representation of the slice and gap thickness.

*From Thomas et al [27].*
### 3.3.2 Image analysis

Images were viewed and analysed using MRIcro software version 1.4 build 1 [39].

Slice images were saved as DICOM files and downloaded sequentially. Total, subcutaneous and visceral fat content was calculated for each slice from the trunk. In the legs, total, subcutaneous and intramuscular fat was calculated.

Using the pixel intensity filter in MRIcro a setting was chosen to allow maximum coverage of fat tissue, with minimal non-fat coverage. This was applied to the entire image sequence. Each slice was then also edited manually to correct areas wrongly identified as fat, and to add those where adipose tissue had not been highlighted. The finished edit of the entire 66 slices were saved as a Region of Interest (ROI). An example is shown below in figure 3.2.

![Abdominal cross-sectional MRI slice from MRIcro viewer.](image)

Subcutaneous fat was calculated by taking the completed total fat ROI and manually deleting the highlighted visceral fat (intramuscular fat in the legs). The non-subcutaneous fat could then be calculated by subtracting the subcutaneous fat from the total.

### 3.3.3 Calculating Adiposity within Body compartments

The fat volume in a single slice was determined by the voxel count ($2 \times 2 \text{ mm}$) multiplied by the slice thickness (10 mm). The fat volume in the ’gap’ was calculated as the average
of the calculated fat volume in the slice above and the slice below the gap. Total body compartment fat could be calculated using the definitions below.

### 3.3.4 Definitions of body compartments

**Total Body Fat**
The entire volume (slice + gap) for 66 slices from tips of toes to top of shoulders. The arms were not included for the purposes of this study.

**Trunk**
The area from slices showing no leg separation until the tips of the shoulders.

**Visceral and Non-visceral internal Fat**
Visceral fat was defined as quantified non-subcutaneous fat present in the slices from the top of the femoral heads until the slice containing the top of the liver or the bottom of the lungs. Subcutaneous fat within this region was termed Subcutaneous Abdominal Fat. All other non-subcutaneous subcutaneous fat was labelled 'non visceral internal fat'.

**Intramuscular Adipose Tissue (IMAT)**
In other studies, IMAT has been defined as the adipose tissue (AT) between muscle groups and beneath the muscle fascia [41], [42], [43]. In this study is non-subcutaneous fat measured in the slices between the ankle and the knee. Bone marrow fat signals were not included.

To calculate fat mass from volume, this total was multiplied by 0.92 kg/l, the assumed density of AT [42].

Selected patient images were analysed blind by an independent subject trained in MRIcro fat analysis to provide reproducibility data.

### 3.4 Magnetic Resonance Spectroscopy

Using a Philips 3T Intrinsa scanner, $^1$H MRS spectroscopy of liver, right gastrocnemius and anterior tibialis muscle were performed following multi-slice whole body fat scan acquisition. Echo time of 38 ms (with spin echo corrected for) and a repetition time of 4000 ms were used. These were phase corrected and no water signal suppression was applied.
3.4.1 Liver Spectroscopy

The scanning field was chosen to exclude macroscopic fat or blood vessels. A $2 \times 2 \times 2$ cm Flex-M surface coil was placed over the liver.

3.4.2 Muscle Spectroscopy

The subject was repositioned in the scanner (supine, feet first). The gastrocnemius and tibialis muscles were identified separately. A $2 \times 2 \times 2$ cm Flex-M coil was placed over the respective muscle during acquisition. The scanning field was chosen to exclude fascia, macroscopic fat or blood vessels.

The spectroscopy sequences took approximately 20 minutes.

3.5 Analysis of Spectra

The MRS data was analysed using jMRUI software version 4.0 [40]. Water signal was used as the reference point for both liver and muscle spectra, and the transmitter frequency set at $1.28 \times 10^8$ Hz.

3.5.1 Liver

Figure 3.3 illustrates a representative liver spectrum. Peak A is H$_2$O and peak B is the CH$_2$ moiety of triglyceride (TG).

![Liver Spectrum](image)

A=water peak B=–CH$_2$ moiety of intra-hepatic triglyceride (IHTG). Y-axis is an arbitrary scale.

Figure 3.3: $^1$H MRS spectra from liver, created on jMRUI software.
To semi-quantify the amount of triglyceride (TG) in the liver, the water peak was used as a reference and set at a frequency of 4.72 parts per million (ppm). The CH₂ Moiety was then manually selected at 1.3 ppm. In some spectra, a mirror image CH₂ peak was observed to the left of the water peak; in this instance the total TG (as represented by the CH₂ moiety) content is the sum of the Area Under the Curve (AUC) of these mirrored CH₂ signals.

### 3.5.2 Muscle

In contrast to the quantification of hepatic triglyceride, ¹H MRS of muscle is more complex. The signals are smaller, and both intra- and extra-cellular triglyceride is present on the spectra produced; furthermore both CH₂ and CH₃ moieties appear as peaks, resulting in 4 traces to be de-convoluted and measured against the reference water peak. Some spectra can have 8 peaks, where, like the liver signals, the triglyceride is mirrored equidistantly from the water signal on the other side. Two spectra from gastrocnemius (Figures 3.4 and 3.5) are shown below (after water peak removal).

![Graph showing muscle spectra](image)

The original trace shows choline and creatine peaks at 3.2 ppm and 3.02 ppm respectively, and the four TG peaks appear as a single deflection. Y-axis has no scale (arbitrary units).

Figure 3.4: ¹H spectra from gastrocnemius after water peak removed.

Setting the water peak to 4.72 ppm and adjusting the phase of the graph to give the optimal view, the water peak was removed using the HSVLD pro-pack function on jMRUI. This was necessary due to the relatively small quantities of triglyceride. The removal of the water peak re-calibrates the scale of the graph and makes identification of the four-triglyceride moieties easier. On each spectra, the peaks and line widths were identified manually, and the mean of each used as the starting value. The prior knowledge with
3.5. ANALYSIS OF SPECTRA

Relative frequencies was also set. In the case of files with 8 peaks, an 8-peak average was generated and applied as starting values.

Labelled A-D in figure 3.5, there are 4 peaks for the triglyceride moieties. These represent, in descending magnitude, extra-myocellular \( \text{CH}_2 \) (EMCH\(_2\)), intra-myocellular \( \text{CH}_2 \) (IMCH\(_2\)), EMCH\(_3\) and IMCH\(_3\) respectively.

![Individual components](image)

After peak identification using the AMARES (Advanced Method for Accurate Robust and Efficient Spectral fitting of MRS data) function of jMRUI (upper), four peaks are found- A=EMCH\(_2\), B=IMCH\(_2\), C=EMCH\(_3\), D=IMCH\(_3\). Y-axis has no scale (arbitrary units).

Figure 3.5: \(^1\text{H}\) spectra from gastrocnemius after peak identification.

The best de-convolution was taken as the result and expressed as a ratio of the peak to the suppressed water peak.

The de-convolution was assessed on several points:

(i) The initial quality of the spectra. One would expect to see a large water peak with smaller peak for triglyceride. Once the water peak had been removed, choline and creatine peaks should be visible. The trace should require as little manipulation (in begin time or phasing) in order to achieve this baseline trace.

(ii) The residue post calculation. The residue should show a flat line in the area of the expected triglyceride signals, indicating that as much of the trace had been quantified in the deconvolution.

(iii) The shape/magnitude of the peaks. The computed trace should reveal 4 (or 8) distinct peaks with EMCH\(_2\) >IMCH\(_2\) >EMCH\(_3\) >IMCH\(_3\). The CH\(_2\):CH\(_3\) should be in the magnitude of 10-14:1.

(iv) The frequency of the found peaks. These should appear at approx.1.5, 1.3, 1.1 and 0.9 ppm. On taking the mean of our spectra, the mean peaks were 1.53, 1.34, 1.13,
0.91 (for 8 peaks also 7.94, 8.11, 8.4 and 8.55) ppm. The ’soft constraints’ i.e. the values which the peaks should fall within were peak A 1.49-1.61 and C 1.28-1.4, with a fixed frequency shift of A $\pm 50.4$Hz and B 1.28-1.4Hz.

### 3.6 Calculations and Statistics

Using AMARES quantification, the respective levels can be calculated as a ratio relative to water. Early published studies used VAPRO (Variable Projection) as a method of de-convolution, but this has been superseded by AMARES [44].

Results for the three groups were analysed using Kruskal-Wallis test, with post-hoc Mann-Whitney U-test. Significance KW was defined as $p<0.05$, and MWU $p<0.02$. 

53
CHAPTER 4

RESULTS - IMAGING STUDY

4.1 Introduction

There were four elements to the study: Tanita Bioimpedance Body Composition measurement, multi slice MRI imaging, $^1$H Magnetic Resonance Imaging of liver and $^1$H MRS of skeletal muscle. While all 5 Healthy Volunteers were studied fully, not all study patients were able to participate in every element. Patient participation is summarised below in Tables 4.1 and 4.2.

<table>
<thead>
<tr>
<th>Acromegaly Patient</th>
<th>Gender</th>
<th>Tanita Bioimpedance</th>
<th>MRI Body Fat</th>
<th>MRS Liver</th>
<th>MRS Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>2</td>
<td>3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>+</td>
<td>4</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 4.1: Imaging details of Acromegaly Patients

Notes to table: (+) indicates a successful scan, numerical value indicates scan not performed or inconclusive, with the following reasons; 1) Unable to analyze spectra, 2) Analyzer out of service, 3) Unable to complete due to claustrophobia/patient discomfort, 4) Format of multi slice image file incompatible with MRICro software.
### 4.1. INTRODUCTION

Cushing’s Patients

<table>
<thead>
<tr>
<th>Cushing’s Patient</th>
<th>Gender</th>
<th>Tanita Bioimpedance</th>
<th>MRI Body Fat</th>
<th>MRS Liver</th>
<th>MRS Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>1</td>
<td>+</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>+</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>+</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 4.2: Imaging details of Cushing’s Patients

Notes to table: (+) indicates a successful scan, numerical value indicates scan not performed or inconclusive, with the following reasons: 1) Analyzer out of service, 2) Unable to analyze spectra, 3) Patient weight greater than acceptable tolerance of scanner pallet, 4) Patient unsuitable for the scanner due to body habitus, 5) Patient unable to attend Christie prior to surgery.
4.2 Body Composition by Bioimpedance

Body composition measured by bioimpedance on a Tanita 3000 scanner. The results are summarised in Table 4.3.

<table>
<thead>
<tr>
<th>Group</th>
<th>BMI</th>
<th>Total Adipose Tissue (kg)</th>
<th>Whole Body Fat (%)</th>
<th>Fat Free Mass (kg)</th>
<th>Whole body fat free mass (%)</th>
<th>Total body water (kg)</th>
<th>Total body water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV</td>
<td>Median 28.1</td>
<td>20.8</td>
<td>24.2</td>
<td>64.2</td>
<td>75.81</td>
<td>47</td>
<td>55.58</td>
</tr>
<tr>
<td></td>
<td>Range 24.9-28.7</td>
<td>18.2-36</td>
<td>22.2-42</td>
<td>47.3-67.3</td>
<td>58.02-77.91</td>
<td>34.6-49.3</td>
<td>42.44-57.04</td>
</tr>
<tr>
<td>AA</td>
<td>Median 27.9</td>
<td>21.4</td>
<td>29.2</td>
<td>68.8</td>
<td>72.8</td>
<td>51.5</td>
<td>60.9</td>
</tr>
<tr>
<td></td>
<td>Range 23.5-38.6</td>
<td>12.4-43.2</td>
<td>14.7-36.4</td>
<td>47.4-75.5</td>
<td>60.8-85.3</td>
<td>37.8-55.8</td>
<td>44.5-86.9</td>
</tr>
<tr>
<td>CD</td>
<td>Median 35.5</td>
<td>51.6</td>
<td>48.2</td>
<td>54</td>
<td>51.8</td>
<td>43.9</td>
<td>42.4</td>
</tr>
<tr>
<td></td>
<td>Range 22.8-43.6</td>
<td>11.7-57.6</td>
<td>15-36.4</td>
<td>45.6-79.8</td>
<td>47.8-85.3</td>
<td>38.6-58.2</td>
<td>35.9-78.1</td>
</tr>
<tr>
<td>Kruskal-Wallis p value</td>
<td>0.3</td>
<td>0.136</td>
<td>0.189</td>
<td>0.215</td>
<td>0.189</td>
<td>0.32</td>
<td>0.479</td>
</tr>
</tbody>
</table>

Table 4.3: Body Composition by Bioimpedance

Table 4.3 details median and range of anthropomorphic measurements from Tanita body composition scanner in Healthy Volunteers, acromegaly and Cushing’s Groups. No significant differences found among groups.

Cushing’s patients had the highest BMI (in kg/m² 35.5 (2.8-43.6) CD vs. 27.9 (23.5-38.6) AA vs. 28.1 (24.9-28.7) HV) and Whole Body Fat Mass (in kg 51.6 (11.7-57.6) CD vs. 27.9 (23.5-38.6) AA vs. 20.8 (18.2-36) HV). Total Body Water was however highest in the AA group (in kg 51.5 (37.8-55.8) AA vs. 47 (34.6-49.3) HV vs. 43.9 (38.6-58.2) CD) as was Fat Free Mass (in kg 68.8 (47.4-75.5) AA vs. 64.2 (47.3-67.3) HV vs. 54 (45.6-79.8) CD).

None of these differences observed were significant. Scatter plots of individual values are shown in Figures 4.1, 4.2, 4.3 and 4.4.
4.2. BODY COMPOSITION BY BIOIMPEDANCE

Figure 4.1: BMI of Healthy Volunteers, Acromegaly and Cushing’s Patients
*Horizontal bars represent median values. HV n=5, acromegaly n=7, CD n=5, p=0.3, Kruskal-Wallis test.*

Figure 4.2: Total Adipose Tissue (TAT) by Bioimpedance
*Horizontal bars represent median values. HV n=5, acromegaly n=7, CD n=5, p=0.1, Kruskal-Wallis test.*
4.2. BODY COMPOSITION BY BIOIMPEDANCE

Figure 4.3: Total Body Water (TBW) by Bioimpedance
*Horizontal bars represent median values. HV n=5, AA n=7, CD n=5, p=0.32, Kruskal Wallis test.*

Figure 4.4: Fat Free Mass (FFM) by Bioimpedance
*Horizontal bars represent median values. HV n=5, AA n=7, CD n=5, p=0.21, Kruskal-Wallis test.*
4.3 Multi Slice Magnetic Resonance Imaging

The results for body fat estimation by multi-slice MRI for Healthy Volunteers, acromegaly and Cushing’s are summarized in Table 4.54. Total body fat content was similar in HV and acromegaly (35 (31.6-40.6) vs. 34.9 (21.1-45.2) kg) while the HV group were younger and had twice as many women (1 female in the acromegaly group). Cushing’s patients had higher fat content in all body compartments, but only total visceral fat was significantly raised (p=0.02). Two Cushing’s patients (5CD and 6CD) were physically unable to be scanned due to weight and body habitus.

Scatter plots comparing the relative fat distribution of each compartment are shown in Figures 4.5, 4.6 and 4.7. Figure 4.9 shows a comparison of TAT measurement by both Bioimpedance and MRI.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Subcutaneous fat (Kg)</th>
<th>Subcut. Fat Mass (%)</th>
<th>Total Visc. Fat (Kg)</th>
<th>Visc. Fat Mass (%)</th>
<th>Total Intramuscular fat (Kg)</th>
<th>Intramuscular Fat Mass (%)</th>
<th>Total body fat (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV</td>
<td>Median 26.1</td>
<td>74.5</td>
<td>5.0</td>
<td>15.9</td>
<td>0.12</td>
<td>0.3</td>
<td>35.0</td>
</tr>
<tr>
<td>Range</td>
<td>17.6-35.2</td>
<td>66.6-86.6</td>
<td>3.2-5.7</td>
<td>8.0-18.9</td>
<td>0.07-0.25</td>
<td>0.2-0.9</td>
<td>31.6-40.6</td>
</tr>
<tr>
<td>AA</td>
<td>Median 25.7</td>
<td>72.6</td>
<td>5.09</td>
<td>17.4</td>
<td>0.38</td>
<td>0.7</td>
<td>34.9</td>
</tr>
<tr>
<td>Range</td>
<td>14.6-39.6</td>
<td>62.3-75.7</td>
<td>3.42-14.42</td>
<td>11.2-22.7</td>
<td>0.09-0.56</td>
<td>0.0-1.1</td>
<td>21.1-45.2</td>
</tr>
<tr>
<td>CD</td>
<td>Median 42.8</td>
<td>65.1</td>
<td>12.7</td>
<td>26.7</td>
<td>0.31</td>
<td>0.8</td>
<td>65.8</td>
</tr>
<tr>
<td>Range</td>
<td>17.4-61.3</td>
<td>52.7-86.3</td>
<td>6.73-18.0</td>
<td>9.47-32.1</td>
<td>0.15-0.39</td>
<td>&lt;0.01-1.2</td>
<td>32.9-71.0</td>
</tr>
</tbody>
</table>

Kruskal-Wallis p value 0.169 0.156 0.02 0.06 0.12 0.99 0.06

Table 4.4: Adipose tissue compartments by total body MRI

Table 4.5 details median and range values for AT compartments in Healthy Volunteers, acromegaly and Cushings Patients by Total body MRI. 1) p=0.02 by Kruskal-Wallis. Post-hoc Mann-Whitney U test p=0.01. Increased visceral fat in Cushing’s Disease group cf Healthy Volunteers.
4.3. MULTI SLICE MAGNETIC RESONANCE IMAGING

Figure 4.5: Total Adipose Tissue (TAT) by MRI
Horizontal bars represent median values. HV n=5, AA n=6, CD n=5, p=0.06, Kruskal-Wallis test.

Figure 4.6: Subcutaneous Adipose Tissue (SAT) by MRI
Horizontal bars represent median values. HV n=5, AA n=6, CD n=5, p=0.17, Kruskal-Wallis test.
4.3. MULTI SLICE MAGNETIC RESONANCE IMAGING

Figure 4.7: Visceral Adipose Tissue (VAT) by MRI
*Horizontal bars represent median values. HV n=5, AA n=7, CD n=5, p=0.02 by Kruskal-Wallis test. Post-hoc Mann-Whitney U test p=0.01 Increased visceral fat in Cushing’s Disease group cf Healthy Volunteers.*

Figure 4.8: Intra-Muscular Adipose Tissue (IMAT) by MRI
*Horizontal bars represent median values. HV n=5, acromegaly n=6, CD n=5, p=0.12, Kruskal-Wallis test.*
Figure 4.9: Mean Total Adipose Tissue Bioimpedance vs. MRI

Error bars represent ± 2 SD. TAT was measured higher by MRI (green) than Bioimpedance (blue).
4.4 $^1$H Magnetic Resonance Spectroscopy

The results from $^1$H MRS scanning of liver and skeletal muscle are summarized in Table 4.5.

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Fat:Water</th>
<th>IMCH$_2$ $\times 10^{-3}$</th>
<th>IMCH$_3$ $\times 10^{-3}$</th>
<th>IMCH$_2$ $\times 10^{-3}$</th>
<th>IMCH$_3$ $\times 10^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV</td>
<td>0.12</td>
<td>8.14</td>
<td>0.685</td>
<td>22.0</td>
<td>4.39</td>
</tr>
<tr>
<td></td>
<td>0.02-0.22</td>
<td>5.19-9.3</td>
<td>0.48-27.0</td>
<td>16.6-44.9</td>
<td>1.9-7.4</td>
</tr>
<tr>
<td>AA</td>
<td>0.0</td>
<td>13.2</td>
<td>1.3</td>
<td>20.4</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>0.0-0.05</td>
<td>7.86-25.3</td>
<td>0.93-10.1</td>
<td>9.33-28.7</td>
<td>0.9-12.5</td>
</tr>
<tr>
<td>CD</td>
<td>0.16</td>
<td>12.8</td>
<td>2.13</td>
<td>29.3</td>
<td>5.65</td>
</tr>
<tr>
<td></td>
<td>0.00-0.47</td>
<td>8.0-65.0</td>
<td>0.3-30.0</td>
<td>19.3-71.6</td>
<td>2.4-13.8</td>
</tr>
</tbody>
</table>

Kruskal-Wallis p value: 0.037$^1$, 0.852, 0.744, 0.185, 0.31

Table 4.5: $^1$H Magnetic Resonance Spectroscopy results

Table 4.5 details results from liver, anterior tibialis and gastrocnemius, expressed as a ratio of fat: water. 1) Decreased liver triglyceride in acromegaly group vs. Healthy Volunteers (p=0.037 by 3-group Kruskal-Wallis test; p=0.01 post-hoc Mann-Whitney U test for HV vs. acromegaly. CD vs. HV and acromegaly vs. CD p= not significant.

4.4.1 Liver

Five Healthy Volunteers (HV), 7 acromegaly (AA) patients and 4 Cushing’s (CD) patients were successfully scanned. These are shown in Figure 4.10. Liver fat: water ratio (as measured by intrahepatocellular CH$_2$) was un-recordable in 3 of 7 patients studied, and significantly lower than HV. Median and (range): 0 (0-0.05) acromegaly vs. 0.12 (0.02-0.22) HV vs. 0.16 (0-0.47) CD, p=0.037 by Kruskal-Wallis test; p=0.01 post-hoc Mann-Whitney U test for HV vs. acromegaly. CD vs. HV and acromegaly vs. CD p= not significant.
4.4. ¹H MAGNETIC RESONANCE SPECTROSCOPY

4.4.1 Hepatic Triglyceride : water ratio by ¹H MRS

Horizontal bars represent median values. HV n=5, AA n=7, CD n=4, p=0.037 by 3-group Kruskal-Wallis test; p=0.01 post-hoc Mann-Whitney U test for HV vs. AA. CD vs. HV and AA vs. CD p= not significant.

4.4.2 Skeletal Muscle

No significant differences were noted among the groups in CH₂ and CH₃ : Water ratios, in either Anterior Tibialis or Gastrocnemius muscle. In Anterior Tibialis, median CH₂ : Water ratios were highest in acromegaly patients 13.2 (7.86-25.3) AA vs. 8.14 (5.19-93) HV vs. 12.8 (8.0-65) CD, whereas these were lowest in acromegaly patients in Gastrocnemius muscle. Wide variation was observed in each study group, in both CH₂ and CH₃ moieties in both muscle groups. Scatter plots are shown in Figures 4.11, 4.12, 4.13 and 4.14.
4.4. \textsuperscript{1}H MAGNETIC RESONANCE SPECTROSCOPY

Figure 4.11: Triglyceride CH\textsubscript{2}:water ratio in Tibialis Anterior by \textsuperscript{1}H MRS

Horizontal bars represents median values. HV n=5, AA n=5, CD n=5, \( p=0.85 \) by Kruskal-Wallis test.

Figure 4.12: Triglyceride CH\textsubscript{2}:water ratio in Gastrocnemius by \textsuperscript{1}H MRS

Horizontal bars represents median values. HV n=5, AA n=5, CD n=5, \( p=0.19 \) by Kruskal-Wallis test.
4.4. $^1$H MAGNETIC RESONANCE SPECTROSCOPY

Figure 4.13: Triglyceride CH$_3$ :water ratio in Tibialis Anterior by $^1$H MRS
Horizontal bars represents median values. HV $n=5$, AA $n=5$, CD $n=5$, $p=0.74$ by
Kruskal-Wallis test

Figure 4.14: Triglyceride CH$_3$ :water ratio in Gastrocnemius by $^1$H MRS
Horizontal bars represents median values. HV $n=5$, AA $n=5$, CD $n=5$, $p=0.31$ by
Kruskal-Wallis test.
4.5 Summary of Results

4.5.1 Bioimpedance

- No significant differences amongst groups studied.
- Highest BMI in Cushing’s patients.
- Total Body Water highest in acromegaly patients.
- Total Adipose Tissue highest in Cushing’s patients; similar TAT in Healthy Volunteers and Acromegaly patients.

4.5.2 Multi-Slice MRI

- Highest VAT in Cushing’s patients (p=0.02 KW, p=0.01 post-hoc MWU).
- Highest median IMAT in acromegaly patient group despite lowest median TAT of the three groups (p>0.05).
- Mean TAT estimation higher by MRI than Bioimpedance in all three groups studied.

4.5.3 Magnetic Resonance Spectroscopy

- Lower Hepatic Fat:Water ratio in Acromegaly patients (p=0.037 KW, p=0.01 post-hoc MWU).
- 3 of 7 acromegaly patients had no recorded Hepatic fat by MRS.
- No significant differences amongst intramyocellular Triglyceride was noted in Gastrocnemius nor Tibialis Anterior amongst Healthy Volunteers, acromegaly and Cushing’s patients.
- 2 Cushing’s patients were unable to participate in either MRI or MRS imaging due to issues relating to weight and body habitus.
5.1 Methods of Measuring Insulin sensitivity (and $\beta$ cell function)

There are a number of validated methods for quantification of insulin sensitivity. The method most suitable for a study depends on a number of factors, including the number of subjects involved, the degree of anticipated spread of the sensitivity results, the necessity for information regarding cell function and whether one wishes to assess central and peripheral sensitivity separately. Even using the most sophisticated multi compartmental modelling and stable isotopes of glucose requires assumptions to be made; these models, while providing meaningful data, are not entirely representative of the physiology involved. An ideal test would involve the least number of blood samples, fewest modelling assumptions, yet provide detailed and accurate information regarding central and peripheral insulin sensitivity, glucose disposal and $\beta$ cell function. All currently validated methods compromise on at least one of these principles.

5.2 Fasting Measures of Insulin Sensitivity

These are the simplest measures, involving only a sample of plasma glucose and insulin. Several indices have been validated in population studies including HOMA and QUICKI
5.3 Oral Glucose Tolerance Test

This is a well established clinical and research method, and is used in the diagnosis of T2DM (measuring glucose only). To determine insulin sensitivity, fasting, and then a number of insulin/glucose levels are taken over a period of two to three hours after the 75g oral glucose load. It has been demonstrated that the oral method of glucose administration leads to inter- and intra subject variation, due to variable GI absorption of the glucose load which can affect insulin secretion and thus peripheral glucose uptake [46]. In common with the IVGTT, insulin sensitivity can best be determined using an average of plasma concentrations over time, or area under Concentration-Time curves, although single time-point measures have also been employed. Interpretation of the OGTT (oral glucose tolerance test) assumes that post absorptive state glucose uptake occurs by insulin dependent routes alone and that hepatic and peripheral insulin sensitivities are equal.

5.4 Insulin Clamps

Hyperinsulinaemic euglycaemic clamps provide 'gold standard' information regarding insulin sensitivity, but as they over-ride physiological insulin secretion, β cell function cannot be assessed. The use of stable isotopes of glucose allows discreet assessment of peripheral and central insulin sensitivity, and has been used extensively in metabolic studies. However, in a previous study using a clamp technique to examine the effects on insulin sensitivity and lipid metabolism in acromegaly, the insulin dose used resulted in complete suppression of hepatic glucose production [18]
5.5  Frequently Sampled IVGTT

The IV route has advantages over oral loading in that it eliminates the delay in GI passage of the glucose, reducing the intra- and inter-subject variability. When combined with stable isotopes of glucose, central and peripheral insulin sensitivity can be estimated separately.

5.6  Stable Isotope Studies

Isotopes are forms of an element that differ in atomic mass. This is due to differing numbers of neutrons within the nucleus. This difference in mass has no effect on its chemical properties, as the number of protons and electrons are unaltered. The difference in atomic mass affects the physical properties of the isotope, and the altered boiling and freezing point, notwithstanding the mass difference, allow these to be separated. A 'stable isotope' is not subject to radioactive decay. The use of stable isotopes in the measurement of glucose and glycerol metabolism is well established. The deuterated hydrogen molecule in the d5-glycerol and d2-glucose allows for calculation of the rate of appearance (R_a) and disappearance (R_d) of both these substrates. As the rate of hepatic glucose production can be considered a marker of central insulin sensitivity, and the rate of insulin mediated glucose disposal (primarily into skeletal muscle) a marker of peripheral insulin sensitivity, then these can be considered separately for the purposes of investigation [47].

5.7  Modelling Glucose Kinetics

The modelling interpretation of FSIVGTT results was first attempted by Bergman et al in 1979 [48]. The addition of an artificial second insulin response (by exogenous insulin administration) and the addition of stable isotope to the glucose bolus have allowed the separate study of glucose production and utilization. Details of the minimal model techniques used have been described elsewhere [49], [50]. The essence of the model is described in the appendix.

5.7.1  MINMOD

From the data collected in the IVGTT, single compartment 'hot' (labelled) and 'cold' (unlabelled) glucose kinetic data can be calculated. MINMOD [51] is a validated modelling
program which provides data on the following parameters.

**Insulin sensitivity (Si)** is defined as the enhancement of basal glucose effectiveness by an increment in the amount of plasma insulin.

**Glucose effectiveness (Sg)** is defined as the incremental change in the rate of glucose clearance caused by a change in the amount of glucose in the plasma pool. It is therefore calculated from multiple sets of glucose isotope and whole glucose collected over many time points in the IVGTT.

**The Acute Insulin Response to glucose (AIRg)** is a measure of $\beta$ cell function. It calculated from the area under curve (AUC) for insulin between 0 and 20 minutes after injection of glucose, whereby the AUC is calculated by the trapezoidal rule with the mean of the baseline values subtracted.

**The Disposition Index (DI)** is a composite measurement calculated from both AIRg ($\beta$ cell function) and insulin sensitivity (Si). It simple terms, it is thought that the product of these two variables will be constant in subjects with matching glucose tolerance, and it is this product which is the DI.

The advantage of DI is that, while glucose kinetic models can be prone to error, the product of these two calculated parameters could be assumed to cancel each other out.

Previous studies using the MINMOD modelling software have produced Sg and Si data with average CV (coefficient of variation) of 2-9%, typically around 5% [50]. Some later studies have quoted a good parameter fit as having a fractional standard deviation of 0.5 or less [52].

In summary, the FSIVGTT allows assessment of both insulin sensitivity and $\beta$ cell function [53]. When combined with stable isotopes of glucose, both peripheral and central insulin sensitivity can be considered separately. While other methods have been validated, this is currently the best for identifying subtle differences between small population groups.
6.1 Patients

Six patients with active Cushing’s disease, six patients with active acromegaly and two healthy volunteers were recruited for the study. Patient characteristics are described in tables 6.1, 6.2 and 6.3.

**Inclusion Criteria**

- All subjects (Healthy Volunteers, Acromegaly patients, Cushing’s patients): Age >18 years
- Acromegaly patients: active disease as evidenced by elevated Growth Hormone (GH) levels and IGF-I > upper limit of normal (ULN)
- Cushing’s patients: active disease as evidenced by failure to suppress plasma cortisol on Low Dose Dexamethasone Suppression Test (LDDST) and/or raised 24 hour Urinary Free Cortisol (24h UFC) levels ×2.

**Exclusion Criteria**

All subjects:

- Conditions resulting in abnormal GH/IGF-I/cortisol (e.g. severe hepatic disease, severe renal disease, malnutrition, treatment with levodopa, heroin abuse).
6.2. PATIENT CHARACTERISTICS

- Inability, to comprehend the nature of the study, to follow instructions, and/or co-operate with study procedures.

- ALT or AST >3 × ULN or clinically significant hepatic disease.

- Visual field defects or other neurological symptoms due to tumour mass.

- History of relevant drug and/or food allergies or on regular treatment with any medication that may interfere with projected study results.

- Have received another experimental medication that may interfere with projected study results.

- Refusal to use adequate contraception to prevent pregnancy during the study.

- Inability to adhere to the visit schedule outlined in the protocol.

- Known or suspected drug / alcohol abuse.

- Women who were pregnant or lactating.

### 6.2 Patient Characteristics

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<th>Age</th>
<th>Prior therapy</th>
<th>Pituitary source</th>
<th>Diabetes</th>
<th>IGF-I (ng/ml)</th>
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Median 59.5

Range 34-70

Table 6.1: Acromegaly Patients

Notes to table: 1) IGF-I in metabolic study patients were analysed using chemiluminescence method IDS-iSYS (IDS Ltd, UK). Sx=Surgery, XRT=External Beam Radiotherapy.
6.2. PATIENT CHARACTERISTICS

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Median 38.5  Range 18-53  359  257-531

Table 6.2: Cushing’s Patients

Notes to table: 1) Metformin + Gliclazide, 2) Metformin alone, 3) Patient had no 24hr UFC result available as condition diagnosed by unsuppressed plasma cortisol on low and high dose dexamethasone suppression test.

The study was approved by Liverpool Local Research Ethics Committee (LREC) and supported by R&D Dept., The Christie NHS Foundation Trust. Patients were recruited via Manchester endocrine clinics, and the healthy volunteers via approved in-hospital advertising at The Christie NHS Foundation Trust. Approved patient information sheets were provided in advance of obtaining consent and all study procedures were carried out in accordance with International Conference on Harmonisation Good Clinical Guidance (ICH GCP) principles.

<table>
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<th>Volunteer</th>
<th>Sex</th>
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<tr>
<td>HV02</td>
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<td>29.7</td>
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</table>

Table 6.3: Healthy Volunteers
6.3 Study Design

Patients with active acromegaly and Cushing’s disease were studied on two occasions no sooner than fourteen days apart. On the second visit the patients were prescribed 250 mg Acipimox at 2000 hrs, 0000 hrs, 0400 hrs and 0800 hrs. The healthy volunteers were studied on a single occasion without Acipimox. The timeline for the study is outlined below in Figure 6.1.

Figure 6.1: Timeline of study protocol
After sampling was completed at 1100 hrs, the participant was fed and allowed home.
Healthy volunteers were studied on one occasion only.

6.4 Study Protocol

Informed consent was obtained prior to commencing the study. Patients were admitted at 1800 hrs to the Endocrine ward at The Christie NHS Foundation Trust. Height and weight were measured and body composition by bioimpedance was recorded. After receiving a meal at 1900 hrs, the patients remained fasted from 2000 hrs until 1100 hrs the following morning.

An 18 g cannula was inserted in the ante-cubital fossa of each arm using aseptic technique. One cannula was used for venous sampling, and the other administration of glucose and glycerol isotope, glucose and insulin bolus as per the protocol stipulations.
6.5 Overnight Study

Samples were taken hourly overnight (2200-0800) for NEFA, BOH and glycerol. Plasma glucose was measured every 30 minutes and insulin sampled hourly.

At 0000 hrs a primed (1.5µmol/kg) constant $[^2$H$_5$]-glycerol infusion (0.1µmol/kg/min) and a primed 170 mg D- [6,6-$^2$H$_2$]-glucose constant glucose infusion (1.7 mg/min infusion D- [6,6-$^2$H$_2$]-glucose) was commenced. Prior to this at 2350, 2355 and 0000 samples were taken for $[^2$H$_5$] -glycerol and D- [6,6-$^2$H$_2$]-glucose enrichment analysis. Samples for measurement of these isotopes were taken hourly overnight.

6.6 Intravenous Glucose Tolerance Test

At 07:50 the IVGTT protocol was commenced as follows: Baseline blood samples at -10 and -5 minutes. At 08:00 (0 minutes) an intravenous bolus of glucose was administered (0.6 g/kg - 50% dextrose solution) spiked with 20 mg/kg 6,6 $^2$H-glucose At 08:20 (20 minutes) an intravenous bolus of insulin was given at a dose of 0.05 units/kg Blood samples for the measurement of plasma insulin, glucose, D- [6, 6-$^2$H$_2$ ]-glucose, $[^2$H$_5$] glycerol, glycerol, and BOH were taken at regular intervals.

After sampling was completed at 1100 hrs the patient was fed and allowed home.

The same protocol was repeated at a minimum of 14 days later. On the second occasion the patient received an oral dose of 250 mg Acipimox at 2000 hrs, 0000 hrs, 0400 hrs and 0800 hrs.

For the two healthy volunteers, the protocol was performed only once, without Acipimox administration.

6.7 Handling of Blood samples

Blood was drawn via an 18 g intravenous cannula placed in the subject’s left arm at time points described above. The line was kept patent by a 0.9% Saline infusion at approximately 125 ml/hr. The first 2 ml of blood drawn was discarded and the subsequent sample collected in a sterile syringe as detailed above.
6.8 Samples

6.8.1 Glucose and glucose for isotope analysis

1.1 ml Fluoride oxalate treated microtubes were filled with 1 ml of freshly drawn whole venous blood, and mixed gently. Samples for whole glucose measurement were stored at 4°C for analysis the following day. Those taken for analysis of glucose isotope were placed immediately on ice, then centrifuged for 15 minutes @ 15000 rpm. The supernatant was pipetted into plain microtubes and frozen at -80°C for later analysis. Whole glucose was analysed using enzymatic-hexokinase method, Adiva Chemistry (Siemens Diagnostics Inc.). Glucose isotope analysis is described in section 6.8.4.

6.8.2 Glycerol, Free Fatty Acid and BOH measurements

1.1 ml Lithium heparin treated microtubes were filled with 1ml of freshly drawn venous blood, mixed gently and placed immediately on ice. They were centrifuged for 15 minutes @ 15000 rpm, the supernatant then being pipetted into plain microtubes and frozen at -80°C for later analysis. Glycerol isotope analysis is described in section 6.9. Free Fatty Acid and BOH were not analysed at the time of writing.

6.8.3 Insulin

1.1 ml SST Gel containing microtubes were filled with 1 ml of freshly drawn venous blood and placed immediately on ice. They were then centrifuged for 15 minutes @ 15000 rpm. The supernatant was pipetted into plain microtubes and frozen at -80°C for later analysis. As haemolysis of insulin samples became apparent as a potentially significant problem, later samples were allowed to stand for 15 minutes after icing before being spun. These were analysed using electrochemiluminescence method, Roche Modular Analyser (Roche Diagnostic Gmbh, Mannheim, Germany). Within assay mean (SD) 22.5 mU/ml (1.58), 80.5 mU/ml (5.64).

6.8.4 Isotope Samples

Samples collected for isotopic determination were analysed at MRC Human Nutrition Research, Cambridge. Glucose enrichment was measured using a fluorinated methylboronate derivative and glycerol enrichment measured as the tert-butyldimethylsilyl, the
6.9. **GLUCOSE AND GLYCEROL ISOTOPE ANALYSIS**

Both derivatives were analysed in selected ion monitoring by gas chromatography-mass spectrometry (GC-MS, *Agilent MS 5973N with HP6890 GC; Agilent, Stockport, UK*).

### 6.9 Glucose and Glycerol Isotope analysis

This is a summary of the method used from the protocols provided by HNR MRC Cambridge.

**GC-MS analysis:** conventional GC-MS measurements were made using an Agilent GCMS 5973N quadrupole mass spectrometer system (*Agilent, UK*), comprising a HP6890 gas chromatograph with autosampler and injector operated in the splitless mode at 250°C. Chromatographic separation was performed using a DB-1MS capillary column (30 m x 0.25 mm i.d., 0.25µm film thickness, *J&W Scientific, USA*). The mass spectrometer was operated in electron ionisation mode, with electron energy of 70 eV. The transfer line to the mass spectrometer was maintained at 280°C, with the ion source at 250°C and quadrupole at 120°C. For deuterated glucose analysis, the initial oven temperature of 90°C was maintained for 1 minute, before ramping at 30°C/min to 270°C, and then held for 0.5 minute to clear the column. Selected ion monitoring (SIM) of the isotopomer cluster, m/z 238-243, was performed with a nominal dwell time of 50 ms per mass unit. For deuterated glycerol, the initial oven temperature was 100°C, held for 0.5 minute, ramped at 30°C/min to 280°C, and then held for a further 0.5 minute. SIM of the cluster, m/z 377-382 was performed again with a dwell time of 50ms. Isotopomer ratios relative to the most abundant ion in the cluster (m/z 240 for the unlabelled glucose derivative and m/z 377 for the unlabelled glycerol derivative) were calculated.

### 6.9.1 Calculating Isotopic \( R_a \) and \( R_d \)

The use of isotopes for glucose and glycerol allows direct confirmation of the degree of their endogenous production. Glucose appearance (\( R_a \)) and disappearance (\( R_d \)) were calculated using Steele’s equation. This describes two rapidly exchanging pools in conditions of non-steady state, and was specifically derived for studying acute changes in glucose kinetics due to a perturbing hormone infusion. For glycerol, steady-state kinetics have been found more applicable where \( R_a = R_d \). \( R_d \) was calculated as the isotope infusion rate divided by the experimentally determined tracer/tracer ratio found in plasma. Following solvent extraction and derivatisation as described elsewhere, samples for glucose
and glycerol isotope were analysed by GC-MS.

### 6.9.2 Calculations and Statistics

The FSIGTT and basal calculations are established and described as follows [52]; Baseline calculations of glucose and insulin were calculated from the mean of the 0700 hrs and 0759 hrs samples preceeding the intravenous glucose bolus. The Hepatic Insulin Sensitivity (HIS) was calculated as the reciprocal of the product of fasting endogenous glucose production and fasting plasma insulin. The FSIVGTT parameters were calculated from Bergmans’ minimal model [48] using MINMOD Millennium software [51]. Insulin Sensitivity (Si), a measure of the capacity of insulin to promote glucose disposal, is modelled from the relationship of incremental insulin values over baseline to glucose disappearance. Sg, a measure of the capacity of glucose to mediate its own disposal, is modelled from the effect of basal insulin levels on glucose kinetics. Parameter resolution was in the range described by Caumo et al [49]. Modelling data was significantly degraded by haemolysis in the Cushing’s arm of the study. While isotopic glucose data was used in the insulin/glucose kinetics modelling from the FSIVGTTs, data on $R_a$ and $R_d$ of glucose and glycerol (derived from Steele’s equation) were not available at the time of writing.

It was envisaged that repeated measures ANOVA would be used to determine trends across all study visits (parametric vs. non parametric depending on the normal distribution of the data) but these were not available at the time of writing; secondly, while healthy volunteer data will be available for more than the 2 studied in this protocol, it was not available at the time of writing. The results therefore are as described with statistical data from 2-tail students t-tests calculated using SPSS v20 (IBM Inc. USA).
7.1 Results

These are the results of the Overnight and IVGTT sampling studies, performed in two healthy volunteers, six acromegaly and six Cushing’s patients. Patients were studied at baseline (no intervention) and re-attended no sooner than 14 days later to be re-studied with an identical protocol, but with 4 hourly oral Acipimox administration overnight from 2000 hrs until 0800 hrs the following day. Healthy Volunteers were studied at baseline only.

The study protocol is detailed in Chapter 6.

Due to illness in the MRC Human Nutrition Research Laboratory, Cambridge, some sample analysis has yet to be performed. No data for glycerol production rates were available, and some Healthy Volunteer and Cushing’s data remains outstanding.

Missing data due to model failure and/or sample haemolysis are detailed for each parameter below.
7.2 Overnight and Basal

7.2.1 Healthy Volunteers

Overnight Insulin and Glucose

These are shown in Figures 7.1, 7.2, 7.3 and 7.4.

The Area Under the Curve (AUC) for insulin was measured at 137 and 90 mU.hr.l\(^{-1}\) for HV1 and HV2 respectively. Glucose AUC was 51 and 53 mmol.hr.l\(^{-1}\) for HV1 and HV2. These results are plotted in Figures 7.1 and 7.3 (with acromegaly) and Figures 7.8 and 7.10 (with Cushing’s). They are also shown as AUC plots in Figures 7.2, 7.4, 7.9 and 7.11.

HOMA Indices

The two healthy volunteers had a HOMA-IR (-Insulin Resistance) index of 1.4 and 0.8 respectively, and a HOMA-\(\beta\)% of 114 and 83. These are shown in Figures 7.5 and 7.6.

Hepatic Insulin Sensitivity (HIS)

No HV data for Hepatic Insulin Sensitivity were available at the time of writing.

7.2.2 Acromegaly

Overnight Insulin and Glucose

These are shown in Figures 7.1, 7.2, 7.3 and 7.4.
Figure 7.1: Mean Overnight Plasma Insulin in Healthy Volunteers and Acromegaly Patients.

Error bars represent ±2 SD. HV n=2, Acromegaly n=5. HV values are plotted individually. 1) Patient 3 had significant haemolysis affecting insulin samples and these results were not included.
7.2. OVERNIGHT AND BASAL

Figure 7.2: Mean AUC for Overnight Plasma Insulin in Healthy Volunteers and Acromegaly Patients.

Error bars represent ± 2 SD. HV n=2, Acromegaly n=5, p value by 2-tailed paired t-test. 1) Patient 3 had significant haemolysis affecting insulin samples and these results were not included.

Figure 7.3: Mean Overnight Plasma Glucose in Healthy Volunteers and Acromegaly Patients.

HV n=2, Acromegaly n=6. For clarity, only hourly values are shown. Error bars represent ±2 SD. HV values are plotted individually.
Mean AUC for both insulin and glucose were improved with Acipimox in acromegaly patients. Mean and (SD) Insulin AUC (in mU.hr.l-1) 135 (52) with Acipimox vs. 279 (77) Baseline, p=0.042, n=5; Glucose AUC (in mmol.hr.l-1) 52 (4) with Acipimox vs. 63 (9) Baseline, p=0.038, n=6. 1) Patient 3 had significant haemolysis affecting insulin samples and these results were not included.
7.2. OVERNIGHT AND BASAL

Homa Indices

Figure 7.5: HOMA-IR in Healthy Volunteers and Acromegaly Patients. Horizontal bars indicate mean IR values. HV n=2. Acromegaly n=6, p value by 2-tailed paired t-test.

Figure 7.6: HOMA-β% in Healthy Volunteers and Acromegaly Patients. Horizontal bars indicate mean values. HV n=2. Acromegaly n=6, p value by 2-tailed paired t-test.
All 6 acromegaly patients’ HOMA-IR index improved (i.e. lowered) with Acipimox; mean (SD) 1.2 (0.7) vs. 1.8 (0.8) baseline, p=0.018. No significant difference was observed in HOMA-β% with Acipimox 104 (28) vs. 114 (29) baseline, p=0.3.

**Hepatic Insulin Sensitivity**

Hepatic Insulin Sensitivity values, and the parameters used to calculate them, are given in Table 7.1. HIS in acromegaly is shown in Figure 7.7. HIS is the reciprocal of the product of fasting endogenous glucose production and fasting plasma insulin.

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<th>Visit</th>
<th>Gb Mmole. L⁻¹</th>
<th>Ib pmole. L⁻¹</th>
<th>H μmole. min⁻¹</th>
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Table 7.1: Hepatic Insulin Sensitivity (HIS) in Acromegaly Patients.  

In figure 7.7, HIS was higher in all acromegaly patients in the Acipimox arm; mean (SD) 16.3 (8.3) vs. 8.4 (3.3) at baseline measured in Min.L.mmol⁻¹.pmol⁻¹ × 10².
Figure 7.7: Hepatic Insulin Sensitivity (HIS) in Acromegaly Patients. Horizontal bars indicate mean values; n=6, p value by 2-tailed paired t-test.
7.2.3 Cushing’s

Overnight Insulin and Glucose Curves

Figure 7.8: Mean Overnight Plasma Insulin in Healthy Volunteers and Cushing’s Patients.

Error bars represent ±2 SD. n=51. HV values are plotted individually.

1) Patient 4 had significant haemolysis affecting insulin samples and these results were not included.
7.2. OVERNIGHT AND BASAL

Figure 7.9: Mean AUC for Overnight Plasma Insulin in Healthy Volunteers and Cushing’s Patients.

Error bars represent ±2 SD. HV n=2, CD n=5, p value by 2-tailed paired t-test.

1) Patient 4 had significant haemolysis affecting insulin samples and these results were not included.

Figure 7.10: Mean Overnight Plasma Glucose values for Healthy Volunteers and Cushing’s Patients.

Error bars represent ±2 SD. n=6. For clarity, only hourly values are shown. HV values are plotted individually.
There was no change in AUC for insulin or glucose in the baseline or Acipimox arms. Mean and (SD) Insulin AUC (in mU.hr.l\(^{-1}\)) 206 (75) Acipimox vs. 206 (79) Baseline, p=1; Mean Glucose AUC (in mmol.hr.l\(^{-1}\)) 86 (52) vs. 84 (38), p=0.7.

While there was no observed difference between baseline and Acipimox AUCs, both insulin and glucose measurements were lower in the Acipimox arm in the basal state (from 7am onwards). Haemolysis limited the number of valid insulin results in the overnight section.

**HOMA Indices**

HOMA-IR for Cushing’s patients are shown in Figures 7.12.

Five of the 6 Cushing’s patients’ insulin resistance (IR) index was lower in the Acipimox arm (i.e. had improvement in insulin sensitivity) compared with the no treatment arm; 1.8 (0.6) Acipimox vs. 2.2 (0.6) baseline, p=0.018.
7.2. OVERNIGHT AND BASAL

Figure 7.12: HOMA-IR in Healthy Volunteers and Cushing’s Patients.
Horizontal bars indicate mean IR values. N=5; p value by 2-tailed paired t-test. 1) Patient 1 with Acipimox had a mean basal insulin value outwith the range of the HOMA calculation and so was not included.

Figure 7.13: HOMA-β% in Healthy Volunteers and Cushing’s Patients.
Horizontal bars indicate mean values. HV n=2. CD n=5; p value by 2-tailed paired t-test. 1) Patient 1 with Acipimox had a mean basal insulin value outwith the range of the HOMA calculation and so was not included.
### Hepatic Insulin Sensitivity (HIS)

HIS for Cushing’s patients is shown in Table 7.2 and Figure 7.14.

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<th>Weight (kg)</th>
<th>Visit</th>
<th>Gb Mmole. L⁻¹</th>
<th>lb pmole. L⁻¹</th>
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<td>910.9</td>
<td>0.198</td>
</tr>
<tr>
<td>C05</td>
<td>1.78</td>
<td>137.4</td>
<td>1</td>
<td>5.8</td>
<td>22.7</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>5.7</td>
<td>20.1</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>C06</td>
<td>n/a</td>
<td>n/a</td>
<td>1</td>
<td>n/a</td>
<td>n/a</td>
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<td></td>
<td>2</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Table 7.2: Hepatic Insulin Sensitivity (HIS) in Cushing’s Patients.  
Gb= basal glucose, Ib= basal insulin, H= Hepatic glucose production.

Figure 7.14: Hepatic Insulin Sensitivity (HIS) in Cushing’s Patients.  
Lines indicate paired values. n=4, p value by 2-tailed paired t-test.
In four Cushing’s patients, HIS values were higher in the Acipimox arm 25.3 (18.2) vs. 12.5 (8.6) at baseline measured in Min.L.mmol$^{-1}$.pmol$^{-1} \times 10^{-2}$ (but did not reach significance, $p=0.08$). This is illustrated in Figure 7.14. Individual patient values are shown in Table 7.2, with parameters used in the estimation of Hepatic Insulin Sensitivity.

### 7.3 Minimal Modelling Results from Intravenous Glucose Tolerance Test

The parameters measured from the IVGTT modelling are described in Chapter 5, Background to metabolic studies.

Data were produced using MINMOD software for 'hot' (deuterated isotope) glucose and cold (non-isotope) models. The correlation between the two is shown below for insulin sensitivity (Si) and disposition Index (DI).

In these studies, both models are well correlated, but 'hot’ Minimal Modelling has advantages over non-isotopic studies in that the glucose isotope can be considered in terms of disposition only. In order to perform 'cold’ modelling, assumptions need to be made regarding rates of disposition based on basal insulin sensitivity and an assumed basal rate of hepatic glucose production. Single compartment modelling is imperfect (it assumes glucose to be in a single pool), but has been validated in previous studies. The data available from these studies were unsuitable for more complex 2-compartment modelling, mainly due to haemolysis of blood samples, making insulin measurements unreliable.

Figure 7.15: Comparison of (a) Si ($p<10^{-7}$) and (b) Comparison of DI ($p<10^{-7}$)
7.3. MINIMAL MODELLING RESULTS FROM INTRAVENOUS GLUCOSE TOLERANCE TEST

7.3.1 Healthy Volunteers - Cold Model

Only one ‘cold’ model was available at the time of writing. This is summarised in Table 7.3 below.

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Si Value</th>
<th>CV</th>
<th>Sg Value</th>
<th>CV</th>
<th>AIRg Value</th>
<th>CV</th>
<th>DI Value</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV02</td>
<td>11.12 1%</td>
<td>0.0134 4%</td>
<td>91.61 0.1018 1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.3: Data for the ‘cold’ glucose kinetics model for Healthy Volunteers

Si is insulin sensitivity ($10^{-4}$mL.uU$^{-1}$.min$^{-1}$), Sg=glucose effectiveness (min$^{-1}$), AIRg =Acute Insulin Response to glucose (uU ml$^{-1}$ min) and DI=Disposition Index.

Healthy Volunteer 2 had an insulin sensitivity (Si) of $11.12 \times 10^{-4}$mL.uU$^{-1}$.min$^{-1}$, a Sg of 0.0134 min$^{-1}$, an AIRg of 91.61uUml$^{-1}$ and a DI of 0.1018. All CVs were in the range 1-4%.

7.3.2 Acromegaly - Cold Model

Table 7.4 shows a summary of Insulin Sensitivity (Si), Glucose Effectiveness (Sg) Acute Insulin Response to Glucose (AIRg) and Disposition Index (DI) in (active) acromegaly patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Visit</th>
<th>Si Value</th>
<th>CV</th>
<th>Sg Value</th>
<th>CV</th>
<th>AIRg Value</th>
<th>CV</th>
<th>DI Value</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>1</td>
<td>4.01 5%</td>
<td>0.0110 4%</td>
<td>95.50 0.0383 5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.44 1%</td>
<td>0.0050 7%</td>
<td>144.90 0.1223 1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.88 5%</td>
<td>0.0177 2%</td>
<td>29.75 0.0086 5%</td>
<td></td>
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<td></td>
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<td></td>
<td>2</td>
<td>11.83 1%</td>
<td>0.0087 4%</td>
<td>54.00 0.0639 1%</td>
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<td></td>
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<tr>
<td>A02</td>
<td>1</td>
<td>9.46 11%</td>
<td>0.0097 5%</td>
<td>4.45 0.0042 11%</td>
<td></td>
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<td></td>
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<td>2</td>
<td>14.88 1%</td>
<td>0.0140 2%</td>
<td>22.15 0.0330 1%</td>
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</tr>
<tr>
<td>A03</td>
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<td>46.32 1%</td>
<td>0.0125 7%</td>
<td>30.90 0.1431 1%</td>
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<td></td>
<td></td>
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<td>2</td>
<td>37.62 1%</td>
<td>0.0154 6%</td>
<td>57.95 0.2180 1%</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>A04</td>
<td>1</td>
<td>- -%</td>
<td>- -%</td>
<td>- -%</td>
<td>- -%</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>- -%</td>
<td>0.0315 3%</td>
<td>147.25 - -%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A05</td>
<td>1</td>
<td>7.98 2%</td>
<td>0.0102 4%</td>
<td>39.80 0.0317 2%</td>
<td></td>
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<td></td>
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</tr>
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<td></td>
<td>2</td>
<td>10.60 1%</td>
<td>0.0136 3%</td>
<td>52.30 0.0555 1%</td>
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<td></td>
</tr>
</tbody>
</table>

Table 7.4: Data for the ‘cold’ glucose kinetics model for Acromegaly Patients.

Visit number: 1= no treatment arm, 2= Acipimox arm. Si is insulin sensitivity ($10^{-4}$mL.uU$^{-1}$.min$^{-1}$), Sg=glucose effectiveness (min$^{-1}$), AIRg =Acute Insulin Response to glucose (uU ml$^{-1}$ min) and DI=Disposition Index.
Insulin Sensitivity (Si)

Cold insulin sensitivity (Si) was modelled successfully in 10 of the 12 visits, and in 5 of the 6 active acromegaly patients. A05 had model failure in both baseline and Acipimox arms of the study due to excessive sample haemolysis (making insulin estimations unreliable) during the IVGTT. All but one successful model had a CV of 5% or less (A03_1 had a CV of 11%).

Cold Si was higher in the Acipimox arm in 4 of the 5 AA patients. A04 had a lower Si with Acipimox than baseline, but both measurements of Si were > 3× higher than both healthy volunteers and other patients in the group. Mean and (SD) (in $10^{-4}$mL.uU$^{-1}$.min$^{-1}$) 14.1 (18.2) with Acipimox vs. 16.7 (11.9) baseline, $p=0.44$. This is shown in Figure 7.16.

![Cold Model Insulin Sensitivity (Si) In Active Acromegaly](image)

Figure 7.16: Cold Model Insulin Sensitivity (Si) in Acromegaly Patients.

*Horizontal bars indicate mean values. n=5\footnote{1}, p value by 2-tailed paired t-test. 1) Patient 5 had significant insulin haemolysis resulting in model failure in both visits*
Cold Si was higher in 4 of 5 patients with Acipimox than at baseline, but did not reach significance (p=0.4, 2-tailed paired t-test). In Patient 4, Si was lower with Acipimox than at Baseline, but was several magnitudes higher in both arms than the other acromegaly patients.

**Glucose Effectiveness (Sg)**

Cold Sg is shown in Figure 7.17

![Figure 7.17: Cold Model Glucose Effectiveness (Sg) in Acromegaly Patients.](image)

Horizontal bars indicate mean values. n=5, p value by 2-tailed paired t-test.

1) Patient 5 had significant insulin haemolysis resulting in model failure in both visits.

Cold glucose effectiveness (Sg) was modelled successfully in 11 of the 12 visits, and in 5 of the 6 acromegaly patients. Patient 5 had model failure in the baseline arm of the study. CV of Sg values were 2-7%. Cold Sg improved in three of the five acromegaly patients in the Acipimox arm, but was lower in the Acipimox arm in two patients *cf* baseline. Patient 5 had model failure in the *no treatment* visit and the highest Sg of the group (0.0315). Mean and SD was (in min⁻¹×10³): 11.3 (4.3) with Acipimox vs. 12.2 (3.2) baseline (p=0.8).
Acute Insulin Response to glucose (AIRg)

AIRg in acromegaly patients is shown in Figure 7.18.

Figure 7.18: Acute Insulin Response to Glucose (AIRg) in Acromegaly Patients. Horizontal bars indicate mean values. n=5, p value by 2-tailed paired t-test.

AIRg improved with Acipimox compared to baseline arm in all five pairs of successful models [54]. Patient 5 had model failure secondary to insulin sample haemolysis at baseline, and patient 3 had a low calculated AIRg in both arms due to some haemolysed samples. The AIRg measurement is used in the calculation of DI. Statistical analysis of the group does not include patient 5, and values are given both with and without those from patient 3. Mean and (SD) for AIRg (in uU ml⁻¹.min) were: 66.3 (46.2) with Acipimox vs. 40.1 (33.7) at baseline (n=5, p=0.01). Patient 3 was also affected by haemolysis; if removed from statistical analysis AIRg improvement with Acipimox (77.2 (45.1) vs. 48.9 (31.3) at baseline) was still significant for n=4 (p=0.035).

Disposition Index (DI)

Cold DI is shown in Figure 7.19

Cold Disposition Index was successfully modelled in 5 acromegaly patients. In all 5
patients cold DI \( \times 10^{-3} \) was higher; 98.5 (74) with Acipimox vs. 45.2 (56.6) at baseline, p=0.011. CVs for DI were 1-5% other than Patient 3, baseline visit with a CV of 11%. If A03 is also removed the observed improvement in cold DI \( \times 10^{-3} \) (114.9 (59.8) with Acipimox vs. 55.4 (59.8) at baseline) remains significant (p=0.02).

### 7.3.3 Acromegaly - Hot Model

Table 7.5 shows a summary of Insulin Sensitivity (Si), Glucose Effectiveness (Sg) Acute Insulin Response to Glucose (AIRg) and Disposition Index (DI) in (active) acromegaly patients.

**Insulin Sensitivity (Si)**

Hot Si in acromegaly patients is shown in Figure 7.20.

Hot insulin sensitivity (Si) was modelled successfully in 10 of the 12 visits; Patient 3 had a CV of 23% cf 2-7% for other successful models; patient 5 had model failure in the Acipimox arm only. Similar to the Cold model, Si was higher in the Acipimox arm in 4 of the
### 7.3. MINIMAL MODELLING RESULTS FROM INTRAVENOUS GLUCOSE TOLERANCE TEST

<table>
<thead>
<tr>
<th>Patient</th>
<th>Visit</th>
<th>Si Value</th>
<th>Si CV</th>
<th>Sg Value</th>
<th>Sg CV</th>
<th>AIRg Value</th>
<th>AIRg CV</th>
<th>DI Value</th>
<th>DI CV</th>
</tr>
</thead>
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<td>4.73</td>
<td>4%</td>
<td>0.0047</td>
<td>6%</td>
<td>95.50</td>
<td>0.0451</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.63</td>
<td>3%</td>
<td>0.0040</td>
<td>7%</td>
<td>144.90</td>
<td>0.0960</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>A02</td>
<td>1</td>
<td>4.61</td>
<td>7%</td>
<td>0.0082</td>
<td>6%</td>
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<td>0.0137</td>
<td>7%</td>
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<tr>
<td></td>
<td>2</td>
<td>15.08</td>
<td>4%</td>
<td>0.0024</td>
<td>10%</td>
<td>54.00</td>
<td>0.0814</td>
<td>4%</td>
<td></td>
</tr>
<tr>
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<td>1</td>
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<td>23%</td>
<td>0.0081</td>
<td>3%</td>
<td>4.45</td>
<td>0.0005</td>
<td>23%</td>
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<td></td>
<td>2</td>
<td>8.23</td>
<td>2%</td>
<td>0.0072</td>
<td>3%</td>
<td>22.15</td>
<td>0.0182</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>A04</td>
<td>1</td>
<td>54.77</td>
<td>2%</td>
<td>0.0066</td>
<td>3%</td>
<td>30.90</td>
<td>0.1692</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>40.77</td>
<td>2%</td>
<td>0.0103</td>
<td>2%</td>
<td>57.95</td>
<td>0.2362</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>A05</td>
<td>1</td>
<td>-</td>
<td>-%</td>
<td>-</td>
<td>-%</td>
<td>-</td>
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<tr>
<td></td>
<td>2</td>
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<td>4%</td>
<td>0.0223</td>
<td>3%</td>
<td>147.25</td>
<td>0.0824</td>
<td>4%</td>
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<td>7.45</td>
<td>3%</td>
<td>0.0066</td>
<td>3%</td>
<td>39.80</td>
<td>0.0296</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.42</td>
<td>2%</td>
<td>0.0035</td>
<td>6%</td>
<td>82.2</td>
<td>0.0938</td>
<td>2%</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.5: Data for the ‘hot’ glucose kinetics model for Acromegaly Patients.

**Visit number:** 1 = no treatment arm, 2 = Acipimox arm. Si is insulin sensitivity \((10^{-4}\text{mL}\cdot\text{uU}^{-1}\cdot\text{min}^{-1})\), Sg = glucose effectiveness \((\text{min}^{-1})\), AIRg = Acute Insulin Response to glucose \((\text{uU}\cdot\text{ml}^{-1}\cdot\text{min})\) and DI = Disposition Index.

Modelling failed in patient A05 in the first visit due to excessive sample haemolysis, making the plasma insulin results unreliable.

![Graph](image_url)

**Figure 7.20:** Hot Model Insulin Sensitivity (Si) in Acromegaly Patients.

*Lines indicate paired samples. n=5*, p value by 2-tailed paired t-test.

1) Patient 5 had significant sample haemolysis, affecting insulin estimation which resulted in model failure in baseline visit.
5 patients. Patient 4 had a lower Si with Acipimox than baseline, but both measurements of Si were $3 \times$ other patients in the group. Mean and (SD) (in $10^{-4} mL.uU^{-1}.min^{-1}$) 16.4 (14) with Acipimox vs. 14.9 (22.4) baseline, $p=0.7$.

**Glucose Effectiveness (Sg)**

Hot Sg is shown in Figure 7.21.

![Hot Model Glucose Effectiveness (Sg) in Acromegaly Patients.](image)

*Figure 7.21: Hot Model Glucose Effectiveness (Sg) in Acromegaly Patients. Horizontal bars indicate mean values. Marker with* indicates Sg for patient 5. n=5$^l$, $p$ value by 2-tailed paired t-test.

1) Patient 5 had significant sample haemolysis, affecting insulin estimation which resulted in model failure in baseline visit.

Hot glucose effectiveness (Sg) was modelled successfully in 11 of the 12 visits, and in 5 of the 6 acromegaly patients. Patient 5 had model failure in the baseline arm of the study, but the highest Sg measured in the Acipimox arm. Other than patient 2 with a CV of 10%, CV of Sg values were 2-7%. Hot Sg was lower in 4 of the five AA patients in the Acipimox arm. Mean and SD was (in min$^{-1} \times 10^{-3}$): 5.4 (3.2) with Acipimox vs. 6.8 (1.4) at baseline ($p=0.4$). This is shown in Figure 24.
Acute Insulin Response to glucose (AIRg)

AIRg is calculated from the AUC in the first part of the IVGTT and is the same measurement as the cold model AIRg.

Disposition Index (DI)

Hot DI is shown in Figure 7.22

Patient 5 had model failure secondary to insulin sample haemolysis at baseline, and patient 3 had a low AIRg values due to some haemolysed samples. The AIRg measurement is used in the calculation of DI and so both AIRg and DI in patient 3 should be interpreted with caution. Calculation of mean and SD of the group does not include patient 5, and values are given both with and without those from patient 3.

![Acipimox Improves Hot Model Disposition Index (DI) in Active Acromegaly](image)

Figure 7.22: Hot Model Disposition Index (DI) in Acromegaly Patients. Horizontal bars indicate mean values. n=5¹, p value by 2-tailed paired t-test.

¹) Patient 5 had significant sample haemolysis, affecting insulin estimation which resulted in model failure in baseline visit.

Disposition Index in the hot model was higher with Acipimox cf baseline visit in all 5 acromegaly patients with successful models; Mean (SD) in Hot DI (values ×10⁻³) 105 (80) with Acipimox vs. 51 (67) baseline (p<0.01). Modelling failed in patient 5, visit 1, and the CV of 23% in patient 3, visit 1, was higher than the 2-7% range for the other DI results from the hot model in AA patients. Removing patient 3 from the statistical
analysis still yields significance (127 (73) with Acipimox vs. 64 (71) baseline) for n=4 (p=0.01).

### 7.3.4 Cushing’s - Cold Model

Table 7.6 shows a summary of Insulin Sensitivity (Si), Glucose Effectiveness (Sg) Acute Insulin Response to Glucose (AIRg) and Disposition Index (DI) in Cushing’s patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Visit</th>
<th>Si Value</th>
<th>CV</th>
<th>Sg Value</th>
<th>CV</th>
<th>AIRg Value</th>
<th>CV</th>
<th>DI Value</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>C01</td>
<td>1</td>
<td>66.13</td>
<td>1%</td>
<td>0.0093</td>
<td>7%</td>
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<tr>
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<td>0.0265</td>
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</table>

Table 7.6: Data for the ’cold’ glucose kinetics model for Cushing’s Patients. Visit number: 1= no treatment arm, 2= Acipimox arm. Si is insulin sensitivity \((10^{-4}\text{mL.uU}^{-1}\cdot\text{min}^{-1})\), Sg=glucose effectiveness (min\(^{-1}\)), AIRg =Acute Insulin Response to glucose (uU ml\(^{-1}\) min) and DI=Disposition Index.

In the data available, only patient 1 has a complete set of two-study data. Patient 5 visit 2 failed due to venous access problems during IVGTT. Both C06 studies were completed but these results were not yet analysed at time of submission due to illness in the Cambridge lab.

### 7.3.5 Cushing’s - Hot Model

Table 7.7 shows a summary of measured parameters in the hot minimal model in Cushing’s patients.
### 7.4 Summary of Results

#### 7.4.1 Overnight and Basal

- Acipimox reduced mean overnight AUC for insulin \((p=0.04)\) and glucose \((p=0.03)\) in acromegaly patients.

- Acipimox had no significant effect on mean overnight AUC for either insulin or glucose in Cushing’s patients.

- Acipimox improved HOMA-IR in both acromegaly \((p=0.02)\) and Cushing’s \((p=0.01)\) patients. No effect was observed in either patient group for HOMA-\(\beta\)%.

- Acipimox improved Hepatic Insulin Sensitivity (HIS) in acromegaly \((p=0.02)\) and in all four Cushing’s patients analysed to date \((n=4, p=0.08)\).
7.4.2 IVGTT and MINMOD

- Acipimox improved Insulin Sensitivity $S_i$ in 4 of 5 acromegaly patients in both hot and cold minimal model ($p>0.05$).

- Acipimox had no significant effect on glucose effectiveness in acromegaly patients in either hot or cold minimal model ($p>0.05$).

- Acipimox improved $AIR_g$ in acromegaly patients ($p=0.01$).

- Acipimox improved DI in acromegaly patients in both hot ($p<0.01$) and cold ($p=0.01$) minimal model.

- Glucose modeling was degraded by the high incidence of haemolysis of the insulin samples in the Cushing’s patients.
The objective of this thesis is to increase understanding of insulin resistance in acromegaly and Cushing’s disease, with a particular focus on the role of lipid metabolism. The imaging studies quantified both macroscopic and microscopic fat distribution using whole body MRI and MRS respectively, while the manipulation of lipolysis with Acipimox (synthetic nicotinic acid) provide insight into the influence of circulating Free Fatty Acids (FFAs).

8.1 Summary of Findings

8.1.1 Acromegaly

There were no differences observed between Healthy Volunteers and acromegaly patients for macroscopic fat distribution or intra-myocellular triglyceride, but hepatocellular triglyceride concentration, measured by $^1$H Magnetic Resonance Spectroscopy, was significantly lower in acromegaly patients than in Cushing’s or Healthy Volunteers. This reduced liver triglyceride is a novel finding for an insulin resistant state; increased hepatic fat has been shown to be associated with decreased insulin sensitivity in healthy volunteers and Type 2 Diabetes. Even Lipodystrophy, which despite characteristic pathological reductions in subcutaneous and visceral fat, is associated with insulin resistance and high liver fat content [55]. Area under the curve (AUC) for overnight plasma insulin and glu-
cose were both lower with inhibition of lipolysis by Acipimox. Hepatic Insulin Sensitivity (HIS) was improved in patients with acromegaly. HOMA-IR values, another measure of basal insulin sensitivity, were lowered by Acipimox, indicating an improvement in insulin sensitivity. The results from MINMOD, from IVGTT modelling, suggest that peripheral glucose uptake is increased by inhibition of lipolysis, not simply by an improvement in peripheral insulin sensitivity, but by an augmented 1st phase insulin response to a glucose stimulus in patients with acromegaly.

8.1.2 Cushing’s

Cushing’s patients had a significantly higher total visceral adipose tissue content (VAT) than the other study groups. Intra-hepatocellular fat was higher than Healthy Volunteers and acromegaly, although this did not reach statistical significance. No significant difference in intra-myocellular triglyceride was observed among the three groups, in either muscle studied.

In contrast to acromegaly, overnight plasma insulin and glucose AUC were unchanged by inhibition of lipolysis. Hepatic Insulin Sensitivity (HIS) (n=4, ns) and HOMA-IR were improved in every patient by inhibition of lipolysis, although the former did not reach statistical significance. This may indicate that the anti-lipolytic effect was less pronounced in the Cushing’s patients than acromegaly. Due to modelling failure in the IVGTT, no MINMOD analysis or estimation of $\beta$ cell function was possible.

8.2 Implications of these findings

8.2.1 Acromegaly

These results suggest that inhibition of lipolysis improves insulin sensitivity in acromegaly; basal (hepatic) and stimulated (peripheral) insulin sensitivity was improved, and $\beta$ cell function was augmented.

In normal physiology (see introduction), Growth Hormone has complementary actions to insulin; in the fed state, GH levels fall allowing insulin to store glucose and promote lipogenesis. The liver is however more GH sensitive, maintaining IGF-I production which potentiates insulin effects (albeit less potently than insulin). When fasting (or nocturnally) GH is secreted. Insulin levels are low and the potent lipolytic actions of GH provide FFAs as an alternative fuel source for peripheral muscle, sparing protein catabolism.
in the non-fed state [56]. Despite high GH levels, the liver becomes relatively insensitive to its actions, and IGF-I production falls, lowering any antagonistic effect on lipid metabolism.

In acromegaly the yin and yang of the relationship between GH and insulin is disrupted as serum GH is perpetually elevated. The pathological hyper-secretion of GH results in increased lipolysis, insulin insensitivity, hyperinsulinaemia and increased circulating IGF-I [15]. The lipolytic effects of GH dominate, with low levels of visceral and subcutaneous fat [42], and as we have shown for the first time, low intra-hepatic triglyceride.

GH and insulin share common intracellular pathways, and their yin and yang is thought to continue at the intracellular level. Common pathways, via PI3K and JAK-STAT, can allow GH to activate insulin sensitising mechanisms, dependent on the relative concentrations of GH, insulin and state of satiety. Pathological GH excess may also have a directly antagonistic effects via these same pathways as high concentrations of GH switch off these mechanisms via these pathways [57].

These studies concur with previous work on FFA and insulin sensitivity in acromegaly, using Pegvisomant as a tool to block GH effect [18] [17]. Both studies observed an improvement in basal insulin sensitivity, but only Higham et al demonstrated a decrease in FFA production overnight with GH receptor blockade. Lindberg-Larsen did not show any alteration in lipolysis measuring serum palmitate.

Our study corroborates the findings of Higham et al, where GH blockade reduced overnight FFA production and increased hepatic insulin sensitivity. In this study, blocking lipolysis improves insulin sensitivity. While we do not have the results from glycerol production, Acipimox has been shown to be a potent short-term anti-lipolytic agent, and insulin sensitivity alterations appear to be a direct consequence of its administration.

This study also suggests that β cell function in acromegaly is improved by blocking lipolysis. Studies examining the effect of FFA on β cell function in other disease groups have proved conflicting; overnight lowering of FFA with Acipimox improved β cell function in both Type 2 Diabetes and non diabetic obese subject [10] while β cell function was improved in another study by a 48h FFA infusion [58]. Insulin insensitivity induced by GH infusion can be reversed with Acipimox [11] but this was using an insulin clamp study on healthy subjects, and β cell function was not assessed. Studies using HOMA-β% as a measure of β cell function have been reported in acromegaly, with improvements post surgery [59] but our study is the first to report on ‘Gold standard’ β cell assessment.

This study adds to the understanding of the role of body composition and fat distribution to insulin resistance in acromegaly. While no difference in body fat compartments was
found, intra-hepatocellular triglyceride was significantly lower than Healthy Volunteers and Cushing’s patients. There appears to be an increased lipolytic rate yet little or no fat depositing in the liver. This may suggest that, in acromegaly, raised circulating FFAs are being used as the substrate for hepatic glucose production, and this rapid flux impairs the glucose-suppressive effect of insulin on the liver [60]. This flux may also impair insulin effect on enzymes involved with hepatic glucose cycling [60]. It is therefore likely that this FFA flux is implicated in the hepatic insulin resistance observed. Blocking the production of FFA improves basal insulin sensitivity.

Our studies documented an improvement in peripheral insulin sensitivity. Freda et al, in addition to observing decreased subcutaneous and visceral adiposity, noted an increase in Intra-muscular adipose tissue (IMAT) in acromegaly, compared to healthy controls. IMAT is defined as extracellular, macroscopic fat deposition legs within leg muscle, and its volume was proportional to IGF-I levels [42]. GH administration to healthy volunteers has been shown to induce increased intramyocellular lipid and peripheral insulin resistance [35] and Madsen et al have reported a decrease in intramyocellular triglyceride with pharmacological treatment of acromegaly [61]. This study failed to show any difference in IMTG amongst the groups, but this may reflect the small numbers studied.

Taken together, our results would suggest that the increased lipolysis seen in in acromegaly is critical to the development of insulin resistance; it reduces hepatic insulin sensitivity, likely through FFA flux, and the increased circulating FFA may also have an inhibitory effect on pancreatic $\beta$ cells which could contribute to the increased incidence of diabetes and glucose intolerance seen in acromegaly.

Peripheral insulin sensitivity is also improved by anti-lipolysis, and it is therefore likely that GH induced peripheral insulin resistance is largely mediated by FFA.

### 8.2.2 Cushing’s

In normal physiology, lipid metabolism is controlled by insulin and growth hormone (with some modulation by catecholamines). After a meal, insulin promotes fat deposition in adipocytes. During fasting and at night, Growth Hormone’s lipolytic actions increases plasma FFA as an alternative fuel.

Short-term studies have shown that acute hypercortisolaemia has a net lipolytic effect, but this may be augmented by catecholamine release in a ‘stress’ response.

In the longer term, sustained hypercortisolaemia results in characteristic body composition changes, with increased fat deposition, muscle wasting and insulin resistance. Cor-
tisol secretion is increased in Cushing’s disease, antagonising the glucose-suppressing effects of insulin on the liver [20]. It also is involved in growth and differentiation of adipocytes [62]. The suppression of satiety with Cushing’s may exacerbate weight gain [63] and increased food intake also augments circulating TG and FFA.

Unlike acromegaly, overall rates of lipolysis have not been shown to increase [23] [22]. FFA production is dependent on prevailing insulin concentration and fat volume. In Cushing’s, this high volume fat-depot and resistance to the effects of insulin results in higher lipid production, i.e. lower rates from a higher volume store. Hypercortisolaemia causes hepatic insulin resistance, promoting gluconeogenesis [20] and de-novo lipogenesis [64]. Lipid mobilised from subcutaneous stores may be taken up by visceral adipose tissue and deposited in the liver, increasing triglyceride assembly and inhibiting intracellular insulin signalling.

Lipolysis may therefore play a role in this insulin resistance. As hypercortisolaemia does not directly cause lipolysis, it is not be surprising that the effects of inhibiting lipolysis were less pronounced in these patients. Overnight plasma insulin and glucose were unaffected, although basal Hepatic insulin sensitivity and HOMA-IR were improved, the former not reaching statistical significance (n=4). VAT was significantly higher in the Cushing’s group, and had the highest intra-hepatic fat, although the latter again did not reach statistical significance. The imaging studies were limited in Cushing’s patients (only four were scanned) because of weight and body habitus.

As in other insulin resistant states, high FFA concentrations may inhibit insulin release, but we were unable to observe this due to model failure in the IVGTT.

8.3 Limitations of The Studies

8.3.1 Lack Of Control Data

When the study was devised, it was intended that control data from Healthy Volunteers would be gathered and that the same HV cohort would take part in both imaging and metabolic sampling studies.

8.3.2 Numbers of Patients Studied

Ideally, the same patients would have completed both the sampling and the imaging studies. This was not possible in the time available.
8.3.3 Sampling and Modelling

The insulin samples were prone to haemolysis, particularly in the rapid sampling drawn through poor venous access during the IVGTT. This was especially problematic in the Cushing’s patients.

Lack of insulin data meant that two-compartment hot modelling was not possible.

8.4 Further Work

There are several elements of this study which are currently outstanding, and which may prove crucial in the interpretation of the findings to date. In the first instance, the glycerol production rates (calculated from the rate of glycerol isotope appearance) will provide evidence of the effectiveness of Acipimox in blocking lipolysis. It could be anticipated from previous work that acromegaly would have a higher rate of lipolysis than either healthy volunteers or Cushing’s patients. Furthermore, the difference in the lipolytic rate amongst patients may explain the differing levels of improvement in insulin sensitivity both in the basal and stimulated state in acromegaly. It will also provide (albeit in only 2 patients) some control data from the healthy volunteers. It will be interesting to note if lipolysis inhibition occurred later in Cushing’s patients.

Secondly, overnight GH profiling in the acromegaly group could be key; if the inhibition of lipolysis, and a subsequent reduction in circulating FFA’s in some way modulates GH secretion, then this could provide another mechanism by which lipid metabolism influences insulin sensitivity in acromegaly.

The improvement in AIRg (1st phase insulin secretion) with inhibition of lipolysis is a novel finding in acromegaly. Further studies are required to ascertain whether this effect is maintained in the longer term.

The IVGTT and glucose modelling in Cushing’s was unsuccessful; there is a case for attempting a similar study, although the problems with this patient group would remain.

This work was the first use of 1H MRS Manchester for measuring intra-hepatic and intra-myocellular triglyceride levels. This could be used to examine other conditions with altered insulin sensitivity, such as hyperthyroidism or steatohepatitis.

Anti-lipolysis remains a target of interest in the treatment of insulin resistance and diabetes, but Acipimox is not the ideal agent as the short-term inhibition of lipolysis is not sustained; longer term studies show an ‘escape’ of FFA control with a rebound rise in
plasma FFA. Its use is also limited by troublesome side effects of flushing, abdominal pain, and in higher doses, deranged LFTs and impaired glucose tolerance have been reported [65]. Niaspan, a sustained release preparation, was shown to have modest benefits on HbA1C and dyslipidaemia in Type 2 Diabetes despite no long-term reduction in FFA [66].

In summary, these studies have helped elucidate the role of lipid metabolism in altered insulin sensitivity in acromegaly and Cushing’s. It appears to have a key role in acromegaly in particular, where (amongst other effects) the lipolytic effect of Growth Hormone impairs central and peripheral insulin action, and the resulting increases in Free Fatty Acids inhibit β cell function. The products of this increased lipolysis do not appear to deposit in the liver, where higher FFA flux acting as a substrate for augmented hepatic glucose production may explain the low hepatic fat observed.
References


[17] Lindberg-Larsen R, Møller N, Schmitz O, Nielsen S, Andersen M, Ørskov H, et al. The Impact of Pegvisomant Treatment on Substrate Metabolism and In-
REFERENCES


REFERENCES


FIRST PRINCIPLES OF MINIMAL MODELLING - ADAPTED FROM CAUMO

A.1 Minimal Modelling

As this is a ‘model’ and not an exact mimicking of normal physiology, some assumptions must be made.

1) Glucose must be treated as a single pool, with mobilization into the periphery and production by the liver.

2) Insulin is a two-pool model - it is produced by the pancreas, but its plasma concentration does not reflect the ‘active insulin’.
In this model, suppression of glucose production cannot be separated from increased peripheral uptake.

By the addition of isotope (G$^h$):

Endogenous glucose production can be separated from glucose uptake in the periphery (predominantly skeletal muscle uptake). Plasma insulin is transported into the remote pool by fractional rate constant $k_{RI}$, and irreversibly lost from this remote pool with fractional rate constant $k_{OR}$. 
A.1. MINIMAL MODELLING

\[
\frac{dG}{dt} \frac{dG}{dt} = -p_1 G - p_2 G_R \tag{A.1.1}
\]

\[
\frac{dR}{dt} = k_{RI} I - kOKR \tag{A.1.2}
\]

Calling insulin action the mathematical construct \(z\)

\[
z = \lambda R + \mu \quad (\lambda \text{ and } \mu \text{ are by experimenters choice}) \tag{A.1.3}
\]

\[
\frac{dz}{dt} = \lambda \frac{dk}{dt} \tag{A.1.4}
\]

\[
\frac{dz}{dt} = \lambda k_{RI} I - \lambda k_{OR} \tag{A.1.5}
\]

\[
= \lambda k_{RI} I - \lambda k_{OR}(z - \mu) \tag{A.1.6}
\]

\[
\frac{dG}{dt} = -p_1 G - p_2 \frac{G}{\lambda} (z - \mu) \tag{A.1.7}
\]

If we then suggest

\[
\mu = 0 \tag{A.1.8}
\]

\[
\lambda = \frac{k_{OR}}{k_{RI}} \tag{A.1.9}
\]

Then \(\frac{dz}{dt} = k_{OR}(I - z)\)

\[
\frac{dG}{dt} = -p_1 G - p_2 \frac{k_{RI}}{k_{OR}} G^h z \tag{A.1.10}
\]

\(z\) is the blunted and reduced and later version of I (the true, lessened, active insulin)

In the basal state, the differential is zero

\[
I_G = z_G \tag{A.1.11}
\]
A.1. MINIMAL MODELLING

Remembering that \( G_G^h = 0 \)

Glucose effectiveness \( \sigma_G \) is defined as 'the change in glucose disposal due to an incremental change in plasma glucose.'

\[
\sigma_G = -\delta \frac{dG_G^h}{dt} = p_1 + p_2 \frac{k_{RI}}{k_{OR}} z
\]  
(A.1.12)

In the basal state,

\[
\sigma_{G(G)} = p_1 + p_2 \frac{k_{RI}}{k_{OR}} z_g = p_1 + p_2 \frac{k_{RI}}{k_{OR}} I_G
\]  
(A.1.13)

Insulin sensitivity \( \sigma_I^h \) is defined as 'the change in glucose disposal due to an incremental change in plasma glucose.'

\[
\sigma_I^h = \frac{\sigma_{G(G)}^h}{dI_G} = p_2 \frac{k_{Rd}}{k_{OR}}
\]  
(A.1.14)

Resubstituting:

\[
\frac{dG^h}{dt} = -\left(\sigma_G^h - \sigma_I^h I_G\right) G^h + \sigma_I^h G^h z
\]  
(A.1.15)

Ends with 4 parameters

\( \sigma_G^h, \sigma_I^h, k_{OR}, v^h \) where \( v^h \) is the volume of distribution…

The difficulty with this is that the same could then be done for 'cold' glucose, but there are more model failures with \( \sigma_G, \sigma_I < 0 \). The precision of the parameter estimates is poorer with the cold model than the hot, and the overall model is 'ill defined'.

One may expect \( \sigma_G > \sigma_G^h \) and \( \sigma_I > \sigma_I^h \), but almost always \( \sigma_I^h > \sigma_I \).

Therefore, if one examines hot and cold data to look at endogenous production the answers are un-physiological; hence, the model is imperfect. One can either accept the limitations of this single compartment model, or use a more complex 2 compartment minimal model. This two-compartment model (the number of compartments refers to glucose in these matters) takes into account both insulin dependent and independent methods of glucose disposal.

GLUT-1 is independent at \( G_1 \). GLUT-2 is however linear in \( G_1 \).
A.1. MINIMAL MODELLING

The greater complexity of the two-compartment model requires greater assumptions to be made; in addition the sampling data needs to be of the highest quality, and the high incidence of haemolysis (particularly in the Cushing’s patients) made this model unsuitable for the data from these studies. In previous studies, different assumptions are made depending on whether the patient is diabetic or not. Both hot and cold data together solves the endogenous glucose production problem, but in order to be confident in the validity of the data, extremely accurate measurements of glucose and insulin must be made.

In these studies, a single compartment model was used, and results obtained for hot and cold models.