Exercise physiology and the renin-angiotensin system: role of the ACE gene insertion/deletion polymorphism in cardiac growth and endurance exercise

MD Thesis

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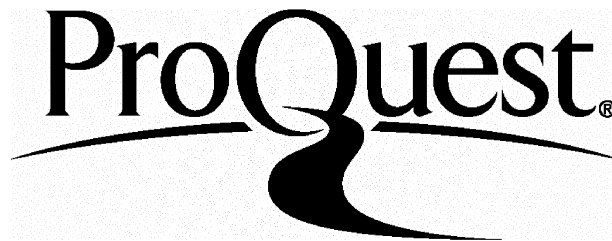

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ABSTRACT

The systemic renin-angiotensin system, an important regulator of cardiovascular homeostasis, is well described, though separate local systems exist in many tissues, the function of which is less well defined. There is evidence for their role in growth responses and cellular metabolism, particularly for the heart, and the understanding of these mechanisms is important, given the detrimental effect of excess cardiac hypertrophy. The examination of these local systems in humans is problematic, though a polymorphism in the human angiotensin converting enzyme (ACE) gene may be utilised to explore the mechanisms involved.

This thesis examines the influence of the ACE gene polymorphism in exercise-induced cardiac hypertrophy. Subjects of DD genotype (associated with higher ACE levels) had greater left ventricular (LV) growth than those of II genotype, and the LV growth in DD subjects was disproportionate to the increase in lean body mass. Blockade of the angiotensin receptor with losartan had no effect on LV growth in either genotype, though the dose may have been inadequate. This and other recent studies support the role of the local renin-angiotensin system in human cardiac growth, with higher ACE levels being associated with greater growth. The appropriate indices for scaling LV mass to body size were also examined and lean body mass was found to be the only suitable index for simple ratio scaling.

The renin-angiotensin system may also play a role in the efficiency of cellular metabolism. The ACE gene polymorphism was examined in elite endurance athletes, and the I allele (associated with lower ACE levels) was found to be more prevalent, suggesting a genetic advantage for endurance. In Olympic runners, the relative frequency of the I allele rose with the distance run. The data suggests greater efficiency associated with lower ACE levels, and the reduced cardiac growth associated with the II genotype may be related to this improved efficiency. Potential mechanisms are discussed.

These results confirm the importance of local renin-angiotensin systems in exercise physiology and may relate closely to disease states. The importance of gene-environment interactions has also been shown.

CONTENTS

CHAPTER 1 - Introduction: renin-angiotensin systems and left ventricular growth

1.1 This thesis – background and aims 10

1.2 The systemic (endocrine) renin-angiotensin system 11

1.2.1 Overview 11

1.2.2 Components of the systemic RAS 12

1.2.2.1 Renin 12

1.2.2.2 Angiotensinogen 13

1.2.2.3 Angiotensin 1-converting enzyme (ACE) 13

1.2.2.4 Angiotensin II 14

1.2.2.5 Angiotensin II receptors 15

1.3 Local renin-angiotensin systems 16

1.3.1 Cardiac renin-angiotensin system 17

1.3.1.1 Renin 17

1.3.1.2 Angiotensinogen 17

1.3.1.3 Angiotensin converting enzyme 18

1.3.1.4 Non-ACE pathways for angiotensin-I conversion 18

1.3.1.5 Angiotensin II receptors 19

1.3.2 Integration of circulating and local renin-angiotensin systems 20

1.4 The RAS and cardiovascular growth 21

1.4.1 Vascular growth 21

1.4.2 Cardiac growth 21

1.4.2.1 Association of LV mass with circulating components of the RAS 21

1.4.2.2 Increased RAS activity in response to growth stimuli 21

1.4.2.3 Direct stimulation of cardiac growth by angiotensin II 22

1.4.2.4 The inhibition of cardiac growth by RAS antagonists 22

1.4.3 The cardiac RAS and human LV growth 23

1.4.4 The human ACE gene insertion/deletion polymorphism 24

1.4.4.1 The ACE I/D polymorphism and LV hypertrophy 25

1.4.4.2 Prospective studies of LV growth 26

1.4.5 Other RAS gene polymorphisms and LV growth 28

1.4.6 RAS-mediated LV growth : mechanisms & rate-limiting steps 28

1.4.6.1 Systemic versus local cardiac RAS 28

1.4.6.2 Angiotensin II or kinin dependant? 29

CHAPTER 2 - Methods

2.1 Genotyping the ACE I/D polymorphism 31

2.1.1 Sample acquisition 31

2.1.1.1 Advantages of the technique 31

2.1.1.2 Disadvantages 31

2.1.2 DNA extraction 32

2.1.2.1 Method 32

2.1.3 Polymerase chain reaction (PCR) 33

2.1.4 Postal DNA sample collection 35

2.2 The assessment of LV mass with cardiovascular magnetic resonance 36

2.2.1 Echocardiography 37

2.2.2 Electron-beam computed tomography 38

2.2.3 Cardiovascular magnetic resonance 38

2.2.3.1 CMR technique used in this thesis 38

2.2.3.2 Accuracy and reproducibility 40

2.2.3.3 Limitations of CMR 41

CHAPTER 3 - Role of ACE genotype and the cardiac renin-angiotensin system in exercise-induced LV growth.

3.1 Background 43

3.1.1 Importance of left ventricular hypertrophy 43

3.1.2 Role of the cardiac RAS in LVH 43

3.1.2.1 Further research 44

3.1.3 Exercise as an appropriate cardiac hypertrophic stimulus 44

3.1.3.1 Scale of hypertrophic response 44

3.1.3.2 Timing of hypertrophic response 44

3.1.3.3 Physiological vs. pathological LVH 45

3.1.4 Military recruits as an appropriate study group 45

3.1.5 Potential mechanisms for cardiac growth 46

3.1.5.1 Direct effects of angiotensin II 46

3.1.5.2 Kinins 46

3.1.5.3 Other genes 47

3.1.5.4 Identifying the mechanism of LVH with RAS inhibitors 47

3.2 Aims of the study 47

3.3 Methods 48

3.3.1 Study group 48
3.3.2 Imaging 49
3.3.2.1 Cardiovascular magnetic resonance (CMR) 49
3.3.2.2 Body composition using magnetic resonance 49
3.3.3 Statistical analysis 49
3.4 Results 50
3.4.1 Study group 50
3.4.2 LV mass 52
3.4.2.1 LV mass index 53
3.4.3 Cardiac volume data 54
3.4.4 Indexing LV mass to LV end-diastolic volume 55
3.4.5 Body composition 56
3.5 Discussion 56
3.5.1 Losartan effect 56
3.5.2 Degree of LV hypertrophy 57
3.5.3 Potential confounding factors 58
3.5.4 Mechanism of effect of the ACE I/D polymorphism 58
3.5.4.1 Cardiac AT_1 receptor 58
3.5.4.2 AT_2 receptor actions 59
3.5.4.3 Kinins 60
3.5.4.4 The sympathetic nervous system 60
3.5.4.5 Other biochemical systems 61
3.5.4.6 Other genetic polymorphisms 61
3.5.5 Recent data from other studies 61
3.5.5.1 ACE gene and LV mass 61
3.5.5.2 AT_1 receptor blockade and ace inhibition: mechanisms and comparisons 62
3.5.6 Limitations 63
3.6 Conclusions 64

CHAPTER 4 - Indexing left ventricular mass to body composition variables
4.1 Introduction 67
4.1.1 The importance of scaling 67
4.1.2 Abbreviations used in this chapter 67
4.1.3 Current limitations of scaling 67
4.1.4 Study aims 68

4.2 Methods 69
4.2.1 Study group 69
4.2.2 Body composition 69
4.2.2.1 Adipose tissue segmentation 69
4.2.2.2 Reproducibility of adipose tissue segmentation 70
4.2.3 Statistical analysis 74
4.3 Results 75
4.4 Discussion 76
4.4.1 Lean body mass 77
4.4.2 Adipose tissue 77
4.4.3 Anthropometric variables 78
4.4.3.1 Body surface area 78
4.4.3.2 Height 78
4.4.3.3 Weight 79
4.4.5 Limitations 80
4.5 Conclusions 80

CHAPTER 5 - Cardiac metabolism and the renin-angiotensin system: effect of the ACE gene polymorphism in elite endurance athletes
5.1 Background 82
5.1.1 Cardiac metabolism and the renin-angiotensin system 82
5.1.1.1 Left ventricular hypertrophy 82
5.1.2 The skeletal muscle RAS and metabolic effects 83
5.1.3 The ACE gene I/D polymorphism 83
5.1.4 Study design 84
5.2 Methods 85
5.2.1 Subjects 85
5.2.1.1 High-altitude mountaineers 85
5.2.1.2 Endurance athletes 85
5.2.1.3 Control group 86
5.2.2 Statistical analyses 86
5.3 Results 87
5.3.1 High altitude mountaineers 87
5.3.2 Olympic athletes 88
5.3.2.1 Runners 88
5.3.2.2 Other disciplines 89

5.4 Discussion 91
5.4.1 Athletes 91
5.4.1.1 Race and sex influences among the runners 91
5.4.1.2 Other sports 91
5.4.2 Climbers 92
5.4.3 Potential confounding factors 93
5.4.3.1 Control group 93
5.4.3.2 Group sampling 93
5.4.4 Potential mechanisms in athletes 93
5.4.4.1 Glucose uptake and substrate use 94
5.4.4.2 Metabolic efficiency 94
5.4.4.3 Summary 95
5.4.5 The kinin-nitric oxide pathway in heart failure 95
5.4.6 Other recent studies in this field 96
5.4.6.1 Frequency distribution of the ACE I/D polymorphism 96
5.4.6.2 ACE I/D polymorphism and survival/cardiac function 96
5.4.6.3 RAS inhibition and survival/cardiac function 97
5.4.7 Limitations 97
5.5 Conclusions 98

CHAPTER 6 - General discussion
6.1 Summary and context 100
6.1.1 ACE I/D polymorphism in the population 100
6.1.1.1 RAS effects on male fertility 100
6.1.2 Other RAS polymorphisms and LVH – recent studies 101
6.1.2.1 Angiotensinogen M235T polymorphism 101
6.1.2.2 Angiotensin AT_1 receptor A1166C polymorphism 102
6.1.2.3 Angiotensin II type 2 receptor gene 102
6.1.2.4 Bradykinin B_2 receptor gene 103
6.2 Strengths and limitations of the research 103
6.2.1 Strengths 103
6.2.2 Limitations 104
6.3 Conclusions 105
6.3.1 Left ventricular hypertrophy 105
6.3.1.1 Systemic vs. local RAS 105
6.3.1.2 Angiotensin vs. kinins 105
6.3.1.3 AT_1 vs. AT_2 receptors 105
6.3.2 Cardiac metabolism and the effect on elite groups 106

6.4 Future research directions 106
6.4.1 LV hypertrophy 106
6.4.1.1 Prognosis in LVH 106
6.4.2 Metabolic efficiency 107
6.4.3 The ACE gene I/D polymorphism in context 107

CHAPTER 7 - References 108

CHAPTER 8 - Appendices
8.1 Publications arising from this research 146
8.2 Supervision of the thesis 147
8.3 Ethical approval for the research 148
8.3.1 LV mass study 148
8.3.2 ACE genotype frequency distribution in athletes 148
8.4 Personal contribution to this research 149
8.4.1 ACE genotyping 149
8.4.2 CMR scanning 149
8.4.3 LV hypertrophy study 149
8.4.4 Athletes 149
8.4.5 Indexing of LV mass 149
8.5 Acknowledgements 150
8.6 Sources of funding 151
8.7 Individual subject data from chapter 3 152
8.7.1 DD placebo group 153
8.7.2 DD losartan group 155
8.7.3 II placebo group 157
8.7.4 II losartan group 159

CHAPTER 1

Introduction:

Renin-angiotensin systems and left ventricular growth

1.1 THIS THESIS – BACKGROUND AND AIMS

The circulating endocrine renin-angiotensin system (RAS) is important in the control of blood pressure, and of circulating blood volume, via its effects on vasoconstriction and salt and water metabolism. These are mediated via the components of the system: renin acts on angiotensinogen to produce angiotensin I (inactive), which is cleaved by angiotensin converting enzyme (ACE) to form angiotensin II - a potent vasoconstrictor which also releases aldosterone, causing salt and water retention. This cardiovascular homeostatic role is controlled mainly by the kidney.

Local renin-angiotensin systems also exist in many tissues – heart, vascular tree, skeletal muscle, adipose tissue, brain, lung, kidney - and there is good evidence for their involvement in growth responses. The local cardiac renin-angiotensin system in particular has strong evidence for its role in left ventricular growth.

This chapter reviews the understanding of local and systemic renin-angiotensin systems at the time the work was initiated and what role genetic polymorphisms in the RAS, particularly the ACE gene insertion/deletion polymorphism, may have for determining responses to physiological stimuli. It will focus on the cardiac RAS and the cardiac growth response.

The thesis explores this area further, including mechanisms of action and potential rate-limiting steps. It will also examine the role of renin-angiotensin systems in metabolic efficiency, particularly cardiac, which may be intricately related to LV growth and to the excess mortality associated with LV hypertrophy.

The investigations will focus on two main areas:

i) The role of the renin-angiotensin system in human cardiac growth, utilising the ACE gene insertion/deletion (I/D) polymorphism. This polymorphism has the greatest evidence for differential effects on LV growth. Potential mechanisms of action for this polymorphism will be explored.

ii) The effect on elite groups, in whom maximal cardiac metabolic efficiency may be crucial to success. The frequency distribution of the ACE gene I/D polymorphism in these groups will be studied and mechanisms examined.

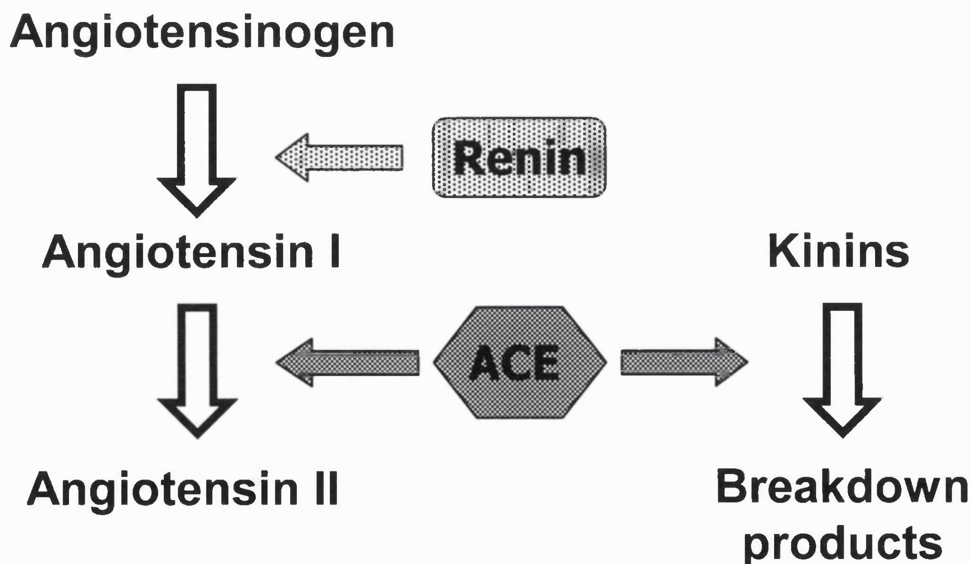
1.2 THE SYSTEMIC (ENDOCRINE) RENIN-ANGIOTENSIN SYSTEM

1.2.1 OVERVIEW

In the systemic renin-angiotensin system (RAS), renin is produced by the juxta-glomerular cells of the kidney. This acts on angiotensinogen (an alpha-2 globulin synthesised in the liver) to form angiotensin I, an inactive decapeptide, which is converted to the active form, angiotensin II, by the enzyme angiotensin-converting enzyme (ACE) - see review.[1] Angiotensin II is a potent vasoconstrictor and also stimulates the release of aldosterone. Thus, the systemic RAS is important in the control of blood pressure and the regulation of salt and water metabolism, and is responsive to changes in these stimuli.[2,3] ACE has a secondary role in the inactivation of bradykinins, and is also known as kininase II. ACE therefore simultaneously generates a potent vasoconstrictor (angiotensin II) and inactivates a potent vasodilator (bradykinin), further enhancing its role in the control of blood pressure (Figure 1.1).

Figure 1.1

Overview of the renin-angiotensin system



1.2.2 COMPONENTS OF THE SYSTEMIC RAS

1.2.2.1 Renin

Renin is an aspartyl protease produced by a complex group of specialised cells in the renal cortex – the juxta-glomerular apparatus (JGA). This consists of:

i) The macula densa, formed from modified mesangial cells and tubular epithelial cells of the ascending loop of Henlę and distal tubule.

ii) Specialised epithelioid smooth muscle cells in the media of the afferent and efferent arterioles of the glomerulus. These manufacture pre-prorenin, converted by the Golgi apparatus into prorenin and then cleaved to form renin, which is secreted into the circulation in response to a decrease in renal perfusion pressure or sodium depletion.[4]

The production and release of renin is thus stimulated by:

- A fall in blood pressure, and the consequent rise in renal sympathetic tone and β–adrenergic stimulation of the JGA.[5]
- Reduced sodium ion concentration, detected by the macula densa[4] or acting directly on JGA cells.[6]

Renin release is inhibited by:

- A rise in renal perfusion pressure and stretching of the afferent arterioles[6]
- Angiotensin II (forming a negative feedback mechanism)[7]
- Atrial natriuretic peptide and antidiuretic hormone (ADH) [8]
- β–adrenergic blockade[5]

The renin gene lies on chromosome 1 and many stimuli (hyponatraemia, reduced circulating volume, sympathetic tone and reduced perfusion pressure due to renal artery stenosis) stimulate both renin gene expression and release of stored renin.[2,3,5,9]

Some of the inactive precursor, prorenin is also released into the circulation - in fact, 80% of circulating renin is in this inactive form, the significance of which is debated.[10] While activation within the plasma seems unlikely,[10] it is possible that tissue uptake and local conversion to renin occurs. The majority of prorenin uptake is by the kidneys, but some uptake occurs in the human heart,[11] raising the possibility of local cardiac formation of renin from its inactive precursor. This transport of renin in the plasma in its inactive form would be an ideal way of delivering renin to the tissues without affecting

cardiovascular homeostasis. This would support a local RAS that lacked the capacity to produce renin but retained all other components.

The persistence of prorenin in the plasma of nephrectomised rats (which resulted in the removal of active renin) has been suggested as evidence for sequestration in peripheral tissues or non-renal synthesis of this form.[10,12] However, it is also possible that the removal of the kidneys removed the main organ of uptake, resulting in the elevated levels. There is also evidence for another circulating renin-like substance other than pro-renin: in nephrectomised rats, a specific renin inhibitor blocked only 20% of the plasma angiotensin I-generating capacity, while a broad sulphydryl enzyme inhibitor blocked up to 90%.[13]

The substrate for renin is angiotensinogen, an alpha-2 globulin synthesised in the liver, which is converted to angiotensin I.

1.2.2.2 Angiotensinogen

The hepatically-derived circulating alpha-2 globulin, angiotensinogen, is cleaved by renin at the first Leu-Leu bond, yielding the inactive decapeptide Angiotensin I (figure 1.2). Angiotensin II exerts negative feedback on angiotensinogen synthesis in the endocrine RAS,[14] in part through inactivation of an unidentified factor stimulating angiotensinogen synthesis.[15] The human angiotensinogen gene is found on chromosome 1 and polymorphisms have been identified, one of which (the mis-sense mutation with methionine⇒threonine substitution at codon 235: M235T) has been associated with human hypertension and coronary artery disease.[16]

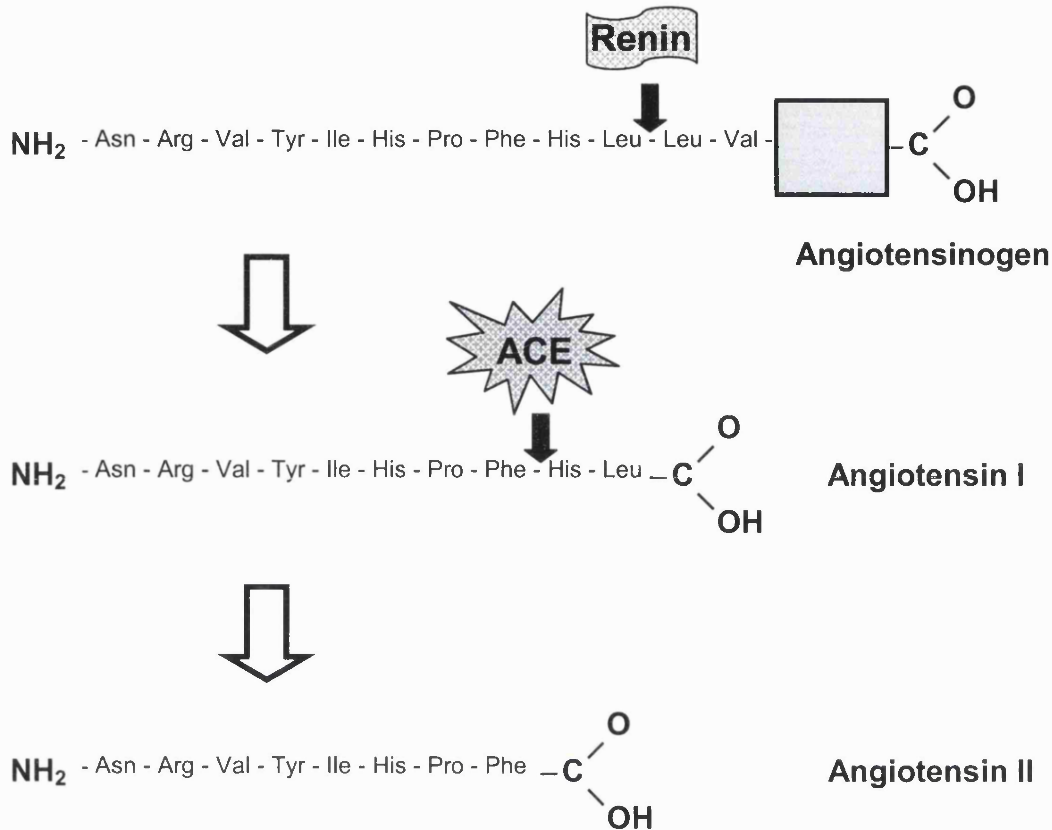
1.2.2.3 Angiotensin 1-converting enzyme (ACE)

This zinc metallo-proteinase catalyses the conversion of angiotensin I (ATI) to angiotensin II (ATII). The his-phe dipeptide at the carboxy terminal end of the inactive decapeptide ATI is removed by hydrolytic cleavage to form the active octapeptide ATII (Figure 1.2). ACE also inactivates bradykinin by two sequential dipeptide hydrolytic steps and is thus additionally known as kininase II.

These two actions are complimentary – the generation of a potent vasoconstrictor (ATII) while inactivating a potent vasodilator (bradykinin) results in the powerful stimulation of increased vascular tone and thus blood pressure, improving renal perfusion.

Figure 1.2

The structures and conversion of angiotensinogen to angiotensin II



The ACE gene is highly expressed in vascular endothelium[17] and 80% of circulating angiotensin I to angiotensin II conversion occurs in the extensive pulmonary capillary network. ACE exists in two forms:

i) Membrane bound - ACE is an integral membrane protein with the C-terminus protruding intracellularly, and the molecule being anchored by a transmembrane hydrophobic component.

ii) Soluble circulating ACE specifically synthesised in some animal cell lines,[18] and also derived partly from pulmonary capillaries.

1.2.2.4 Angiotensin II

ACE acts on ATI to produce the octapeptide ATII, the primary identified biological effector generated by the circulating RAS. Its pressor actions are mediated directly through vasoconstriction, and indirectly through renal salt and water retention (reviewed by Dzau[19]):

- ATII causes preferential efferent glomerular arteriole vasoconstriction, raising glomerular pressure. The increased filtration fraction is offset by higher renal vascular resistance and fall in renal blood flow with a net modest reduction in glomerular filtration rate and a reduction in water clearance.
- Renal sodium retention occurs in the proximal convoluted tubule, mediated by AT_1 receptors.[20]
- Aldosterone synthesis and release occurs through AT_1 receptor activation. This mineralocorticoid hormone is itself a vasoconstrictor causing renal sodium and water retention.
- Paracrine or circulatory ATII actions on the central nervous system increase thirst and salt appetite, and release anti-diuretic hormone.[21]

ATII breakdown products may also have some biological activity, either through AT_1 receptors or others.[22] Angiotensin 1-7 causes neuronal excitation and stimulates ADH release.[23] Angiotensin III (angiotensin 2-8) is a modest vasoconstrictor with affinity for AT_1 and AT_2 receptors.[24] Angiotensin IV (angiotensin 3-8) may have effects on cardiac fibroblastsand endothelial cells via the AT4 receptor subtype.[25,26]

1.2.2.5 Angiotensin II receptors

For many years, angiotensin was thought to act via a single receptor, responsible for all its actions, as no alternative receptor was found. The discovery in 1989 of two subtypes[27] changed this, and angiotensin II receptors are now classified into two main types - type 1 (AT_1) and type 2 (AT_2), defined by the use of subtype-selective antagonists[22] losartan (AT_1) and PD123177 (AT_2), as reviewed by Unger.[28] The AT_1 receptor is highly expressed in vessel walls and transduces the majority of the systemic effects of angiotensin II. It belongs to the family of seven transmembrane domain receptors, coupled to G-proteins, with secondary messenger activity mediated via several mechanisms:

i) Inhibition of adenylate cyclase causing reduced cyclic AMP levels (proximal convoluted tubule).

ii) Phospholipase C activation and the subsequent generation of diacylglycerol, causing protein kinase C activation[29] (hepatocytes and smooth muscle).

iii) Activation of mitogen activated protein (MAP) kinases.

The AT_2 receptor shares 34% homology and a seven-transmembrane domain with the AT_1 receptor,[30] though the functional significance of the AT_2 receptor is still unclear. It is present in adult tissues, but expressed maximally in embryonic tissues,[29] and is more abundant at times of maximal vascular growth,[31] suggesting a role in growth and development.[22,30]

Two other receptors have been suggested – AT_3 in cultured neuroblastoma cells[32] and AT_4 in vascular endothelial cells, specific for angiotensin 3-8 (angiotensin IV).[26] Their existence and functional role has not yet been clarified.

1.3 LOCAL RENIN-ANGIOTENSIN SYSTEMS

There is considerable evidence for local renin-angiotensin systems within several tissues, which act in a paracrine or autocrine fashion. Both localised production of several components of the RAS and feedback response to angiotensin II are present in a diverse variety of tissues:

- Both renin and angiotensinogen and their mRNA are found in the brain, kidneys, adrenal glands, liver, endothelium and heart.[21,33-36]

- Angiotensin I to angiotensin II conversion occurs in these tissues[37-40] and is blocked by ACE inhibition, with corresponding decreases in the actions of angiotensin II.[37,41-44] Local ACE gene transcription and expression also occur.[17,23,45]

- Angiotensin II receptors exist on many cell surfaces and result in local physiological changes which are blocked by angiotensin II receptor antagonists.[22,46-49]

- Differential regulation of the local RAS occurs in different tissues in the renal hypertensive rat.[50] Cardiac angiotensin II receptors are up-regulated in response to renovascular hypertension and contribute to cardiac hypertrophy.[51]

- Nuclear angiotensin II receptors occur and stimulation can result in negative feedback of angiotensin II on its own synthesis.[52]

1.3.1 CARDIAC RENIN-ANGIOTENSIN SYSTEM

Both angiotensin I and II are found in isolated cardiac tissue[42,53] and the isolated perfused rat heart is capable of their generation.[54] Virtually all components of the RAS can be synthesised in cardiac tissue, though some utilisation of plasma components, particularly renin, may occur. A good review, with emphasis on *human* cardiac tissue, can be found in Urata et al.[31]

1.3.1.1 Renin

Renin mRNA is detectable in cardiac tissue,[33,36,55] and is markedly elevated in response to sodium depletion in the heart and kidney (but not in submaxillary glands or testes), suggesting tissue-specific regulation.[33] Cardiac tissue demonstrates renin-like activity[56] and renin protein is detectable in isolated cardiomyocytes.[57] Renin mRNA in the left ventricle is also upregulated in response to volume overload.[58] However, other studies were unable to demonstrate the presence of renin mRNA[35,59] and Lindpaintner showed that in the isolated heart, levels of angiotensin I in the coronary sinus were undetectable until exogenous renin was infused,[54] suggesting the importance of circulating plasma renin, irrespective of any cardiac synthesis. It seems likely that at least a proportion of cardiac renin is plasma-derived, and the relative contribution of circulating and locally produced renin may differ between species, with porcine hearts having little evidence for local production.[60]

1.3.1.2 Angiotensinogen

Cardiac gene expression of angiotensinogen mRNA has been demonstrated in rodents[54,61] and humans.[62] Levels are elevated in pressure-overload cardiac hypertrophy[63] as well as in peri-infarct tissue after myocardial infarction,[64] suggesting a role for the cardiac RAS in myocardial growth. The system is also responsive to external stimuli: glucocorticoids (another growth stimulus), which are known to induce hepatic angiotensinogen expression, can increase cardiac angiotensinogen mRNA levels,[54] as can dietary sodium restriction.[65] In the isolated rat heart, angiotensinogen exhibited a continued low-level release after the initial peak 'washout' of circulating protein.[54] The increase in magnitude of this low-level release with glucocorticoid infusion is in favour of cardiac synthesis. Lastly, the generation of angiotensin I in isolated rat myocytes and fibroblasts also suggests the local synthesis of angiotensinogen substrate.[53]

1.3.1.3 Angiotensin converting enzyme

Physiologically-responsive local cardiac ACE synthesis has been demonstrated. Cultured rat myocytes can synthesise angiotensin II[53] and isolated perfused rat hearts can convert angiotensin I to angiotensin II,[54,66] suggesting the presence of an angiotensin I-converting enzyme. This conversion of angiotensin I is prevented by ACE inhibitors,[42,67] which also block the inotropic and vasoconstrictor actions of infused angiotensin I but not angiotensin II in this model.[42] The presence of active ACE within myocardial cells has subsequently been shown,[53,67,68] with higher levels in hypertrophied hearts[67] and ACE activity is also detectable in human myocardium.[69] ACE levels vary in different regions of the heart, both in humans[69] and rats,[70] with the right ventricle having levels approximately two-fold higher than the left ventricle and levels in the atria three-fold higher than the ventricles, the greatest concentration occurring along the endocardial border of the cardiac chambers and in the coronary arteries. There was also high activity in all four cardiac valves.

1.3.1.4 Non-ACE pathways for angiotensin-I conversion

In the rat heart, most of the basal angiotensin I to angiotensin II conversion is mediated by ACE.[54,67] Its activity is increased in the hypertrophied rat heart[67,71] and 70% of this can be inhibited by enalaprilat, the active metabolite of enalapril. However, this still leaves 30% of cardiac angiotensin I conversion which is mediated by an enzyme other than ACE. ACE-independent angiotensin II formation is involved in the development of volume-overload cardiac hypertrophy,[72] and in another rat model, enalapril prevented the excess LV growth induced by minoxidil but the LV mass was still higher than when enalapril was given alone (without minoxidil).[73] This suggests the stimulation by minoxidil of other LV growth factors which are not inhibited by an ACE inhibitor. In the canine heart, angiotensin II generation does not appear to be ACE-dependent, but is inhibited by serine proteases (e.g. aprotinin) and chymostatin, suggesting the involvement of other non-ACE enzymes.[74,75]

In humans, only a minority of cardiac angiotensin I conversion may be mediated by ACE. Urata found that captopril reduced angiotensin II formation in both normal and diseased human hearts by only 10%, compared to 80% reduction by a serine protease inhibitor.[69] He went on to identify a 30kD glycoprotein chymase, termed human heart chymase, with high efficiency for angiotensin I to angiotensin II conversion.[76] It is highly specific for angiotensin I and was completely inhibited by serine protease inhibitors. Chymases are chymotrypsin-like serine proteases found in the secretory granules of

mast cells and endothelial cells. They are found in a wide variety of human tissues, including skin, oesophagus, stomach and uterus.[77] Human heart chymase is mostly localised to the interstitial region of the myocardium,[78,79] with some additional localisation in mast cells, endothelial and mesenchymal cells.[79] Contrary to cardiac ACE, human chymase activity is greater in the ventricles than the atria.[79,80] This, and the differing micro-localisation of the two enzymes may suggest distinct physiological roles.

Other non-ACE angiotensin I converting enzymes may also exist. Chymases are not inhibited by aprotinin but this serine protease prevented the production of angiotensin II in a canine cardiac model in which ACE inhibitors had little effect on angiotensin II levels.[74] This suggests a non-ACE, non-chymase pathway of angiotensin II generation.

1.3.1.5 Angiotensin II receptors

Angiotensin II receptors have been identified in a number of mammalian hearts,[81-83] including human,[49,84] and also in cultured myocytes.[85] Their stimulation induces positive inotropic effects[86,87] as well as having a role in protein synthesis,[88,89] cardiac fibrosis[90,91] and cellular growth (see below). Although some of the effect of angiotensin II may be mediated via cardiac sympathetic nerves,[92,93] a large component of the positive inotropic effect is direct, occurring in denervated hearts[86] and in the presence of β–adrenoreceptor blockade.[83,87] The presence and function of human angiotensin II receptors appears to be similar.[84,87,94,95] The receptors are more abundant in the atria than the ventricles,[49,84] particularly around the sino-atrial node, and are also present in the extra-cardiac adrenergic nerves.[84]

The two subtypes of angiotensin II receptors – AT_1 and AT_2 – are both present in cardiac tissue. In the adult rat, they are present in equal amounts throughout the heart[96] though in human atria, the AT_2 receptor is twice as abundant.[97] The AT_1 subtype, however, transduces the majority of the known effects of angiotensin II – positive inotropic response, coronary vasoconstriction and cardiac hypertrophy.[22] In rat and mouse hearts, the AT_1 subtype is further classified into AT_{1a} and AT_{1b},[51] with close sequence homology and functional characteristics,[98] though no such sub-division has been identified in humans. At the time of commencing this thesis, the function of the AT_2 subtype was not determined, though it appeared to have a role in cardiac dysfunction, with upregulation of AT_2 and downregulation of AT_1 subtypes in failing hearts.[49,97]

1.3.2 INTEGRATION OF THE CIRCULATING AND LOCAL RAS

While it is clear that both circulating and local systems exist, they have different functions, and the integration of the components may differ according to tissue and pathology. One might speculate that restriction of supply, selective uptake, or selective degradation of RAS components derived from the circulation might allow tissues to control local concentrations of such components and use them in a paracrine fashion. Such interaction might account for the finding that renin-inhibitory peptide infused into dogs causes a hypotensive response, although blood pressure continues to fall long after plasma renin activity is maximally suppressed.[19] Dzau postulates that the two systems, at least in the cardiovascular system, play two independent roles.[19] The short-term regulation of cardiovascular homeostasis is carried out by the endocrine system. Tonic control is due to local systems which regulate thirst and salt appetite (brain), sodium handling (kidney), vascular tone (vascular walls), and cardiac contractility and function (heart). This hypothesis is supported by evidence from the study of acute and chronic systolic cardiac failure. In chronic compensated human cardiac failure (as in renovascular hypertension), plasma renin activity returns to a normal level after an initial rise. Tissue RAS activity remains elevated, however.[99-102] The acute hypotensive response to ACE-inhibition correlates well with the initial plasma renin activity, but this does not remain the case with chronic treatment. ACE-inhibition may also lower blood pressure in patients with low circulating renin levels.[19]

While the liver and kidneys may be able to take up prorenin and convert it to renin,[103] in the cardiac RAS, it seems that renin is primarily derived from the circulation. There is much evidence for cardiac ACE production however (above) and this is supported by the very different chamber distribution of angiotensins I and II and the 3-fold greater angiotensin I to angiotensin II ratio in cardiac tissue relative to plasma,[104] both suggesting against the passive sequestration of plasma ACE in cardiac tissue.

The diffuse presence of the RAS in a variety of tissues might infer a common tissue role. RAS gene expression is high in situations of vascular injury or growth (such as occur in atherosclerosis, hypertension, or endothelial denudation), suggesting that the local RAS may have a growth-promoting function.[105,106]

1.4 THE RAS AND CARDIOVASCULAR GROWTH

1.4.1 VASCULAR GROWTH

The expression of RAS genes and increased RAS activity within vascular tissue is modulated by physiological stimuli, and in particular by stimuli which lead to tissue growth responses, such as hypertension[48] and vascular injury.[107] Known growth factors such as glucocorticoids and fibroblast growth factor can also increase ACE mRNA expression in vascular smooth muscle cells.[108,109]

In cultured rat aortic cells, angiotensin II can directly stimulate protein synthesis,[88] including α–actin gene expression,[89] and induce cell proliferation[110,111] and hypertrophy.[112] These effects were prevented by the AT_1 receptor blocker, losartan.[111]

Finally, growth responses (e.g. intimal hyperplasia following vascular injury) in animal models can be limited by the use of ACE-inhibitors at doses which suppress tissue ACE activity whilst having no effect on circulating ACE activity or blood pressure.[113,114] The same may be true of angiotensin II receptor blockers.[115,116]

1.4.2 CARDIAC GROWTH

Evidence for involvement of the RAS in cardiac growth comes from a number of sources:

1.4.2.1 Association of LV mass with circulating components of the RAS

In Schunkert's study of 615 normal subjects, serum concentrations of ACE were independently associated with increased LV mass in both univariate and multivariate analyses.[117] The same was also true of serum aldosterone levels, although the association was less strong in men.

1.4.2.2 Increased RAS activity in response to growth stimuli

Cardiac ACE and angiotensinogen gene expression increase during LV hypertrophy, particularly in pressure-overload models,[63,68,71] and angiotensin II receptor numbers increase.[49,51] Other models of growth stimuli have also shown involvement of the RAS. Plasma and cardiac renin activity are increased in minoxidil-induced[73] and volume-overload cardiac hypertrophy,[58,72] in which increased levels of angiotensin II have also

been shown, both in plasma and myocardium.[118] In acute myocardial infarction, the cardiac RAS is upregulated in the peri-infarct tissue undergoing significant growth, with increased expression of angiotensinogen mRNA[64] and ACE activity.[119] There is some evidence that the degree of activation correlates with the size of the infarct, with the largest infarcts having persistent elevation of angiotensinogen mRNA.[64] Finally, levels of the effector hormone, angiotensin II, are increased, both in the plasma and cardiac tissue, in response to volume overload.[118]

1.4.2.3 Direct stimulation of cardiac growth by angiotensin II

The stretch-mediated hypertrophy of *in vitro* cardiomyocytes is angiotensin II mediated[120] and angiotensin II stimulates protein synthesis in isolated rat hearts, the effect being abolished with losartan.[121] Angiotensin II can induce both cardiac and aortic hypertrophy *in vivo* in a mechanism unrelated to its effect on blood pressure.[122] Finally, sub-pressor doses of a chronic (1 week) angiotensin II infusion caused LV hypertrophy without any blood pressure changes.[123] Further increases in LV mass were observed at pressor doses, though normalisation of the blood pressure with hydralazine did not affect the increased LV mass, suggesting that the angiotensin II effect is via non-pressor mechanisms.

1.4.2.4 The inhibition of cardiac growth by RAS antagonists

ACE inhibitors have been shown to prevent the LV hypertrophy in several models of cardiac growth – in pressure-overload,[63] volume-overload[118] and ventricular remodelling post myocardial infarction.[119,124] They can also reduce the interstitial fibrosis in the latter (post MI) model.[119] Physiological cardiac hypertrophy in the porcine heart is impaired by RAS antagonists, particularly angiotensin II receptor blockers.[125]

Angiotensin II receptor blockers significantly suppress the genetic upregulation of growth factors post myocardial infarction[126] and are also effective at preventing or reducing LV growth in several models.[73,119,126,127] There is some evidence that they are better than ACE inhibitors in this task: losartan was better than enalapril at attenuating volume-overload and minoxidil-induced hypertrophy.[72,73] However, the effects seem to be comparable in the myocardial infarction studies.[119,124]

The data suggests that the majority of this effect is mediated via the cardiac rather than the systemic RAS.[128] In the myocardial infarction model, both an ACE inhibitor and AT_1 blocker reduced cardiac angiotensin II levels, with associated reductions in cardiac

growth, with no reduction or even an increase (in the case of the AT_1 blocker) in plasma levels.[124] Losartan prevented minoxidil-induced LV hypertrophy without changes in cardiac haemodynamics[73] and in the volume-overload model, regression of LV hypertrophy with ACE inhibitors only occurred in the presence of decreased cardiac angiotensin II levels.[118] Enalapril decreased LV mass with no effect on blood pressure (either carotid or tail) in aortic banding.[63] In a similar model, ramipril significantly reduced LV mass while equivalent antihypertensive doses of nifedipine and dihydralazine had no effect.[128] This LV regression also occurred with non-hypotensive doses of ramipril.[128] Lastly, the upregulation of secondary cellular messengers important for growth by *in vitro* stretching of cardiomyocytes was diminished by candesartan, again suggesting local cardiac RAS effects.[127]

1.4.3 THE CARDIAC RAS AND HUMAN LV GROWTH

The difficulties in obtaining left ventricular tissue from normal individuals impedes examination of the importance of human cardiac renin-angiotensin systems to cardiac growth. Nevertheless, several studies have provided data which suggest their importance.

In Dahlof's meta-analysis of 109 anti-hypertensive trials with nearly 2400 patients,[129] ACE inhibitors were associated with a much larger regression in LV mass (45g) than β-blockers (23g), calcium antagonists (27g) and diuretics (21g), despite the same reduction in mean arterial pressure. The degree of regression of LV mass with ACE inhibitors was not related to the change in blood pressure. This has been confirmed in a subsequent meta-analysis.[130]

The HYCAR study[131] demonstrated the effect of a 'low' (non-hypotensive) dose of ramipril in reducing LV mass in hypertensive individuals with LVH. Three groups were compared: placebo, 1.25mg and 5mg of ramipril for 6 months, and both the 1.25mg and 5mg doses were effective in reducing LV mass (-13g and –20g respectively), though the 5mg dose was more effective. Interestingly, all three groups had the same reduction in blood pressure (between 7.6 and 11.8 mmHg), suggesting perhaps that the conventional 5mg dose of ramipril was not effective at reducing blood pressure, or that some other factor (such as increased awareness of cardiovascular health and greater levels of exercise during the study) had a larger influence on blood pressure. In either case, multiple regression analysis showed the change in LV mass to be dependent on treatment with either dose of ramipril, and not on the change in blood pressure.

The effect of ACE inhibitors on reducing ventricular remodelling (LV growth) in patients with left ventricular dysfunction or post-myocardial infarction is well established. In the SOLVD study (Studies of LV Dysfunction), enalapril completely abolished the LV growth with no changes in ejection fraction and no blood pressure changes in the symptomatic group.[132] The prevention (asymptomatic) group had a reduction in systolic and diastolic blood pressure with enalapril, though interestingly, this was only evident at 12 months, but not 4 months into the study, suggesting a long term effect on blood pressure via reduced aortic and other vascular endothelial growth. Several huge trials have examined the beneficial effect of ACE inhibitors post myocardial infarction,[133-136] These have shown a reduction in LV remodelling with ACE inhibitors,[137-140] associated with an improvement in survival and cardiac haemodynamics.

Lastly, recent evidence in humans following myocardial infarction showed increased ACE activity in the viable peri-infarct tissue,[141] suggesting an important role in ventricular remodelling. The increased activity occurred both in myocytes and other surrounding cells (fibroblasts, macrophages, endothelial cells & vascular smooth muscle cells).

There are difficulties in investigating further the role of the cardiac RAS in human LV growth, due to the inability to obtain left ventricular tissue routinely. However, the discovery of a functional polymorphism in the human ACE gene has opened new routes for research.

1.4.4 THE HUMAN ACE GENE INSERTION/DELETION POLYMORPHISM

Large inter-individual differences in plasma ACE levels exist, but levels are similar within families,[142] suggesting a strong genetic influence in the control of ACE levels. The human ACE gene is found on chromosome 17q23[143] and in 1990, Rigat described a restriction fragment length polymorphism of the gene consisting of the presence (insertion 'I' allele) or absence (deletion 'D' allele) of a 287 base pair alu repeat sequence[144] in intron 16.[145] D allele frequency is approximately 0.57-0.59 in western populations[143,146] and is 0.51 in a healthy male British population.[147]

Serum ACE levels were 65-140% higher in those with DD genotype (compared to II),[144,148,149] with the polymorphism accounting for 47% of the variance in plasma ACE, though considerable overlap existed between groups.[144] Local ACE activity was also increased in T-lymphocytes (39% higher in the DD genotype),[148] and there was a suggestion of increased activity in human internal mammary arteries.[150] Lastly, and

most importantly, Danser has shown higher ACE activity in human myocardium in DD relative to ID and II genotypes (39% vs II; 46% vs ID).[151] These data suggest an influence of the I/D polymorphism on tissue and plasma ACE activity, with DD>ID>II genotypes. Any phenotype critically-regulated by a tissue RAS may be more prominent within a population amongst those of DD genotype if tissue ACE levels are the rate limiting step in the tissue RAS. The mechanism by which the polymorphism influences ACE levels is unknown. If simply an intron polymorphism, it may mark another polymorphism elsewhere in the gene, such as in the promoter region where it might influence transcription kinetics. However, part of the polymorphism might be translated and incorporated into mRNA, altering mRNA stability or splicing, or ACE protein stability.[144]

Many physiological stimuli cause induction of tissue RAS (including ACE) gene expression, as discussed above. Prospective studies of the polymorphism's influence on the phenotypic response to a physiological stimulus might therefore allow examination of the important rate-limiting step in the tissue RAS. The use of RAS antagonists might further enhance the identification of the important factors in the control of physiological responses by tissue RAS's. This concept has been utilised in the studies described later in this thesis.

1.4.4.1 The ACE I/D polymorphism and LV hypertrophy

If a cardiac RAS controls left ventricular growth in humans, then we might expect those with higher ventricular ACE levels to exhibit greater cardiac growth responses, and for such growth consequently to be associated with the D allele. There is evidence of an association of the D allele with vascular growth, in the form of coronary atherosclerosis,[152,153] though this association was not seen in a large prospective study.[154] Studies of the association of ACE genotype with LV mass have also shown an association of LV hypertrophy with the D allele,[155-158] though again, one large study suggested that there was no such association,[159] and these results thus appear to conflict. There are, though, several reasons why this might be so, and which make the data from such studies unreliable. All of these studies have been retrospective or cross-sectional in design, examining LV mass at a single time-point. LV mass is a continuous variable, under the influence of a number of environmental and biological effects (e.g. exercise, age, sex, blood pressure burden, race),[160-162] and any genetic effect on LV mass within a population might be obscured by the uncontrolled and unquantifiable influence of such factors. To study the effect of the ACE gene on a phenotypic response (LVH) requires a 'levelling of the playing field' with removal of the

other known influences on LV mass. This is hard to achieve in practice but is vital for comparing the differences in what may be a modest mediator rather than the most crucial factor regulating LV growth.

Furthermore, the ACE gene, if associated, is likely to be a modulator of the LV growth response to an external stimulus rather than a cause of LV hypertrophy in itself – a gene-environment interaction. A larger stimulus would result in greater LV growth and for this reason, it is important that the stimulus is the same when comparing different genetic groups. In retrospective analyses, we have no way of measuring the hypertrophic burden on the left ventricle that has occurred over the preceding decades. It is an inherent assumption that with a large group, the average burden is similar between groups but there may be so much 'white noise' that any genetic effect is hidden. A large, heterogeneous, group of subjects, while good for statistical power because of the size, would also involve widely differing environmental stimuli. This is compounded by the excess mortality associated with LV hypertrophy, and possibly with the D allele,[152,153,163] both of which would result in a selection bias in retrospective analyses.

A good example of the importance of surrounding influences is found in a study examining the relationship of the ACE gene with the degree of LVH in patients with hypertrophic cardiomyopathy,[164] a genetic disease with a strong LV growth stimulus, albeit a genetic one in this case. This showed an association of the D allele with increasing LVH, but the strongest effect occurred in patients from the same family, in which other genetic and environmental influences on LVH would be likely to be similar.

A second consequence of the ACE gene being a mediator rather than a cause in itself is that a growth stimulus needs to exist in the first place. Without one, there may be no difference between genetic groups, as witnessed by studies in which there was no association of the ACE gene I/D polymorphism with LV mass in normal populations, without hypertension or coronary disease.[165]

To evaluate a gene-environment interaction such as this, prospective studies looking at LV growth in a homogenous group need to be undertaken.

1.4.4.2 Prospective studies of LV growth

Two studies of LV remodelling post-myocardial infarction showed greater levels of growth in those with the D allele,[166,167] which was prevented with captopril,[166] providing

good confirmatory evidence that the I/D polymorphism plays an important role in cardiac growth.

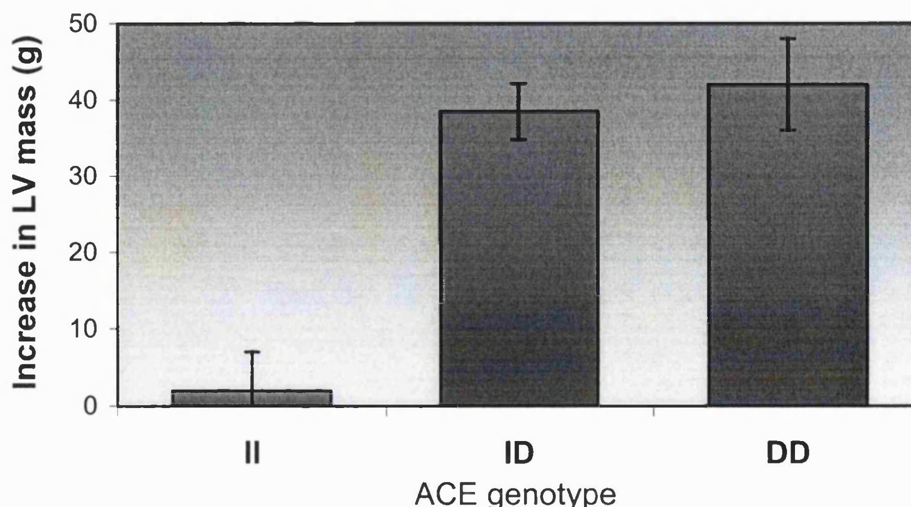
Further prospective studies of pathological left ventricular hypertrophy (such as sustained untreated hypertension) would require a very long period of follow up and be deemed unethical if such hypertension were to be left untreated. The application of pharmacological agents (such as pressor agents or thyroxine) as a short-term prospective model of LV hypertrophy would be ethically unacceptable. Physical training, however, increases left ventricular mass,[162,168] through both an increase in wall thickness and end-diastolic diameter,[169] and is ethically acceptable.

The association of the ACE gene I/D polymorphism and exercise-induced LV growth has been examined in a previous study by Montgomery.[170] One hundred and forty UK military recruits were examined during their basic military training, with left ventricular mass measured by echocardiography at the start and end of the training period. LV mass increased overall by 18% and this response was strongly correlated with ACE genotype: LV mass rose by 2g, 38g, and 42g for the II, ID and DD genotypes respectively (figure 1.3). The data suggests a strong influence of ACE genotype on left ventricular growth, though needs to be confirmed, particularly as the reproducibility of echocardiography is poor, and this is important for serial measurements of LV mass.

<u>Figure 1.3</u>

Physical training in military recruits: increase in LV mass after 10 weeks, by ACE genotype. (data from Montgomery et al[170])

Values are mean ±SEM; Overall $p<0.0001$



1.4.5 OTHER RAS GENE POLYMORPHISMS AND LV GROWTH

Two other RAS gene polymorphisms had been studied at the time of commencing this research :

- The angiotensinogen gene M235T polymorphism (single substitution of threonine for methionine at position 235). This has been associated with raised blood pressure[171,172] though with minimal or no increased plasma angiotensinogen levels.[149,171] Iwai's study suggested an association of the TT genotype with increased LV mass, probably due to effects on blood pressure, but there was no such association in another population cohort[149] or in a study of prospective growth post-myocardial infarction.[167]

- The angiotensin AT_1 receptor polymorphism A1166C (cytosine for adenine at position 1166). There was no association with LV mass in the one study that existed,[165] although this suffered from the same limitations as before, being a cross-sectional study in normal individuals without a stimulus for LV hypertrophy. There may be other effects of this polymorphism than on LV mass, with one study suggesting a synergistic effect with the ACE gene on the risk of myocardial infarction.[173]

1.4.6 RAS-MEDIATED LV GROWTH : MECHANISMS & RATE-LIMITING STEPS

Although the sections above summarise the strong evidence for RAS involvement in cardiac hypertrophy, questions remain about the contributing roles of the various components of the system:

1.4.6.1 Systemic versus local cardiac RAS

There is debate over which has the strongest influence on cardiac growth. The evidence points to the local cardiac system – the reduction of LV mass post-MI is associated with changes in cardiac but not plasma RAS,[124] and ACE inhibitors decrease LV mass at non-hypotensive doses.[63,73,131] There may well be interactions however. Hypertension - a very powerful stimulus for LV growth – may be mediated by the systemic RAS, though the mechanism by which the raised blood pressure induces LV growth may involve up-regulation of the local cardiac RAS.[71]

The rate-limiting steps in each RAS may also differ – in the systemic RAS, it does not appear that ACE is the limiting factor – rather, renin may be the key component. In cardiac tissue however, the situation may differ - could ACE be the limiting factor? This may also vary between species: in rat hearts, there is good evidence for the importance of ACE, though the much lower mRNA levels of renin compared to angiotensinogen[33] imply that renin may be the limiting factor in rodents. Does human heart chymase[76] play a significant role in humans? And what of the non-ACE, non-chymase pathway in dogs?

1.4.6.2 Angiotensin II or kinin dependant?

Most attention has been focussed on angiotensin II as the effector protein in LV hypertrophy. Only losartan attenuated the LV hypertrophy in a volume overload model (no effect of enalapril), suggesting the importance of angiotensin II and the possibility of non-ACE conversion of angiotensin I.[72] Kinin inhibitors, however, appeared to be better at abolishing the effects of ramipril in preventing the development of LV hypertrophy while the effect of ramipril on regression of established LVH was not affected.[128]

There are other potential pathways - aldosterone is often overlooked as a potential effector protein and is a component of the renin-angiotensin-aldosterone system. Its production is stimulated by angiotensin II and there is evidence for its effect on LV hypertrophy.[174]

CHAPTER 2

General methods

2.1 GENOTYPING THE ACE I/D POLYMORPHISM

2.1.1 SAMPLE ACQUISITION

Cell samples from subjects were obtained using a mouth-rinse technique. A small amount (5 ml) of 0.9% saline is taken into the mouth and swirled around before being replaced in a sterile specimen container. Many buccal cells from the outer (dead) layer of the epithelium, containing the subject's DNA, are washed into the solution. Trials in the laboratory suggested that a 'swish' time of 30 seconds in the mouth was sufficient to obtain enough cells for reliable DNA extraction and amplification.

2.1.1.1 Advantages of the technique

The technique is simple, requiring no special training, and removes the need for venepuncture. The acceptability to participants is increased and uptake is far greater. This is important for large population screening, both for potential trial subjects and for other groups in whom genotyping is the only study performed. It has the particular advantage of allowing collection of samples by the subjects themselves at a distance from a research centre without medical supervision. This enables the postal collection of samples (see below) and also sampling at an army barracks with a military (non-medical) officer supervising.

The method is also fast, both for collection, where many samples can be obtained at the same time, and for DNA extraction (see below) in which the protocol is simplified as the need to extract haem from the sample is removed.

2.1.1.2 Disadvantages

Obtaining sufficient DNA for amplification is less reliable than extraction using peripheral blood leucocytes (the standard method). This is mostly due to fewer cells being obtained, often from individual differences in the vigour with which subjects swirl the saline around, and this resulted in a ten-fold difference in the quantity of DNA obtained between the best and worst samples. Despite this, in laboratory testing over 95% of samples were successfully genotyped.

Eating or drinking prior to sampling can dramatically reduce the number of cells obtained as the dead epithelial cells are removed by this. Chewing gum is particularly

efficient at removing any chance of DNA extraction. To overcome this, subjects were always sampled first thing in the morning, before breakfast or tooth-brushing.

Bacterial, and occasionally food, contamination of the sample is inevitable though of variable amounts, and the DNA from these would also be extracted. While it seems unlikely, though unproven, that bacteria contain ACE (and thus an ACE gene), most animal species do, which has a potential confounding effect. However, these studies examined the I/D polymorphism of the ACE gene. This has only been identified in humans and the primers for this are specific to the polymorphism. The PCR amplification technique results in amounts of replicated DNA many orders of magnitude higher than any background DNA which renders the problem of contamination negligible in practice.

Freezing the samples results in lysis of the cells on thawing, and the loss of the DNA. The samples thus need to be processed within a few days and kept refrigerated (but not frozen) until then to reduce bacterial overgrowth.

2.1.2 DNA EXTRACTION

This was performed using a technique developed in the Centre for Cardiovascular Genetics at UCL, employing a modified version of a whole blood extraction technique. It was designed to produce high yields of high-molecular weight DNA in a short time. The technique was refined to enable large numbers of samples to be processed together.

2.1.2.1 Method

The specimen containers were centrifuged for 1 minute (3,000 rpm), and the supernatant discarded, leaving a pellet of buccal cells. To this was added 500μl of lysis buffer (ingredients below), incorporating both cell membrane and nuclear lysis in the same step; the sample was agitated and transferred to a 1.5ml polypropylene microfuge tube (Epindorf). 150μl of 5M sodium perchlorate solution was added along with 500μl chloroform and the mixture was agitated vigorously. This was then centrifuged for 1 minute (10,000 rpm) and the supernatant, containing DNA in solution, transferred to a fresh Epindorf. The DNA was precipitated with twice the volume (1000μl) of 100% ethanol and the solution centrifuged a last time, leaving the pellet of DNA at the bottom once the supernatant had been discarded. This was re-dissolved in 1ml distilled, sterile water and the samples refrigerated at 4°C until use.

Lysis buffer solution:	400mM Tris HCl (pH 8.0)
	120 mM EDTA
	300 mM sodium chloride
	2% sodium dodecacyl sulphate

Twenty four samples were processed at one time, requiring approximately one hour for each run - a time of just over 2 minutes per sample.

2.1.3 POLYMERASE CHAIN REACTION (PCR)

The ACE I/D polymorphism was identified by polymerase chain reaction amplification (PCR) of the insertion sequence region and subsequent separation of fragments by electrophoresis.

A triple primer method was used because the original two-primer method,[146,175] with each primer flanking the insertion sequence, was prone to preferential amplification of the shorter (D) allele, resulting in some mis-typing of heterozygous genotypes as DD.[176] The third primer anneals to the insertion sequence itself, creating a shorter fragment for the I allele (65 base-pairs) compared to 84 base-pairs for the D allele (figure 2.1). There is less competitive inhibition of the longer fragment with this method, enabling accurate detection of both alleles.[153] The shorter fragment is still preferentially amplified however and there remains a small possibility that the longer fragment (D allele) may be inhibited in heterozygotes, resulting in misclassification as an II genotype. To overcome this, all II subjects had replica PCR amplification performed without the insertion-specific primer to ensure accuracy. Six subjects out of several hundred initially classified as II genotype were subsequently discovered to be of ID genotype by this technique and removed from the analyses.

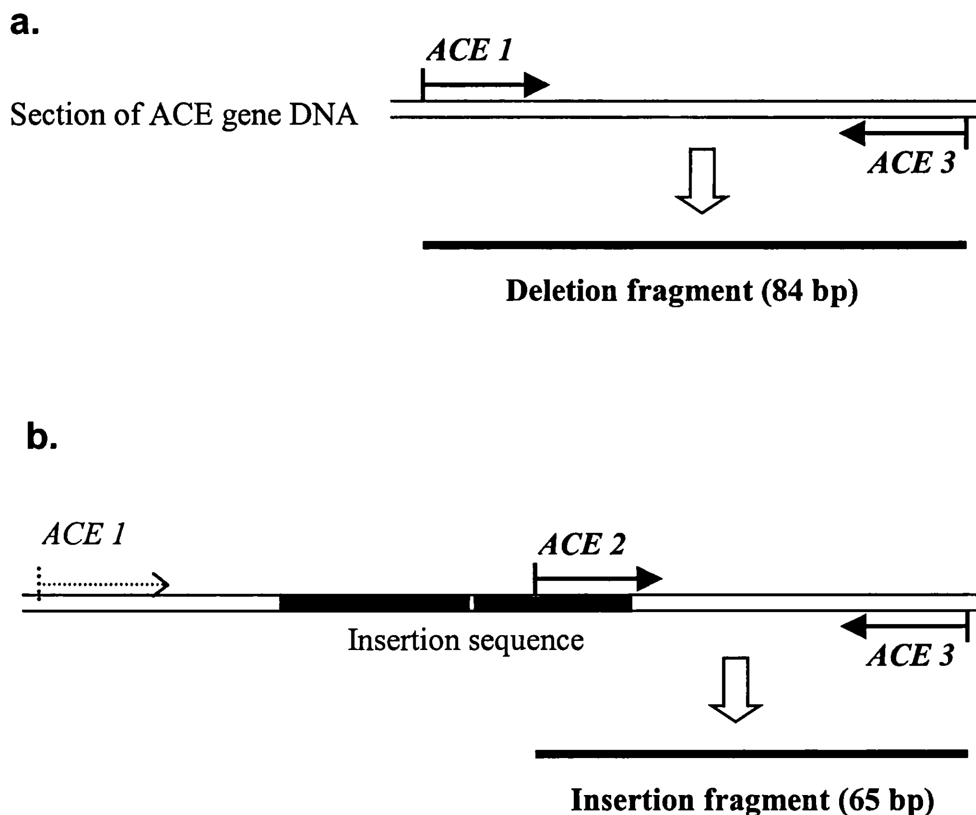
Primer ratios corresponded to 10 ρmol *ACE1* (deletion-specific oligonucleotide), 2 ρmol *ACE2* (insertion-specific oligonucleotide) and 8 ρmol *ACE3* (common oligonucleotide), in a 20μl reaction, similar to that used by Evans[153] and subsequently validated.[177] The amplification conditions were based on O'Dell's method, optimised to increase specificity and reduce time:

1 cycle	95°C 5 mins
40 cycles	95°C 1 min; 50°C 30 secs; 72°C 1 min

Each 20μl PCR reaction contained 50mM KCl, 10mM Tris HCl pH 8.3, 1.5 mM $MgCl_2$, 0.2 units Taq polymerase, 10 ρmol primer *ACE1*, 2 ρmol *ACE2* and 8 ρmol *ACE3*, overlaid with 20μl mineral oil.

Figure 2.1

Diagrammatic representation of the position of the 3 oligonucleotide primers for the ACE I/D polymorphism and the resulting amplified DNA fragments. a) Deletion allele; b) Insertion allele.



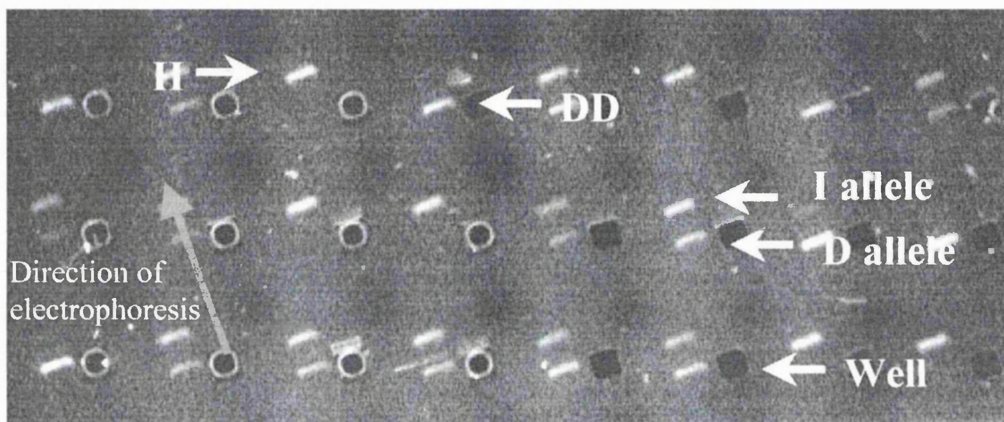
The PCR products were visualised using 7.5% polyacrylamide gel electrophoresis to separate the target alleles. To maximise throughput, microtitre array diagonal gel electrophoresis (MADGE) plates were used.[177] These are horizontal polyacrylamide gels attached to a glass support, which contain 96 wells matching the configuration of the wells on the PCR block, but arranged diagonally, allowing a longer track for electrophoresis. They facilitate easy transfer of the PCR product from the sequencing plate to the gel using a multi-channel pipette, and simultaneous electrophoresis of 96 samples. The gels were pre-stained with ethidium bromide and 5μl of PCR product was mixed with 2μl xylene cyanol/bromophenol blue loading buffer and 5μl of the resulting mixture (containing 3.57μl PCR product) loaded into the MADGE gel wells. 45 minutes

electrophoresis time was allowed and the gel viewed under ultraviolet light to identify the bands (figure 2.2).

Positive (of known genotype) and negative DNA controls were included in all analyses. This PCR method and electrophoresis has been previously validated.[177]

Figure 2.2

Example of ACE gene polymorphism PCR product on a MADGE gel, viewed under ultraviolet light with ethidium bromide staining. The deletion bands are slightly weaker than the insertion bands in heterozygotes, though still clearly identifiable.



2.1.4 POSTAL DNA SAMPLE COLLECTION

The mouthwash DNA sampling technique was utilised in order to carry out sampling of the ACE gene polymorphism in elite groups of people (see chapter 5). These individuals were either a large cohort, widely spread throughout the UK (in the case of the athletes), or smaller in number but living in inaccessible areas in a variety of locations (climbers). For these reasons, access to them was not possible without a postal service.

The Royal Mail in the UK was contacted for advice on sample handling and a package was put together containing the specimen container inside a hard plastic protective casing inside a padded envelope. This was acceptable to the Royal Mail in terms of the small infective risk to their employees. The Post Office also agreed to contribute funding towards the project.

The samples were obtained by posting the specimen containers with 5 mls of sterile saline to the recipients with simple instructions on how to swish the saline around the mouth and back into the container first thing in the morning on a day chosen by the subject (Monday to Wednesday). They were instructed to place the container back into the protective casing which was then placed into a pre-paid padded envelope addressed to our research laboratory and posted the same day. With the first class post in the UK, over 95% of the samples were received by the next day. In this way, we received the samples on a working day and could extract the DNA immediately, avoiding degradation of the sample and bacterial overgrowth. A few samples could not be processed the same day and were refrigerated overnight until processing the following day. The samples were individually numbered, corresponding to a database, such that the DNA extraction and ACE genotype identification were undertaken using only this number and preserving the blind nature of the genotyping in respect to other details of the subject.

The packages were designed to be extremely simple to understand and process, requiring minimal effort on the part of the subject and undertaken in less than a minute. In this way, participation was maximised.

Over 1100 packages were sent out with a response rate of 53% in the athletes and 76% in the climbers – a high figure for any postal survey and even more so for a scientific study in which there was no benefit to the subjects. All but one of the containers were received intact and there was sufficient DNA in the samples for the ACE genotype to be identified in 86% of the athletes and 100% of the climbers.

2.2 THE ASSESSMENT OF LV MASS WITH CARDIOVASCULAR MAGNETIC RESONANCE (CMR)

The accurate measurement of LV mass has in the past been difficult, partly due to the oblique angle at which the heart lies within the chest, its continuous movement and the lack of a technique for imaging the whole left ventricle. Initial measurements with electrocardiogram (ECG) data were surrogate markers for LV mass, with values affected by positioning of the leads, orientation of the heart and amount of subcutaneous fat.[178-180] Nevertheless, criteria were developed for identifying LV hypertrophy with ECG[181-183] which correlated to an extent with true LV hypertrophy but were insensitive and non-specific (specificity 6-56%). [184-188] The extent of the importance of LVH only became clear by including very large numbers of subjects in

studies, such as Framingham,[189] where the inaccuracy of the technique was overcome by weight of numbers.

2.2.1 ECHOCARDIOGRAPHY

Echocardiography (echo) was a distinct advance for LV mass measurement, with direct visualisation of the myocardium and real-time imaging. Sensitivity for LVH has been reported at 85%.[190] However, obtaining good quality images is very dependent on a skilled operator, patient position and angle of the transducer beam.[191,192] In addition, patient anatomy and obesity may prevent the acquisition of images of sufficient quality for LV mass measurement in up to 1/3 of cases,[192-195] perhaps more in real clinical settings. In Montgomery's study on the effect of the ACE gene on exercise-induced LV growth,[170] 27% of echocardiograms had to be excluded at each time point due to poor image quality. Moreover, both M-mode and two-dimensional echo calculate LV mass using LV wall thickness, chamber diameter and an assumed geometric shape. Variations in wall thickness and ventricular geometry affect calculated LV mass[190] and further reduce the accuracy of the measurement.

M-mode is the commonest echocardiographic method for measuring LV mass, the images being easier to obtain and the calculations straightforward. Although originally well validated,[196,197] it suffers most from the assumption of geometric shape and this variability in measurement is reflected in the poor accuracy of the technique, with standard errors of the estimate (SEE) of 29 – 97g (95% confidence intervals 57-190g).[196-199] Inter-study reproducibility is also poor, with standard deviations of the difference between successive measurements of 22 to 49g (95% CI: 45-96g).[197,200-203] The importance of operator skill is underlined by the large inter-observer variability of a similar degree (SEE 28-41g; 95% CI: 55-80g).[197,200,201]

Two-dimensional (2D) echo has theoretical advantages over M-mode echo, as measurement is made of the ventricular length and minor axis in two planes (4-chamber and 2-chamber views), utilising the same formulae used in angiographic ventriculography. It still however assumes a prolate ellipsoid shape of the left ventricle and, to an extent, uniform wall thickness and is thus prone to similar inaccuracies as M-mode echo. The accuracy (SEE 31-39g)[198,199] and reproducibility[204,205] are moderately improved over M-mode though the increased difficulty in obtaining suitable quality images for evaluation may further limit the ability to determine LV mass.

2.2.2 ELECTRON-BEAM COMPUTED TOMOGRAPHY

Recently, the faster image acquisition time of electron beam computed tomography (EBCT), when coupled with blood-pool contrast agents, has facilitated three-dimensional cardiac imaging precise enough to measure LV mass. The technique is similar to cardiovascular magnetic resonance (CMR), with multiple contiguous image planes from which the myocardial mass in each slice is summed to achieve the total. There is good reported accuracy (SEE 16g)[206] and reproducibility,[207] though validation studies are limited. The disadvantages are the exposure to ionising radiation and need for an intravenous contrast agent to delineate the cardiac blood pool. In addition, the image slices are not true short axes but an approximation due to the limitations of available image planes, which decreases accuracy due to partial volume effects.

2.2.3 CARDIOVASCULAR MAGNETIC RESONANCE

CMR overcomes most of the problems associated with echo, and does not involve the ionising radiation or need for contrast agents with EBCT. The free choice of imaging planes and excellent tissue visualisation mean that virtually all images are of sufficient quality for LV mass determination, and the technique has been well validated. The method involves imaging a stack of contiguous slices through the left ventricle, with the myocardial volume measured from each image slice summed to obtain the total myocardial volume, employing Simpson's rule [208] (figure 2.3). When multiplied by the density of myocardial tissue (1.05 g/cm^3), the LV mass is obtained.

2.2.3.1 CMR technique used in this thesis

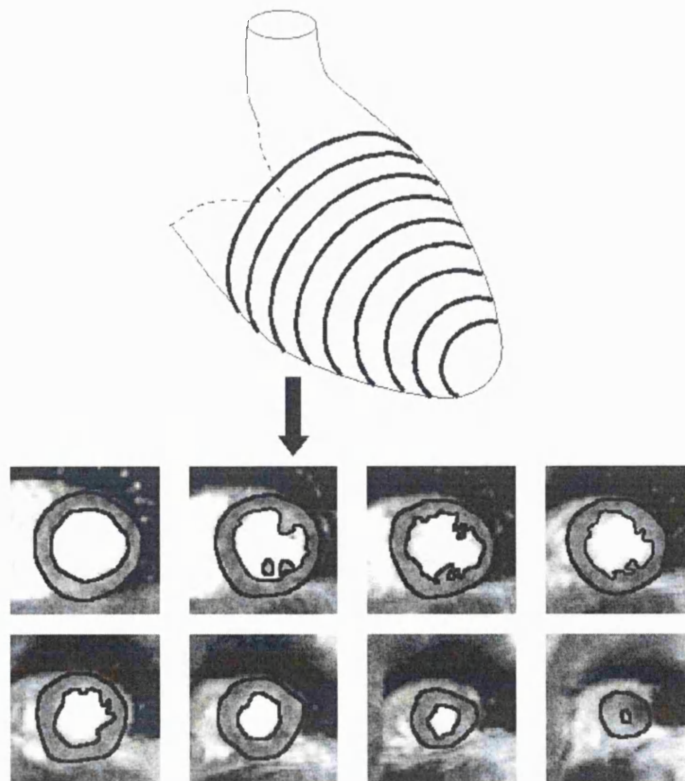
For the most accurate and reproducible measurements, the image stack should be parallel to the true LV short axis, which minimises errors due to partial volume effects of the myocardium within the image plane. The short axis was identified by first piloting a vertical long axis (VLA) plane from transaxial images, which passes through the centre of the mitral valve and apex of the LV. A horizontal long axis (HLA) plane was then obtained by imaging a plane perpendicular to the VLA, again passing through the centre of the mitral valve and apex. From the HLA, a stack of short-axis images was obtained, covering the length of the LV. ECG-gated cine-MR images were required in order to measure the LV mass at a single time point within the cardiac cycle (the standard is end-diastole – the frame immediately at the QRS of the ECG). Each cine image slice was acquired within a single breath-hold, removing motion artefact due to respiratory movement.

Studies were performed with a custom-built mobile cardiac magnetic resonance scanner (0.5 Tesla; imaging software: Surrey Medical Imaging Systems, Surrey, UK) belonging to Royal Brompton Hospital. The major advantage of this particular unit was its portability which allowed scanning of the subjects at their own base, minimising disruption to their usual schedule and maximising the uptake. It would not have been possible to include as many subjects as were obtained at the army training regiment in Bassingbourn, Hertfordshire (see chapter 3) without the presence of the scanner on-site.

Dr. Myerson performed all the CMR scans, having been trained in the technique at the Royal Brompton Hospital, and conducted the image analysis with in-house software, CMRTools (©Imperial College), blinded to genotype and drug status, over a single time period.

Figure 2.3

Diagrammatic representation of a left ventricle with short axis imaging planes and typical CMR images obtained.



2.2.3.2 Accuracy and reproducibility

The accuracy of CMR measurements of LV mass has been validated using post-mortem hearts, imaged *in vivo* in the case of animal studies [209-214] or *ex-vivo* (post-autopsy) for human hearts [202,215] (table 2.1). These show excellent agreement between the CMR-obtained and true LV masses, with differences of 3-5% and standard deviations of the order of 10g (95% CI ≈ 19g) in the canine studies and 8g (95% CI ≈ 15g) in the human ones. Given the 95% confidence intervals for echo-derived LV mass of 57-190g,[196-198] this represents a significant improvement in accuracy.

Table 2.1

Accuracy of CMR-determined LV mass in human and animal studies. Values are compared to post-mortem derived LV mass. SDD = standard deviation of the difference between the two measurements; C.I.= confidence interval (=1.96 x SDD); MI = myocardial infarction; LVH = left ventricular hypertrophy. *Standard error of the estimate from regression equation.

Study	n	SDD	95% C.I.	Mean difference	Mean % difference	Notes
Bottini [202]	6	8.9 g	± 17.5 g	0.7 g	4.0 %	Human
Katz [215]	10	7.4 g	± 14.5 g	10.2 g	5.3 %	Human
McDonald [209]	10	1.8 g	± 3.5 g	4.4 g	5.2 %	Canine
Shapiro [210]	10	6.7 g*	± 13.1 g			Canine normal
	8	8.7 g*	± 17.1 g			Canine post-MI
Caputo [211]	13	13.7 g*	± 26.9 g		10.0 %	Canine normal + LVH
Keller [212]	10	3.5 g	± 6.9 g	6.8 g	13.3 %	Canine
Maddahi [213]	8	4.9 g*	± 9.6 g			Canine; *in vivo*
	9	3.4 g*	± 6.7 g			Canine; dead (*in-situ*)
Florentine [214]	11	13.1 g*	± 25.7 g			Canine + feline
Canine studies (Total & means)	**79**	**7.0 g**	**± 13.7 g**			

Of greater importance for assessing changes in LV mass, both for individuals and research studies, is the reproducibility of LV mass measurements by CMR. This encompasses inter-study (i.e. test-retest reliability) and inter- and intra-observer variability in values. Again these are excellent for CMR, particularly for human studies (table 2.2). Inter-study variability is reported at ~5% with a standard deviation of the difference (SDD) of ~10g (95% CI ≈19g). Intra and inter-observer variability are also

good, being 6.1% and 5.1% (average) respectively. These values are equivalent, if not better, using the newer breath-hold fast-acquisition technique used for this work.[216]

Table 2.2

Reproducibility of CMR-derived LV mass measurements from human studies. Values are standard deviations of the difference (SDD) between successive scans (g) or % variability. LVH = left ventricular hypertrophy; DCM = dilated cardiomyopathy; MI = myocardial infarction.

Study	n	Inter-study	Intra-observer	Inter-observer	Notes
Bottini [202]	4	8.2 g			Normal subjects
Germain [203]	20	11.2 g (6.7 %)			Normal subjects
Yamaoka [217]	10		5.8 g	17.8 g	Normals, LVH & DCM
Katz [215]	10		6.1 %	7.2 %	
Semelka [218]	11	5.2 %		4.4 %	Normal subjects
Semelka [219]	11	4.7 – 6.1 %		3.4 %	DCM
	8	3.5 - 4.8 %		5.5 %	LVH
Matheijssen [220]	8		3.6 %	3.6 %	MI
Bellenger [216]	15	2.8 %	1.6 %	2.4 %	Normal subjects
	15	3.0 %	2.7 %	3.1 %	DCM
	15		2.2 %	3.2 %	LVH
Bogaert [221]	12	4.4 %	4.1 %	4.2 %	Normal subjects
Mean values		**4.5 % (n=92)**	**3.4 % (n=75)**	**4.1 % (n=105)**	

2.2.3.3 <u>Limitations of CMR</u>

Patient factors can sometimes limit the usefulness of the technique. Due to the enclosed nature of the MR scanner, some people find this too claustrophobic though in practice, only two subjects found this to be a problem.

The same restrictions as for any MR scanner apply for patients with cranial aneurysm clips, ocular foreign bodies and pacemakers though none of these conditions occurred in the study groups.

CHAPTER 3

Role of ACE genotype and the cardiac renin-angiotensin system in exercise-induced LV growth.

3.1 BACKGROUND

3.1.1 IMPORTANCE OF LEFT VENTRICULAR HYPERTROPHY

The importance of left ventricular hypertrophy (LVH) in cardiovascular risk is often overlooked. Although serving to increase myocardial work capacity and normalise wall stress in hypertensive subjects,[222] it is associated with a poor outcome. The Framingham Study, amongst others, showed that increased LV mass is associated with a significant excess cardiovascular mortality and morbidity.[189] The relative risk of cardiovascular death was increased in men by 72% *per 50g/m increment* in LV mass, indexed to height. This excess risk is independent of the presence of coronary artery disease (CAD)[223] or hypertension,[189] with a tripling of the mortality rate in subjects with[189,223,224] and without[223] either of these. In fact it has a greater effect on mortality than either age or the presence of hypertension.[225] Overall, LVH is associated with an increase in mortality rate of up to 10-fold.[226] Not only are cardiac morbidity and mortality raised, but so too are the risks of coronary, peripheral, or cerebrovascular disease, even amongst asymptomatic normotensive subjects with LVH.[189,227,228]

Why some individuals and not others develop LVH given an appropriate stimulus remains a mystery. In addition, why *should* a thicker left ventricular wall predispose to an increased risk of coronary or cerebrovascular disease? Understanding the mechanisms regulating myocardial growth might shed light on the pathophysiology of this response, and of the hypertensive state, leading to new therapeutic strategies.

3.1.2 ROLE OF THE CARDIAC RAS IN LVH

The mechanisms which regulate myocardial mass and the genetic elements involved in their control are poorly understood.[229] The development of LVH in hypertension and its regression with therapy correlate poorly with blood pressure[230,231] and some hypotensive agents are associated with either failure of regression of LVH (e.g. hydralazine and nifedipine[226]), or with progression of LVH (e.g. minoxidil and tiapamil[226,232]). These findings focus attention on the local trophic mechanisms regulating LV mass and the evidence for a strong influence of the cardiac RAS was reviewed in chapter 1.

3.1.2.1 Further research

The inaccessibility of normal human left ventricular tissue has impaired further investigation of this hypothesis, though Montgomery's study of the ACE I/D polymorphism[170] has suggested new routes for research. The mechanism of effect of this polymorphism on LV growth is unclear, in particular whether it acts via the cardiac RAS, and this study attempts to clarify this further. The elimination from the environment of blood pressure, a potent stimulus to LV hypertrophy, is important and the ACE gene I/D polymorphism is not associated with differences in blood pressure amongst normal individuals,[144] or hypertensives.[233,234]

3.1.3 EXERCISE AS AN APPROPRIATE CARDIAC HYPERTROPHIC STIMULUS

As discussed in chapter 1, a suitable prospective model for human cardiac hypertrophy is difficult to obtain and physical training appears to be the only ethically acceptable model that increases LV mass.[162,168]

3.1.3.1 Scale of hypertrophic response

The degree of LV hypertrophy is variable and influenced by training intensity.[162] Rowers, cyclists and canoeists had the greatest LV mass in a study of 947 elite athletes,[235] which may be as much as 45% greater compared to control groups.[169] Prospective studies however, assessing athletes pre and post training, have suggested a more modest increase in LV mass of 12-28%[168,236,237] and this may be more representative than comparing athletes to non-athletes. Given the influence of the pattern of exercise undertaken on LV hypertrophy, a uniform exercise stimulus is important for this study.

3.1.3.2 Timing of hypertrophic response

Changes in LV end-diastolic diameter can be seen after 1 week of training,[238] with consequent increases in calculated LV mass, but wall thicknesses take slightly longer – from 3 weeks in Ehsani's study[238] to 6,10 or 11 weeks in others.[168,236,237] Regression of LV mass on cessation of training can be shown in a shorter timescale – as little as 1-3 weeks.[169,238-240]

3.1.3.3 Physiological vs. pathological LVH

It is assumed that the mechanism of LV growth with physical training is the same in pathological states. Differences in histology outlined above may suggest differing processes but the concept of a completely different growth mechanism seems unlikely. The RAS appears to be involved for all causes of LV hypertrophy and the previous study on the ACE gene[170] suggested its involvement in exercise-induced hypertrophy.

It remains possible that exercise-induced LVH is a separate entity from hypertensive (and other pathological) LVH, with differences in the control mechanisms, and that the assumption above may not be correct. There is no other prospective model available for study in humans however and the results have to be interpreted with these assumptions in mind.

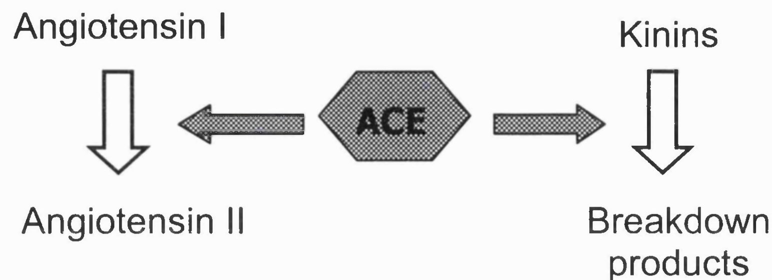
3.1.4 MILITARY RECRUITS AS AN APPROPRIATE STUDY GROUP

Military recruits undergoing training should represent a uniform group with the same exercise stimulus. They are a homogenous group with respect to environmental and other influences - all are of similar age, live under identical environmental conditions, have the same daily patterns (such as sleep patterns and meals) and groups of single sex can be examined. In the UK they undergo an identical supervised 10-week physical training programme comprising 69 periods of physical training (each of 40 minutes). Frick[241] has demonstrated cardiac growth amongst Finnish military recruits, data supported by Montgomery's study,[170] which also looked at ACE genotype. This data suggests a strong influence of ACE genotype on left ventricular growth, though there are several limitations to this study. Echocardiography is an inaccurate tool in the assessment of LV mass, as discussed in chapter 2, and in the above study 27% of echocardiograms were excluded at each stage because of poor image quality, which could bias results. In addition, exercise-induced LV hypertrophy can be associated with morphological changes[169,242] which further reduce the accuracy.[190]

By contrast, cardiovascular magnetic resonance (CMR) has been shown to be more accurate and reproducible than echocardiography in the assessment of LV mass (section 2.2.3.2) and is not influenced by morphological changes. The ability of MRI to detect very small changes in LV mass would allow the identification of even modest modulating genetic influences on LV growth.

3.1.5 POTENTIAL MECHANISMS FOR CARDIAC GROWTH

The mechanism by which the ACE I/D polymorphism may influence cardiac growth via the local RAS is unproven, though there are three main possibilities – direct effects of angiotensin II, growth inhibitory effects of kinins, or through other genes. A reminder of the position of ACE in the RAS is below:



3.1.5.1 Direct effects of angiotensin II

Angiotensin II could be acting as a growth factor (section 1.4.2.3) and the increased levels associated with the D allele could stimulate greater cardiac growth. This action could be via either AT_1 or AT_2 receptors, though the majority of known effects are via the AT_1 site. Angiotensin II stimulates DNA synthesis and the production of growth factors platelet-derived growth factor (PDGF) and transforming growth factor beta 1 (TGF-β1), which are associated with vascular smooth muscle cell proliferation.[111,243] The mechanism may be via expression of growth-associated nuclear proto-oncogenes,[243-246] which can induce expression of TGF-β1. These effects of angiotensin II were inhibited by losartan, but not by specific neutralising antibodies for PDGF and TGF-β1, suggesting at least some direct effects of angiotensin II.[111]

3.1.5.2 Kinins

Kinins may act as growth inhibitors and the increased breakdown of these associated with the higher ACE levels of the D allele could allow greater cardiac growth. ACE inhibitors can attenuate vascular growth to a greater degree than losartan[247,248] and this extra effect was reduced to that of losartan by a specific bradykinin β_2-receptor antagonist, HOE 140, suggesting the importance of bradykinin. The effect of ramipril in preventing pressure-induced LVH and cardiac remodelling was also blocked by HOE 140.[249,250] However, the results from the pressure overload model were not reproduced in a separate study from this group[251] and another similar study also found no effect of the bradykinin inhibitor on ramipril-attenuated LVH.[252]

3.1.5.3 Other genes

Lastly, the I/D polymorphism is within an intron and as such, has no direct effect on the structure of ACE. It is assumed that it is in linkage disequilibrium with another mutation in a promoter sequence of the gene which affects the rate of production of ACE. It is feasible that the linkage disequilibrium is with a mutation in a different, nearby gene, such as for growth hormone,[233] which is the mechanism of the observed effects.

3.1.5.4 Identifying the mechanism of LVH with RAS inhibitors

By blocking the RAS pathway at specific points and examining how this affects the growth response, the mechanism of action of the I/D polymorphism (and of the cardiac RAS) may be elicited. The RAS pathway can be inhibited at several sites, though as yet only two classes of drugs are licensed for use in the UK - ACE inhibitors and AT_1 receptor blockers. ACE inhibitors reduce the production of angiotensin II and also reduce the breakdown of bradykinins. In this respect, they are less useful as a research tool because the mechanism of action could be via either route, and because they do not differentiate between the angiotensin II receptors. AT_1 receptor blockers are therefore more ideal due to their specific site of action.

Losartan was the first licensed AT_1 receptor antagonist and with its specific site of action, does not affect the breakdown of kinins. It is licensed for the treatment of hypertension at a dose of 50-100 mg daily (Losartan ("Cozaar") data sheet; Merck Sharp and Dohme Pharmaceuticals). At a dose of 25mg daily, it does not have any significant effect on systemic blood pressure[253] but does increase renin levels,[254] suggesting a local effect. It has a good safety profile.

3.2 AIMS OF THE STUDY

We sought to confirm the association of the D allele of the ACE gene I/D polymorphism with exaggerated LV growth in response to exercise, seen in the previous study,[170] using the more accurate and reproducible technique of cardiovascular magnetic resonance.

In addition, the study intended to help clarify the mechanism underlying this association by examining the role of the angiotensin II AT_1 receptor using an antagonist, losartan, at a non-hypotensive dose.

In doing so, the study aimed to confirm the importance of the local cardiac RAS in human LV growth and identify the exact pathway through which it exerts its effect. It also aimed to determine the strength of genetic influences on the cardiac RAS and whether these could be modified by pharmacological means.

3.3 METHODS

3.3.1 STUDY GROUP

The study group was comprised of Caucasian male recruits from the Army Training Regiment, Bassingbourn, UK over a 18 month period. All were normotensive and free from cardiovascular disease and underwent an identical 10-week intensive physical training programme, with additional duties often involving further physical exercise.

ACE genotype was determined using mouthwash sample DNA as described in chapter 2. II genotype was confirmed by repeat PCR in the absence of the primer for the *I* allele (identifying heterozygotes in whom the *D* allele had been suppressed by competitive amplification of the shorter *I* allele fragment). All those homozygous for the ACE gene (II and DD) were invited to participate in the trial and written informed consent was obtained. Homozygotes were chosen to maximise the difference in LV growth between groups, as the number of subjects that could be scanned was limited by time constraints.

Subjects were studied at the beginning and end of the training period. At both time points, height, weight and blood pressure (mean of 3 manual measurements 1 min apart, after 20 minutes supine) were recorded using a mercury sphygmomanometer and cardiac magnetic resonance (CMR) was performed. After baseline scans, each homozygote group was independently randomised to receive either 25mg losartan or placebo daily. The tablets were continued throughout training, until follow-up scans, and tablet-taking was supervised by the recruits' senior officers, ensuring compliance. Both the subjects and staff were blinded to the nature of the tablets, thus conforming to a prospective parallel-arm double-blind randomised controlled trial protocol. Merck Sharp & Dohme (manufacturers of losartan) have developed a 'triple-blind' technique in which the company is also unaware of the randomisation details until the end of the trial. The randomisation details are generated by computer onto a card with an opaque upper layer. This was peeled off when the trial was un-blinded at its completion.

Overall, 34% of subjects failed to complete the training course at the first attempt, the majority of whom merely had to repeat sections of the training. None experienced any side effects from either placebo or losartan and the drop-out rates were no different between genotypes (DD=30%, II=39%; p=0.15) or between drug/placebo arms (losartan=37%, placebo=32%; p=0.39). Baseline physical characteristics and LV mass did not differ between those who completed and those who dropped out of training (p>0.05 by ANOVA for all parameters). Only those that completed training at the first attempt were included in the analysis.

Ethical approval was obtained from the Royal Brompton Hospital Ethics Committee and the Army Medical Services Research Executive (ethics committee).

3.3.2 IMAGING

3.3.2.1 Cardiovascular magnetic resonance (CMR)

Studies were performed at the training regiment using the technique described in section 2.2.3.1. All image analysis was performed blinded to genotype and drug status, over a single time period.

3.3.2.2 Body composition using magnetic resonance

Whole body MR scans were performed in a subset of 117 recruits to quantify adipose tissue, and thus calculate lean body mass. In this way, changes in lean and adipose tissue could be measured. Lean mass was also used to adjust LV mass for body size, as discussed below. Studies were performed using the same scanner according to a standard protocol,[255] which is described in more detail in section 4.2.2. The fat mass was subtracted from each subject's weight to obtain the lean body mass. This technique is highly reproducible, with the mean difference in adipose tissue measurements between repeated scans being 0.17 Kg (2.9%), with 95% confidence limits of ±0.4 Kg (section 4.2.2.2).

3.3.3 STATISTICAL ANALYSIS

A power calculation was undertaken to determine the group sizes needed. A change in LV mass of 5% of the total mass would represent a reasonable change in mass over time and the power calculations were based on this. In Montgomery's study,[170] the mean pre-training LV mass was 167g and a 5% change in this would be 8.35g. The standard deviation of the difference between successive MR scans is reported at

8.9g.[202] To establish statistical significance, a two-sided paired test was used with both alpha and beta levels of 0.05 (a 95% power to detect a significant change, with 95% confidence). With this, the number of subjects needed to demonstrate a 5% (8.35g) change in LV mass was 30 in each group. With four subject groups, a total of 120 subjects was needed.

Baseline and follow-up values were compared using the paired Student's t-test. Baseline values and mean changes between groups were compared using unpaired t-tests and analysis of variance (ANOVA). Values were considered statistically significant at $p<0.05$. All analyses were performed using Statview 5.0 (SAS Institute, Cary, USA). Results are shown as means ±1 standard deviation, unless otherwise stated.

3.4 RESULTS

3.4.1 STUDY GROUP

1248 recruits were screened for ACE genotype, and the frequency distribution is displayed in table 3.1, together with a control group of 1906 British male subjects free of cardiovascular disease.[147] The genotype distribution in the study group was in Hardy-Weinberg equilibrium and the relative frequencies were not significantly different from those of the controls.

Table 3.1

ACE genotype distribution and (relative frequency) in the study and control groups.

	DD	ID	II
Study group	332 (0.27)	615 (0.49)	301 (0.24)
Control group	496 (0.26)	953 (0.50)	457 (0.24)

Of the 633 recruits homozygous for the ACE gene *I* or *D* allele, 383 passed initial selection and 212 chose to take part in the study and had baseline scans. 141 recruits completed training and were available for follow-up scans. A summary of the study participants is in figure 3.2. Data from individual subjects are included in appendix 8.7; results shown here are mean values for each group. Baseline parameters were not significantly different between genotypes nor between losartan and placebo groups of either genotype (table 3.2). There was no change in blood pressure and a slight fall in pulse rate between pre and post-training, with no inter-group differences (table 3.3).

Figure 3.2

Overview of study subjects

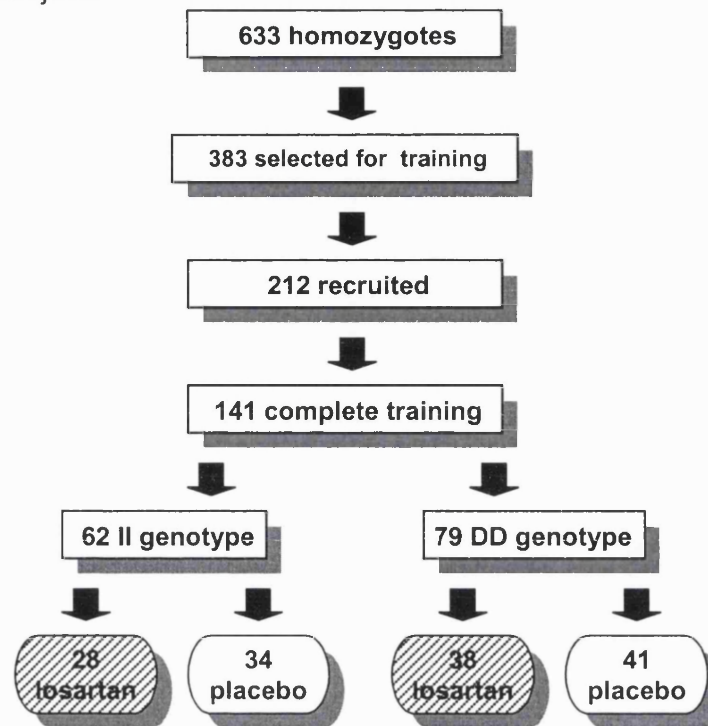


Table 3.2

Baseline parameters for the 141 subjects with paired data sets. Values are means ± standard deviation. BMI = Body mass index; BP = blood pressure.

	DD genotype		II genotype		P value
	Placebo	Losartan	Placebo	Losartan	(ANOVA)
n	41	38	34	28	
Age (years)	19.6 ± 2.7	19.6 ± 2.5	19.3 ± 2.5	20.4 ± 2.2	0.26
Height (cm)	175.5 ± 5.9	175.0 ± 5.9	175.2 ± 6.0	175.3 ± 6.6	0.80
Weight (Kg)	70.4 ± 8.2	71.7 ± 9.2	70.1 ± 7.6	70.9 ± 8.4	0.86
BMI (kg/m^2)	22.9 ± 2.6	23.3 ± 2.0	22.9 ± 1.8	23.1 ± 2.3	0.74
Resting pulse (bpm)	65.8 ± 13.0	68.4 ± 11.8	64.7 ± 12.0	63.8 ± 13.5	0.48
Systolic BP (mmHg)	118.7 ± 13.0	118.3 ± 11.8	117.0 ± 11.3	115.7 ± 9.5	0.69
Diastolic BP (mmHg)	65.6 ± 10.8	65.1 ± 9.4	65.0 ± 10.0	69.0 ± 11.9	0.23

Table 3.3

Pre and post-training pulse and blood pressure (± standard deviation); n=141.

	Pre-training	Post-training	P value
Pulse rate (beats/min)	65.8 ±12.5	63.6 ±10.4	0.02
Systolic BP (mmHg)	117.4 ±11.7	117.6 ±12.0	0.85
Diastolic BP (mmHg)	66.0 ±10.5	65.5 ±10.4	0.55

3.4.2 LEFT VENTRICULAR MASS

LV mass increased significantly with training (mean ±standard error: +8.4 ±1.2g, p<0.0001), with data for the four individual groups shown in table 3.4 and figure 3.3. The increase in LV mass was modest amongst those of II genotype though increased significantly more for those of DD genotype (+4.3 ±1.8g vs. +11.6 ±1.5g respectively; p=0.002). Losartan had no effect on the increase in LV mass: DD = 11.0 ±2.1g; II = 3.7 ±2.7g (p=0.69 and 0.75 respectively compared to placebo in each genotype group).

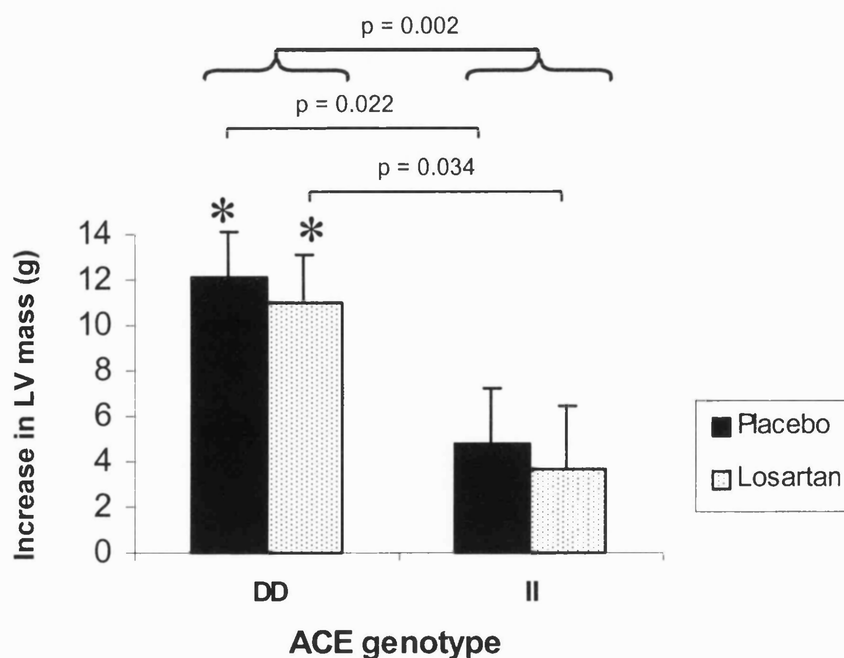
Table 3.4

LV mass measurements pre and post training. Values are means ± standard error.

Genotype	Drug	n	Baseline	Follow-up	Change	% Increase	P value (paired t-test)
DD	Placebo	41	185.0 ± 4.2	197.1 ± 4.2	12.1 ± 2.0	6.5%	<0.0001
	Losartan	38	178.1 ± 4.1	189.0 ± 3.6	11.0 ± 2.1	6.2%	<0.0001
II	Placebo	34	186.1 ± 3.8	190.9 ± 4.1	4.8 ± 2.4	2.6%	0.057
	Losartan	28	189.0 ± 4.5	192.6 ± 4.4	3.7 ± 2.7	2.0%	0.18
P (ANOVA)	Genotype		0.16	0.75	0.002		
	Drug		0.62	0.52	0.80		

Figure 3.3

Change in LV mass by ACE genotype and drug status (values are mean ± SEM). *p<0.0001 from baseline by paired t-test. Group comparisons by unpaired t-test.



3.4.2.1 LV mass index

It has been suggested that LV mass may relate best to lean body mass.[256] The ratio of LV mass to lean body mass was thus calculated in a subset of 117 recruits in whom body composition data were available (section 3.4.5). Baseline LV mass indexed to lean mass was similar for all groups (p=0.16 by ANOVA) and increased by 0.063 ±0.023 g/kg (+2.4%); p=0.008 (table 3.5 and figure 3.4). The modest LV growth in II subjects was completely attenuated when indexed to lean mass while the growth in DD subjects remained. There was still no effect of losartan on the change in LV mass index within either genotype (p=0.70 and 0.81 respectively compared to placebo).

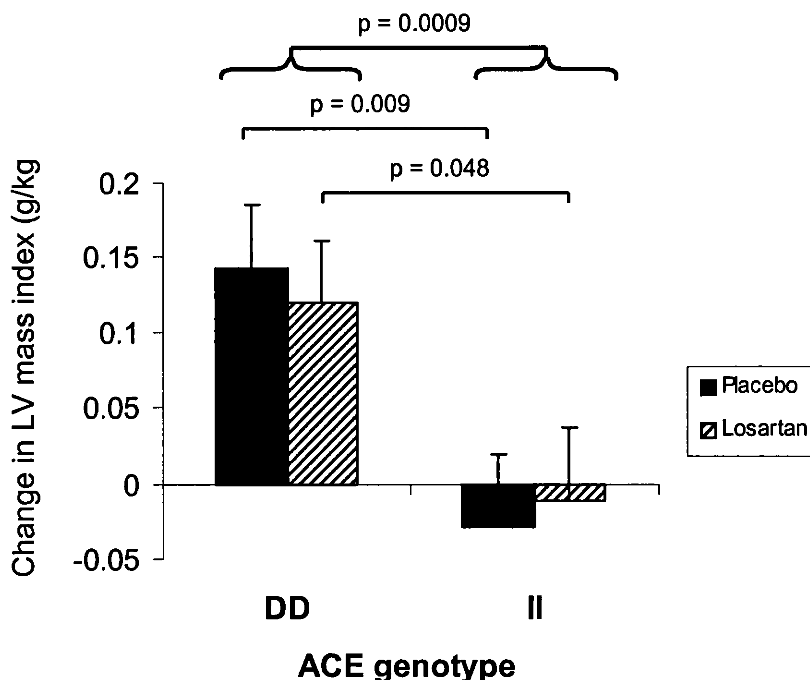
Table 3.5

LV mass indexed to lean mass, pre and post-training. Values are in g/kg (mean ± standard error); p values for paired t-test.

		n	Baseline	Follow-up	Change	% Change	p value
DD	Placebo	31	3.195 ± 0.058	3.338 ± 0.063	+ 0.143 ± 0.042	+ 4.5 %	0.002
	Losartan	34	3.023 ± 0.052	3.143 ± 0.046	+ 0.120 ± 0.041	+ 4.0 %	0.007
II	Placebo	31	3.197 ± 0.065	3.169 ± 0.068	- 0.029 ± 0.047	- 0.9 %	0.56
	Losartan	21	3.192 ± 0.073	3.181 ± 0.084	- 0.011 ± 0.048	- 0.3 %	0.81

Figure 3.4

Change in LV mass index by ACE genotype and drug status (values are mean ±SEM)



3.4.3 CARDIAC VOLUME DATA

Ventricular volume data are shown in table 3.6. The group as a whole showed an increase in LV end-diastolic volume with little change in end-systolic volume, and thus an increase in stroke volume. Due to the small decrease in resting pulse rate, the resting cardiac output was unchanged. LV end-diastolic volume is shown by genotype and drug group in table 3.7.

Table 3.6

Ventricular volume data, pre and post training. Values are means ±standard error in mls (cardiac output in L/min); p values for paired t-test. LV = left ventricle; RV = right ventricle; EDV = end diastolic volume; ESV = end-systolic volume; SV = stroke volume.

	Baseline	Follow-up	Change	%Change	p value
LV EDV	108.2 ±2.1	114.7 ±2.2	+ 6.5 ±1.8	+ 6.0 %	0.0004
LV ESV	34.2 ±0.8	35.9 ±0.9	+ 1.7 ± 0.8	+ 5.0 %	0.03
LV SV	73.9 ±1.4	78.8 ±1.6	+ 5.0 ±1.4	+ 6.8 %	0.0005
Cardiac output	4.85 ±0.16	4.80 ±0.14	- 0.05 ± 0.16	- 1.0 %	0.72
RV EDV	130.6 ±2.2	139.5 ±2.4	+ 8.8 ±1.8	+ 6.7 %	<0.0001
RV ESV	57.1 ±1.2	60.9 ±1.3	+ 3.8 ±1.1	+ 6.6 %	0.0004
RV SV	73.6 ±1.4	78.5 ±1.5	+ 4.9 ±1.2	+ 6.7 %	<0.0001

Table 3.7

LV end-diastolic volume pre and post-training. Values are means ± standard deviation in mls. P values for paired t-test.

LVEDV		n	Baseline	Follow-up	Change	% Change	p value
DD	Placebo	41	110.6 ± 26.0	117.5 ± 27.6	+ 6.8 ± 18.7	+ 6.1 %	0.02
	Losartan	38	103.9 ± 22.7	108.9 ± 23.9	+ 4.9 ± 23.0	+ 4.7 %	0.19
II	Placebo	34	106.3 ± 23.1	116.4 ± 30.1	+ 10.1 ± 22.3	+ 9.5 %	0.01
	Losartan	28	113.1 ± 26.5	115.5 ± 23.0	+ 2.4 ± 16.9	+ 2.1 %	0.46

There were no significant differences between genotypes or between losartan and placebo groups in any of the volume parameters, though there was a non-significant trend for losartan to attenuate the increases in LVEDV (unpaired t-tests for placebo vs.

losartan groups were p=0.69 and p=0.13 for DD and II genotypes respectively; p=0.20 for the group as a whole).

3.4.4 INDEXING LV MASS TO END-DIASTOLIC VOLUME

Finally, to determine if the increase in LV mass was due to an increase in end-diastolic volume rather than wall thickness per se, the LV mass was indexed to LVEDV. Results are shown in table 3.8:

Table 3.8

LV mass indexed to LV end-diastolic volume. Values are g/ml (mean ±standard deviation).

LV mass / LVEDV		n	Baseline	Follow-up	Change	% Change	p value
DD	Placebo	41	1.75 ± 0.43	1.75 ± 0.41	0.00 ± 0.30	0.0 %	0.98
	Losartan	38	1.78 ± 0.41	1.80 ± 0.39	+0.02 ± 0.36	+1.1 %	0.75
II	Placebo	34	1.80 ± 0.39	1.72 ± 0.45	-0.08 ± 0.32	-4.4 %	0.12
	Losartan	28	1.75 ± 0.40	1.72 ± 0.35	-0.02 ± 0.23	-1.1 %	0.49

There were no significant changes in the ratio of LV mass to LVEDV for any group, though a trend towards a reduction in the ratio was noticed in the II genotype groups. This data suggests that the greater LV growth in DD subjects observed in section 3.4.2 was proportional to increases in LVEDV. Although the II subjects increased LVEDV to a similar degree (table 3.7), this was not accompanied by a proportional increase in LV mass, resulting in the downward trend in the ratio observed above.

In summary, all subjects tended to increase LVEDV with training, with those of DD genotype increasing LV mass proportionately while those of II genotype achieved an increase in LVEDV with a smaller increase in LV mass.

3.4.5 BODY COMPOSITION

The results of the body composition analysis are shown in table 3.9. All subjects increased lean mass and decreased fat mass, but there were no significant differences between genotype or drug groups, either at baseline or in the change over the 10-week period.

Table 3.9

Body composition data for all genotype and drug groups; values are means ± standard deviation. The changes were significantly different from baseline ($p<0.01$ by paired t-test) for all values except *.

Group		n	Lean mass (kg) Pre	Lean mass (kg) Post	Lean mass (kg) Change	Fat mass (kg) Pre	Fat mass (kg) Post	Fat mass (kg) Change
DD	Placebo	31	58.2 ± 6.1	59.9 ± 6.2	+1.7 ± 2.0	12.2 ± 4.2	11.2 ± 3.5	-1.0 ± 1.8
DD	Losartan	34	59.5 ± 6.9	60.7 ± 6.9	+1.2 ± 1.7	12.0 ± 3.8	10.8 ± 2.8	-1.2 ± 1.9
II	Placebo	31	57.9 ± 5.8	59.7 ± 5.7	+1.9 ± 2.0	12.0 ± 3.3	11.3 ± 2.3	-0.7 ± 2.1*
II	Losartan	21	60.2 ± 5.0	61.7 ± 4.1	+1.4 ± 1.9	12.9 ± 4.2	11.6 ± 3.1	-1.4 ± 2.0
ANOVA p value		Gene	0.92	0.67	0.70	0.99	0.68	0.66
ANOVA p value		Drug	0.19	0.14	0.35	0.98	0.66	0.27

3.5 DISCUSSION

These results confirm the ACE genotype effect on LV growth seen in the previous study,[170] and this was consistent in both the losartan and placebo groups. The absolute amount of LV growth in this study was lower than previously reported, though the improved reproducibility of CMR still allowed significant genotype differences to be shown on a smaller sample size.

3.5.1 LOSARTAN EFFECT

Losartan at the 25mg dose had no effect on LV growth. It was important that the dose of losartan chosen should have biological effects through the AT_1 receptor, yet have minimal effect on blood pressure. A dose of 25mg/day seemed to satisfy these criteria[257-259] and it has been reported that doses of up to 50mg/day are without blood

pressure effect on either salt-replete[260] or salt-deplete[254,261] normal subjects. Even if this dose had produced some degree of hypotensive effect, then this would only have confounded the data detrimentally, by reducing the LV growth response in a pressor-interactive fashion. In fact, despite potential negative confounders, there was no evidence of any effect on this response.

Nonetheless, a small biological effect of losartan on the LV growth response could have been missed. Post-hoc analysis demonstrated an 80% power to detect a 2.6% reduction in LV growth by losartan, and a 95% power to detect a change of 4.8%. The nearly three-fold differences in growth effect between genotypes, however, was scarcely affected by losartan treatment.

3.5.2 DEGREE OF LV HYPERTROPHY

The earlier study[170] showed a much greater mean increase in LV mass for subjects of DD genotype than observed here (42g vs. 12.1g) and there are several reasons why this may be so. Firstly, the measurement of LV mass by echocardiography, as used in the first study has several problems. It uses LV wall thickness and an assumed geometric shape to calculate LV mass,[196,197] which may be inaccurate. In addition, the equations were not designed to assess alterations in LV mass within individuals. M-mode echo tends to over-estimate LV mass (and hence prospective change in mass) when compared to CMR[202] or 3D echo.[198] A small increase in LV thickness might be exaggerated by the cubing involved in the calculations. These factors are exhibited in the poor reproducibility of M-mode echo[200,202] which increases the confidence intervals for detecting serial changes in LV mass and repeated measures may thus vary considerably from (and potentially over-amplify) actual changes. Although echocardiographic measurement of mass may have its place in large studies, it is less applicable to prospective (serial) studies of LV growth in small samples. The measurement of LV mass with CMR is direct, removing any error associated with volume changes or ventricular shape.

Secondly, military training has changed slightly since the first study. It is now of reduced intensity, with less resistance and more endurance exercise, and this balance between the two types may be important in determining LV growth.[162,239,262] This and the previous study[170] have demonstrated a substantial gene-environment interaction between ACE genotype and physical exercise in determining the scale of the LV hypertrophic response. It thus seems likely that changes in the nature of the

environmental stimulus (exercise) will interact with genotype to produce differences in the nature and scale of phenotypic response.

The more modest amount of LV growth seen in this study may however still be important. The figures are group means and a *population* difference of 7g (as seen between II and DD groups) may have much greater clinical significance than individual changes of this amount, given the profound detrimental effects of LV hypertrophy on morbidity and mortality.[189,223,226] Additionally, the suggestion that for DD subjects, the LV growth was out of proportion to the change in lean mass, as seen in this study, may be more important than absolute changes.

3.5.3 POTENTIAL CONFOUNDING FACTORS

There was no significant difference in baseline parameters between any of the groups (table 3.2). In particular, baseline LV mass was not significantly different between groups and the percentage increase in LV mass showed the same pattern as the absolute values (table 3.4). Additionally, when indexed to lean body mass, the (non-significant) increase in LV mass seen in the II subjects was completely attenuated while the LV growth in DD subjects remained. The results suggest that the LV growth in DD subjects is in excess of the increase in lean body mass, while the small change in II subjects is proportional to increased lean body (skeletal muscle) mass.

3.5.4 MECHANISM OF EFFECT OF THE ACE I/D POLYMORPHISM

3.5.4.1 Cardiac AT_1 receptor

By avoiding blood pressure changes, any effect would be likely to have been via cardiac AT_1 receptors rather than haemodynamic changes due to the systemic RAS. Further evidence also exists for RAS effects on cardiac growth distinct from the haemodynamic effects: i) Angiotensin II infusion increased LV mass in a pressure independent fashion, which was inhibited by hypotensive doses of losartan, but not the equivalent hypotensive dose of hydralazine.[123] ii) AT_1 blockade can reduce LV hypertrophy following myocardial infarction,[263] but this may be only at higher doses which also reduce LV end-diastolic pressure.[264] iii) Finally, despite equal blood pressure reduction, AT_1 antagonism caused greater LV mass regression than atenolol (21g/m^2 vs. 10g/m^2 respectively)[265] and hydralazine (which failed to prevent LV hypertrophy).[127] An exercise-induced model of LV hypertrophy has even been developed in rats, which were subjected to an 8-week swimming regime![266] LV weight increased compared to

sedentary controls, and two thirds of this increase was diminished by an AT_1 antagonist, though at a slightly hypotensive dose (126 vs. 140mmHg for untreated).

The specific role of the AT_1 receptor in the development of LVH independent of blood pressure has been shown in a recent study from Canada.[267] Over-expression of myocardial AT_1 receptors in transgenic mice resulted in marked LV hypertrophy and cardiac failure without changes in blood pressure. These changes were prevented by an AT_1 receptor inhibitor.

At the other extreme, AT_{1a} receptor knockout mice did not exhibit LV hypertrophy with sub-pressor doses of angiotensin II, while the wild-type mice increased LV mass.[268] The knockout mice were able to increase LV mass in response to a pressor stimulus (aortic banding), indicating the presence of other, non-AT_1 dependant, mechanisms in pressure overload and the importance of AT_{1a} receptors for the sub-pressor effects of angiotensin II. It is possible however that this pressor response occurred via the AT_{1b} receptors in the absence of AT_{1a} receptors,[269] raising the possibility of AT_{1b}-mediated LV growth effects in the pressure-dependant model.

These and the above study suggest that the majority of the LV growth response is mediated via cardiac AT_1 receptors, but that the growth stimulus is powerful enough to require hypotensive doses of AT_1 receptor blockers. Any effect that higher doses of losartan might have on cardiac AT_1 receptors would be difficult to distinguish from haemodynamic effects in the above study.

3.5.4.2 AT_2 receptor actions

The evidence for the AT_2 receptor suggests that stimulation results in anti-growth effects and inhibition of AT_1-mediated effects (reviewed by Matsubara[270]). Stimulation of the AT_2 receptor resulted in inhibition of AT_1 receptor cell signalling,[271] and AT_2 receptor blockade enhanced the angiotensin II-mediated growth response in hypertrophied isolated rat hearts[272] and rat myocytes.[273] Genetic over-expression of the AT_2 receptor resulted in attenuation of AT_1-mediated pressor and chronotropic effects.[274] The balance between the two receptors is probably the key to the effect of angiotensin II.[275] The proportion of the AT_2 subtype is increased in hypertrophied isolated rat hearts[276] and failing human hearts,[277,278] suggesting the upregulation of AT_2 receptors in pathological hypertrophy may suppress the stimulatory effects of angiotensin II.

3.5.4.3 Kinins

The evidence for the growth-inhibitory properties of kinins is reviewed in section 3.1.5.2. There is far less evidence for kinin-mediated reduction in LVH, and although some studies have shown the anti-hypertrophic effect of ACE inhibitors can be abolished by bradykinin inhibitors,[250,279] these effects may be indirect.

Cyclosporin-induced LVH, in which hypertension is part of the mechanism, was reduced to a greater extent by angiotensin II antagonists than by an ACE inhibitor and the kinin antagonist, HOE140, had no effect on LVH.[280] Bradykinin β2 receptor knockout mice had greater LVH than wild-type[281] but the increase was modest (9%) and accompanied by chamber dilation (+46%) and increased LV end-diastolic pressure (25mmHg), suggesting haemodynamic rather than direct effects. Furthermore, ACE inhibition attenuated the pressure-induced LVH in these knockout mice, suggesting that the β2 receptor was not involved.[282]

3.5.4.4 The sympathetic nervous system

It has been suggested that an interaction exists between the RAS and the autonomic nervous system (ANS) and that angiotensin II in particular has sympatho-stimulatory effects.[283,284] The results are conflicting however, and a recent review suggested little evidence for direct effects.[285] Although there is some evidence for a direct central nervous system effect of angiotensin II, such as the attenuation of the bradycardic response to volume overload,[286] the studies have been largely animal-based, often in unusual conditions such as pithed rabbits[283] or transgenic, renin over-expressing rats (TG(mREN2)27)[287] which may bear little resemblance to human *in vivo* situations. The effects have been modest and indirect (such as heart rate variability)[288,289] and it is difficult to distinguish these effects as directly related to angiotensin II or to changes in cardiovascular homeostasis, such as through blood pressure. In some older trials, angiotensin II appears to have both inotropic and chronotropic effects,[83,92] though the effect of β–blockade on this was minimal, suggesting little involvement of the sympathetic nervous system. Several of the human trials showed no effect of ACE inhibition on sympathetic activity,[290,291] though numbers were limited.

Furthermore, the direct link between raised catecholamine levels/sympathetic activity and LV hypertrophy has been questioned.[292] The effects may be impossible to distinguish from changes in cardiovascular haemodynamics, with which the autonomic nervous system is closely involved.

3.5.4.5 Other biochemical systems

Like RAS components, levels of cardiac insulin-like growth factor-1 (IGF-1) and its receptor are upregulated during pressure overload-induced LVH.[293] This has lead some to suggest that these two systems may be related,[294] and that angiotensin II stimulates IGF-1 activation.[294] However, it is not clear that these effects are directly related, and an angiotensin II infusion may exert its effect indirectly through effects on blood pressure.[295] There is also evidence that IGF-1 may attenuate angiotensin II formation, adding to the uncertainty.[296]

An interaction between the endothelin-1 and renin-angiotensin systems has also been suggested (reviewed by Rossi[297]), though a specific effect on cardiac hypertrophy has not been proven. One study suggested a down-regulation of endothelin-1 by an activated RAS, in contrast to the previous pro-hypertrophic effects of endothelin-1.[298]

3.5.4.6 Other genetic polymorphisms

The failure of losartan in preventing the excess LV growth associated with the ACE DD genotype means that the effector mechanism may not involve ACE/angiotensin II. The neighbouring growth hormone gene is a good candidate for a mutation in linkage disequilibrium with the ACE I/D polymorphism though studies to date have failed to provide any evidence for this.[233,299] Other potential mutations remain to be investigated.

3.5.5 RECENT DATA FROM OTHER STUDIES

3.5.5.1 ACE gene and LV mass

Several further cross-sectional studies have shown no association of the ACE gene I/D polymorphism with LV mass in both normal populations[300-302] and in the presence of hypertension.[302-304] Two studies did show a positive association in hypertensive[305] or diabetic[306] males, but not females. All these studies however suffer from the same problems of cross-sectional design and relatively small numbers of subjects measured with echocardiography as discussed previously (section 1.4.4.1) Any effect of the ACE gene is likely to be swamped by the great heterogeneity of the subjects or absent due to the lack of a growth stimulus. The general lack of association of LV mass with ACE genotype is thus not surprising, particularly for the normal subjects. These effects are compounded in a recent meta-analysis[307] in which the studies and subject groups were widely heterogeneous, included both hypertensives and normals together and used

very differing methods of evaluating LV mass and differing cut-off points for LV hypertrophy. Unsurprisingly, there was no overall association of the I/D polymorphism with LV mass. However, when the untreated hypertensives were analysed separately, the DD polymorphism showed a modestly raised LV mass compared to the II genotype. Finally, one cross-sectional study was able to show a small but significant association of ACE genotype with LV mass by using highly accurate LV mass measurement (measured directly at post-mortem examination): DD vs. ID vs. II = 250g vs. 230g vs. 227g.[308]

Other studies, in which the subject group had a suitable hypertrophic stimulus and were more closely defined (reducing extraneous 'white noise'), did show an association. In patients with similar degrees of aortic stenosis,[309] the DD genotype was associated with greater LV mass (DD vs. ID vs. II = 197g vs. 175g vs. 155g) pre-operatively. Post-operatively, the DD and ID genotypes showed a greater degree of regression in LV mass, resulting in similar values for all genotypes at 15 months post-operatively. This neatly exemplifies the role of the ACE genotype as a *mediator* of LV mass in the setting of a hypertrophic stimulus which, when removed, also removes the differences between genotypes. Candy examined the ACE genotype in dilated cardiomyopathy with reduced LV function (ejection fraction <40%)[310] and found the DD genotype to be associated with a greater end diastolic diameter (67.1mm vs. 64.2mm vs. 65.9mm) and reduced ejection fraction (23.5% vs. 26.3% vs. 28.0%) for DD, ID and II genotypes respectively. This reduced function has important consequences for prognosis and may reflect differences in myocardial cellular efficiency – a subject examined in chapter 5. The DD genotype was also associated with increased end diastolic diameter and LV mass in a group of long-distance runners – another study of exercise-induced LV hypertrophy.[311]

3.5.5.2 AT_1 receptor blockade and ACE inhibition: mechanisms and comparisons

Effective blockade of the RAS at both these key points together appears to be much more effective than either alone. Menard showed that combinations of these two inhibitors at low doses were better than either agent alone in higher doses, both for lowering blood pressure and reducing LV mass.[312]

The different mechanisms within cardiac tissue of these two classes of drugs were examined in an elegant study by Danser's group in a porcine post-MI model.[313] Cardiac angiotensin II levels increased post-MI due to increased uptake from the circulation. This uptake was attenuated by eprosartan suggesting that the process was AT_1 receptor-mediated – that is, angiotensin mediates its own uptake into cardiac tissue.

Captopril prevented the rise in circulating angiotensin II levels, as expected, by reducing angiotensin I to II conversion, but did not affect uptake into cardiac tissue. Cardiac angiotensin II levels increased however in both captopril and eprosartan groups, due to cardiac production of angiotensin I and conversion to angiotensin II. The failure of captopril to prevent this conversion may reflect the importance of other converting enzymes (e.g. chymases) in the cardiac RAS. Despite this rise in cardiac angiotensin II levels, both eprosartan and captopril prevented the increase in LV mass. This might be due to unopposed stimulation of anti-hypertrophic AT_2 receptors by the increased angiotensin II levels, together with diminished AT_1 receptor stimulation. In the captopril group, the AT_1 receptor density was reduced which may explain some of its beneficial effects though the exact mechanism is unclear. Both treatment groups had reduced mean arterial pressures (6-10 mmHg lower), indicating that the dose of RAS inhibitors required for effective cardiac inhibition in this study had haemodynamic effects. The specific effects of this blood pressure-reduction were not addressed by the study.

There may also be different effects on the histology of myocardial tissue. In a rat post-MI study,[314] both drug classes reduced LV hypertrophy equally but enalapril reduced fibroblast proliferation and fibrosis, while losartan had minimal effects.

3.5.6 LIMITATIONS

The mechanism of the ACE gene I/D polymorphism effect on LV hypertrophy remains unclear after this study. It is likely that the dose of losartan used was too low, and that angiotensin may have effects on LV hypertrophy via the local RAS, but the effective dose of losartan to block this is too high to avoid haemodynamic effects. Alternatively, any effect of the ACE gene may be due to haemodynamic changes. Although there was no difference in resting blood pressure between genotypes, exercise-induced BP changes might be greater in subjects with DD genotype.[315]

Secondly, there was no heterozygote group, as the aim was to confirm an ACE genotype-association with LV growth, and to explore the potential role of the AT_1 receptor in such genotype-dependence. The study did not set out to explore the potential additive or synergistic effects of the presence of more than one allele.

Caution is required if these conclusions are to be extended to disease states and to other groups. The myocardial growth response to exercise is influenced by sex and age[316,317] and the ACE I/D polymorphism may not be associated with differential ACE levels in black subjects.[318] Exercise-related hypertrophy differs from that associated

with disease states in that it is associated with improved myocardial function.[319] An association of ACE-genotype with such physiological hypertrophy does not necessarily imply an association with pathological hypertrophy. However, these findings are consistent with a role for paracrine renin-angiotensin systems in the control of LV growth,[63,101,231,320] whose inhibition may partly account for the effect of ACE inhibitors in reducing myocardial mass.

Furthermore, while the mechanism of left ventricular hypertrophic responses to a physiological stimulus may not apply to a pathological state, the prospective study of ventricular hypertrophy in disease states is problematic, as mentioned previously, and a physiological model provides a good basis for understanding which is likely to have many similarities.

3.6 CONCLUSIONS

The association of ACE I/D genotype with exercise-induced LV hypertrophy observed by Montgomery[170] has been confirmed, though with lower absolute changes in LV mass. Losartan in low dose had no effect on LV growth, suggesting either that higher doses are required which may also affect blood pressure or that this association is mediated via mechanisms other than cardiac angiotensin II AT_1 receptors.

Other studies however have suggested that the AT_1 receptor is an important mediator of LV hypertrophy, occurring by both pressure-dependent and independent mechanisms, with a probable inhibitory effect of the AT_2 receptor. The balance between the two receptors is important and AT_1 receptor blockers may have some of their effect by unopposed stimulation of anti-proliferative AT_2 receptors. The dose of AT_1 receptor inhibitor required to achieve adequate cardiac receptor blockade (and thus prevention of LVH) may also have hypotensive effects. The importance of the local cardiac RAS is supported however by the regression of LVH by AT_1 receptor blockers and ACE inhibitors at hypotensive doses being greater than other similarly hypotensive agents, and the ability of sub-pressor doses of angiotensin II to increase LV mass. This local effect may be overwhelmed in the presence of significant hypertrophic stimuli (particularly continued pressure overload), in which there may be other mechanisms which induce or maintain LV hypertrophy.

Additionally, the complete attenuation of LV growth in II subjects when indexed to lean mass, while not markedly reducing the LV growth in those of DD genotype, suggests

that the LV growth in DD subjects is disproportionate to the increase in lean body (skeletal muscle) mass, which may be more important.

The indexation of LV mass to body mass indices, as used above, is important for determining the true LV mass range and whether any hypertrophy is excessive. It may also be useful in understanding the stimuli to LV hypertrophy. Finding the appropriate index with which to scale for LV mass is thus important for both clinical practice and research and this is the subject of the next chapter.

The mechanism of cardiac growth could also involve cellular metabolism – more efficient use of the available substrates might result in increased output, in response to greater demand, without the need to hypertrophy. Evidence exists for a role of the RAS in metabolic efficiency, particularly cardiac (reviewed in section 5.1.1), and the ACE gene I/D polymorphism may also be used to investigate this subject, which will be explored in chapter 5.

CHAPTER 4

Indexing left ventricular mass to body composition variables

4.1 INTRODUCTION

4.1.1 THE IMPORTANCE OF SCALING

The study of left ventricular hypertrophy, and its role in clinical practice, requires the scaling of left ventricular (LV) mass to account for individual differences in body size. This has traditionally been accomplished by simple ratio scaling using indices such as weight, height and body surface area (BSA). However, this scaling procedure (e.g. LV mass/BSA) assumes a linear relationship between LV mass and the scaling variable and there is a growing body of evidence suggesting that most of these relationships are non-linear.[321-328] If this is the case then previous cardiac literature that has reported ratio scaled data must be questioned on theoretical grounds. The research in chapter 3 suggested a negation of LV mass changes in the II genotype group when indexed to lean mass and it may be that this is the most appropriate index for LV mass. This chapter will explore the scaling of LV mass to various body composition variables using data from the same study.

4.1.2 ABBREVIATIONS USED IN THIS CHAPTER

LV	Left ventricle
LBM	Lean body mass
FM	Fat mass
VFM	Visceral fat mass
SCFM	Subcutaneous fat mass
BSA	Body surface area
BMI	Body mass index

4.1.3 CURRENT LIMITATIONS OF SCALING

It is clear that appropriate scaling is important for correct inter and intra-group comparisons and the construction of reference standards for normality.[329] Great care, therefore, must be given to the determination of the most appropriate scaling procedures. Much of the recent scientific literature has suggested a curvi-linear model, of the general form $\mathbf{Y = aX^b}$, as the most appropriate scaling procedure (where $\mathbf{X}$ = body size variables, $\mathbf{Y}$ = cardiac dimensions, $\mathbf{a}$ = constant multiplier and $\mathbf{b}$ = scaling exponent). This allows the formation of a power function ratio $\mathbf{Y/X^b}$, which is more likely to provide a size-independent cardiac index than the simple per ratio standard $\mathbf{Y/X}$.[330]

Most studies examining scaling have utilised echocardiography to estimate cardiac indices and standard anthropometry and/or bioelectrical impedance to estimate body size and composition. Whilst these procedures are common within clinical practice they are prone to error in the estimation of data. Error in the echocardiographic estimation of LV mass has been discussed in section 2.2.1 and may be largely due to the use of crude mathematical models to describe the shape of the left ventricle as well as the availability of different regression equations that produce divergent LV mass estimates.[331] Likewise, the estimation of body size and composition variables using anthropometry or bioelectrical impedance relies on prediction equations that may be based on erroneous assumptions as well as having been validated in different groups to the one being studied.

Whether these potential errors in estimation would impact upon the relationship between LV mass and body size variables, and thus the scaling procedure adopted, has not been determined. Furthermore, the importance of accurate body composition analysis has been highlighted by the association of LV mass with fat mass (FM) and lean body mass (LBM), sometimes referred to as fat-free mass. Some researchers have argued that FM may have an independent effect on LV mass[327,332] while others have argued that the effect is negligible.[324,333,334] Part of the problem may be related to the definition and method of assessment of FM. The use of body mass index (BMI) to assess adiposity[327] may be problematic because of the influence of LBM in the determination of BMI.

Magnetic resonance imaging (MRI) has become the “gold-standard” technique for measurement of both LV mass and body composition. It is more accurate and reliable than echocardiography (section 2.2.3) because of greater resolution and more sophisticated modelling of the left ventricle. Similarly, the MRI estimation of body compositional indices has been reported in animal[335] and human[336] studies and has been validated against dissection and chemical analysis.[337] The accurate determination of both LBM and FM can be achieved, which may be further segmented into visceral fat mass (VFM) and subcutaneous fat mass (SCFM).

4.1.4 STUDY AIMS

The study intended to investigate the relationship between LV mass and body composition variables using MRI. The relationships between LV mass and the anthropometrically determined variables of height, body mass and body surface area (BSA) were also examined. The use of BSA is open to many sources of error as an indirect estimation is used. However, we could not find a “gold-standard” approach to the

estimation of BSA and thus adopted a standard anthropometric approach[338] on the basis of its extremely common use.

4.2 METHODS

4.2.1 STUDY GROUP

The cohort consisted of 117 male army recruits (age range 17-28) from the study in chapter 3, in whom whole body MR images were taken for determination of body composition variables at the start of military training. All subjects were free of cardiovascular disease and none were taking any form of medication. Height and weight were recorded and body surface area was calculated from these using the equation of Dubois and Dubois.[338]

LV mass was determined as described in section 2.2.3.1.

4.2.2 BODY COMPOSITION

Whole body imaging was performed using 40 transaxial spin echo image slices (TE40, TR500, field of view 45 x 45 cm, slice thickness 10mm), covering the whole body from wrists to ankles in subjects lying prone with arms outstretched. The image slices were non-contiguous, with a gap of 40mm between slices such that the leading edge of each slice was 50mm from the next. To reduce respiratory motion artefact, signal averaging was performed, with 4 averages for the abdominal region and 2 for the pelvic region (L4/5 and below).

4.2.2.1 Adipose tissue segmentation

Segmentation of adipose tissue was performed using an automated technique developed specifically for this purpose. Adipose tissue can be differentiated by its high signal relative to other tissues on spin echo (T1-weighted) images, with minimal overlap of tissue intensities, and thus a threshold technique can be employed for segmenting this tissue from others. However, signal intensity varies on different acquisitions and also across a single image due to a non-linearly varying bias field and this variation prevents the use of an absolute signal intensity threshold. Attempts to overcome this have included using a reference phantom, either during all image acquisitions and employing a relative threshold,[335,336] and/or prior to imaging for bias

field estimation and correction.[339,340] The automated analysis was designed to overcome these variations in signal intensity without the use of a phantom. It uses a mosaic of multiple overlapping regions (tiles) within each image, in which the adipose tissue threshold is determined using the pixel intensity histogram for each tile. The tile with the greatest absolute difference in intensity (greatest contrast) is used as the reference tile and the four adjacent tiles are compared to this using the overlapping regions. If a neighbouring tile has a conflicting segmentation result with the reference tile within the overlapped region, the threshold for the neighbouring tile is reclassified to concur with the reference. This process is systematically propagated to all the tiles using the overlapping regions to ensure consistent segmentation across the whole image (figure 4.1). Once segmented, the total area of adipose tissue in each image is measured by summing the number of voxels identified as adipose tissue, with the voxel size calculated from the known field of view (45 x 45 cm) and matrix size (256 x 256).

Adipose tissue was measured in each image by this automated technique, with manual inspection to ensure correct segmentation. Separation into visceral and subcutaneous compartments was performed using a manual tracer to delineate the peritoneal cavity (figure 4.2). The high signal intensity from trabecular bone, due to the lipid content of bone marrow, resulted in the need for manual delineation and exclusion of bone from the analysis. The total volumes of subcutaneous and visceral fat mass were calculated independently, using the Cavalieri technique of averaging the area of adipose tissue between two images, using a truncated pyramid formula.[341] The volume was multiplied by the density of adipose tissue (0.95 g/cm^3)[342] to obtain the mass.

4.2.2.2 Reproducibility of adipose tissue segmentation

The reproducibility of the technique has been examined in 10 volunteers (body mass index 19.2 to 29.2 kg/m^2 who had MR scans of the abdominal region (diaphragm to perineum) performed twice on the same day. Adipose tissue quantification was determined and the paired sets from each subject compared (test-retest reliability). The mean values and mean of the absolute differences between repeated scans are shown in table 4.1.

Figure 4.1

The processing results of a transverse image acquired at the upper thigh level of one of the subjects studied. (a) The original acquired image; (b),(c) segmentation results based on manual thresholding; (d) Bias corrected image using overlapping mosaics; (e) initial three-class segmentation result; (f) final segmented adipose tissue distribution.

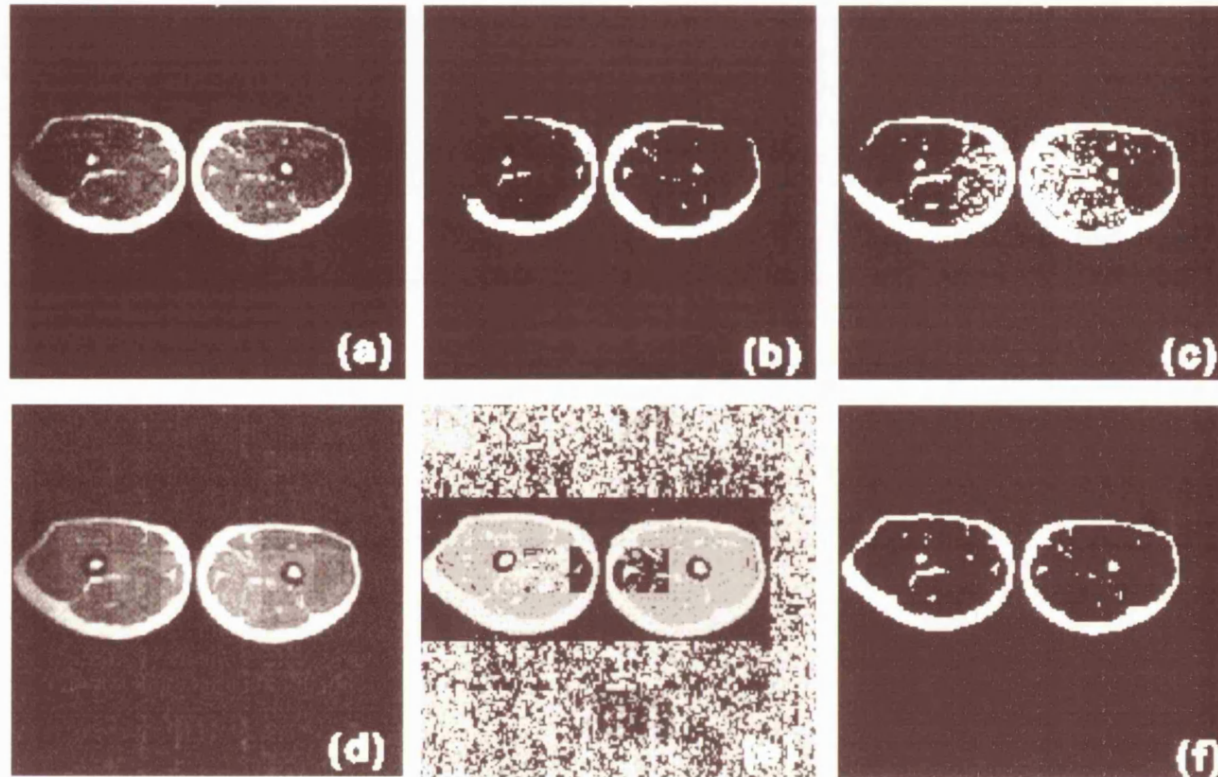


Figure 4.2

a) Abdominal magnetic resonance image with b) segmentation of adipose tissue by automated image analysis, delineated into visceral (white) and subcutaneous (black) compartments.

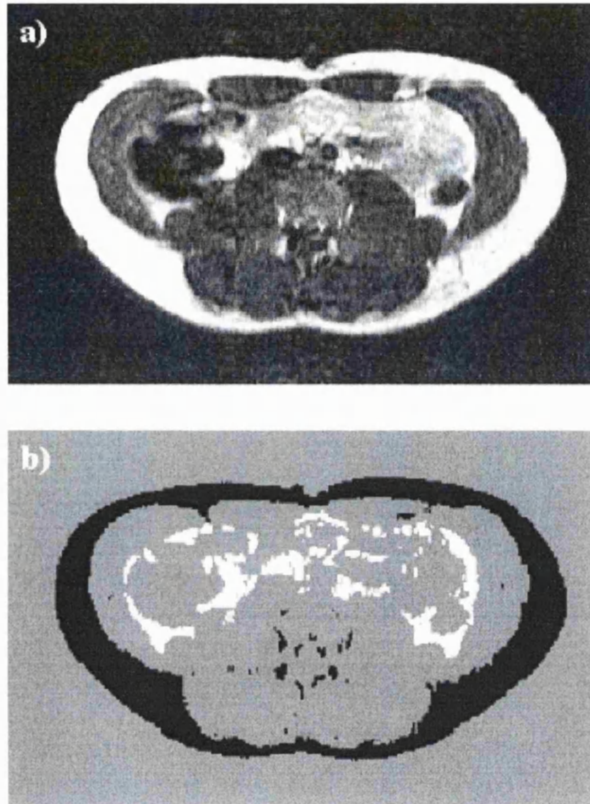


Table 4.1

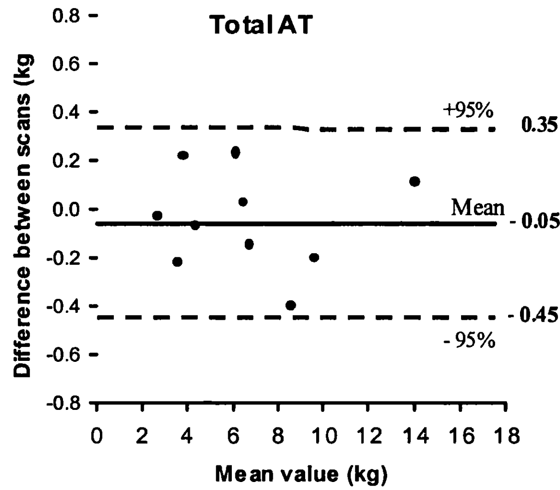
Adipose tissue mass measurements from repeated abdominal MR scans for 10 subjects. Values are in kg. AT = adipose tissue.

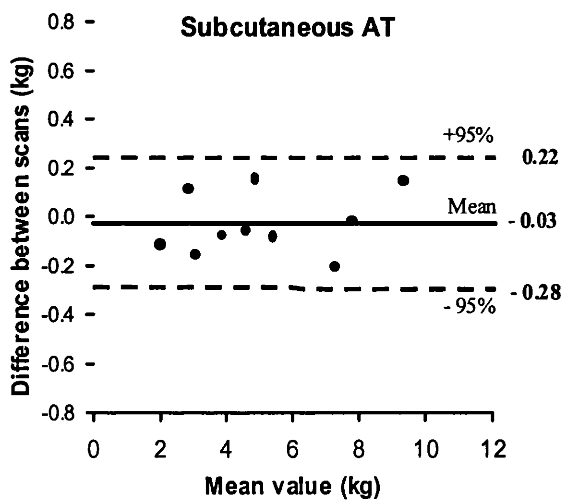
	1st scan	2nd scan	Mean absolute difference	% difference
Total AT	6.65	6.60	0.17	2.8 %
Subcutaneous AT	5.16	5.13	0.11	2.7 %
Visceral AT	1.48	1.47	0.09	8.0 %

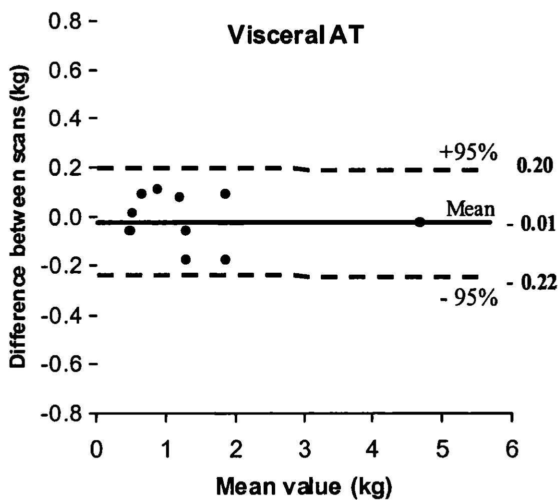
The repeated values were highly correlated ($r>0.99$; $p<0.0001$ for all three measurements), with Bland-Altman plots showing 95% confidence limits for total AT: –0.44 to +0.35, subcutaneous AT: -0.29 to + 0.22 and visceral AT: -0.22 to + 0.20 kg (figure 4.3). These results show excellent reproducibility of this technique, with differences of < 0.2 kg for total AT and < 0.1 kg for visceral AT between scans.

Figure 4.3

Bland-Altman plots showing 95% limits of agreement for repeated scans. a) total, b) subcutaneous and c) visceral adipose tissue.







4.2.3 STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS for Windows 6.0 (Tulsa, Oklahoma) with the critical alpha set at 0.05. The relationships between body size and composition variables and LV mass were initially checked for linearity (with zero intercept) using Tanner's 'special circumstance' calculation.[343] In this procedure the correlation coefficient, **r**, for each combination of body size and composition variable and LV mass was compared with the ratio of the coefficient of variations for the same two variables **cvX/cvY.** Where **r** is equal, or roughly equivalent (arbitrarily chosen as ±0.05) to **cvX/cvY,** a linear relationship with a zero intercept is evident. Conversely if these two terms are not similar then either a linear relationship does not exist or the linear relationship has a positive or negative **y** intercept - a theoretically and practically implausible situation.

Subsequently, allometric procedures were investigated to describe the relationship between each combination of LV mass with a body composition or size variable. The general allometric equation $\mathbf{Y = aX^b}$ can be linearised by taking natural logarithms of both sides of the equation: $\log\mathbf{Y} = \log\mathbf{a} + \mathbf{b}\log\mathbf{X} + \log\mathbf{e}$, where **e** is the multiplicative residual error term. This makes the data amenable to simple linear regression (log-log linear regression) where **b** is the slope of the log-log plot. Regression analysis utilised the ordinary least-squares technique. This approach, despite being the most commonly adopted in cardiac research does have an assumption that error and/or biological variation between subjects is only associated with the dependent variable, making accurate methods of assessment for LV mass all the more important.

4.3 RESULTS

The mean, standard deviation and range of values for LV mass and body composition and size variables are represented in Table 4.1. The computations of Tanner's 'special circumstance' data are reported in Table 4.2. Close similarity between the ratio of coefficient of variation data and the correlation coefficient was noted between LV mass and LBM. The use of ±0.05 as a comparison cut-off figure is arbitrary and individual comparisons should be subjectively interpreted for their degree of similarity. However, it is noticeable that the majority of other relationships produced very divergent ratios that would suggest either a non-linear relationship or a positive or negative **y** intercept. The ratio that came closest to, but outside, the arbitrary 0.05 cut-off was that for LV mass and height (0.09). This is supported by the data in column 3 where, although the

b exponent for height is 1.41, the confidence intervals are broad (±0.60) and thus include 1.0. Despite this, the scatter-plot of LV mass and height would not suggest a best-fit line that is linear and passes through the origin whilst optimising the reduction in residual error (see Figure 4.4a).

Table 4.1

Left ventricular mass (LVM) and body composition/size variables in the whole cohort. S/C = subcutaneous; SD = standard deviation.

	Mean ± SD	Range
LV mass (g)	183 ±27	122-255
Height (m)	1.75 ±0.07	1.59-1.91
Body mass (kg)	70.6 ±9.0	45.2-97.2
Body surface area (m^2)	1.85 ±0.14	1.48-2.22
Lean body mass (kg)	58.8 ±6.3	41.7-76.4
Fat mass (kg)	11.8 ±4.1	3.5-24.5
Visceral fat mass (kg)	0.97 ±0.22	0.34-2.95
S/C fat mass (kg)	10.86 ±3.80	2.92-22.68
Leg fat mass (kg)	3.81 ±1.33	1.15-7.78
Arm fat mass (kg)	1.11 ±0.15	0.35-2.58
Torso fat mass (kg)	6.90 ±2.58	1.90-14.56

Table 4.2

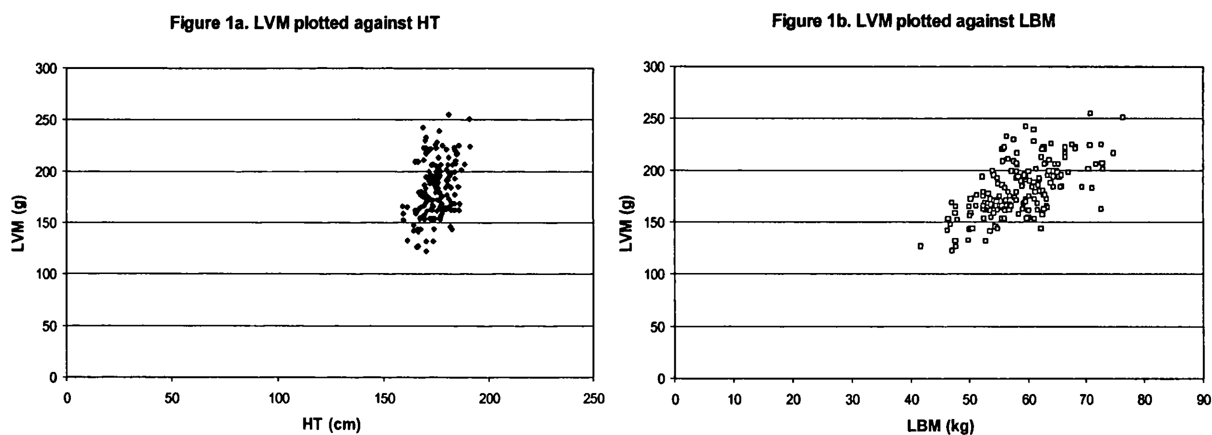
The relationships between left ventricular mass (LVM) and body size & composition variables. Column 2: A comparison of the correlation coefficient and the ratio of coefficient of variation (***r*** : ***cvX/cvY***); ratios are **BOLD** if the difference is ≤0.05. Column 3: The **b** exponents for the relationships (Data are mean ± 95% confidence intervals with r^2 values in brackets).

LVM vs:	***r* : *cvX /cvY***	**b** exponent ± 95% CI
Height (m)	0.34 : 0.25	1.41 ±0.60 (0.36)
Body mass (kg)	0.67 : 0.86	0.80 ±0.13 (0.66)
Body surface area (m^2)	0.62 : 0.50	1.30 ±0.24 (0.64)
Lean body mass (kg)	**0.65 : 0.70**	0.90 ±0.15 (0.66)
Fat mass (kg)	0.46 : 2.39	0.20 ±0.06 (0.48)
Visceral fat mass (kg)	0.30 : 3.34	0.12 ±0.05 (0.35)
S/C fat mass (kg)	0.46 : 2.33	0.20 ±0.06 (0.48)
Leg fat mass (kg)	0.44 : 2.39	0.19 ±0.06 (0.46)
Arm fat mass (kg)	0.36 : 2.83	0.13 ±0.06 (0.35)
Torso fat mass (kg)	0.44 : 2.53	0.19 ±0.06 (0.48)

The log-log linear regression analyses produced a range of **b** exponents describing the slope of the best-fit lines (Table 4.2). Most **b** exponents were significantly different from 1.0 and therefore represented curvi-linear relationships. Scatter-plots for LV mass against LBM and height are presented in Figures 4.4a and 4.4b, respectively. The figures visually demonstrate an essentially linear relationship between LV mass and LBM (**b**=0.90±0.16). This is compared to a curvi-linear relationship between LV mass and height (**b**=1.41±0.60), that has already been alluded to. A range of r^2 scores is represented in table 4.2 suggesting a degree of variability in the strength of association between relationships. The lowest r^2 was 0.36 for LV mass-height, suggesting greater residual variability in this relationship.

Figure 4.4

Scatter plots for left ventricular mass (LVM) against height (a) and lean body mass (b)



For adipose tissue, the relationship between LV mass and a range of FM variables is consistently low (**b** range – 0.12 to 0.20). The r^2 scores were generally lower for the fat variables than weight, BSA and LBM, again suggesting a greater degree of dispersion of data points from the best-fit line.

4.4 DISCUSSION

The data suggest that there are very few linear relationships between LV mass and body size and composition variables. This questions the widespread use of ratio scaling using these indices.

4.4.1 LEAN BODY MASS

The association between LBM and LV mass was the closest to a linear relationship, with the **b** exponent (0.90) not significantly different from 1.0 and similar to previous studies.[321] As may be predicted, the potential for a linear relationship occurs when the three-dimensional LV mass is paired with a three-dimensional body composition variable (LBM). This geometric similarity has been supported in a range of previous cardiac investigations.[321,323] Interestingly, the r^2-value for the LV mass-LBM relationship was one of the highest in this analysis suggesting the potential for a reduction in residual error with this scaling variable. This data supports the previous statements of Daniels[323] and Roman[344] that LBM is potentially the best scaling variable for LV mass. This may be intuitively sensible on the basis that LBM (mostly composed of skeletal muscle) represents a large, but variable, component of total mass that may place significant haemodynamic loads on the left ventricle (e.g. during exercise). Recent research has supported the use of LBM as it produced the strongest relationship to LV mass.[345] Hense also reported a strong relationship between LV mass-LBM[256] that, importantly, was independent of the effect of blood pressure, removed gender differences in LV mass as well as reducing the influence of FM. The advantage of using LBM is further enhanced by the ability to scale LV mass to LBM as a simple ratio.

The ability to measure LBM with MRI is severely limited however, and for most situations is impractical. Bioimpedance estimates of LBM are reasonably accurate when compared to MRI (5-6% differences; unpublished data from this study), are very quick to obtain, and may be a solution for practical purposes. The small inaccuracy of the technique is outweighed by the convenience and this would be a better index than any other scaling variable examined here, given the linearity of the relationship and the highlighted problems with other variables. Bioimpedance analysis has already been used in this way.[256,346]

4.4.2 ADIPOSE TISSUE

The association between MRI-derived adiposity variables and LV mass revealed an overall pattern of small positive relationships with LV mass. The partitioning of total FM into various sub-components, that has not been previously investigated, had no noticeable impact upon the nature of the relationship with LV mass. The range of **b** exponents supports the contention that LBM is a more important predictor of LV mass than FM.[256,345] However, unlike previous research[322,324,333,334] which reported no significant association between LV mass and FM (determined anthropometrically or via

bioelectrical impedance), the small positive relationship in the current study is statistically significantly different from zero. One explanation for this may be a confounding effect of LBM – if % body fat remains constant, a higher LBM would result in a proportionately raised FM. The increased LV mass (due to increased LBM) would be incorrectly associated with the FM. It remains possible however that FM has a small positive association with LV mass and the greater accuracy of FM estimation by MRI has elicited this.

4.4.3 ANTHROPOMETRIC VARIABLES

4.4.3.1 Body surface area

Of the anthropometrically determined scaling variables, only $BSA^{1.5}$ fits the concept of geometric similarity and supports similar previous findings,[347] though the similarity to other data[328] is not surprising given the similar sample demographics and the fact that the same BSA estimation equation was adopted. The widespread use of BSA as a ratio scaling variable must be seriously questioned however, given the non-linear relationship with LV mass in this and these other studies. If BSA was to be used, there would be some significant caveats. Ratio scaling using BSA may only be appropriate for cardiac areas and should be avoided unless empirically supported for the scaling of LV mass. It is likely that linear cardiac dimensions will scale to BSA raised to the power of 0.5 and LV mass will scale to the power of 1.5 but this requires further study. Furthermore, the validity of BSA computation, although simple, must be questioned as general prediction formulae are derived from limited sample sizes (9 subjects in the paper of Dubois and Dubois[338]). A definitive, accurate method has not been developed, nor accurate validation of this BSA calculation been done.

4.4.3.2 Height

The final scaling variable analysed was height. The data reported here could not preclude a ratio scaling approach for LV mass and height given the breadth of the confidence intervals. However, visual inspection of the data (Figure 4.4b) would not realistically support this but it does demonstrate a problem with the LV mass-height relationship. Geometric similarity would predict a **b** exponent for LV mass-height of 3.0 and some research has supported this relationship,[323] though the **b** exponent in the current study (1.41 $\pm$0.6) did not include this within the confidence intervals. Inspection of scaling research illuminates two key points. Firstly, empirical evidence[323,348] has reported a wide range of **b** exponents from 1.97 to 3.0 which is hard to defend from a theoretical

perspective. Secondly, data for the relationships between height and cardiac variables often display broad confidence intervals and high residual error,[321,322,328] similar to the current study. It has been postulated before[328] that in studies with a homogenous sample, primarily in terms of age, that the limited variability in height comparative to other scaling variables restricts the value of the statistical analysis. This argument could be applied to our sample of young, healthy adult males where the standard deviation for height was only 14 cm. On the basis of the findings in this study caution would be advised with the use of height as a scaling variable in cardiac research.

4.4.3.3 Weight

If a geometrically consistent relationship between LV mass-LBM could have been predicted then a similar association may also have been constructed for LV mass-weight. Although the upper confidence interval for LV mass-weight approaches linearity (0.93), it is significantly different from 1.0 and we could not support the ratio scaling of LV mass by weight. This finding is similar to previous research[321] and may be explained by the similarity in the subjects (young, healthy adults). Whilst some studies[328] have reported a **b** exponent around 1.0, it is not surprising that weight is different from 1.0 if LBM includes a linear relationship within its confidence intervals. For both weight and LBM to include the possibility of linearity, the difference between them must be relatively constant across the cohort. Given the broad range of body composition within the current sample this is unlikely to be true. Furthermore, the relatively lean study group (mean body fat 16.6%) meant that the effect of adipose tissue (which has a poor correlation with LV mass, see below) was minimised. Other groups with higher body fat content would be likely to have a poorer association between weight and LV mass. The ratio scaling of LV mass by weight would result in the underestimation of cardiac size in subjects who are heavier as a result of greater adiposity.

The nature of the relationship between LV mass and the scaling variables of weight and LBM has some striking parallels with research that has attempted to appropriately scale maximum oxygen uptake (VO_2max). Recent studies reported a similar range of **b** exponents for VO_2max-weight and VO_2max-LBM[349,350] The intuitive link between cardiac output, muscle bulk and muscle function variables is appealing and supported by these data.

4.4.4 LIMITATIONS

Whilst the current study has produced some extra insight into the relationship between LV mass and body composition and size variables, some limitations have to be noted. This study utilised a homogenous sample of young, healthy adult males. Whether the relationships described here persist in other groups is worthy of future study.

Based on this knowledge of scaling and cardiac - body composition relationships, there is a need to develop normal ranges for the allometrically scaled cardiac indices. These will be numerically different from any previous cardiac indices based on ratio scaling. For example, only with large scale analysis of normal and clinical populations will we be aware of what constitutes a critical cardiac index for LV mass/$BSA^{1.5}$. Finally, the practical issue of MRI availability for the determination of cardiac and body composition data in routine clinical practice must be noted. Most clinical work will continue to utilise echocardiography and relatively simple body composition techniques.

4.5 CONCLUSIONS

In conclusion this study provides the first analysis of the relationship between LV mass and body composition variables, determined using MRI. The calculated **b** exponent for LV mass-LBM is both geometrically consistent and supports previous scaling research that has used echocardiography and anthropometry or bioelectrical impedance. Bioelectrical impedance may represent a practical solution to measurement of LBM.

The relationship between LV mass-BSA was also found to be geometrically consistent and similar to previous research, though significant caveats remain. In particular, the use of BSA in a simple ratio standard cannot be supported due to the non-linearity of the relationship with LV mass. The ratio standard LV mass/$BSA^{1.5}$ may be acceptable, though further studies are required to determine normal values for this measure.

On the basis of this study, caution should be expressed with regard to the use of weight and especially height as scaling variables. Neither weight nor height were geometrically consistent, and height produced the weakest relationship with LV mass. Indices of adiposity produced only small positive relationships with LV mass. Finally, simple ratio standard procedures should only be used with LBM as the denominator. The use of other variables should be discouraged due to the non-linearity of the relationships.

CHAPTER 5

Cardiac metabolism and the renin-angiotensin system:

Effect of the ACE gene polymorphism in elite endurance athletes

5.1 BACKGROUND

5.1.1 CARDIAC METABOLISM AND THE RENIN-ANGIOTENSIN SYSTEM

Evidence for a role of the RAS in cardiac metabolism comes from the large trials of ACE inhibitors in heart failure – CONSENSUS,[351] SAVE,[134] SOLVD,[352] and AIRE.[133] All these trials showed a beneficial effect of ACE inhibitors on survival that was over and above the effect on blood pressure – the ACE inhibitors were in addition to conventional treatment including other hypotensive agents (e.g. hydralazine) and in the VheFT II study, hydralazine/isosorbide dinitrate did not share the same survival advantage. Given that a major problem in heart failure is the failure/reduction of tissue oxygen delivery, a beneficial effect would be likely to accrue from better use of the available substrate, both in the myocardium (resulting in increased cardiac contractile function) and in the peripheral tissues. This may be a mechanism by which ACE inhibitors have their effect.

Further evidence for a role in cardiac metabolism comes from the additional observation in these trials that the risk of ischaemic events (heart attacks and unstable angina) was reduced in the ACE inhibitor groups. Might an ACE inhibitor protect the heart from ischaemia by increased efficiency in the myocardial cells?

Other studies support the hypothesis that ACE inhibition has beneficial effects in ischaemia and cardiac function, particularly in LVH. Captopril reduced post-myocardial infarction ischaemia and improved cardiac output in humans[140] and in an isolated ischaemic rat heart model, improved myocardial oxygen consumption, cardiac haemodynamics and reduced arrhythmias.[353] In post-myocardial infarction heart failure, ACE inhibitors improved the decline in myocardial high energy phosphates & mitochondrial oxidation.[354]

5.1.1.1 Left ventricular hypertrophy

LVH increases the susceptibility of the myocardium to ischaemia, through increased diffusion distances and reduced coronary vasodilator reserve.[355] ACE inhibitors improve cardiodynamics, increase myocardial energy reserves (ATP and creatine phosphate) and reduce lactate, even at low doses.[251] These effects were prevented by concomitant bradykinin inhibition and although losartan had similar effects, this was at hypotensive (vasodilating) doses. These data suggest the mechanisms for the

improved cellular metabolism involve both increased blood flow (angiotensin and kinin mediated) and direct intracellular effects of bradykinin.

The risk of arrhythmias also increases with LVH,[356] which may be a manifestation of the increased ischaemic susceptibility. Both ACE inhibitors and angiotensin II receptor blockers can reduce the risk of arrhythmias and sudden death (presumed to be arrhythmic) in LVH,[357,358] though the mechanism remains unclear – it may be a consequence of the reduction in LV mass per se rather than a direct anti-arrhythmic effect of the RAS antagonists. Baxter and Yellon showed a similar reduction in arrhythmias associated with a reduction in LV mass without RAS antagonists.[359]

Given the detrimental effects of left ventricular hypertrophy on cardiac metabolism, it is difficult to distinguish specific effects of the RAS on metabolism from those on LVH, as the two may be interdependent.

Cardiac growth might also be related to cellular metabolism in a different way – if a heart made better use of the available substrate, it might be able to cope with higher demand without the need to hypertrophy. This raises the question of whether cardiac growth is beneficial or a maladaptive process due to an inability to cope with increased workload. Skeletal muscle might also have an effect on cardiac growth – a less efficient muscle mass would require greater cardiac output to maintain the same external workload, placing an increased hypertrophic burden on the heart.

5.1.2 THE SKELETAL MUSCLE RAS AND METABOLIC EFFECTS

There is increasing evidence for a local RAS involved in skeletal muscle metabolism, particularly via kinin-mediated pathways.[360] Bradykinin can increase glucose uptake which is independent of blood flow and inhibited by a bradykinin β_2 receptor inhibitor.[361] It also appears to have a direct effect on reducing mitochondrial oxygen consumption, mediated by nitric oxide.[362] Both of these may provide mechanisms by which ACE inhibitors could be beneficial, particularly in cardiac failure in which tissue substrate delivery is poor. The increased bradykinin could improve skeletal muscle substrate use and thus also reduce the burden placed on cardiac output.

5.1.3 THE ACE GENE I/D POLYMORPHISM

The study of human tissue renin-angiotensin systems and their importance in metabolism is restricted by access to cardiac tissue and difficulties with *in vivo*

experiments. Genetics may again be a useful tool for examining this area, and the ACE gene is a good candidate. There were no studies of the ACE gene I/D polymorphism and cardiac metabolism prior to commencing this work, though one study showed an increased mortality in DD patients with idiopathic heart failure.[363] The mechanism is unclear however - it may be related to cardiac metabolism or to other effects of the increased left ventricular hypertrophy also seen in the DD group. Although tissue levels of ACE are influenced by the ACE genotype,[151,364] no studies in skeletal muscle have been done.

Some of the effect of the ACE gene I/D polymorphism on LV mass might be via cardiac metabolism – if the II genotype (and reduced ACE levels) was associated with increased metabolic efficiency, this might result in reduced cardiac growth via the mechanisms outlined above. Similarly, better substrate use in skeletal muscle in those with the II genotype might reduce the burden on the heart.

5.1.4 STUDY DESIGN

How might any effects of the ACE gene polymorphism on metabolic efficiency be further examined? Any phenotypic differences between genotypes are likely to be small, particularly in normal individuals, because great efficiency is unnecessary in modern daily living. Only in extreme situations might a small advantage have noticeable effects.

Physical exertion at the extremes of endurance would provide such an environment, where a small genetic advantage in metabolic efficiency might exert a detectable effect on LV function. If the physical endurance was at an extreme such that only the most efficient were able to achieve success, particularly in a competitive environment, then we might expect to see dropout of the least favourable genetic groups and a selection bias in favour of the more efficient genotype. Such a bias would result in a disproportionately high number of the 'efficient' genotype in this elite group. Two suitable subject groups for study were identified who were exposed to extreme endurance conditions and a potential selection bias:

i) High altitude mountain climbers – they perform hard physical exertion at altitudes with up to 1/3 less oxygen. Survival is the greatest challenge in this harsh environment, where mortality is high and any small advantage could make an important difference. Those with a less favourable genetic predisposition would

be likely to drop out from continuing, either through choice (exhaustion) or from increased mortality.

ii) Olympic endurance athletes. Inherited ability (i.e. a favourable genetic predisposition) is one factor which is important for a successful international athlete, though no specific gene had been identified which had an effect. Even a small competitive advantage would make a noticeable difference in this highly competitive world.

The above two groups were studied to determine if a difference in the frequency distribution of the ACE I/D genotype existed compared to a control group, suggesting a genetic advantage for one particular genotype. This would suggest a role for ACE and local renin-angiotensin systems in cardiac and/or skeletal muscle energetics.

5.2 METHODS

5.2.1 SUBJECTS

5.2.1.1 High-altitude mountaineers

The British Mountaineering Council were contacted to identify all potential British climbers with a history of ascending beyond 7,000 metres without the use of supplementary oxygen. The thirty three candidates were contacted by post, enclosing a mouthwash sample for buccal cell collection described in chapter 2. There were 25 male respondents and DNA was extracted and ACE genotype determined using the three-primer polymerase chain reaction amplification described in chapter 2. Genotype distribution was compared with that of the control group.

5.2.1.2 Endurance athletes

All 1086 elite athletes selected by the British Olympic Association as potential Olympic competitors were contacted enclosing the same mouthwash sample as above. We were particularly interested in long distance runners and sprinters in whom a good comparison can be made due to similar muscle use over differing distances, thus involving differing types of metabolism (relative proportions of aerobic/anaerobic). Of the 576 respondents (response rate: 53%), 495 had sufficient DNA for PCR amplification, of which 91 were runners (48 men, 43 women; 79 Caucasian) who

competed over twelve distances ranging from 100m to 100km (sprinters to ultra-marathon runners). There were 404 respondents from other sports (219 men; 185 women) participating in 19 disciplines. ACE genotype was determined using the same three-primer polymerase chain reaction as above.

The frequencies of the *I* allele amongst the runners as a group was compared with that of the control subjects. Within-group analysis of allele frequency was also performed across the 12 distances run. These were grouped according to the type of muscle metabolism involved:[365]

≤ 200m	predominantly anaerobic, or power-generating
400-3000m	mixed aerobic & anaerobic
≥ 5000m	predominantly aerobic or endurance-trained

This segregation was confirmed by two experts in the field (David Jones, professor of sport and exercise physiology, Birmingham University and Richard Godfrey, chief physiologist, British Olympic Medical Centre).

Finally, the allele frequency amongst the remaining 404 elite athletes was compared to that of the controls.

5.2.1.3 Control group

A control group of 1,906 British males free from clinical cardiovascular disease was identified[147] in whom the ACE gene I/D frequency was known: relative frequency II=0.24, ID=0.50, DD=0.26; *I* allele = 0.49. The same control group is used in section 3.4.

5.2.2 STATISTICAL ANALYSES

Genotype and allele frequencies between control and subject groups were compared by chi squared test. The frequency of the *I* alleles across the twelve competitive distances were compared by chi squared test for linear trend using the distance run as the categorical variable. P values of <0.05 were considered statistically significant.

5.3 RESULTS

5.3.1 HIGH ALTITUDE MOUNTAINEERS

Mean age was 40.6 ±6.5 years in the 25 subjects, and 55.6 ±3.2 years for the control group. The distribution of genotypes in both groups were in Hardy-Weinberg equilibrium. Both genotype distribution and allele frequency differed significantly between climbers and controls (table 5.1 and figure 5.1) with a relative excess of II genotype and deficiency of DD genotype found in the climbers. Among the 15 climbers who had ascended beyond 8,000 m without oxygen, none were homozygous for D (6 II and 9 ID. *I* allele frequency = 0.65). Further, ranked by number of ascents above 8,000 m without oxygen, the top performer was homozygous for *I* (5 ascents, compared with a mean of 2.4 ± 0.3 ascents), as were the top two in this group for number of additional 7,000 m ascents (>100 and 18, compared with a mean of 10.3 ± 6.5 ascents).

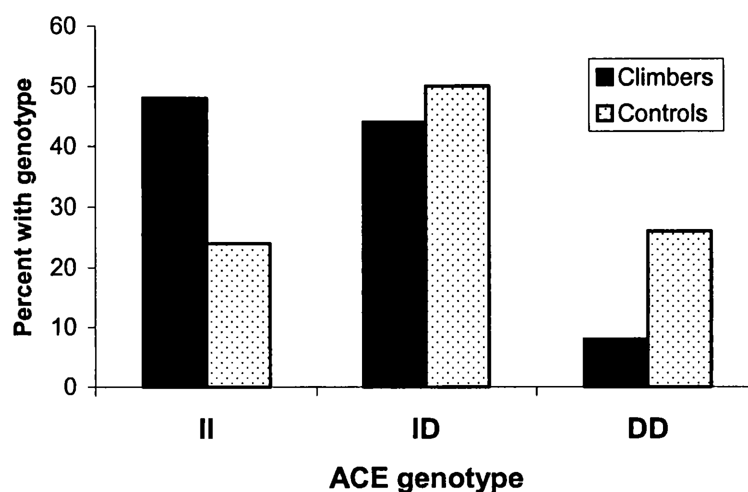
Table 5.1

ACE genotype distribution and *I* allele frequency among high altitude climbers and controls. Values are absolute numbers with relative frequency in brackets for genotype.

	Genotypes			*I* allele frequency
	II	**ID**	**DD**	(95% confidence intervals)
Climbers	12 (0.48)	11 (0.44)	2 (0.08)	0.70 (0.57 – 0.83)
Controls	457 (0.24)	953 (0.50)	496 (0.26)	0.49 (0.47 – 0.51)

Figure 5.1

Distribution of ACE I/D genotypes in 25 high altitude climbers and 1906 controls



5.3.2 OLYMPIC ATHLETES

5.3.2.1 Runners

Analysis of the runners revealed a linear trend of increasing *I* allele frequency with distance run, when grouped into ≤ 200m (predominantly anaerobic), 400-3000m (mixed aerobic and anaerobic) and ≥ 5000m (predominantly aerobic); see table 5.2. The proportion of *I* alleles increased from 0.35 to 0.53 and 0.62 amongst those running ≤ 200m (n=20), 400-3000m (n=37) and ≥ 5000m (n=34) respectively: p = 0.009 for linear trend (Figure 5.2). This association remained when the thirteen runners who competed in hurdling events were excluded (*I* allele proportions 0.32, 0.62 and 0.62; n=14, 30 and 34 respectively for the three distance groups: p = 0.020 for linear trend).

When analysed with a conventional chi squared test, the observed numbers of both genotypes and alleles in each group were significantly different from expected values (p = 0.019 and 0.012 for genotypes and alleles respectively). This was due to higher frequencies of the *I* allele in the ≥ 5,000m group and of the *D* allele in the ≤ 200m group.

Table 5.2

a) The relative *I* allele frequency of 91 Olympic-standard runners and 1906 controls (p=0.009 for linear trend). b) The same analysis for the 79 Caucasians alone (p=0.180 for linear trend). Values are relative frequency ± standard error

All runners

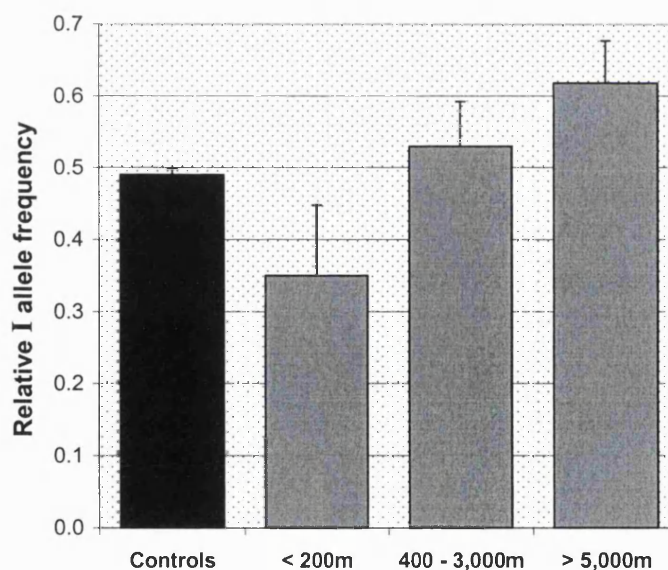
Distance run		ACE genotype			I Allele frequency	
		DD	ID	II	(95% confidence limits)	
≤ 200m	(n =20)	0.45	0.40	0.15	0.35	(0.20-0.50)
400 - 3,000m	(n =37)	0.19	0.57	0.24	0.53	(0.41-0.64)
≥ 5,000m	(n =34)	0.18	0.41	0.41	0.62	(0.50-0.73)
All distances	(n = 91)	0.24	0.47	0.29	0.52	(0.45-0.59)

Caucasians

Distance run		ACE genotype			I Allele frequency	
		DD	ID	II	(95% confidence limits)	
≤ 200m	(n =13)	0.31	0.46	0.23	0.46	(0.27-0.65)
400 - 3,000m	(n =32)	0.16	0.56	0.28	0.56	(0.44-0.69)
≥ 5,000m	(n =34)	0.18	0.41	0.41	0.62	(0.50-0.73)
All distances	(n = 79)	0.19	0.48	0.33	0.57	(0.49-0.65)

Figure 5.2

The relative *I* allele frequency of 91 Olympic-standard runners and 1906 controls



5.3.2.2 Other disciplines

The *I* allele frequency in other sports as a whole was no different from controls: 0.50 vs. 0.49 respectively (p = 0.526). Individual sports did not show any significant excess of the *I* allele (Table 5.2):

Table 5.2

ACE genotype distribution and *I* allele frequency among 414 Olympic-standard athletes. P value refers to the chi-squared analysis compared to the expected values from the control group (values <0.05 in bold).

SPORT		ACE genotype (relative frequency)				*I* allele frequency	
		DD	ID	II	p value		p value
Discus, hammer, javelin	(n = 12)	0.25	0.50	0.25	1	0.50	1
High, long & triple jumps	(n = 9)	0	1.00	0	**0.027**	0.50	1
Pole vault	(n = 7)	0.14	0.86	0	0.147	0.43	0.593
Shot putt	(n = 7)	0.14	0.71	0.14	0.535	0.50	1
Triathlon	(n = 10)	0.20	0.60	0.20	0.766	0.50	1
Badminton, tennis	(n = 17)	0.18	0.71	0.12	0.325	0.47	0.732
Canoeing	(n = 28)	0.25	0.54	0.21	0.898	0.48	1
Diving/gymnastics	(n = 23)	0.17	0.52	0.30	0.659	0.57	0.376
Hockey	(n = 53)	0.25	0.47	0.28	0.768	0.52	0.560
Ice Hockey	(n = 34)	0.26	0.50	0.24	1	0.49	1
Figure skating	(n = 13)	0.15	0.69	0.15	0.538	0.50	1
Speed skating	(n = 12)	0.25	0.50	0.25	1	0.50	1
Judo/taekwondo	(n = 20)	0.20	0.40	0.40	0.301	0.60	0.206
Rowing	(n = 61)	0.20	0.52	0.28	0.522	0.54	0.277
Skiing	(n = 12)	0.17	0.33	0.50	0.135	0.67	0.102
Swimming	(n = 64)	0.39	0.42	0.19	0.076	0.40	**0.034**
1500m swimming	(n = 4)	0.25	0.25	0.50	0.472	0.63	0.480
Weightlifting	(n = 12)	0.17	0.50	0.33	0.717	0.58	0.414
Wrestling	(n = 6)	0.50	0.50	0.00	0.472	0.25	0.083
TOTALS	**(n = 404)**	0.24	0.52	0.24	0.650	0.50	0.526

5.4 DISCUSSION

These results support the hypothesis that the insertion (*I*) allele of the ACE gene may be associated with improved endurance performance. The data suggests *I* allele frequency is greater in successful high altitude mountaineers and longer-distance runners than controls, and rises with the distance run in the athletes.

5.4.1 ATHLETES

5.4.1.1 Race and sex influences among the runners

The racial mix of the cohort studied may have influenced our results, as ACE genotype distribution differs between races.[366] The association of the *I* allele with endurance performance is, however, unlikely to have been due to the effects of race alone. Firstly, only blacks and Caucasians were represented amongst the athletes studied, and it has been shown that ACE genotype distributions in healthy African and Caribbean blacks in the UK are no different from healthy Caucasians in a British population sample (II,ID,DD = 0.18, 0.50, 0.32 for both ethnic groups; *I* allele frequency = 0.43).[367] Secondly, when only the 79 Caucasian runners are included in the chi squared analysis, allele frequency still differed significantly from controls (p = 0.039). This was again due to an increasing frequency of the *I* allele in those running longer distances, with the proportion of *I* alleles rising from 0.46 amongst those running ≤ 200m, to 0.56 and 0.62 in those running 400-3000m and ≥ 5000m respectively (p = 0.180 for linear trend). Chi squared for Caucasian *genotypes* was not significantly different from controls (p = 0.091). The association of *I* allele frequency with running distance amongst those of different races requires further study, given the small number of non-Caucasians represented in this sample, on which no conclusion can be drawn.

Men and women were included together in the groups as the ACE gene is not sex-linked, and allele frequency is therefore the same in males and females. However, again due to limitations of sample size, we were unable to examine any differential impact of genotype on performance between sexes.

5.4.1.2 Other sports

The *I* allele of the ACE gene may thus be associated with improved endurance performance, and such an effect might influence general sporting prowess in the potential Olympians engaged in other disciplines. The *I* allele frequency in other sports

as a whole was no different from controls: 0.50 vs. 0.49 respectively ($p = 0.526$) and individual sports did not show any significant excess of the *I* allele (Table 6.2). These results should be interpreted with caution however, as the limitations in sample size imposed by the elite nature of the athletes studied reduces the number of athletes represented in each of the 19 sporting disciplines. In particular, there may be sports in which endurance is an important but not prime determinant of success. In these, any effect of the *I* allele may be masked in such a small group. Nonetheless, it is interesting to note the excess of the *D* allele in swimmers: *D* allele frequency 0.60 vs. 0.51 for swimmers and controls respectively ($p = 0.034$). Most swimming events are undertaken in under 2 minutes and thus power, rather than pure endurance characteristics, may play a key role (personal communication: Richard Godfrey, chief physiologist, British Olympic Medical Centre). The suggestion of a possible advantageous effect of the *D* allele in power sports is supported by the presence of an excess frequency of the *D* allele amongst sprinters when compared to controls and elite endurance runners (*D* allele frequency 0.62 in those running $\leq$ 200m). Given the recognised growth-stimulating properties of angiotensin II (section 1.4.2.3), the higher levels associated with the *D* allele might partly account for this postulated effect through an increase in skeletal myocyte fibre size.

5.4.2 CLIMBERS

The data showed a marked increase in *I* allele frequency in the climbers studied, compared to controls, suggesting an endurance advantage. This selection bias may occur through greater drop-out of DD subjects who cannot continue with climbing at this high level, or in those who do continue, a higher death rate. High altitude mountaineering is a highly dangerous activity, with many deaths each year. Although environmental conditions (weather, ice, snow, rocks) play an important part in the risks, physical fatigue is important for coping with these extremes and reduced fitness (either mental or physical) can have dire consequences in the harsh environment. Demonstrating a higher death rate in DD subjects would be difficult. It would require obtaining DNA from the deceased or a prospective study following the relatively small group of elite climbers over many years which would lack statistical power. Further attempts to investigate these groups might look at physiological data in addition to genetic (e.g. VO_2max, work capacity).

The small size of the sample is a potential criticism, though it is difficult to strike a balance between the need for an elite group (necessarily small) with a larger but more heterogeneous group, less likely to demonstrate an endurance advantage. Using a

larger group (e.g. of international climbers) leads to problems in identifying an adequate control group (see below).

5.4.3 POTENTIAL CONFOUNDING FACTORS

5.4.3.1 Control group

The studies were limited to British climbers and athletes as it is important to have a good control group for comparison. Including international subjects would have led to problems identifying an adequate comparison group, which is crucial in comparing genotype frequencies. The control group in this study had a mean age of 55.6 ± 3.2 years, compared to 40.6 ± 6.5 years in the climbers, though this 15 year difference is unlikely to have affected the results. For a difference in ACE genotype frequency with advancing age to occur, we must assume the attrition (through death) of one or other allele. If this occurred in those with the *D* allele, the higher *D* allele frequency in a younger control group would exacerbate the difference between these and the climbers, making the data stronger. Only attrition of the *I* allele would confound the results, though it is the *D* allele and increased ACE levels which has been associated with coronary disease,[152] LVH and increased mortality,[363,368] making any attrition of the *I* allele unlikely.

5.4.3.2 Group sampling

The studies presented here relied upon the return of samples from an unsolicited mailshot. With this type of 'survey', the rate of return can often be very low, particularly as we were asking for a genetic sample from top athletes – potentially sensitive information, even with the strict anonymity applied. If only a small proportion of subjects responded, it may have reduced the validity of the results. The 75% return in the climbers and a 53% return in the athletes was higher than usual for postal surveys, and probably reflects the subjects' interest in the area. There is no reason that the response rates should differ between ACE I/D genotypes, given that the subjects were unaware of their genetic identity.

5.4.4 POTENTIAL MECHANISMS IN ATHLETES

There is little direct evidence for the mechanism of association of the *I* allele with endurance performance but several possibilities exist. The effects may not be related to the ACE gene polymorphism itself but rather to a close genetic polymorphism with

which the ACE polymorphism is in linkage disequilibrium, as discussed for LV hypertrophy (section 3.5.4.6). There is no current evidence for linkage disequilibrium with factors influencing the expression of the neighbouring growth hormone gene – the most likely candidate.[299] Assuming that ACE is part of the mechanism, several pathways may be involved:

5.4.4.1 Glucose uptake and substrate use

The local RAS may alter the sensitivity of skeletal muscle cells to insulin and increase the uptake of glucose - the optimal fuel for metabolism - from the circulation. Both systemic and local paracrine ACE inhibition can increase insulin-mediated glucose uptake in skeletal muscle[369,370] which is independent of capillary flow.[370] These effects appear to be bradykinin mediated[371-374] as bradykinin can directly stimulate glucose uptake[374] and GLUT 4 receptor upregulation,[372] which are inhibited by bradykinin β2 receptor inhibition.[374] Some of the effect may be via bradykinin stimulation of endothelial-derived nitric oxide (NO)[375,376] and thus increased capillary flow. However, there is also evidence for a direct role of nitric oxide in controlling substrate utilisation – it increased the uptake of glucose, decreased that of free fatty acids, and reduced myocardial oxygen consumption, effects which were reversed by a NO synthase inhibitor.[377] The same study also showed angiotensin II increased myocardial oxygen consumption and lactate uptake.

Thus, lower ACE levels associated with the *I* allele (or ACE inhibition) could be associated with reduced myocardial oxygen consumption and changes in substrate use by both kinin and angiotensin II-mediated mechanisms, though kinins appear to have the dominant role. In the skeletal myocyte, in which a local RAS also exists,[360,378] the increased availability of high-energy fuel could enhance performance.

5.4.4.2 Metabolic efficiency

The effect of the local RAS on cellular metabolic efficiency has mostly been studied in the heart where, by modulating kinin levels, tissue ACE activity may influence metabolic efficiency. During ischaemia, locally administered bradykinin can improve energy-rich phosphate levels (adenosine triphosphate and creatine phosphate), reduce lactate concentration and preserve glycogen stores while improving cardiodynamics (left ventricular pressure and contractility).[353,379] It can also limit infarct size, an effect which is abolished by bradykinin inhibitors.[380] Ramipril improved several indices of cardiac function and metabolism, including decreased lactate and increased ATP and

creatine phosphate, the effect of which was blocked by the bradykinin β2 receptor antagonist, HOE140.[251] This evidence strongly suggests an improvement in myocardial metabolic efficiency mediated by bradykinin through a local RAS. Similar studies are required in skeletal muscle.

The action of bradykinin is likely to be mediated via coronary endothelial nitric oxide. Bradykinin, kallikrein and kininogen all increased nitric oxide release from isolated coronary vessels, which was inhibited by kinin antagonists and N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase (NOS).[381] This endothelial-derived nitric oxide (NO) reduces myocardial oxygen consumption via its actions on the mitochondrial respiratory chain,[382,383] an effect which is mimicked by bradykinin.[382] Further evidence for this bradykinin-nitric oxide mediated control mechanism comes from several knockout gene studies. Bradykinin induced a reduction in myocardial oxygen consumption in wild type (inhibited by L-NAME) but not endothelial NOS knockout mice.[384] It also had no effect on oxygen consumption in isolated hearts from ecNOS knockout mice, though its action was restored by the addition of coronary microvessels capable of producing NO.[385] In a bradykinin β2 receptor knockout model, bradykinin and an ACE inhibitor had no effect on oxygen consumption, compared to a reduction in wild-type mice, inhibited by L-NAME.[386] In all these studies, an exogenous NO donor, S-nitroso-N-acetyl-penicillamine (SNAP) had the same effects as bradykinin on both the wild-type and knockout mice, confirming the importance of NO.

5.4.4.3 Summary

The decreased tissue ACE levels associated with the *I* allele would increase local bradykinin levels and may improve performance via the mechanisms highlighted above. Under normal circumstances, when nutrient supply is adequate, this efficiency is not necessary and therefore no advantage would accrue. Only under situations of extreme metabolic stress, such as during extreme endurance exercise, would this genotype be of an advantage and a selection bias exist.

5.4.5 THE KININ-NITRIC OXIDE PATHWAY IN HEART FAILURE

Baseline NO production in endothelium from failing hearts appears to be reduced,[387] and this may be one of the pathological mechanisms involved in heart failure. ACE inhibitors can partially restore this imbalance: they can increase NO levels within ischaemic[388] or failing[389] myocardium and the NO-mediated reduction in myocardial oxygen consumption with bradykinin and ACE inhibitors remains intact in failing human

hearts,[390] though NO levels are still reduced compared to normal controls.[387] ACE inhibition has also been shown to improve myocardial energy metabolism post myocardial infarction, using *in vivo* cardiac spectroscopy.[391]

This mechanism may be one way in which ACE inhibitors have their beneficial effect in heart failure. It is interesting to note similar beneficial effects of left ventricular assist devices in improving mitochondrial function in end-stage heart failure.[392] One study has suggested involvement of the kinin-NO pathway in the improvement with these devices.[393]

5.4.6 OTHER RECENT STUDIES IN THIS FIELD

5.4.6.1 Frequency distribution of the ACE I/D polymorphism

Two studies have examined the ACE gene and exercise physiology. The first examined the I/D frequency in 60 professional cyclists, runners and handball players and found a similar increased frequency of the *I* allele compared with controls.[394] This agrees with our data, though the group studied are much more heterogeneous. The second study looked at a very small group of Kyrghyz highland men, living at altitude, to determine if the incidence of high altitude pulmonary hypertension differed between genotypes.[395] The result was a higher prevalence of the *I* allele in those subjects with pulmonary hypertension – a surprising finding given the vasoconstrictive effects of angiotensin II and the previous beneficial evidence for the *I* allele. However, the study is seriously flawed by the small number of subjects – 21 with and 16 without pulmonary oedema, and the only real difference was between the 9 II genotypes with and the 1 without pulmonary hypertension – a finding that could easily have been due to chance.

5.4.6.2 ACE I/D polymorphism and survival/cardiac function

In idiopathic dilated cardiomyopathy,[310] the DD genotype was associated with greater LV dilation and reduced ejection fraction – a similar situation to Andersson's earlier study showing greater LV mass, reduced ejection fraction and a markedly reduced 5 year survival rate in DD subjects with idiopathic heart failure (49% vs. 72%), despite similar levels of ACE inhibitor treatment.[363] While it could be argued that the increased LV mass (153 vs. 134 g/m^2 in DD vs. ID/II; p=0.019) and reduced ejection fraction (0.39 vs. 0.43 in DD vs. ID/II; p=0.12) was a significant factor in the survival, despite the lack of statistical significance for the latter, it is precisely these physiological parameters that may be affected by ACE genotype and separating these would be difficult.

5.4.6.3 RAS inhibition and survival/cardiac function

A meta-analysis confirmed the reduction in sudden cardiac death with ACE inhibitors post myocardial infarction[368] and van Veldhuisen's study[396] suggested greater benefit in exercise capacity with higher vs. lower doses of ACE inhibitors used in heart failure, though plasma ACE levels were not different between the differing dosage groups – supporting evidence for the importance of the local RAS.

Angiotensin II blockade also had beneficial effects on survival in post-MI rats.[264] The mechanism for the latter effect was not commented on, apart from favourable haemodynamics. This debate is interesting as there is recent evidence to support the benefits of angiotensin II receptor blockade in heart failure – the Elite II trial[397] showed no difference in survival between ACE inhibitors and angiotensin II receptor blockers and the ValHeFT trial[398] showed an improvement with the addition of valsartan to standard heart failure therapy, including ACE inhibitors. This is supported by a porcine pacing-induced heart failure model suggested the combination of both agents was superior to either alone, which was accounted for primarily by the improvement in haemodynamic parameters.[399] These studies suggest a beneficial mechanism for angiotensin II receptor blockade, distinct from the kinin-NO mediated pathways for ACE inhibitors outlined above. Potential mechanisms include reduced sympathetic nervous system stimulation[287] and a prevention of the decline in membrane-bound GLUT4 glucose transporters,[400] resulting in improved glucose uptake.

5.4.7 LIMITATIONS

These studies measured only genotype frequencies, with inferences made about mechanisms for increase in one genotype. No physiological measurements were made which would have strengthened the data. However, access to both groups studied was difficult as they live in all areas of the UK, often in remote locations (particularly the climbers) and this limits the potential for physiological assessment. The postal sample collection system enabled us to study an otherwise poorly accessible group of elite endurance athletes who would have been almost impossible to reach by other means.

In addition, we postulate that much of the ACE gene effect is through local tissue renin-angiotensin systems (cardiac & skeletal muscle), which are difficult to study in humans. Examination of tissue biochemistry and physiology would have required tissue biopsies which are likely to have been declined by the participants and are probably unethical (cardiac biopsy particularly). Furthermore, the mechanisms involved are exercise

related and phenotypic differences between genotypes may well have been similar at baseline, with differences only apparent during hard exercise. Further studies are required to study these processes, perhaps utilising magnetic resonance spectroscopy to quantify high energy phosphates or fluoro-deoxy glucose positron emission scanning (FDG-PET) to study glucose uptake in both cardiac and skeletal muscle.

It is important to note that factors other than the ACE gene are important for endurance performance. In both the high altitude climbers and the long distance runners, there were subjects of DD genotype, indicating their ability to perform at a top level. It is likely that there are many other genes containing polymorphisms which may affect athletic ability, and all of them interacting with environmental stimuli such as training and conditioning to have a combined effect on performance.

5.5 CONCLUSIONS

The hypothesis that elite groups subject to extreme endurance conditions would have a higher frequency of the II genotype has been confirmed. In both high altitude mountaineers and Olympic long distance runners, there was a greater than expected proportion of the II genotype and *I* allele. Additionally, in the track athletes, there was an increasing frequency of the *I* allele with distance run. These findings are supported by the effectiveness of ACE inhibitors in heart failure and ischaemic heart disease, both conditions where additional efficiency would be beneficial.

The mechanism for this is unclear but may involve increased metabolic efficiency of cardiac and/or skeletal myocytes with the lower ACE levels associated with the *I* allele. There is considerable evidence for involvement of a kinin-nitric oxide pathway affecting mitochondrial oxygen consumption, and some evidence for increased kinin-mediated glucose uptake. Angiotensin II receptor blockers also appear to be beneficial in heart failure and the mechanism here is unclear – modulation of the sympathetic nervous system is a possibility.

The understanding of the mechanisms of cellular efficiency has important applications beyond the world of extreme endurance sports. The management of heart failure and myocardial infarction has improved with RAS antagonists and a greater understanding of the mechanisms involved would be of major benefit in these and other situations of extreme metabolic stress, such as unstable angina, adult respiratory distress syndrome and severe pancreatitis, where intervention with appropriate pharmacological agents, such as ACE inhibitors, may one day improve outcome.

CHAPTER 6

General discussion

6.1 SUMMARY AND CONTEXT

The research shows that the RAS is an important system for determining the response to exercise training, particularly for LV growth but also for endurance exercise. A likely mechanism for the latter involves metabolic efficiency and the control of mitochondrial respiration via a kinin-NO pathway. This may explain the beneficial effects of ACE inhibitors in heart failure and ischaemic heart disease and the increased mortality of the *D* allele.

The ACE gene I/D polymorphism is not the only factor involved however, as shown by the presence of the DD genotype in the elite endurance groups, albeit at a lower frequency. The polymorphism exists in the context of other genetic polymorphisms and differing environmental stimuli, all of which play a role in the gene-environment interaction.

6.1.1 ACE I/D POLYMORPHISM IN THE POPULATION

One important unanswered question is why the ACE gene I/D polymorphism should exists in the (Caucasian) population in a roughly 1:2:1 frequency if the *D* allele is associated with a greater mortality? One might expect a much reduced frequency of the *D* allele with such an evolutionary disadvantage. The answer may be that the diseases for which ACE has been shown to be important (LV hypertrophy, heart failure, ischaemic heart disease) occur predominantly over the age of 45. Not only has reproduction occurred for most people by this age (removing any evolutionary effect), but it is also only within the last hundred years of human history that life expectancy has been much beyond this age. This amount of time (3-5 generations) is too short to expect a reduction in population frequency of one allele. Additionally, the postulated effect of the ACE gene on metabolic efficiency would only have advantages in situations of extreme stress – the majority of the population have no need for increased efficiency in times of adequate food and oxygen supply.

6.1.1.1 RAS effects on male fertility

An alternative explanation may lie in the role of a local testicular RAS in spermatozoal function. ACE inhibitors reduce sperm motility and ovum penetration in rats[401] and humans,[402] which appears to be mediated via angiotensin II type 2 receptors,[402] and suggests that high ACE levels may be beneficial for male fertility. ACE exists in two isoforms – somatic and testicular - encoded from the same gene by different

transcription enzymes in the differing locations.[403] In ACE gene knockout mice, the sperm exhibit greatly reduced oviduct transport and ovum binding, but restoration of the testicular isozyme with gene targeting results in the restoration of sperm function and fertility,[404,405] confirming the importance of testicular ACE. Humans also exhibit these two isozymes[17] and if the ACE gene I/D polymorphism affected levels of the testicular isozyme in a similar way to the somatic form, the increased fertility associated with the *D* allele could explain its persistence in the population. The only study to date however has shown no effect of the I/D polymorphism on sperm (testicular) or seminal fluid (somatic) ACE levels.[406]

6.1.2 OTHER RAS POLYMORPHISMS AND LVH – RECENT STUDIES

6.1.2.1 Angiotensinogen M235T polymorphism

Several polymorphisms have been identified in the angiotensinogen gene, though only the M235T variant (substitution of amino acid threonine for methionine at position 235) has good evidence for functionality.[407] Another polymorphism – the substitution of methionine for threonine at position 174 (T174M) actually occurs in a subset of the M235T polymorphism and is thus in complete linkage disequilibrium.[408]

The *T* allele is associated with increased plasma angiotensinogen levels,[407,409] with a meta-analysis suggesting 11% higher levels for the TT genotype and 7% higher for the TM genotype compared to MM.[407] Being the substrate for renin and the rate-limiting step in the systemic RAS, these differences may be biologically significant, particularly for blood pressure. This was found to be the case, with the T allele associated with higher blood pressure,[171,172] greater prevalence of hypertension[407] and use of anti-hypertensive drugs.[171]

There is also an effect on left ventricular mass, with greater hypertrophy associated with the *T* allele in hypertensives[172,410] and also in endurance athletes.[411] It is difficult to distinguish, however, the direct effect of the *T* allele on LVH from that mediated via blood pressure.[172] as the TT genotype is associated with hypertension, the intermediate phenotype (raised angiotensinogen levels) and the end result, LV hypertrophy. Some insight is gained through a study using transgenic mice carrying a gene over-expressing cardiac angiotensinogen, in which the excess LV growth was independent of blood pressure, though hypertension increased the LV mass further.[412] One study found synergy between the ACE I/D and the angiotensinogen M235T polymorphisms, with the DD/TT genotypes having the highest LV mass and the II/MM

genotypes the lowest, even after adjusting for blood pressure, age and body mass index.[413] Two other studies did not suggest an association of LVH with the *T* allele however.[301,302]

6.1.2.2 Angiotensin AT_1 receptor A1166C polymorphism

Several polymorphisms in the AT_1 receptor gene have been identified,[414,415] though the A1166C polymorphism (cytosine for adenine at position 1166) has been the most studied, possibly due to the early suggestion of a weak association of the *C* variant with hypertension.[415] The polymorphism does not result in an amino acid change and would therefore presumably be a marker for other polymorphism(s) with direct effects, if any.

Studies in normal populations showed no consistent association with hypertension or LV mass[165,416] and there is no strong evidence either in patients with hypertension or LVH,[417,418] including the initial study provoking interest, in which the association was weak.[415] The *C* allele was associated with increased LV mass in patients with hypertrophic cardiomyopathy, where the degree of hypertrophy was related to the genotype: LV mass index 168 vs. 187 vs. 205 g/m^2 for the AA, AC and CC genotypes respectively. However, the numbers were limited (57, 39 and 6 respectively) and this necessitated the pooling of both *C* allele groups (AC and CC) together for statistical analysis to show a significant result.

In summary, there is little evidence for an effect of this polymorphism on cardiac mass, hypertension or other cardiovascular pathology.

6.1.2.3 Angiotensin II type 2 receptor gene

The angiotensin II type 2 receptor gene is located on the X chromosome and has an identified polymorphism at position 1675 (guanine to adenine), G1675A. In the only study to date, there was a significant association of the *A* allele with greater LV mass in mildly hypertensive (systolic BP 140-160 mmHg) but not normotensive subjects, in keeping with a modulating effect.[419] The hypertensive subjects with the *A* allele had a mean LV mass index of 138g/m^2 compared to 120g/m^2 for the *G* allele ($p<0.001$).

6.1.2.4 Bradykinin B_2 receptor gene

Several polymorphisms have been identified in the bradykinin B_2 receptor gene[420] and the functional importance of these remains to be determined. Of particular interest is the identification of a nine base-pair insertion/deletion polymorphism in the promoter region which is associated with higher gene transcriptional activity[421] and greater contractile response to kinins.[422] Interestingly, it is B_1 receptor activity that it appears to affect, despite being within the B_2 gene,[422] which can only be explained by linkage disequilibrium with another polymorphism in the B_1 gene, to which it is closely related on chromosome 14.

6.2 STRENGTHS AND LIMITATIONS OF THE RESEARCH

6.2.1 STRENGTHS

The use of a functional genetic polymorphism allows the precise study of biological mechanisms. If the environment is as uniform as possible, then the only difference between genotypes is the gene and its product, allowing the phenotypic (e.g. anatomical, physiological) differences between genotypes to be attributed to the product (in this case, ACE levels). The principle of gene-knockout mice is similar in this respect - the mice are identical apart from the single gene deletion and the effect this has on physiology, anatomy, mortality etc. highlights the role of that specific element.

In addition, the use of genetic variation allows insight into local tissue mechanisms which are otherwise exceedingly difficult to study. While this includes assumptions about the consistent association of the ACE genotype with tissue ACE levels, and is thus an indirect approach, it is a straightforward and elegant technique for examining human systems in particular.

The use of a postal sampling technique for DNA collection greatly facilitated access to otherwise difficult groups for study and increased the speed with which these groups could be studied.

Cardiovascular magnetic resonance is an accurate and reproducible tool for measuring LV mass (see chapter 2) and the use of this technique allowed the identification of small differences in LV growth which would otherwise have required much larger sample sizes.

6.2.2 LIMITATIONS

The research concentrated on only one genetic trait when several are likely to be involved, perhaps others in the RAS (see above). To investigate several polymorphisms independently would have required much larger sample sizes and would have been difficult to achieve. The inclusion of only homozygotes in the LV growth study (chapter 3) was to maximise the power of the study given the limitations in time and accessibility of the subjects.

The ACE I/D polymorphism exists in an intron, and thus is unlikely to affect the structure of the enzyme. To alter ACE levels, it must have effects on the promoter region or be in allelic association with another polymorphism within the ACE gene promoter region. The mechanism is currently unknown, though the latter (linkage disequilibrium) hypothesis is supported strongly by the fact that different racial groups (such as Afro-Caribbeans) possess the polymorphism but do not share the same association with serum ACE levels,[318] suggesting the absence of the proposed allelic association in these racial groups.

The research did not include biochemical evidence (such as serum ACE levels) which would have been extremely difficult (in the case of the elite endurance groups) and would have resulted in significantly reduced acceptance to participate in the army recruits, in whom venepuncture is greatly disliked. The ACE gene polymorphism has thus not been associated with ACE levels in this study. However, the local RAS in cardiac and skeletal muscle has little correlation with serum levels, making the measurement of these less relevant.

The exercise-induced model of LV growth assumes that the growth mechanism is the same as in pathological hypertrophy. There are several recent pieces of research which suggest that exercise-induced (or 'athletic') hypertrophy may differ from pathological hypertrophy in function, though differences in the growth process have not been examined. High energy phosphate levels have been shown to be normal in athletic hearts compared with healthy controls using magnetic resonance spectroscopy.[423] Reduced levels were observed in both hypertrophied[424] and dilated[425] hearts that had reduced function or poor prognosis, though not in those with normal function.[424] There may be differences in the degree of collagen laid down[426,427] and thus differing degrees of fibrosis, which may well affect prognosis, and there is little evidence that athletic hypertrophy is detrimental to health in the same way as

pathological hypertrophy. One characteristic of athletic hypertrophy is the rapid return to normal LV size once the exercise stimulus is removed.[238,428]

6.3 CONCLUSIONS

6.3.1 LEFT VENTRICULAR HYPERTROPHY

6.3.1.1 Systemic vs. local RAS

Although the study in chapter 3 showed no effect of low-dose losartan on LV growth, there were clear differences between ACE genotypes at similar blood pressures, suggestive of activity in the local RAS. Other recent studies have also supported a greater role for the local RAS. The effective concentration of RAS inhibitors may be at hypotensive doses, but the reduction in cardiac mass is greater than with other non-RAS hypotensive agents and the agents are effective in isolated animal heart models where haemodynamic loads remain unchanged.

6.3.1.2 Angiotensin vs. kinins

The majority of the evidence suggests angiotensin II-mediated LV growth. Kinins appear not to have a direct effect on myocardial hypertrophy, though may have indirect effects via blood pressure changes. The majority of this growth effect appears to be through the AT_1 receptor, though this is not mandatory for LV growth at least in pressure-overload models, as exhibited by the absence of effect of AT_1 antagonists in the presence of continued severe pressure load and the ability to induce LV hypertrophy in AT_{1a} knockout mice with a pressor stimulus.[268] The presence of AT_{1a} receptors, however, appeared to be necessary for the sub-pressor LV growth-promoting effects of angiotensin II in this study.

6.3.1.3 AT_1 vs. AT_2 receptors

There is good evidence for cardiac growth stimulatory effects of angiotensin AT_1 receptors and the opposite growth inhibitory effects of AT_2 receptors. This difference in effect provides a balanced control mechanism with the relative proportions of each subtype important for the overall effect.

Other receptors, such as the putative AT_4 receptor for the angiotensin II metabolite angiotensin IV (peptides 3-8), have low affinity for angiotensin II[429] and there is insufficient evidence to determine their role in LV growth.

6.3.2 CARDIAC METABOLISM AND THE EFFECT ON ELITE GROUPS

The insertion allele of the human ACE gene I/D polymorphism has been shown to be more prevalent in elite groups subject to extreme endurance conditions where a selection bias may exist. This suggests a beneficial effect of this allele, and of the associated lower ACE levels, on metabolic efficiency. This may be mediated via a kinin-nitric oxide pathway acting on the mitochondria of myocardial cells. The beneficial effects of ACE inhibitors in heart failure may be partially explained by this process, although angiotensin II antagonists have also shown to be of benefit in these patients.

6.4 FUTURE RESEARCH DIRECTIONS

6.4.1 LV HYPERTROPHY

The specific mechanism of effect of the ACE gene polymorphism remains unclear, although there is significant evidence supporting the importance of angiotensin II via the AT_1 receptor. It would be interesting to repeat the LV growth study using an ACE inhibitor versus an angiotensin II antagonist, perhaps at slightly higher doses (e.g. 50mg losartan, which still has little effect on blood pressure in normal subjects).

6.4.1.1 Prognosis in LVH

It may be possible to determine if the ACE gene I/D polymorphism has prognostic significance with a re-analysis of the data in the large heart failure prognosis trials (SOLVD, SAVE) to examine the polymorphism if DNA or whole blood samples exist. It would be interesting to note if, within each treatment group (ACE inhibitor/placebo), there was a divergence in mortality with the ACE genotypes.

The LIFE study examining prognosis in hypertensive LVH is due to report in 2002.[430] This is comparing losartan with atenolol on the mortality rate, for the same reduction in blood pressure. If losartan proves to be superior, questions will remain over the mechanism of effect as the LV mass is not being measured – LVH was diagnosed on ECG criteria.

No study has been set up to examine prospectively the specific effect of LV mass reduction on prognosis and whether RAS antagonists have a greater effect on mortality for the same reduction in LV mass. It remains possible that the benefits of ACE inhibition in heart failure are due to a reduction in LV mass per se rather than through reduced RAS activity. The reduced numbers required for a comparative study with cardiac magnetic resonance would facilitate this, though the limiting factor remains the relatively low event rate in the population, particularly with treatment. In previous studies,[431,432] the event rate was 3-4 per 100 patient-years.

6.4.2 METABOLIC EFFICIENCY

Magnetic resonance spectroscopy is a useful tool for examining human cardiac energetics and could be used to examine this in relation to LVH. Decreased high energy phosphates are associated with poor outcome in dilated cardiomyopathy[425] and aortic valve disease.[424] Could the same be true for LVH? The lower death rate in asymptomatic LVH would require increased numbers but it is a feasible study.

It may also be worth studying the association of the ACE gene polymorphism with myocardial and skeletal muscle phosphocreatine/ATP ratios, to examine whether this is a potential mechanism for the differences in genotype frequency observed in chapter 5 and the putative increased mortality associated with the *D* allele. It would also be possible to examine the changes in skeletal muscle with exercise to determine if a difference exists between ACE genotypes.

6.4.3 THE ACE GENE I/D POLYMORPHISM IN CONTEXT

The renin-angiotensin system is clearly important for cardiac disease and possibly for endurance exercise, and as such remains an important area for future research. Genetic polymorphisms are useful tools for examining systems such as this, as well as identifying genetic determinants of the severity of disease and exercise-induced physiological changes. While the ACE I/D polymorphism is important, other genetic polymorphisms are likely to be found which affect the RAS, as well as other physiological systems. Cardiac metabolism and diseases are subject to multiple genetic and environmental influences, of which the ACE gene is but one, and future work is likely to identify several others.

CHAPTER 7

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CHAPTER 8

Appendices

8.1 PUBLICATIONS ARISING FROM THIS RESEARCH

Left ventricular hypertrophy with exercise and the angiotensin converting enzyme gene i/D polymorphism: a randomised controlied triai with iosartan.
Myerson SG, Montgomery HE, Whittingham M, Jubb M, World MJ, Humphries SE, Pennell DJ.
Circulation 2001; 103: 226-30

The human angiotensin I-converting enzyme gene and endurance performance.
Myerson S, Hemingway H, Budgett R, Martin J, Humphries S, Montgomery H.
Journal of Applied Physiology 1999; 87(4): 1313-6

Human gene for physical performance.
Montgomery HE, Marshall R, Hemingway H, Myerson S, Clarkson P, Dollery C, Hayward M, Holliman DE, Jubb M, World M, Thomas EL, Brynes AE, Saeed N, Barnard M, Bell J, Prasad K, Rayson M, Talmud PJ, Humphries SE
Nature 1998 May 21; 393(6682):221-2

Angiotensin-converting enzyme genotype affects the response of human skeletal muscie to functional overioad.
Folland J, Leach B, Little T, Hawker K, Myerson S, Montgomery H, Jones, D
Experimental Physiology 2000; 85: 575-9

The assessment of left ventricular mass by cardiac magnetic resonance (review article)
Myerson SG, Bellenger NG, Pennell DJ.
Hypertension 2002; 39: 750-5

The relationships between MRI-determined ieft ventricular mass and body composition variables.
George KP, Myerson SG, Birch KM, World MJ and Pennell, DJ
Submitted to Am J Cardiol

8.2 SUPERVISION OF THE THESIS

Supervision was provided by:

Professor Steve Humphries
British Heart Foundation chair of cardiovascular genetics and Director, Centre for cardiovascular genetics, University College London

Dr. Hugh Montgomery
Lecturer in cardiovascular medicine and consultant in intensive care medicine Centre for cardiovascular genetics, University College London

Professor Dudley Pennell
Professor of Cardiology, National Heart and Lung Institute, Imperial College and Clinical Director, Cardiovascular Magnetic Resonance unit, Royal Brompton Hospital

8.3 ETHICAL APPROVAL FOR THE RESEARCH

8.3.1 LV MASS STUDY

The study protocol was approved by the ethical committees of both the Royal Brompton Hospital and the British army. In addition, funding was obtained from the British Heart Foundation whose detailed analysis includes consideration of the ethical foundation for any approved research.

Separate consent forms were signed by the subjects for obtaining genetic samples and two further consent forms for participation in the study, once ACE genotype had been identified (one each for the Royal Brompton Hospital and the British army).

8.3.2 ACE GENOTYPE FREQUENCY DISTRIBUTION IN ATHLETES

The British Olympic Association considered the ethics of the study in detail and approved the protocol. Particular attention was paid to the anonymity of genetic data and protection of athletes' details. The study was also approved by the UCL ethics committee.

8.4 PERSONAL CONTRIBUTION TO THIS RESEARCH

8.4.1 ACE GENOTYPING

Dr. Myerson extracted the DNA and performed the ACE genotyping for all the subjects in these research projects.

8.4.2 CMR SCANNING

Dr. Myerson performed all the cardiac and whole body MR scans, having been trained in the technique at the Royal Brompton Hospital. Technical assistance with the running of the scanner, including transporting to and from the army base, was provided by Steve Collins, Ray Hughes and Peter Gatehouse.

8.4.3 LV HYPERTROPHY STUDY

The overall co-ordination of the study, including consenting and organising subjects, liasing with the troop commanders, other administration and data analysis, was undertaken by Dr. Myerson. Additional support was provided by Capt. Martin Whittingham who assisted with the sample collection and communication within the army base. The study was facilitated by Lt. Col. Mike World, Professor of military medicine, who organised the administration within the Army Medical College.

8.4.4 ATHLETES

Study design was by Hugh Montgomery and Dr. Myerson. The British Mountaineering Council and British Olympic Association provided contact details for the subjects. Postal packs were prepared by Dr. Myerson, Maj Mutch, Helen McGloin and Hugh Montgomery. The collation of samples, genotyping and data analysis was performed by Dr. Myerson.

8.4.5 INDEXING OF LV MASS

The cardiac and whole body data was acquired by Dr. Myerson. Detailed mathematical analysis was performed by Keith George, Manchester Metropolitan University.

8.5 ACKNOWLEDGEMENTS

Grateful help was received from the following:

- The staff of the Cardiovascular Magnetic Resonance Unit, Royal Brompton Hospital, in particular Steve Collins and Ray Hughes for their help with the mobile scanner. Technical assistance was also received from Peter Gatehouse and Permi Jhooti, and G.Z.Yang and Francois Chabat provided invaluable computing expertise. Thanks too to Karen Taylor for countless administrative tasks.

- All the staff of the UCL cardiovascular genetics laboratory, in particular Ian Day, Minolas Spanakis and Ros Whittall for showing me initially how it all worked.

- Maj Mutch and Helen McGloin for their help with the postal sampling.

- The British Olympic Association and Dr. Richard Budgett, medical director.

- The British Mountaineering Council.

- Keith George, Manchester Metropolitan University, for the mathematical analysis of LV mass and body composition scaling.

I would also like to thank my friends and family who supported me through the completion of this thesis. In particular, I would like to thank:

Matthew Myerson
Louise Elliott
Mark Kon
Danny Lawes
Linda Porter

8.6 SOURCES OF FUNDING

- **British Heart Foundation**
  Funded a junior research fellowship for Dr.Myerson for 2 years (£70, 000).

- **Coronary Artery Disease Research Association (CORDA)**
  Provided funds to build and maintain the mobile CMR scanner.

- **The British army medicai research executive**
  A grant of £30,000 towards the costs of the LV mass study.

- **Merck Shape & Dohme pharmaceuticais**
  Provided initial start-up funds of £8,000 and supplied the randomised packs of losartan/placebo.

- **British United Provident Association (BUPA)**
  An unrestricted programme grant for genetic research at UCL.

- **The Post Office**
  A grant of £550 towards the costs of postal sampling.

8.7 INDIVIDUAL SUBJECT DATA FROM CHAPTER 3

Notes:

- These tables are organised according to ACE genotype and drug status.
- The number in bold in the left-most column refers to an individual subject.
- Under each heading, the terms 'pre' and 'post' refer to the values for that parameter at the beginning and end of the 10-week training programme.

DD genotype subjects (placebo group) - cardiac data

	Age	Pulse (bpm)		BP (mmHg)		Height (m)	Weight (kg)		LV mass (g)			LVEDV (mls)			LVESV (mls)			LVSV (mls)			RVEDV (mls)			RVESV (mls)			RVSV (mls)			LV mass/EDV (g/ml)		
		pre	post	pre	post		pre	post	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1	17	85	78	123/72	125/68	177.1	72.3	73.8	**174**	**189**	**15**	104	133	29	45	52	7	59	81	22	145	158	13	82	78	-4	63	80	17	1.67	1.42	-0.25
2	17	58	55	112/60	110/60	181.0	81.2	80.3	**146**	**164**	**18**	99	124	25	25	35	10	74	89	15	113	138	25	43	52	9	69	87	18	1.47	1.32	-0.15
3	19	70	65	116/62	114/63	170.0	62.9	64.8	**154**	**160**	**6**	110	113	3	36	36	0	74	77	3	145	162	17	73	81	8	72	81	9	1.40	1.42	0.02
4	22	60	64	115/43	115/50	169.0	60.1	60.3	**166**	**167**	**1**	98	82	-16	29	19	-10	69	63	-6	131	109	-22	58	46	-12	73	64	-9	1.69	2.04	0.34
5	19	70	54	105/56	125/61	177.4	65.6	67.5	**200**	**216**	**16**	97	130	33	32	45	13	64	84	20	131	148	17	68	69	1	62	79	17	2.06	1.66	-0.40
6	20	63	65	142/65	125/53	181.0	77.0	77.2	**198**	**206**	**8**	115	134	19	31	31	0	84	104	20	138	156	18	59	62	3	79	94	15	1.72	1.54	-0.18
7	17	80	77	115/61	117/55	171.5	78.5	81.6	**220**	**206**	**-14**	100	88	-12	37	23	-14	63	65	2	138	137	-1	74	69	-5	64	68	4	2.20	2.34	0.14
8	17	70	49	117/50	107/51	191.1	81.9	80.8	**224**	**228**	**4**	139	134	-5	48	56	8	91	78	-13	164	175	11	75	90	15	90	85	-5	1.61	1.70	0.09
9	17	85	72	126/64	133/62	176.0	64.2	67.8	**171**	**173**	**2**	114	111	-3	42	36	-6	71	75	4	142	151	9	67	78	11	75	72	-3	1.50	1.56	0.06
10	18	65	64	141/61	129/58	173.1	71.4	68.2	**162**	**199**	**37**	90	106	16	25	36	11	64	71	7	113	133	20	51	59	8	62	75	13	1.80	1.88	0.08
11	20	50	60	131/79	115/53	186.0	72.8	68.8	**162**	**173**	**11**	106	109	3	40	43	3	66	66	0	120	139	19	54	66	12	67	73	6	1.53	1.59	0.06
12	17	51	66	103/54	118/63	170.0	69.3	67.9	**183**	**182**	**-1**	107	104	-3	41	27	-14	66	77	11	143	128	-15	69	55	-14	73	73	0	1.71	1.75	0.04
13	25	58	55	124/58	106/53	170.0	73.1	75.2	**223**	**233**	**10**	149	149	0	46	48	2	103	101	-2	168	192	24	77	78	1	91	114	23	1.50	1.56	0.07
14	20	75	65	132/62	135/55	176.6	68.5	66.5	**198**	**210**	**12**	96	146	50	34	55	21	62	90	28	103	157	54	46	69	23	57	87	30	2.06	1.44	-0.62
15	21	60	63	138/66	142/60	166.4	67.0	70.3	**209**	**225**	**16**	134	124	-10	34	29	-5	100	95	-5	166	169	3	69	72	3	97	97	0	1.56	1.81	0.25
16	20	69	70	134/56	137/60	170.0	72.0	70.3	**217**	**225**	**8**	73	78	5	15	19	4	59	59	0	85	108	23	31	43	12	54	65	11	2.97	2.88	-0.09
17	17	66	43	104/53	101/51	176.5	61.5	63.4	**181**	**192**	**11**	104	85	-19	26	18	-8	78	67	-11	115	124	9	45	49	4	70	75	5	1.74	2.26	0.52
18	20	70	72	111/64	123/54	184.1	67.0	71.8	**199**	**212**	**13**	134	125	-9	48	43	-5	86	81	-5	177	174	-3	94	83	-11	83	91	8	1.49	1.70	0.21
19	21	81	75	126/61	119/78	176.8	70.3	70.4	**196**	**237**	**41**	121	121	0	25	42	17	97	79	-18	138	162	24	44	79	35	94	83	-11	1.62	1.96	0.34
20	18	78	76	103/58	106/63	170.1	55.1	53.4	**122**	**134**	**12**	51	60	9	18	20	2	33	40	7	71	79	8	42	32	-10	30	47	17	2.39	2.23	-0.16
21	18	43	48	117/64	111/58	170.0	68.3	67.9	**233**	**216**	**-17**	106	76	-30	35	28	-7	71	47	-24	136	105	-31	60	54	-6	76	51	-25	2.20	2.84	0.64
22	20	57	55	129/66	126/71	172.6	79.6	78.1	**206**	**198**	**-8**	151	153	2	47	45	-2	104	109	5	178	183	5	68	74	6	110	109	-1	1.36	1.29	-0.07
23	27	58	60	122/51	127/55	181.0	94.6	91.0	**255**	**290**	**35**	151	182	31	50	44	-6	101	138	37	187	194	7	84	62	-22	103	132	29	1.69	1.59	-0.10
24	17	62	60	105/71	127/81	174.0	70.0	76.2	**166**	**174**	**8**	72	93	21	25	34	9	47	59	12	91	111	20	38	50	12	52	61	9	2.31	1.87	-0.43
25	18	50	52	109/69	115/74	182.0	78.0	80.4	**162**	**191**	**29**	124	109	-15	33	36	3	91	74	-17	130	123	-7	44	44	0	86	79	-7	1.31	1.75	0.45
26	24	54	50	108/60	136/83	173.0	65.4	69.6	**154**	**178**	**24**	87	108	21	23	28	5	63	80	17	118	119	1	51	50	-1	67	68	1	1.77	1.65	-0.12
27	17	97	82	111/48	120/82	178.0	61.6	64.8	**159**	**181**	**22**	57	84	27	22	38	16	35	46	11	76	99	23	35	45	10	41	54	13	2.79	2.15	-0.63
28	22	45	47	100/60	122/76	172.0	77.8	78.4	**188**	**210**	**22**	75	103	28	22	36	14	53	67	14	123	140	17	59	67	8	64	73	9	2.51	2.04	-0.47
29	18	83	80	122/81	117/77	178.0	81.4	78.4	**220**	**214**	**-6**	87	83	-4	22	19	-3	65	65	0	113	121	8	52	60	8	61	61	0	2.53	2.58	0.05
30	20	57	55	104/71	108/74	178.0	67.0	67.6	**161**	**173**	**12**	96	109	13	26	36	10	69	73	4	120	121	1	47	48	1	73	72	-1	1.68	1.59	-0.09
31	24	52	54	107/67	94/64	184.0	61.6	64.6	**168**	**186**	**18**	113	93	-20	38	31	-7	75	62	-13	121	119	-2	55	54	-1	66	65	-1	1.49	2.00	0.51
32	18	90	80	115/73	113/72	181.0	69.4	70.8	**175**	**193**	**18**	128	132	4	36	33	-3	92	99	7	120	121	1	43	47	4	77	75	-2	1.37	1.46	0.09
33	19	55	70	113/75	110/79	170.0	57.2	59.4	**173**	**203**	**30**	113	122	9	35	39	4	78	83	5	131	144	13	54	60	6	77	84	7	1.53	1.66	0.13
34	17	74	70	115/80	111/79	169.6	69.7	70.1	**203**	**207**	**4**	124	120	-4	47	47	0	77	74	-3	156	138	-18	72	59	-13	84	79	-5	1.64	1.73	0.09
35	24	64	62	135/83	117/79	175.8	72.5	72.5	**167**	**176**	**9**	134	121	-13	48	32	-16	86	89	3	139	138	-1	50	51	1	89	87	-2	1.25	1.45	0.21
36	18	62	60	119/79	109/77	161.3	60.2	57.2	**165**	**162**	**-3**	120	127	7	33	31	-2	87	97	10	137	139	2	47	45	-2	90	94	4	1.38	1.28	-0.10
37	25	60	60	137/90	120/88	172.1	85.8	80.0	**196**	**214**	**18**	103	147	44	30	49	19	72	98	26	144	196	52	74	109	35	70	86	16	1.90	1.46	-0.45
38	23	78	74	123/82	96/67	176.2	70.6	73.2	**189**	**201**	**12**	169	189	20	63	73	10	107	116	9	148	186	38	58	75	17	90	111	21	1.12	1.06	-0.05
39	18	60	63	146/76	114/75	180.4	76.8	75.8	**177**	**198**	**21**	124	152	28	29	45	16	94	107	13	124	162	38	51	65	14	73	97	24	1.43	1.30	-0.12
40	18	50	51	95/59	105/80	170.0	66.0	68.6	**194**	**212**	**18**	156	137	-19	52	46	-6	104	92	-12	154	171	17	52	73	21	103	98	-5	1.24	1.55	0.30
41	19	84	76	109/71	100/60	181.0	63.2	67.4	**167**	**172**	**5**	105	120	15	30	32	2	75	88	13	124	137	13	49	49	0	75	88	13	1.59	1.43	-0.16
Mean:				118/65	117/67	175.5	70.4	71.0	185.0	197.1	12.1	110.6	117.5	6.8	34.7	37.0	2.2	75.8	80.6	4.8	132.1	143.1	11.0	57.7	62.2	4.6	74.4	80.8	6.4	1.75	1.75	0.00
Standard deviation:						5.9	8.2	7.4	26.9	27.1	12.6	26.0	27.6	18.7	10.6	11.5	9.4	18.1	19.7	13.2	25.9	27.9	17.4	14.5	15.4	11.9	16.4	17.3	11.5	0.43	0.41	0.30

DD genotype subjects (placebo group) - body composition data

	Lean mass (kg)			Fat mass (kg)			% Fat			V mass indexed to lean mass (g/k		
	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1												
2												
3												
4	52.4	53.0	0.6	7.7	7.3	-0.4	12.8	12.1	-0.7	3.17	3.15	-0.02
5	57.2	59.4	2.2	8.4	8.1	-0.3	12.8	12.1	-0.7	3.50	3.64	0.14
6	65.0	67.6	2.6	12.0	9.6	-2.4	15.6	12.5	-3.1	3.05	3.05	0.00
7	62.7	65.5	2.8	15.8	16.1	0.3	20.2	19.8	-0.4	3.51	3.15	-0.36
8	70.6	71.7	1.1	11.3	9.1	-2.2	13.8	11.3	-2.5	3.17	3.18	0.01
9	56.2	57.4	1.2	8.0	10.4	2.4	12.4	15.4	3.0	3.04	3.01	-0.03
10	54.8	54.7	-0.1	16.6	13.5	-3.1	23.2	19.8	-3.4	2.96	3.64	0.68
11	61.5	60.3	-1.2	11.3	8.5	-2.8	15.5	12.4	-3.1	2.63	2.87	0.23
12	56.1	55.7	-0.4	13.2	12.2	-1.0	19.1	17.9	-1.2	3.26	3.27	0.01
13	62.6	61.1	-1.5	10.5	14.1	3.6	14.3	18.8	4.5	3.56	3.81	0.25
14	56.7	56.2	-0.5	11.8	10.3	-1.5	17.2	15.4	-1.8	3.49	3.74	0.24
15	55.7	59.0	3.3	11.3	11.3	0.0	16.9	16.1	-0.8	3.75	3.81	0.06
16	58.1	59.0	0.9	13.9	11.3	-2.6	19.3	16.1	-3.2	3.73	3.81	0.08
17												
18	57.9	60.9	3.0	9.1	10.9	1.8	13.6	15.2	1.6	3.44	3.48	0.04
19	59.3	60.1	0.8	11.0	10.3	-0.7	15.6	14.6	-1.0	3.31	3.94	0.64
20	47.0	48.3	1.3	8.1	5.1	-3.0	14.6	9.6	-5.0	2.60	2.77	0.18
21												
22	62.8	63.5	0.7	16.8	14.6	-2.2	21.1	18.7	-2.4	3.28	3.12	-0.16
23	73.8	78.2	4.4	23.8	20.7	-3.1	25.2	22.8	-2.4	3.46	3.71	0.25
24												
25	56.7	62.3	5.6	21.3	18.1	-3.2	27.4	22.5	-4.9	2.86	3.07	0.21
26	56.0	59.9	3.9	9.4	9.7	0.3	14.4	14.0	-0.4	2.75	2.97	0.22
27	54.1	57.8	3.7	7.5	7.0	-0.5	12.2	10.8	-1.4	2.94	3.13	0.19
28	63.8	66.5	2.7	14.0	11.9	-2.1	18.0	15.1	-2.9	2.95	3.16	0.21
29												
30	56.1	56.7	0.6	10.9	10.9	0.0	16.3	16.1	-0.2	2.87	3.05	0.18
31	53.2	56.0	2.8	8.4	8.6	0.2	13.7	13.3	-0.4	3.16	3.32	0.16
32												
33	50.2	53.8	3.6	7.0	5.6	-1.4	12.2	9.4	-2.8	3.45	3.77	0.33
34												
35												
36	47.7	47.1	-0.6	12.5	10.1	-2.4	20.8	17.6	-3.2	3.46	3.44	-0.02
37	65.7	63.5	-2.2	20.1	16.5	-3.6	23.5	20.6	-2.9	2.98	3.37	0.39
38	60.9	63.5	2.6	9.7	9.7	0.0	13.7	13.3	-0.4	3.10	3.17	0.06
39	62.9	62.6	-0.3	13.9	13.2	-0.7	18.1	17.4	-0.7	2.81	3.16	0.35
40	52.2	57.4	5.2	13.8	11.2	-2.6	20.9	16.3	-4.6	3.72	3.69	-0.02
41	53.7	56.9	3.2	9.5	10.5	1.0	15.0	15.6	0.6	3.11	3.02	-0.09
Mean:	58.2	59.9	1.7	12.2	11.2	-1.0	17.1	15.6	-1.5	3.195	3.338	0.143
Standard deviation:	6.1	6.2	2.0	4.2	3.5	1.8	4.1	3.5	2.1	0.321	0.330	0.211

DD genotype subjects (losartan group) - cardiac data

	Age	Pulse (bpm)		BP (mmHg)		Height	Weight (kg)		LV mass (g)			LVEDV (mls)			LVESV (mls)			LVSV (mls)			RVEDV (mls)			RVESV (mls)			RVSV (mls)			LV mass/EDV (g/ml)		
		pre	post	pre	post	(m)	pre	post	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1	18	72	70	122/60	120/64	183.5	78.8	83.1	**223**	**215**	**-8**	80	113	33	25	38	13	55	75	20	116	144	28	61	73	12	55	71	16	2.79	1.90	-0.88
2	21	82	80	116/62	117/63	182.0	77.3	77.1	**160**	**161**	**1**	96	94	-2	28	23	-5	68	70	2	119	122	3	57	58	1	62	64	2	1.67	1.71	0.05
3	18	70	57	108/66	139/59	174.2	66	68.8	**154**	**171**	**17**	109	99	-10	39	37	-2	70	61	-9	122	106	-16	52	49	-3	70	57	-13	1.41	1.73	0.31
4	18	60	52	129/66	127/67	181.1	82.5	82.1	**213**	**236**	**23**	97	156	59	34	55	21	63	101	38	138	169	31	74	73	-1	65	96	31	2.20	1.51	-0.68
5	19	90	63	125/68	136/75	172.3	72.4	78.4	**163**	**182**	**19**	114	115	1	42	48	6	72	67	-5	110	133	23	37	61	24	73	72	-1	1.43	1.58	0.15
6	17	70	79	116/70	117/59	161.5	58.4	55.7	**159**	**168**	**9**	121	101	-20	32	31	-1	90	70	-20	134	124	-10	42	50	8	92	74	-18	1.31	1.66	0.35
7	18	70	56	124/68	114/58	169.4	65.6	65.4	**186**	**181**	**-5**	103	103	0	40	40	0	64	63	-1	113	128	15	58	65	7	55	64	9	1.81	1.76	-0.05
8	26	90	70	130/74	127/61	182.0	76.3	75.2	**173**	**185**	**12**	123	122	-1	38	41	3	85	80	-5	132	139	7	53	60	7	79	79	0	1.41	1.52	0.11
9	21	76	68	117/57	108/54	173.3	64.5	68.5	**167**	**189**	**22**	74	89	15	26	30	4	48	59	11	110	129	19	59	65	6	51	64	13	2.26	2.12	-0.13
10	21	86	55	129/60	106/53	180.5	77.3	75.7	**206**	**216**	**10**	152	142	-10	55	50	-5	96	92	-4	164	184	20	65	96	31	99	88	-11	1.36	1.52	0.17
11	19	83	86	117/54	114/54	182.4	73.8	71.6	**144**	**177**	**33**	69	94	25	21	31	10	48	63	15	84	119	35	43	52	9	41	67	26	2.09	1.88	-0.20
12	21	49	64	106/65	142/60	175.4	87.7	82.7	**184**	**207**	**23**	123	122	-1	35	36	1	87	86	-1	182	176	-6	90	91	1	91	85	-6	1.50	1.70	0.20
13	18	51	50	117/50	135/55	174.5	70.7	69.4	**184**	**201**	**17**	91	106	15	26	27	1	65	78	13	115	136	21	50	53	3	65	83	18	2.02	1.90	-0.13
14	20	58	67	122/62	141/51	168.6	60.4	60.4	**156**	**155**	**-1**	110	90	-20	37	22	-15	73	68	-5	139	106	-33	72	46	-26	67	60	-7	1.42	1.72	0.30
15	19	73	64	102/54	105/59	171.4	68.2	65.5	**175**	**190**	**15**	101	105	4	31	31	0	70	74	4	114	125	11	48	56	8	66	69	3	1.73	1.81	0.08
16	18	74	68	107/58	111/59	180.0	60.8	63.1	**166**	**182**	**16**	114	136	22	44	47	3	70	89	19	121	125	4	59	56	-3	62	69	7	1.46	1.34	-0.12
17	17	60	62	125/62	116/68	170.1	55.8	56.3	**132**	**151**	**19**	59	63	4	17	12	-5	42	50	8	74	84	10	29	34	5	45	50	5	2.24	2.40	0.16
18	17	68	80	116/56	114/53	178.4	80.2	74.8	**181**	**187**	**6**	61	55	-6	22	22	0	39	33	-6	77	70	-7	39	32	-7	39	38	-1	2.97	3.40	0.43
19	21	58	67	98/54	116/54	169.6	68.5	66.5	**179**	**196**	**17**	83	94	11	29	25	-4	53	69	16	105	136	31	49	73	24	57	63	6	2.16	2.09	-0.07
20	21	64	66	132/64	113/69	182.0	87.9	87.9	**201**	**217**	**16**	109	98	-11	25	21	-4	84	78	-6	143	127	-16	58	50	-8	85	77	-8	1.84	2.21	0.37
21	18	78	74	140/55	123/67	184.2	94.2	93.1	**207**	**234**	**27**	99	149	50	32	39	7	68	109	41	137	172	35	63	68	5	74	104	30	2.09	1.57	-0.52
22	19	76	72	112/52	109/43	176.5	73.8	72.4	**194**	**200**	**6**	107	80	-27	28	26	-2	79	54	-25	122	119	-3	54	58	4	68	61	-7	1.81	2.50	0.69
23	19	50	52	113/56	112/74	172.0	67.2	64.1	**166**	**182**	**16**	100	94	-6	30	26	-4	70	68	-2	120	130	10	53	59	6	68	71	3	1.66	1.94	0.28
24	16	64	63	113/59	115/71	168.5	65	64.4	**175**	**174**	**-1**	102	94	-8	23	27	4	80	67	-13	136	127	-9	57	62	5	80	65	-15	1.72	1.85	0.14
25	20	62	60	111/67	129/78	167.5	59	66.4	**144**	**169**	**25**	72	107	35	18	25	7	55	83	28	92	127	35	35	54	19	57	73	16	2.00	1.58	-0.42
26	19	60	62	105/65	96/59	169.0	59.6	62.2	**176**	**177**	**1**	102	108	6	28	29	1	73	79	6	133	124	-9	57	48	-9	76	76	0	1.73	1.64	-0.09
27	23	84	78	122/69	138/73	183.0	74.5	76.6	**194**	**211**	**17**	81	127	46	24	39	15	57	88	31	114	150	36	53	59	6	61	92	31	2.40	1.66	-0.73
28	18	81	75	117/69	145/91	175.0	76.4	79.8	**200**	**216**	**16**	97	129	32	25	54	29	72	75	3	124	138	14	53	68	15	71	70	-1	2.06	1.67	-0.39
29	22	64	60	109/63	101/52	184.1	78.9	78.9	**220**	**208**	**-12**	136	156	20	46	40	-6	90	116	26	150	182	32	61	67	6	90	115	25	1.62	1.33	-0.28
30	24	66	64	96/73	97/75	166.3	61.1	60.0	**141**	**170**	**29**	84	79	-5	21	27	6	62	52	-10	107	116	9	40	52	12	67	63	-4	1.68	2.15	0.47
31	19	52	50	125/73	119/64	167.9	59.7	61.6	**165**	**165**	**0**	117	116	-1	35	36	1	83	80	-3	146	148	2	66	62	-4	80	86	6	1.41	1.42	0.01
32	18	76	70	113/62	106/63	176.0	66.9	64.5	**144**	**170**	**26**	102	122	20	32	47	15	70	75	5	107	121	14	39	49	10	68	72	4	1.41	1.39	-0.02
33	18	70	82	136/68	123/75	176.2	70.7	70.0	**179**	**173**	**-6**	130	109	-21	32	37	5	98	72	-26	145	122	-23	55	47	-8	91	75	-16	1.38	1.59	0.21
34	18	65	84	127/88	131/77	182.5	78.9	80.3	**198**	**181**	**-17**	138	86	-52	49	32	-17	88	54	-34	154	110	-44	62	60	-2	92	51	-41	1.43	2.10	0.67
35	28	48	45	125/85	117/78	180.0	83.9	78.3	**221**	**221**	**0**	134	141	7	41	52	11	92	89	-3	164	169	5	69	74	5	95	92	-3	1.65	1.57	-0.08
36	19	50	50	149/80	156/77	174.6	73.2	72.6	**226**	**239**	**13**	121	139	18	28	31	3	93	108	15	159	159	0	64	45	-19	95	114	19	1.87	1.72	-0.15
37	18	70	71	107/76	108/60	177.4	73.1	72.5	**157**	**161**	**4**	92	89	-3	29	26	-3	63	63	0	104	108	4	38	42	4	66	66	0	1.71	1.81	0.10
38	18	81	72	118/81	111/57	177.0	75.8	72.6	**154**	**168**	**14**	146	115	-31	45	45	0	100	70	-30	147	126	-21	53	59	6	94	67	-27	1.05	1.46	0.41
Mean:				118/65	120/64	175.5	71.7	71.5	178.1	189.1	11.0	103.9	108.9	4.9	31.9	34.3	2.4	72.0	74.4	2.4	125.6	132.4	6.8	54.4	58.6	4.2	71.4	73.8	2.4	1.78	1.80	0.02
Standard deviation:						5.9	9.2	8.7	25.2	23.4	12.0	22.7	23.9	23.0	8.9	10.3	8.8	15.8	17.0	17.6	24.2	24.9	19.9	12.2	13.1	10.9	15.9	16.3	15.8	0.41	0.39	0.36

DD genotype subjects (losartan group) - body composition data

	Lean mass (kg)			Fat mass (kg)			% Fat			LV mass indexed to lean mass (g/kg)		
	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1	68.3	70.4	2.1	10.5	12.7	2.2	13.4	15.3	1.9	3.27	3.05	-0.21
2												
3	59.7	61.5	1.8	6.3	7.3	1.0	9.5	10.7	1.2	2.58	2.78	0.20
4	66.3	67.4	1.1	16.2	14.7	-1.5	19.6	17.9	-1.7	3.21	3.50	0.29
5	62.6	66.5	3.9	9.8	11.9	2.1	13.6	15.1	1.5	2.60	2.74	0.13
6	47.5	46.0	-1.5	10.9	9.7	-1.2	18.7	17.4	-1.3	3.35	3.65	0.30
7	55.5	55.1	-0.4	10.1	10.3	0.2	15.3	15.7	0.4	3.35	3.28	-0.07
8	63.1	64.1	1.0	13.2	11.1	-2.1	17.2	14.7	-2.5	2.74	2.89	0.14
9	56.9	59.4	2.5	7.6	9.1	1.5	11.7	13.3	1.6	2.93	3.18	0.25
10												
11	62.1	61.8	-0.3	11.7	9.8	-1.9	15.9	13.7	-2.2	2.32	2.86	0.55
12	65.5	65.2	-0.3	22.2	17.5	-4.7	25.3	21.1	-4.2	2.81	3.17	0.37
13	60.6	60.4	-0.2	10.1	9.0	-1.1	14.2	13.0	-1.2	3.04	3.33	0.29
14	50.1	52.3	2.2	10.3	8.1	-2.2	17.0	13.4	-3.6	3.11	2.96	-0.15
15	55.1	55.4	0.3	13.1	10.1	-3.0	19.2	15.4	-3.8	3.18	3.43	0.25
16	54.4	56.1	1.7	6.4	7.0	0.6	10.6	11.1	0.5	3.05	3.24	0.19
17	47.6	48.1	0.5	8.2	8.2	0.0	14.6	14.5	-0.1	2.77	3.14	0.37
18	62.5	62.2	-0.3	17.7	12.6	-5.1	22.0	16.9	-5.1	2.90	3.01	0.11
19	56.6	56.1	-0.5	11.9	10.4	-1.5	17.3	15.6	-1.7	3.16	3.49	0.33
20	72.9	73.7	0.8	15.0	14.2	-0.8	17.1	16.1	-1.0	2.76	2.94	0.19
21	72.8	73.6	0.8	21.4	19.5	-1.9	22.7	21.0	-1.7	2.84	3.18	0.34
22	58.2	60.3	2.1	15.6	12.1	-3.5	21.1	16.7	-4.4	3.33	3.32	-0.02
23	54.0	54.9	0.9	13.2	9.2	-4.0	19.7	14.4	-5.3	3.07	3.32	0.24
24	52.4	53.9	1.5	12.6	10.5	-2.1	19.3	16.4	-2.9	3.34	3.23	-0.11
25	50.2	56.9	6.7	8.8	9.5	0.7	14.9	14.3	-0.6	2.87	2.97	0.10
26	51.3	54.7	3.4	8.3	7.5	-0.8	13.9	12.0	-1.9	3.43	3.24	-0.19
27	65.3	69.1	3.8	9.2	7.5	-1.7	12.3	9.8	-2.5	2.97	3.05	0.08
28	63.5	66.0	2.5	12.9	13.8	0.9	16.9	17.3	0.4	3.15	3.27	0.12
29	62.3	64.4	2.1	16.6	14.5	-2.1	21.0	18.4	-2.6	3.53	3.23	-0.30
30												
31	49.9	51.5	1.6	9.8	10.1	0.3	16.4	16.3	-0.1	3.31	3.20	-0.10
32	54.8	54.1	-0.7	12.1	10.4	-1.7	18.2	16.1	-2.1	2.63	3.14	0.51
33	61.2	61.6	0.4	9.5	8.4	-1.1	13.4	11.9	-1.5	2.92	2.81	-0.12
34	66.9	68.6	1.7	12.0	11.7	-0.3	15.2	14.6	-0.6	2.96	2.64	-0.32
35	68.1	67.8	-0.3	15.8	10.5	-5.3	18.8	13.4	-5.4	3.25	3.26	0.01
36	64.0	63.4	-0.6	9.2	9.2	0.0	12.6	12.7	0.1	3.53	3.77	0.24
37	62.4	62.6	0.2	10.7	9.9	-0.8	14.6	13.7	-0.9	2.52	2.57	0.06
38												
Mean:	59.5	60.7	1.2	12.0	10.8	-1.2	16.6	15.0	-1.6	3.023	3.143	0.120
Standard deviation:	6.9	6.9	1.7	3.8	2.8	1.9	3.6	2.6	2.0	0.303	0.272	0.220

II genotype subjects (placebo group) - cardiac data

	Age	Pulse (bpm)		BP (mmHg)		Height	Weight (kg)		LV mass (g)			LVEDV (mls)			LVESV (mls)			LVSV (mls)			RVEDV (mls)			RVESV (mls)			RVSV (mls)			LV mass/EDV (g/ml)		
		pre	post	pre	post	(m)	pre	post	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1	19	55	50	118/70	118/65	171.0	74.0	71.0	**210**	**226**	**16**	106	117	11	38	43	5	61	74	13	133	134	1	53	54	1	80	80	0	1.98	1.93	-0.05
2	18	65	60	125/65	120/60	181.0	65.0	64.1	**181**	**184**	**3**	160	138	-22	53	54	1	107	84	-23	165	139	-26	64	69	5	101	70	-31	1.13	1.33	0.20
3	18	80	80	110/60	115/62	170.0	62.5	66.9	**169**	**151**	**-18**	110	97	-13	31	28	-3	79	68	-11	129	121	-8	52	50	-2	77	71	-6	1.54	1.56	0.02
4	21	45	60	112/70	110/70	175.5	79.7	75.3	**228**	**248**	**20**	139	145	6	61	45	-16	78	100	22	184	188	4	105	91	-14	79	97	18	1.64	1.71	0.07
5	18	85	55	133/60	132/64	181.3	75.9	75.8	**195**	**208**	**13**	117	164	47	46	42	-4	71	122	51	146	213	67	55	102	47	91	111	20	1.67	1.27	-0.40
6	20	53	48	111/46	113/38	173.0	68.5	70.3	**196**	**201**	**5**	105	121	16	38	46	8	67	74	7	116	133	17	55	58	3	61	75	14	1.87	1.66	-0.21
7	17	80	67	120/65	130/57	175.4	70.6	70.5	**172**	**179**	**7**	93	79	-14	29	22	-7	63	54	-9	112	94	-18	55	43	-12	57	51	-6	1.85	2.27	0.42
8	24	70	61	147/72	123/60	172.6	68.4	68.9	**171**	**172**	**1**	96	96	0	27	18	-9	69	77	8	133	145	12	67	70	3	66	75	9	1.78	1.79	0.01
9	17	60	45	135/58	117/55	178.0	77.3	74.2	**225**	**227**	**2**	87	93	6	29	33	4	59	59	0	124	133	9	62	74	12	62	59	-3	2.59	2.44	-0.15
10	18	58	47	132/62	115/53	166.9	61.6	60.7	**179**	**193**	**14**	139	134	-5	44	40	-4	95	94	-1	177	197	20	79	98	19	98	100	2	1.29	1.44	0.15
11	18	67	50	124/68	102/60	169.5	52.5	54.9	**153**	**162**	**9**	88	96	8	19	27	8	69	69	0	116	121	5	50	56	6	66	65	-1	1.74	1.69	-0.05
12	17	68	58	124/65	113/60	169.0	77.4	70.3	**211**	**204**	**-7**	76	110	34	22	44	22	54	66	12	121	166	45	64	104	40	56	62	6	1.85	1.46	-0.39
13	21	52	57	120/65	137/65	170.4	68.2	69.7	**220**	**209**	**-11**	98	135	37	31	27	-4	67	107	40	121	168	47	59	64	5	62	105	43	2.24	1.55	-0.70
14	19	51	47	106/60	112/62	181.9	72.9	75.0	**166**	**187**	**21**	55	64	9	13	17	4	42	47	5	87	98	11	42	45	3	45	52	7	3.02	2.92	-0.10
15	20	80	66	118/53	140/57	176.6	76.5	75.3	**195**	**209**	**14**	87	68	-19	24	22	-2	63	46	-17	144	109	-35	79	56	-23	65	53	-12	2.24	3.07	0.83
16	24	63	76	119/55	109/61	180.5	73.5	74.2	**186**	**173**	**-13**	128	91	-37	35	25	-10	93	67	-26	153	114	-39	55	48	-7	98	66	-32	1.45	1.90	0.45
17	17	73	70	119/61	103/63	159.5	54.1	54.1	**152**	**156**	**4**	81	87	6	26	20	-6	55	67	12	90	100	10	34	35	1	56	65	9	1.88	1.79	-0.08
18	23	50	50	130/51	120/67	180.5	70.5	74.4	**191**	**207**	**16**	85	93	8	27	28	1	58	66	8	114	108	-6	54	49	-5	61	59	-2	2.25	2.23	-0.02
19	17	65	64	119/59	126/62	166.8	65.7	66.1	**163**	**157**	**-6**	84	103	19	26	24	-2	58	78	20	108	122	14	55	44	-11	53	77	24	1.94	1.52	-0.42
20	16	94	85	116/83	128/79	176.0	69.0	73.4	**163**	**192**	**29**	94	117	23	29	41	12	65	76	11	115	129	14	54	52	-2	61	77	16	1.73	1.64	-0.09
21	17	48	45	113/63	119/78	184.5	81.0	79.0	**210**	**210**	**0**	149	172	23	43	64	21	106	108	2	166	174	8	63	76	13	104	98	-6	1.41	1.22	-0.19
22	21	54	53	105/53	138/88	175.0	69.0	74.4	**205**	**225**	**20**	121	179	58	35	49	14	85	130	45	144	179	35	58	75	17	86	104	18	1.69	1.26	-0.44
23	17	74	70	107/71	107/77	169.0	58.0	64.8	**169**	**177**	**8**	78	115	37	35	34	-1	43	81	38	103	131	28	52	56	4	50	75	25	2.17	1.54	-0.63
24	19	60	58	111/63	125/87	180.0	70.2	69.8	**163**	**175**	**12**	99	106	7	30	45	15	68	61	-7	127	133	6	59	68	9	68	65	-3	1.65	1.65	0.00
25	18	65	70	114/85	120/81	185.0	89.6	90.8	**217**	**211**	**-6**	142	181	39	55	64	9	86	117	31	160	189	29	77	80	3	84	109	25	1.53	1.17	-0.36
26	20	53	53	105/63	105/71	184.0	75.2	76.8	**162**	**188**	**26**	108	115	7	33	42	9	74	73	-1	110	125	15	40	44	4	70	82	12	1.50	1.63	0.13
27	21	60	55	117/80	109/64	175.3	70.1	71.1	**197**	**217**	**20**	111	143	32	43	53	10	68	90	22	121	173	52	44	70	26	77	103	26	1.77	1.52	-0.26
28	23	58	50	127/73	115/69	170.5	64.8	68.5	**170**	**171**	**1**	117	115	-2	25	26	1	92	89	-3	115	125	10	36	51	15	79	73	-6	1.45	1.49	0.03
29	21	90	78	121/88	119/73	173.6	70.4	74.9	**172**	**179**	**7**	122	91	-31	37	30	-7	85	61	-24	122	95	-27	39	35	-4	83	59	-24	1.41	1.97	0.56
30	17	70	65	103/59	100/49	178.0	68.2	69.2	**168**	**175**	**7**	114	159	45	31	46	15	83	113	30	128	184	56	47	68	21	81	116	35	1.47	1.10	-0.37
31	18	65	70	101/62	112/66	169.0	71.8	70.6	**223**	**188**	**-35**	100	101	1	35	34	-1	65	68	3	123	142	19	50	63	13	73	79	6	2.23	1.86	-0.37
32	20	63	60	105/76	111/73	184.0	70.4	73.8	**193**	**196**	**3**	109	116	7	36	42	6	73	74	1	130	134	4	53	56	3	77	79	2	1.77	1.69	-0.08
33	22	65	60	103/67	121/70	177.0	76.0	77.8	**179**	**188**	**9**	90	97	7	28	22	-6	62	76	14	119	129	10	59	56	-3	60	73	13	1.99	1.94	-0.05
34	21	61	58	104/63	96/68	177.0	64.0	65.2	**173**	**146**	**-27**	125	120	-5	45	52	7	79	67	-12	129	135	6	42	63	21	86	72	-14	1.38	1.22	-0.17
Mean:				117/65	118/66	175.2	70.1	70.9	186.1	190.9	4.8	106.3	116.4	10.1	34.1	36.7	2.6	71.8	79.5	7.7	129.0	140.6	11.6	56.2	62.4	6.2	72.7	78.1	5.4	1.80	1.72	-0.08
Standard deviation:						6.0	7.5	6.8	22.2	23.9	14.3	23.1	30.1	22.3	10.3	12.8	9.0	15.6	21.0	19.4	22.5	31.5	24.6	14.0	17.7	14.3	15.3	18.3	17.2	0.39	0.45	0.32

II genotype subjects (placebo group) - body composition data

	Lean mass (kg)			AT mass (kg)			% Fat			LV mass indexed to lean mass (g/kg)		
	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1												
2												
3	52.8	54.5	1.7	9.7	12.4	2.7	15.5	18.6	3.1	3.20	2.77	-0.43
4	60.9	63.2	2.3	18.8	12.1	-6.7	23.6	16.1	-7.5	3.74	3.92	0.18
5	63.7	63.3	-0.4	12.2	12.5	0.3	16.0	16.5	0.5	3.06	3.29	0.22
6	54.1	57.3	3.2	14.4	13.0	-1.4	21.1	18.5	-2.6	3.62	3.51	-0.12
7	61.0	63.0	2.0	9.6	7.5	-2.1	13.7	10.6	-3.1	2.82	2.84	0.02
8	54.1	56.6	2.5	14.3	12.3	-2.0	20.9	17.9	-3.0	3.16	3.04	-0.12
9												
10	52.3	51.8	-0.5	9.3	8.9	-0.4	15.1	14.7	-0.4	3.42	3.73	0.30
11	46.4	48.1	1.7	6.1	6.8	0.7	11.6	12.4	0.8	3.30	3.37	0.07
12	56.6	55.1	-1.5	20.8	15.2	-5.6	26.9	21.6	-5.3	3.73	3.70	-0.03
13	55.6	56.9	1.3	12.6	12.8	0.2	18.4	18.3	-0.1	3.96	3.67	-0.28
14	63.2	65.1	1.9	9.7	9.9	0.2	13.3	13.2	-0.1	2.63	2.87	0.25
15	63.3	63.5	0.2	13.2	11.8	-1.4	17.3	15.7	-1.6	3.08	3.29	0.21
16	61.7	62.6	0.9	11.8	11.6	-0.2	16.1	15.7	-0.4	3.01	2.76	-0.25
17	48.0	46.9	-1.1	6.1	7.2	1.1	11.2	13.3	2.1	3.17	3.33	0.16
18	61.9	65.1	3.2	8.6	9.3	0.7	12.1	12.5	0.4	3.09	3.18	0.09
19	52.4	53.5	1.1	13.7	12.6	-1.1	20.8	19.0	-1.8	3.11	2.93	-0.18
20	55.9	61.8	5.9	13.1	11.6	-1.5	19.0	15.8	-3.2	2.92	3.11	0.19
21	63.5	65.2	1.7	17.5	13.8	-3.7	21.6	17.4	-4.2	3.31	3.22	-0.09
22	58.1	63.2	5.1	10.9	11.2	0.3	15.7	15.0	-0.7	3.53	3.56	0.03
23	47.0	55.1	8.1	11.0	9.7	-1.3	19.0	15.0	-4.0	3.60	3.21	-0.38
24	59.0	60.8	1.8	11.2	9.0	-2.2	16.0	12.8	-3.2	2.76	2.88	0.12
25	74.8	74.5	-0.3	14.8	16.3	1.5	16.5	17.9	1.4	2.90	2.83	-0.07
26	61.6	62.0	0.4	13.6	14.8	1.2	18.1	19.3	1.2	2.63	3.03	0.40
27	59.4	60.9	1.5	10.7	10.2	-0.5	15.2	14.4	-0.8	3.32	3.56	0.25
28	54.6	57.7	3.1	10.2	10.8	0.6	15.7	15.8	0.1	3.11	2.96	-0.15
29	59.5	61.9	2.4	10.9	13.0	2.1	15.4	17.4	2.0	2.89	2.89	0.00
30	59.2	60.4	1.2	9.0	8.8	-0.2	13.3	12.7	-0.6	2.84	2.90	0.06
31	56.0	58.0	2.0	15.8	12.6	-3.2	22.0	17.9	-4.1	3.98	3.24	-0.74
32	61.8	64.4	2.6	8.6	9.4	0.8	12.2	12.8	0.6	3.12	3.04	-0.08
33	62.3	64.2	1.9	13.7	13.6	-0.1	18.1	17.5	-0.6	2.87	2.93	0.06
34	53.5	55.1	1.6	10.5	10.1	-0.4	16.4	15.5	-0.9	3.23	2.65	-0.58
Mean:	57.9	59.7	1.9	12.0	11.3	-0.7	17.0	15.9	-1.2	3.197	3.169	-0.029
Standard deviation:	5.8	5.7	2.0	3.3	2.3	2.1	3.7	2.5	2.4	0.360	0.331	0.261

II genotype subjects (losartan group) - cardiac data

	Age	Pulse (bpm)		BP (mmHg)		Height	Weight (kg)		LV mass (g)			LVEDV (mls)			LVESV (mls)			LVSV (mls)			RVEDV (mls)			RVESV (mls)			RVSV (mls)			LV mass/EDV (g/ml)		
		pre	post	pre	post	(m)	pre	post	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1	24	55	77	140/90	117/64	171.5	77.2	76.7	**200**	**182**	**-18**	103	100	-3	22	33	11	81	68	-13	132	119	-13	55	49	-6	77	71	-6	1.94	1.82	-0.12
2	20	48	50	95/50	116/68	180.0	57.0	58.3	**164**	**182**	**18**	113	115	2	37	35	-2	76	80	4	133	138	5	61	60	-1	72	78	6	1.45	1.58	0.13
3	17	80	77	122/63	132/49	177.1	61.0	66.5	**200**	**211**	**11**	133	151	18	51	50	-1	81	101	20	137	162	25	61	58	-3	76	103	27	1.50	1.40	-0.11
4	23	75	52	108/59	103/54	177.2	80.4	76.6	**206**	**190**	**-16**	145	138	-7	50	43	-7	94	95	1	170	166	-4	75	68	-7	94	98	4	1.42	1.38	-0.04
5	20	70	55	113/66	105/51	163.4	53.5	54.3	**161**	**171**	**10**	120	109	-11	42	53	11	78	56	-22	123	119	-4	52	63	11	71	56	-15	1.34	1.57	0.23
6	20	75	63	130/70	114/69	169.0	68.1	71.2	**180**	**175**	**-5**	79	106	27	22	32	10	57	74	17	105	125	20	42	46	4	62	79	17	2.28	1.65	-0.63
7	22	48	54	111/61	112/56	181.0	82.3	82.1	**199**	**202**	**3**	86	90	4	31	30	-1	55	60	5	128	133	5	69	70	1	59	63	4	2.31	2.24	-0.07
8	20	50	61	104/54	134/60	179.9	67.0	68.0	**191**	**199**	**8**	101	111	10	30	35	5	71	76	5	113	139	26	48	63	15	65	76	11	1.89	1.79	-0.10
9	23	57	45	114/55	134/60	168.5	71.7	71.4	**242**	**270**	**28**	107	124	17	24	31	7	82	93	11	168	192	24	87	100	13	81	91	10	2.26	2.18	-0.08
10	19	54	64	104/65	106/59	173.4	68.2	68.4	**194**	**194**	**0**	129	136	7	55	42	-13	74	94	20	157	179	22	82	88	6	74	91	17	1.50	1.43	-0.08
11	22	93	80	109/64	108/60	177.5	72.6	72.6	**175**	**191**	**16**	72	82	10	21	21	0	51	61	10	105	109	4	43	51	8	61	59	-2	2.43	2.33	-0.10
12	18	56	55	109/56	113/67	188.3	71.4	74.4	**207**	**219**	**12**	81	92	11	24	26	2	57	66	9	126	122	-4	69	58	-11	57	65	8	2.56	2.38	-0.18
13	22	54	55	111/56	98/62	170.5	63.2	64.9	**170**	**172**	**2**	82	92	10	26	30	4	57	63	6	97	97	0	37	40	3	60	57	-3	2.07	1.87	-0.20
14	17	60	56	124/57	115/70	176.4	74.1	72.8	**239**	**225**	**-14**	168	138	-30	49	40	-9	118	98	-20	200	164	-36	88	57	-31	113	107	-6	1.42	1.63	0.21
15	24	92	75	112/69	113/77	172.0	72.0	74.0	**174**	**197**	**23**	75	99	24	18	28	10	57	72	15	98	116	18	38	52	14	60	65	5	2.32	1.99	-0.33
16	19	86	55	110/67	137/82	172.0	66.0	70.2	**154**	**176**	**22**	101	133	32	32	38	6	68	95	27	105	141	36	34	43	9	70	98	28	1.52	1.32	-0.20
17	20	50	52	107/69	127/63	158.0	65.8	62.6	**166**	**169**	**3**	112	98	-14	40	27	-13	73	71	-2	132	118	-14	57	54	-3	75	64	-11	1.48	1.72	0.24
18	18	68	60	120/59	120/68	176.0	71.6	72.8	**213**	**230**	**17**	139	140	1	37	34	-3	102	106	4	157	170	13	61	54	-7	97	116	19	1.53	1.64	0.11
19	19	72	70	105/83	106/69	179.0	64.8	68.4	**169**	**159**	**-10**	121	99	-22	50	31	-19	71	69	-2	136	125	-11	67	55	-12	70	70	0	1.40	1.61	0.21
20	24	65	70	118/73	113/76	167.0	64.4	66.6	**154**	**177**	**23**	70	86	16	22	27	5	48	58	10	86	101	15	33	40	7	53	61	8	2.20	2.06	-0.14
21	22	50	70	131/93	125/76	174.8	65.1	69.0	**179**	**176**	**-3**	129	122	-7	42	39	-3	87	83	-4	129	152	23	56	64	8	74	87	13	1.39	1.44	0.06
22	20	60	60	127/89	105/76	184.6	79.8	76.2	**216**	**195**	**-21**	133	152	19	57	54	-3	76	98	22	147	153	6	67	67	0	80	86	6	1.62	1.28	-0.34
23	18	75	73	116/75	113/65	180.6	75.2	74.7	**164**	**178**	**14**	91	86	-5	38	31	-7	52	54	2	132	114	-18	77	53	-24	55	60	5	1.80	2.07	0.27
24	20	45	60	129/86	113/65	176.0	79.0	77.4	**186**	**182**	**-4**	147	119	-28	41	30	-11	106	89	-17	187	163	-24	91	76	-15	96	87	-9	1.27	1.53	0.26
25	18	73	70	115/83	117/83	178.0	68.8	70.2	**175**	**183**	**8**	130	133	3	40	41	1	90	92	2	143	151	8	46	61	15	97	90	-7	1.35	1.38	0.03
26	21	55	62	103/72	116/68	174.0	72.0	73.6	**187**	**175**	**-12**	136	140	4	43	36	-7	93	104	11	184	162	-22	80	67	-13	104	94	-10	1.38	1.25	-0.13
27	22	60	60	119/73	111/55	186.0	95.2	93.2	**225**	**205**	**-20**	120	88	-32	40	27	-13	80	61	-19	155	136	-19	73	58	-15	82	78	-4	1.88	2.33	0.45
28	20	60	50	113/74	124/61	176.0	77.2	75.2	**201**	**209**	**8**	145	156	11	49	45	-4	96	111	15	162	191	29	60	74	14	102	117	15	1.39	1.34	-0.05
Mean:				116/69	116/66	175.3	70.9	71.5	189.0	192.6	3.7	113.1	115.5	2.4	36.9	35.3	-1.6	76.1	80.3	4.2	137.4	141.3	3.9	61.0	60.3	-0.7	76.3	81.0	4.6	1.75	1.72	-0.02
Standard deviation:						6.6	8.4	7.3	24.1	23.3	14.3	26.5	23.0	16.9	11.4	8.3	8.2	18.0	17.4	13.1	28.9	26.3	18.7	17.0	13.4	12.2	16.4	18.0	11.3	0.40	0.35	0.23

II genotype subjects (losartan group) - body composition data

	Lean mass (kg)			AT mass (kg)			% Fat			LV mass indexed to lean mass (g/kg)		
	pre	post	change	pre	post	change	pre	post	change	pre	post	change
1	64.9	65.4	0.5	12.3	11.3	-1.0	15.9	14.7	-1.2	3.08	2.78	-0.30
2												
3												
4	64.6	64.9	0.3	15.8	11.7	-4.1	19.6	15.2	-4.4	3.19	2.93	-0.26
5												
6												
7	64.2	65.9	1.7	18.1	16.2	-1.9	22.0	19.7	-2.3	3.10	3.07	-0.03
8												
9	59.5	60.4	0.9	12.2	11.0	-1.2	17.0	15.4	-1.6	4.07	4.47	0.40
10	59.6	58.4	-1.2	8.6	10.0	1.4	12.6	14.6	2.0	3.26	3.32	0.07
11	56.9	58.7	1.8	15.7	13.9	-1.8	21.6	19.1	-2.5	3.08	3.25	0.18
12	63.0	65.3	2.3	8.4	9.1	0.7	11.8	12.2	0.4	3.29	3.35	0.07
13	55.9	57.5	1.6	7.3	7.4	0.1	11.6	11.4	-0.2	3.04	2.99	-0.05
14	60.9	60.7	-0.2	13.9	12.1	-1.8	18.5	16.6	-1.9	3.92	3.71	-0.22
15	57.7	61.8	4.1	14.3	12.2	-2.1	19.8	16.4	-3.4	3.02	3.19	0.17
16	52.5	60.0	7.5	13.5	10.2	-3.3	20.5	14.5	-6.0	2.93	2.93	0.00
17	51.1	53.4	2.3	14.7	9.2	-5.5	22.3	14.7	-7.6	3.25	3.16	-0.08
18	62.2	63.3	1.1	9.4	9.5	0.1	13.1	13.1	0.0	3.42	3.63	0.21
19												
20	55.5	56.9	1.4	9.1	9.7	0.6	14.1	14.5	0.4	2.77	3.11	0.34
21	58.0	60.4	2.4	7.1	8.6	1.5	11.0	12.5	1.5	3.09	2.91	-0.17
22	66.4	65.3	-1.1	13.4	10.9	-2.5	16.8	14.4	-2.4	3.25	2.99	-0.27
23	63.3	63.5	0.2	11.9	11.2	-0.7	15.9	15.0	-0.9	2.59	2.80	0.21
24												
25	60.1	60.6	0.5	8.7	9.6	0.9	12.6	13.7	1.1	2.91	3.02	0.11
26	54.9	57.5	2.6	17.1	16.1	-1.0	23.7	21.9	-1.8	3.41	3.04	-0.36
27	72.7	71.9	-0.8	24.5	21.3	-3.2	25.2	22.9	-2.3	3.09	2.85	-0.24
28	61.3	63.8	2.5	15.9	11.4	-4.5	20.6	15.1	-5.5	3.28	3.28	0.00
Mean:	60.2	61.7	1.4	12.9	11.6	-1.4	17.4	15.6	-1.8	3.192	3.181	-0.011
Standard deviation:	5.0	4.1	1.9	4.2	3.1	2.0	4.4	3.0	2.5	0.333	0.384	0.218