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**INSULIN-LIKE GROWTH FACTORS  
IN THE ISCHAEMIC HEART**

**A thesis submitted in partial fulfilment of the  
requirements for the degree of**

**Doctor of Philosophy**

**at the**

**University of Auckland**

**by**

**KENNETH GEORGE MATTHEWS**



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## ***Abstract***

Cardiac muscle has very limited ability to regenerate following injury. Loss of muscle due to myocardial infarction (MI) is therefore compensated for by hypertrophy of the surviving cardiac muscle. Deposition of inflexible scar tissue in the infarct zone leads to subsequent dysfunction of the heart, often culminating in chronic morbidity and eventual death due to heart failure.

Insulin-like growth factor I (IGF-I) and insulin-like growth factor II (IGF-II) are members of a family of insulin-related peptides involved in tissue development, repair and replacement. Their involvement in these roles has been clearly demonstrated in skeletal muscle, but remains unclear in cardiac muscle. The aim of this thesis, therefore, was to undertake a comprehensive evaluation of the insulin-like growth factor axis following induced MI in sheep, in order to determine the relationship between time-based changes in the myocardium and insulin-like growth factor levels, and subsequently to determine the therapeutic effect of GH or IGF-I treatment on cardiac function following MI.

To achieve the aims of the thesis, a model of MI was developed from the existing cardiology technique of percutaneous transluminal coronary angioplasty. Using this model, MI was induced in adult ewes by selected coronary artery occlusion under fluoroscopic guidance. Subsequently, the model was further developed into one of stable heart failure, utilising serial microembolisations targeted by echocardiography.

Results showed that, in the cardiomyocytes bordering the infarct, IGF-I mRNA, protein and receptor binding increased, whereas IGF-II mRNA and protein levels did not vary, although IGF-II receptor binding increased.

Following these findings, IGF-I levels were manipulated by subcutaneous injections of either growth hormone or IGF-I, and by intra-pericardial IGF-I delivery via catheter.

Results showed that neither growth hormone nor subcutaneous IGF-I were able to effect an improvement in cardiac function, although there were indications that a longer duration of treatment with subcutaneous IGF-I might have done so. Intra-pericardial IGF-I, however, resulted in a significant and sustained improvement in cardiac function.

In conclusion, surviving cardiomyocytes at the infarct border show marked changes in IGF-I localisation, production and specific binding, indicating that IGF-I is directly involved in post-infarct events, possibly in the maintenance of cardiac function by the induction of hypertrophy. Delivery of IGF-I directly into the pericardium results in a significant improvement in left ventricular ejection fraction which is sustained after cessation of treatment. Such a result indicates that IGF-I may have a role as a therapy in the failing heart.

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# Table of Contents

<i>Abstract</i> .....	ii
<i>Acknowledgements</i> .....	iv
<i>Table of Contents</i> .....	vi
<i>List of Figures</i> .....	xii
<i>List of Tables</i> .....	xiv
<i>List of Abbreviations</i> .....	xv
<b>CHAPTER 1</b> .....	<b>1</b>
1.1 THE INSULIN-LIKE GROWTH FACTORS .....	1
1.1.1 <i>A Brief History of IGF-I &amp; IGF-II</i> .....	2
1.1.2 <i>IGF Structure</i> .....	3
1.2 THE HEART .....	5
1.2.1 <i>Pre-Natal Heart Development</i> .....	6
1.2.2 <i>The Cardiomyocyte</i> .....	7
1.3 THE IGFs IN THE HEART .....	11
1.3.1 <i>IGF Message and Protein</i> .....	11
1.3.2 <i>IGF Receptors in the Heart</i> .....	12
1.3.3 <i>The IGFs and the Cell Cycle</i> .....	15
1.3.4 <i>The IGFs and Post-Natal Cardiac Development</i> .....	21
1.3.5 <i>IGF Regulation</i> .....	24
1.4 MYOCARDIAL INFARCTION .....	32
1.4.1 <i>The IGF Axis Post-Infarct</i> .....	36
1.4.2 <i>Possible Post-Infarct Roles of the IGFs</i> .....	37
1.5 HEART FAILURE .....	41

1.5.1	<i>Therapeutic Roles for IGF-I in Ischaemic Heart Failure</i> .....	43
1.6	SUMMARY .....	46
1.7	THE AIM OF THIS THESIS .....	46
1.7.1	<i>Achieving the Aim of the Thesis</i> .....	47
<b>CHAPTER 2</b>	.....	<b>48</b>
2.1	MATERIALS .....	48
2.1.1	<i>Peptides and Hormones</i> .....	48
2.1.2	<i>Antibodies</i> .....	49
2.1.3	<i>Molecular Probes</i> .....	49
2.1.4	<i>Enzymes</i> .....	51
2.1.5	<i>Radioisotopes</i> .....	51
2.1.6	<i>Other Reagents</i> .....	52
2.2	COMPOSITION OF SOLUTIONS .....	53
2.2.1	<i>In Situ Hybridisation Buffers</i> .....	54
2.2.2	<i>Northern Hybridisation Buffer</i> .....	54
2.2.3	<i>Receptor Autoradiography Buffers</i> .....	55
2.2.4	<i>Immunohistochemistry Buffers</i> .....	55
2.2.5	<i>Other Solutions</i> .....	56
2.3	EXPERIMENTAL METHODS .....	57
2.3.1	<i>In Situ Hybridisation (Molenaar et al. 1992)</i> .....	57
2.3.2	<i>Immunohistochemistry (Hsu et al. 1981)</i> .....	61
2.3.3	<i>Receptor Binding Studies (Elliott et al. 1992)</i> .....	64
2.3.4	<i>Radioimmunoassay (Hodgkinson et al. 1991a)</i> .....	66
2.3.5	<i>Western Ligand Blot (Hossenlopp et al. 1986)</i> .....	69
2.3.6	<i>Northern Analysis (Sambrook et al. 1989)</i> .....	71



2.3.7	<i>Image Analysis</i> .....	73
2.3.8	<i>Photomicrography</i> .....	74
2.3.9	<i>Statistical Analyses</i> .....	74
<b>CHAPTER 3.....</b>		<b>75</b>
3.1	THE AIM OF THE DEVELOPMENT PHASE.....	75
3.2	MYOCARDIAL INFARCTION .....	76
3.2.1	<i>The Sheep</i> .....	76
3.2.2	<i>Induction of Myocardial Infarction</i> .....	78
3.3	STABLE HEART FAILURE.....	86
3.3.1	<i>Method of Induction</i> .....	86
3.3.2	<i>Echocardiography</i> .....	89
3.4	RESULTS.....	92
3.4.1	<i>Induction of Infarction</i> .....	92
3.4.2	<i>Echocardiography</i> .....	102
3.4.3	<i>Conclusions</i> .....	103
<b>CHAPTER 4.....</b>		<b>105</b>
4.1	INTRODUCTION.....	105
4.2	THE AIM OF THIS CHAPTER.....	107
4.3	METHODS.....	107
4.3.1	<i>The Animal Model</i> .....	107
4.3.2	<i>Descriptive Histology</i> .....	108
4.3.3	<i>Immunohistochemistry</i> .....	108
4.3.4	<i>Receptor Binding Studies</i> .....	109
4.3.5	<i>In Situ Hybridisation</i> .....	109

4.3.6	<i>Western Ligand Blot - IGFbps</i> .....	110
4.3.7	<i>Statistical Analysis</i> .....	110
4.4	RESULTS.....	111
4.4.1	<i>Descriptive Histology</i> .....	111
4.4.2	<i>Immunohistochemistry</i> .....	115
4.4.3	<i>In Situ Hybridisation</i> .....	120
4.4.4	<i>Receptor Binding</i> .....	122
4.4.5	<i>Western Ligand Blot - IGFbps</i> .....	127
4.5	DISCUSSION.....	129
4.5.1	<i>IGF-I</i> .....	129
4.5.2	<i>IGF-II</i> .....	132
4.5.3	<i>IGF Binding Proteins</i> .....	135
4.5.4	<i>Conclusions</i> .....	136
<b>CHAPTER 5</b>	.....	<b>138</b>
5.1	INTRODUCTION.....	138
5.2	THE AIM OF THIS CHAPTER.....	139
5.3	METHODS.....	140
5.3.1	<i>The Animal Model</i> .....	140
5.3.2	<i>Descriptive Histology</i> .....	141
5.3.3	<i>Radioimmunoassay – IGF-I Protein</i> .....	141
5.3.4	<i>Northern Analysis</i> .....	143
5.3.5	<i>Western Ligand Blot - IGFbps</i> .....	144
5.3.6	<i>Echocardiography</i> .....	145
5.3.7	<i>Statistical Analysis</i> .....	145
5.4	RESULTS.....	146

5.4.1	<i>Induction of Myocardial Infarction</i> .....	146
5.4.2	<i>Descriptive Histology</i> .....	147
5.4.3	<i>Radioimmunoassay – IGF-I Protein</i> .....	150
5.4.4	<i>Northern Analysis</i> .....	154
5.4.5	<i>Western Ligand Blot - IGF-BPs</i> .....	159
5.4.6	<i>Echocardiography</i> .....	163
5.5	DISCUSSION.....	164
5.5.1	<i>Plasma IGF-I Levels</i> .....	164
5.5.2	<i>Cardiomyocyte Hypertrophy</i> .....	164
5.5.3	<i>Cardiac IGF-I</i> .....	166
5.5.4	<i>Cardiac Function</i> .....	168
5.5.5	<i>Conclusions</i> .....	171

**CHAPTER 6.....173**

6.1	INTRODUCTION.....	173
6.2	THE AIM OF THIS CHAPTER.....	174
6.3	METHODS.....	175
6.3.1	<i>The Animal Model</i> .....	175
6.3.2	<i>Radioimmunoassay – IGF-I Protein</i> .....	179
6.3.3	<i>Western Ligand Blot - IGF-BPs</i> .....	180
6.3.4	<i>Echocardiography</i> .....	180
6.3.5	<i>Statistical Analysis</i> .....	182
6.4	RESULTS.....	182
6.4.1	<i>Induction of Stable Heart Failure</i> .....	182
6.4.2	<i>Radioimmunoassay of Plasma IGF-I</i> .....	182
6.4.3	<i>Western Ligand Blot - IGF-BPs</i> .....	188

6.4.4	<i>Echocardiography</i> .....	190
6.5	DISCUSSION.....	197
6.5.1	<i>Plasma IGF-I Levels</i> .....	197
6.5.2	<i>Cardiac Function</i> .....	199
6.5.3	<i>Conclusions</i> .....	205
<b>CHAPTER 7</b>	.....	<b>206</b>
7.1	SUMMARY OF THE RESEARCH .....	206
7.2	CONCLUSIONS .....	216
7.3	FUTURE WORK.....	223
	<i>References</i> .....	224
	<i>Publications Arising From This Thesis</i> .....	248

## *List of Figures*

Figure 1-1 Cyclins and Cyclin-Dependent Kinases in the Cell Cycle .....	18
Figure 3-1 Sheep Coronary Arteries .....	77
Figure 3-2 The Waves of the ECG.....	83
Figure 3-3 Typical Sampling Areas .....	85
Figure 3-4 Post-Infarct Changes in ECG Pattern .....	93
Figure 3-5 Post-Infarct Myocardial Enzyme Profiles .....	95
Figure 3-6 Hearts Displaying Evidence of Myocardial Infarction.....	97
Figure 3-7 Normal and Remodelled Sheep Heart .....	99
Figure 3-8 Heart 60 Days Post-Infarct .....	100
Figure 3-9 Dilated and Normal Hearts .....	101
Figure 4-1 Time Course of Post-Infarct Tissue Changes.....	112
Figure 4-2 Normal and Hypertrophied Cardiomyocytes.....	113
Figure 4-3 Cardiomyocyte Area of Non-Infarcted and Infarcted Animals .....	114
Figure 4-4 Immunohistochemistry Controls .....	116
Figure 4-5 Increased IGF-I Immunostaining at Infarct Border.....	117
Figure 4-6 Immunostaining for IGFBP-2.....	117
Figure 4-7 IGF-I Immunohistochemistry Time Course .....	118
Figure 4-8 IGF-I mRNA .....	121
Figure 4-9 Comparison of Displacement by IGF-I and by des(1-3) IGF-I.....	123
Figure 4-10 IGF-I Receptor Binding.....	124
Figure 4-11 IGF-II Receptor Binding .....	126
Figure 4-12 Ligand Blot of Myocardial IGFBPs .....	128
Figure 4-13 Islands of Tissue .....	133
Figure 5-1 Heart 30 Days Post-Infarct .....	146

Figure 5-2 Cardiac Tissue 30 Days Post-Infarct .....	147
Figure 5-3 Cardiomyocyte Area – Control and Growth Hormone Treated .....	149
Figure 5-4 IGF-I Protein in Peri-Infarct and Distal Tissue .....	151
Figure 5-5 Plasma IGF-I .....	153
Figure 5-6 IGF-I Northern Blot.....	155
Figure 5-7 IGF-I mRNA Abundance .....	157
Figure 5-8 Western Ligand Blot, IGF-BPs - Plasma Samples .....	159
Figure 5-9 Western Ligand Blot, IGF-BPs - Plasma and Tissue Extract.....	161
Figure 5-10 Western Ligand Blot, IGF-BPs - Cardiac Tissue Extract.....	162
Figure 5-11 Left Ventricular Ejection Fraction.....	163
Figure 6-1 Subcutaneous Group Acute Plasma IGF-I .....	184
Figure 6-2 Subcutaneous Group Long-Term Plasma IGF-I.....	185
Figure 6-3 Intrapericardial Group Acute Plasma IGF-I.....	186
Figure 6-4 Intrapericardial Group Long-Term Plasma IGF-I .....	187
Figure 6-5 Western Ligand Blot - Subcutaneous Group.....	188
Figure 6-6 Western Ligand Blot - Intrapericardial Group .....	189
Figure 6-7 Subcutaneous Group Acute EF Response .....	191
Figure 6-8 Subcutaneous Group Long-Term Ejection Fraction.....	193
Figure 6-9 Intrapericardial Group Acute EF Response.....	194
Figure 6-10 Intrapericardial Group Long-Term EF .....	196
Figure 7-1 Schematic Diagram of Possible IGF-I Pathways .....	222

## *List of Tables*

Table 3-1 Left Ventricular Ejection Fraction .....	102
Table 5-1 Heart Weight Index .....	146
Table 5-2 IGF-I Receptor – Mean Optical Densities .....	157
Table 5-3 Growth Hormone Receptor – Mean Optical Densities .....	158

## *List of Abbreviations*

<b>ALS</b>	acid-labile subunit
<b>ATP</b>	adenosine-5-triphosphate
<b>BP</b>	binding protein
<b>BSA</b>	bovine serum albumin
<b>C</b>	Celsius
<b>cDNA</b>	complementary deoxyribonucleic acid
<b>Ci</b>	Curie
<b>cm</b>	centimetre
<b>CPM</b>	counts per minute
<b>C-terminal</b>	carboxyl terminal
<b>CV</b>	coefficient of variation
<b>Cx</b>	circumflex (coronary artery)
<b>Da</b>	Dalton
<b>DAB</b>	diaminobenzidine
<b>DEPC</b>	diethyl pyrocarbonate
<b>DNA</b>	deoxyribonucleic acid
<b>DSS</b>	dissucinimidyl suberate
<b>E.coli</b>	Escherichia coli
<b>ECG</b>	electrocardiograph
<b>EDTA</b>	ethylene diaminetetra-acetic acid disodium salt
<b>EF</b>	ejection fraction
<b>EDD</b>	end diastolic diameter
<b>ESD</b>	end systolic diameter
<b>g</b>	gravity
<b>g/l</b>	grams per litre
<b>GH</b>	growth hormone
<b>h</b>	hour
<b>HCl</b>	hydrochloric acid
<b><sup>125</sup>I</b>	iodine-125
<b><sup>131</sup>I</b>	iodine-131
<b>iv</b>	intravenous
<b>IGF</b>	insulin-like growth factor
<b>IGF-I</b>	insulin-like growth factor I
<b>IGF-II</b>	insulin-like growth factor II
<b>IGFBP</b>	insulin-like growth factor binding protein
<b>kDa</b>	kilo Dalton
<b>LAD</b>	left anterior descending (coronary artery)
<b>LV</b>	left ventricle



<b>LVEDD</b>	left ventricular end diastolic diameter
<b>LVESD</b>	left ventricular end systolic diameter
<b>M</b>	molar, moles per litre
<b>MI</b>	myocardial infarction
<b>m</b>	metre
<b>mM</b>	millimolar, millimoles per litre
<b>mm</b>	millimetre
<b>mg</b>	milligram
<b>mg/ml</b>	milligrams per millilitre
<b>min</b>	minute
<b>MGF</b>	mechanogrowth factor
<b>mRNA</b>	messenger ribonucleic acid
<b>MSA</b>	multiplication stimulating activity
<b>MW</b>	molecular weight
<b>nmol</b>	nanomolar, nanomoles per litre
<b>NSILA</b>	non-suppressible insulin-like activity
<b>N-terminal</b>	amino terminal
<b>P</b>	probability
<b>pH</b>	hydrogen ion concentration
<b>PAGE</b>	polyacrylamide gel electrophoresis
<b>PBS</b>	phosphate buffered saline
<b>PMSF</b>	phenyl methyl sulphonyl fluoride
<b>PTCA</b>	percutaneous transluminal coronary angioplasty
<b>r</b>	recombinant
<b>rh</b>	recombinant human
<b>ro</b>	recombinant ovine
<b>RIA</b>	radioimmunoassay
<b>RT</b>	room temperature
<b>RV</b>	right ventricle
<b>SD</b>	standard deviation
<b>SDS</b>	sodium dodecyl sulphate
<b>SED</b>	standard error of the difference
<b>SEM</b>	standard error of the mean
<b>SERCA</b>	sarcoplasmic reticulum calcium ATP-ase
<b>TCA</b>	trichloroacetic acid
<b>vol</b>	volume
<b>v/v</b>	volume per volume
<b>w/v</b>	weight per volume

*This Thesis Is Dedicated To*  
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