Cardiovascular Consequences of Anabolic Steroid Use

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A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the Degree of Doctor of Philosophy

November 2011

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ACKNOWLEDGEMENTS

The process of completing this PhD has been a challenging, demanding and thoroughly enjoyable process (well mostly). It has given me the opportunity to continue learning and has helped me grow as a person. Although this has demanded much of myself, I have had help from far too many people to mention but I honestly believe I could not have done it without the help and support of a lot of people.

Firstly, Keith, I couldn't have asked for more support and guidance from a supervisor. You have always been there to help and I truly appreciate all the support you have given me over the years. I look forward to continuing to work with you in the future.

To my other supervisors Greg and Danny, despite the long distances you have always helped me out when I needed and have contributed significantly to my understanding and development as a researcher. I also need to make a special thank you to my other 'unofficial' supervisor Neil, who made a significant contribution to my research and to helping my understanding. I look forward to continuing to work with you all.

To Mum, Dad, Matt and Claire, I count myself extremely lucky to have a family that is so close and offers so much love, encouragement and support. Without you guys supporting and believing in me throughout my life I don't think I would ever have achieved half as much as I have and for that I will always be grateful I can never thank you enough. I also have to thank Philippa, Eliott, Ettienne, Esme, Rob and the rest of my family for all contributing to where I am now. You all really are the best family anyone could ever ask for.

To Jane, your love and support helped me through what should have probably been one of the most stressful periods of my life but you helped to make it easier than it ever should have been. Thank you for being there for me and always listening to me moan, that alone deserves a medal. I can't ever say it enough but thank you, you've been amazing.

To all of my friends both near and far, some old and some new, you have all helped in your own way by just being there to help me escape the stresses and strains of a PhD in some shape or form and to everyone one of you I owe a lot and will always be grateful to have so many good people around to provide so much positive support and encouragement.

And Finally, I couldn't have done it without you all so...Thank you!

ABSTRACT

With the increase in the prevalence of anabolic steroid (AS) use for non-medical performance and image enhancement purposes, the impact of AS on cardiovascular health is an issue of growing public concern. AS use has long been associated with a number of negative CV outcomes such as acute myocardial infarction, stroke, thrombo-embolisms and sudden death. These associations, however, are largely assumed from case study findings where no cause and effect can be apportioned. Small cohort studies have suggested a negative effect of AS on a number of established CV disease risk factors including, blood pressure (BP), lipid profiles (e.g total cholesterol, LDL and HDL), C-reactive proteins and homocysteine, although data is limited both in number of studies and number of participants and is often contradictory. Another potential mechanism to link AS use to cardiac events has been to assess cardiac structure and function. Available data is contradictory but recent developments in imaging technologies suggest that new mechanistic insights could be developed and/or tested.

In study one we investigated the impact of AS use on a broad profile of CV risk factors as well as an in-depth cardiac assessment utilising speckle tracking echocardiography which can assess regional and global cardiac deformation in multiple planes. In AS users we observed an increase in resting heart rate and low-density lipoprotein concomitant to a decrease in high-density lipoprotein levels. 2D and speckle-tracking echocardiography revealed a significant effect of AS use on cardiac function, most notably a decreased diastolic (relaxation) function that suggests a stiffer left ventricle in the AS users. This imposes a greater workload on the heart and increases the risk of CV events.

In study two we developed the assessment of cardiac structural assessment in AS users by adopting state-of-the-art magnetic resonance imaging that provides a greater accuracy in morphology assessment as well as allowing us to determine the presence of perfusion defects and interstitial fibrosis. We observed that AS users had a hypertrophy of both the left and right ventricles that was concomitant with diastolic dysfunction in the left ventricle and a reduction in right ventricular contractility. Despite these findings no AS user presented with any perfusion defect or evidence of interstitial fibrosis, suggesting that these pathways to CV events were not apparent in the current group of AS users.

The final study comprised two pilot or feasibility studies of the impact of resistance exercise on left ventricular function in AS users. Trial 1 assessed blood pressure and cardiac tissue velocities during an acute leg press with and without valsalva. Systolic blood pressure was significantly elevated even after one repetition and this was mediated by AS use. In trial 2 cardiac function and blood biomarkers of cardiac cell damage were assessed before and after a standard resistance exercise training session. Left ventricular systolic function was maintained in recovery in both AS and NAS participants. Diastolic function was reduced in both groups with some evidence that AS use exaggerated this effect. Multiple technical, design and participant lessons were gained to take these feasibility studies forward to full studies.

Overall we add new data to the concept that AS use can place the participant at an increased CV risk. It is probably not obligatory that AS use leads to increased CV risk but in some the route from AS use to CV event may be mediated by changes in structure and function of both the LV and RV. Exercise stress may add an additional CV risk to the AS user.

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Chapter 1. Introduction

1.1 Anabolic Steroids (AS): Development and Use

Testosterone is a steroid hormone that is part of a group of androgen hormones. As the principal male sex hormone it is responsible for the development of male reproductive tissue as well as the development of secondary characteristics such as muscle/bone growth and the development of body hair. Most testosterone is produced in the Leydig cells of the testes with a small amount released from the adrenal cortex. A young adult male will produce between 3-10 mg of testosterone per day (Basaria and Dobs, 2007). In stark contrast, an 80 year old male will produce less than 50% of that of a 20 year old (Evans, 2004).

Anabolic-androgenic steroid (AAS), or anabolic steroid (AS) hormones as they have become more commonly known, are synthetic derivatives of the male sex hormone testosterone (van Amsterdam et al., 2010). These synthetic hormones have been developed as a clinical therapy choice as testosterone has been identified as influencing/controlling a number of physiological mechanisms such as muscle metabolism, sexual and cognitive functions, kidney function, liver function and bone metabolism (Cellotti and Cesi, 1992). AS mimic the effects of testosterone and are primarily used in clinical scenarios to increase protein synthesis in muscle tissue and therefore lead to an increase in muscle size and strength. The therapeutic use of testosterone has been shown to have positive effects in patients where muscular atrophy is a component of the disease, such as AIDS and HIV (Mulligan and Schambelan, 2002), and has also been shown to have a positive effect on cardiovascular (CV) disease risk (Ong et al., 2000).

Male hormones have long been of interest to individuals looking to improve both their physical and mental performance. As far back as ancient Greece there are reports suggesting athletes ate a concoction of substances including animal testes in order to improve performance (Higgins, 2006). The first modern case report related to "testosterone" use by the famous physiologist, Charles E. Brown-Sequard. In the late 19th Century Brown-Sequard claimed that he had extracted a substance from dog and guinea pig testicles that had increased his strength and improved his intellect (Basaria et al., 2001). It wasn't until the 1930's, however, that the active anabolic component that Brown-Sequard had consumed was isolated as testosterone by Adolf Butenandt in 1936, following identification of testosterone in 1935 by Dingemanse, Freud and Laquer (Hall, 2005, Freeman et al., 2001). The claims made by Brown-Sequard were supported by evidence from scientists through the 1940's and in particular in a book, *The Male Hormone*, by Paul du Kruif (Basaria et al., 2001).

Whilst AS were developed, and continue, to be used to treat a number of conditions including depression, impotence and starvation (Dhar et al., 2005), their use outside clinical treatment regimens has grown substantially. Historically, some commentators have suggested that soldiers in Nazi Germany, at the 1936 Munich Olympics, were subjected to systematic doping with AS to enhance their physical performance and aggression, although this is unsubstantiated (Yesalis et al., 1989). By the 1950's AS had entered the sporting realm. After dominating the World Weightlifting Championships in Vienna, 1954, anecdotal reports claim that the then Soviet team doctor revealed to the US team doctor, a Dr Ziegler, that the Soviet team had used AS (Fair, 1993). Ziegler returned to the US and promptly began testing the efficacy of AS on himself and his athletes. Since then the use of AS has remained a

constant and controversial issue within the world of elite sport highlighted by high profile cases such as those of Ben Johnson in the 1988 Seoul Olympics and the Bay Area Laboratory Co-Operative (BALCO) scandal involving a number of athletes including British sprinter Dwain Chambers. Many sports including cycling, baseball, American football and combat sports have all experienced problems with AS use but AS use is, perhaps, most often associated with bodybuilding where a culture of AS use has become accepted, maybe more so than in any other sport (Yesalis et al., 2000).

With the increase in media and societal pressures for individuals to improve their physical appearance and performance in line with certain ideals, it is perhaps unsurprising that AS use is no longer confined to those within elite sport. In fact, a large majority of users of AS are recreational exercisers (Yesalis and Bahrke, 2005). AS users now include; models, security personnel as well as prison and police officers (Evans-Brown et al., 2008). The effect of AS on muscular growth and repair in particular has led to its increased use in non-elite sport where performance and image enhancement can be achieved from supra-physiological doses (Bhasin et al., 1996, Bahrke and Yesalis, 2004). Indeed, it could even be argued that image enhancement now supersedes performance enhancement as the ultimate training outcome for many recreational users.

Despite the apparent increase in AS use, data on prevalence of use is difficult to obtain and verify (McVeigh et al., 2003). In many countries, including the UK, AS use falls within a legal grey area where possession is not illegal but supply or intent

to supply is. This can make users reluctant to admit their use and therefore makes quantification of the number of users, both nationally and internationally, very difficult. However, reports from needle and syringe programmes (NEP's) have indicated a large and continuing growth of AS use within the UK. Some estimates have shown that the number of AS-injecting individuals attending NEP's has increased 2000% from 1991-2006 (Evans-Brown et al., 2008). Surveys of gym's have previously shown that around 5% of members (Korkia et al., 1993) were using AS whilst the prevalence of use, or previous use, in gyms equipped for competitive bodybuilding has been shown to be between 25-50% (Korkia and Stimson, 1997). At a sporting level, 45% of all adverse findings from WADA-accredited laboratories in 2006, tested positive for AS (Kicman, 2008).

1.2 Pharmacokinetics

The mechanism by which AS are thought to exert their effect is important. All AS exert both anabolic (increased protein synthesis) and androgenic (development of secondary male sexual characteristics) effects on the body in differing proportions. This has meant that there is a constant quest by manufacturers, both legal and illegal, to maximise the anabolic whilst minimising the androgenic components of AS. A purely anabolic agent has yet to be discovered (Bahrke and Yesalis, 2004). Whilst AS come in many different forms, they are predominantly taken via either oral ingestion or intra-muscular injection (transdermal patches/gels have also been used). Due to the rapid breakdown of testosterone within the body one key development with respect to AS has been to slow the rate of metabolism. This has been done by

esterification of the 17 β- hydroxyl group for intramuscular injection or alkylation at the 17α position for oral administration.

Figure 1.1. 17α alkylation and 17 β esterification of testosterone. (Srinivas-Shankar and Wu, 2006)

The addition of an ester at the 17 β-hydroxy position causes an increase in the hydrophobic/lipophilic properties of the AS that helps to slow down its absorption and degradation when injected intramuscularly, with the length of the chain determining the rate of breakdown. An AS that has a decanoate ester, for example, will have a longer half-life than a phenylpropionate (Minto et al., 1997), and testosterone undecanoate has a longer half-life than testosterone enanthate (Weinbauer et al., 2003). Whilst there is limited scientific data concerning the active half-lives of different AS, much of the values given come from anecdotal sources

(Table 1.1). Those AS that have an ester added at the 17 α-hyrdoxy position are used for oral ingestion as this minimises metabolism in the liver. It is believed that this change to the structure of AS, to allow for oral ingestion, has a side effect linked to hepatic and renal toxicity (Welder et al., 1995b, Applebaum-Bowden et al., 1987).

Table 1.1. Half-lives of different oral and injectable AS and esters.

Oral Steroids

Drug	**Active half-life**
Dianabol (methandrostenolone, methandienone)	5 to 6 hours
Anavar (oxandrolone)	9 hours
Anadrol / Anapolan50 (oxymetholone)	8 to 9 hours4
Winstrol (stanozolol)	9 hours
Methyltestosterone	4 days

Injectable steroids

Drug	**Active half-life**
Winstrol (stanozolol)	1 day
Testosterone Suspension	1 day
Finaject (trenbolone acetate)	3 days
Testosterone Propionate	4.5 days
Testosterone Enanthate	10.5 days
Primobolan (methenolone enanthate)	10.5 days
Testosterone Cypionate	12 days
Equipoise	14 days
Deca-durabolin (Nandrolone decanate)	15 days
Sustanon or Omnadren	15 to 18 days

Steroid esters:

Drug	Active half-life
Formate	1.5 days
Acetate	3 days
Propionate	4.5 days
Phenylpropionate	4.5 days
Butyrate	6 days
Valerate	7.5 days
Hexanoate	9 days
Caproate	9 days
Isocaproate	9 days
Heptanoate	10.5 days
Enanthate	10.5 days
Octanoate	12 days
Cypionate	12 days
Nonanoate	13.5 days
Decanoate	15 days
Undecanoate	16.5 days

Once inside the body there are a number of different metabolic fates for AS including reduction to dihydrotestosterone or aromatization to estradiol. In this latter case they can then exert oestrogenic effects (secondary female sexual characteristics) on the target cell.

The effects of AS on muscle cells (skeletal or cardiac) is mediated primarily by the presence of androgen receptors (AR) present within the cytoplasm of cells (Inoue et al., 1994). AS that are reduced to dihydrotestosterone can still illicit an androgenic or anabolic effect on the cell via the AR (Kicman, 2008). Androgen-receptor complexes

regulate the transcription of target genes that may control the accumulation of DNA responsible for muscle growth. AS are relatively small molecules, thereby allowing them to diffuse into cells and bind with the receptors present in the target cells. Following activation of the AR, it is translocated into the nucleus and triggers the formation of a transcription complex. Whilst it was previously thought that AR's were saturated at physiological levels it is important to note that, despite previous contradictory findings, AS concentrations have been shown to cause an up-regulation of AR's (Carson et al., 2002). It is also believed that strength training can increase the number of AR's (Bamman et al., 2001) suggesting a possible complimentary impact of AS use and strength training.

Whilst the direct anabolic effect of AS on skeletal muscle cells has been shown, additional complimentary anabolic effects have been suggested. AS may also stimulate the secretion of growth hormone (GH) or insulin-like growth factor (IGF-1) both of which have documented anabolic effects (Kuhn, 2002). It has also been postulated that behavioural effects of AS, such as increased aggression and drive/determination, could influence training intensity thereby indirectly increasing muscle size and strength (Yates, 2000).

1.3 Current AS Use: Practices and Side Effects

In order to maximise the positive effects of AS use, a number of techniques are employed by users. Users often combine a series of 2 or more oral and/or injectable AS preparations in a process referred to as 'stacking'. These combinations, with

varying ratios of different AS used at different time points, are administered in 'cycles' which typically last 4-12 weeks (Evans-Brown et al., 2008) although there is large individual variation with some users continually using for extremely long periods of time. Doses can vary substantially with anecdotal evidence suggesting doses ranging between less than 500 mg per week up to 2000 mg per week with additional anabolic agents also used. Evans (1997) reported doses ranging between 250 mg and 3200 mg per week of testosterone or its equivalent. Following an 'on' cycle, users will often have a period of abstinence ranging from 2 to 6 weeks, although, as with 'on' cycle lengths, there is large variation. This period of abstinence is used to "clear the system" and allow the body to normalise ready for another period of usage. 'Stacks' and 'cycles' are 'designed' in order to maximise the effect of each AS on skeletal muscle growth, based on the different pharmacokinetics and pharmacodynamics that each steroid can exert, despite having little scientific underpinning (Evans-Brown et al., 2008). In addition to AS, a number of other drugs are often taking in combination. These drugs include anabolic agents such as insulin and IGF-1, stimulant and fat loss agents such as ephedrine and clenbuterol as well as drugs to limit and prevent issues of aromatisation such as tamoxifen. Further to this, many AS users undertake a post-cycle therapy in order to help stimulate the natural production of testosterone, using substances such as tamoxifen and clomid to help stimulate the production of follicle stimulating hormone and luteinizing hormone to in turn increase production of testosterone from the testes.

Not surprisingly, given the supraphysiological or supraclinical doses that are used, case studies and case-series research has indicated a number of negative side effects

associated with AS use/abuse. Some of the negative side-effects that are associated with AS abuse include acne, gynaecomastia and testicular atrophy (Evans-Brown et al., 2008) and have also been associated with certain psychological disturbances such as mania, hypomania and depression (Pope Jr and Katz, 1994). The abuse of anabolic steroids (AS), outside of therapeutic use, has raised significant concerns about negative CV health consequences (Melchert and Welder, 1995). Despite the commonly held belief that AS are detrimental to CV health the scientific data detailing CV disease presence, morbidity, mortality as well as risk are controversial (Korkia and Stimson, 1997). If AS use has a direct impact on cardiovascular risk it may then precipitate cardiovascular disease and events (e.g. heart attack). Alterations in CV risk factors such as lipid profiles (Lane et al., 2006, Hartgens et al., 2004). and cardiac events (Bispo et al., 2009, Santamarina et al., 2008) have been reported with AS use but are often contradictory (Hartgens and Kuipers, 2004). There has been conflicting research regarding the effects of AS on both structural and functional parameters of the heart. Whilst there has been evidence of increases in left ventricular mass and wall thicknesses in AS users (Sachtleben et al., 1993, Kasikcioglu et al., 2009) there have also been findings that have observed no differences (Hartgens et al., 2003, Baggish et al., 2010). Research concerning alterations in cardiac function in AS users, and more specifically the relaxation capabilities of the ventricle, have also had conflicting outcomes (Dickerman et al., 1998, Kasikcioglu et al., 2009, Baggish et al., 2010). Despite this, reductions in the relaxation capacity of the ventricles in AS users would imply a consistent increased cardiac workload that may signify an increased CV risk.

In order to develop our knowledge further, this thesis will focus on the cardiovascular consequences of AS use/abuse. Specifically, it will first present a review of previous case study and case series research related to a number of cardiovascular risk factors. The thesis will then progress to 4 empirical studies detailing; 1) the effects of AS use on cardiac structure and function using novel, state-of-the-art regional deformation analysis, 2) the impact of AS use on both left and right ventricular structure and global function using the gold standard technique of magnetic resonance imaging (MRI) which will also facilitate the assessment of perfusion defects or interstitial fibrosis in the hearts of AS users, 3) the impact of AS use on the dynamic response to resistance exercise either in the form of the integrated cardiovascular response to a single set of exercise, or 4) the cardiac functional and biomarker response to a single training session. These latter 2 studies will move the assessment of the cardiovascular consequences of AS use away from resting studies to dynamic scenarios where the heart of the AS user will be placed under greater stress.

Aims

The aims of this Thesis are:

1. To examine the effect of AS upon global and regional cardiac structure and function using new "state-of-the-art" echocardiographic technologies.
2. To determine the impact of AS use on both left and right ventricular global structure and function as well as perfusion and fibrosis using the "gold standard" MRI technique.

3. To explore the effect of AS use on the integrated cardiovascular response to a single set of dynamic resistance exercise.
4. To explore the effect of AS use on cardiac function and markers of cardiomyocyte damage following a full body resistance exercise workout.

Chapter 2. Literature Review

2.1 Introduction

The use and abuse of anabolic steroids (AS) gains popular and media attention when elite athletes fail "doping" tests. Although significant, in some respects, these cases underplay the pervasive nature of AS use in Western societies. Whilst official figures from the British Crime Survey (2008) suggested a decrease in anabolic steroid (AS) use, reports from needle exchange programmes and other public health initiative's have pointed towards a growing use of AS in the UK (McVeigh et al., 2003). The clandestine nature of AS use makes an accurate assessment of the prevalence of use difficult. Despite this, some studies have attempted to quantify the level of use in specific groups. Yesalis et al. (2000) found that between 4 and 6% of high school males and 1-2% of high school females had tried AS. Bahrke and Yesalis (Bahrke and Yesalis, 2004) remarked that studies from countries including Canada, Sweden, England, Australia and South Africa suggest 1-3% of high school students had tried AS at some time. The widespread use of AS has been documented in professionals working in emergency services, casual fitness enthusiasts, as well as sportsmen and women from a wide variety of different sports (Korkia and Stimson, 1997). Although the exact health consequences of AS use are largely undefined, AS use represents a growing public health concern.

The therapeutic use of testosterone is somewhat controversial with conflicting data, although some studies have indicated limited positive effects on isolated cardiovascular (CV) risk factors in CV disease states (Ong et al., 2000). The use of AS outside of therapeutic prescription has raised significant concerns about negative CV health consequences (Melchert and Welder, 1995). Despite the commonly held

belief that AS are detrimental to CV health, scientific data detailing CV disease prevalence, morbidity and mortality are limited and controversial (Korkia and Stimson, 1997).

This review considers the current evidence-base regarding AS use in humans and the presence of CV disease or elevated risk of developing CV disease. We seek to present the latest data as well as limitations and future directions for work in this area. For the purposes of this review, data has been delimited to available human studies. Whilst much can be learned from animal models (LeGros et al., 2000) and recent data suggests a direct link between AS use and coronary lesion formation (Belhani et al., 2009), such studies rarely reflect the reality of human AS use for performance or image enhancement. Throughout the review we will seek to critique available evidence, discuss potential mechanism(s) where relevant human and/or animal data provide insight.

Search Strategies

In order to locate relevant studies for this review, a literature search was performed through the Liverpool John Moores University electronic library which covers a number of databases including Sport Discus, Web of Science, Science Direct, Emerald, Cinahl Plus, PubMed and Academic Search. The search terms used were 'anabolic steroids', 'anabolic androgenic steroids', with additional operators of 'cardiovascular effects', 'cardiac' and 'vascular' with the search period being set

between 1970-2010. Inclusion of studies was delimited to human and cellular studies, with animal studies only being used to infer possible mechanisms of action.

2.2 Anabolic Steroid Use and the Presence of CV disease

Assessment of the presence of CV disease in any population can be made in a number of ways. Epidemiological approaches, which follow large cohorts over prolonged follow-up periods, assess specific end-points including cardiac-related death, acute myocardial infarction, cardiac failure and presence of coronary disease on coronary angiography. Such studies have been performed to assess the impact of various risk factors on CV disease presentation (e.g. physical [in]activity; (Morris et al., 1953, Paffenbarger and Hale, 1975)). In many sporting sub-cultures AS use is banned, partially because of concerns over adverse health effects as well as the fact that it constitutes "cheating" and a breakdown of the "Olympic-ideal" (Pipe and Hebert, 2008). These issues would make many users, some of whom are involved in competitive sport, reluctant to admit their use.

Alternative ways to determine the presence of cardiovascular disease include the assessment of the presence and nature of any atherosclerotic load or burden within an individual. Assessments can be made before a significant CV event occurs, providing another option in the assessment of the effects of AS on CV health. The National Institute for Clinical Excellence (NICE) guidelines for assessment of chest pain use an algorithm starting with the assessment of the presence of CV risk factors (smoking, diabetes, hyperlipidaemia) coupled with sex and age. This is then followed by non-invasive and invasive testing as required, depending on the

perceived degree of risk. Of the tools available, invasive coronary angiography remains the gold-standard for assessment of coronary atherosclerosis. However, the use of an invasive test in AS users without a history of chest pain, coupled with the use of radiation, cost and complexity in addition to the semi-invasive nature of angiography are why this is not routinely performed outside of a clinical setting and why there appears to be no controlled human trials of AS use using these techniques. Non-invasive computerised tomography (CT) coronary angiography is an alternative technique, but again cost, radiation and limited resolution available until recently has led to its use being limited. Other assessments exist including CT coronary calcium scores, which indicate the overall atherosclerotic burden without indicating the degree of stenosis in the coronary arteries, but again we are unaware of any such data in AS users. This would be a useful technique given the rapid acquisition time, but also requires low dose radiation. Another option is the assessment of the carotid artery intima-media thickness (cIMT), which measures the width or depth of the wall of a conduit artery, most often the carotid artery. The sub-intimal space is where plaque formation typically occurs in arterial disease and assessment of cIMT is easily and accurately performed using ultrasound technology which thus provides a non-invasive assessment of atherosclerotic burden (Salonen and Salonen, 1993). Despite being assessed in a peripheral artery, the measurement of cIMT has been shown to reflect atherosclerotic burden in the coronary arteries (Bots et al., 2002). Sader et al. (2001) reported no difference in the cIMT of AS using and non-AS using subjects, despite a greater cIMT observed in both bodybuilding groups compared to the sedentary control cohort.

The only substantial and growing database related to the association between AS use and the presence of CV disease is provided by case study reports (Hartgens and Kuipers, 2004) (see Table 2.1). Case studies can present CV events or disease end-points (e.g. sudden cardiac death, acute myocardial infarction etc.) as well as autopsy data that directly determine atherosclerotic burden. The developing list of case studies provides evidence of the possible significant, and maybe fatal, association between AS and CV disease (Lau et al., 2007, Murai et al.). Although not completely exhaustive, Table 2.1 documents the occurrence of a number of significant CV disease end-points, such as strokes, myocardial infarctions and thrombo-embolism. Often these events are fatal, and even if not, can lead to significant disability and functional impairment. The physical, psychological and sociological ramifications are likely significant in those who survive an initial "event".

Few common themes emerge from consideration of the case studies presented in Table 2.1. A broad spectrum of specific events is observed in a range of individuals. A noticeable pattern of disease presence and/or risk is hard to deduce. It is also difficult to be sure of accurate reporting of a broad spectrum of physiological or behavioural risk factors for CV disease for each subject.

Whilst case studies provide a useful initial insight into the effects of AS that may help drive case-series or case-control studies, they are by no means conclusive and cannot be considered strong evidence of causal relationships. Case studies are also reliant on self-reporting of substance use, which can often be unreliable, with a lack

of independent confirmation of what the person believes they are taking, as well as ignoring the co-abuse of other substances.

Table 2.1 Case study events in AS users and reported presence of risk factors.

Event	Case Studies [CV Disease Risk Factors Present]
Stroke	Frankle et al. (1988) [↓ HDL, ↑ Liver enzymes], Mochizuki et al. (1988), Kennedy et al. .(1993), Sahraian et al. (2004), Santamarina et al. (2008) [↑ Apo B].
Myocardial Infarction	McNutt et al. (1988) [↑LDL, ↓HDL], Bowman (1990) [↑TC, ↓HDL], Ferenchick and Adelman (1992) [Family History], Kennedy (1993) [↑TC, ↓HDL, Smoker], Appleby et al. (1994) [Smoker, ↑BP], Huie (1994) [Family History, ↑ALT, ↑ASP, ↑CK], Fisher et al. (1996), Goldstein et al. (1998), Fineschi et al. (2001), Gunes et al. (2004) [↓HDL], Angelilli et al. (2005) [↑Trig, history of hypertension], Wysozcanski et al. (2008) [↑BP, ↑CK, ↓HDL], Lunghetti et al. (Lunghetti et al., 2009).
Sudden Death	Luke et al. (1990), Campbell et al. (1993), Hausmann et al. (Hausmann et al., 1998), Madea and Grellner (1998) [↑BP, ↑TC & LDL, ↓HDL↑Trig], Fineschi et al. (2007) Laroche (1990) [Smoker], Gaede & Montine (1992), Jaillard et al. (1994), Mewis et al. (1996) [↑ LDL], Nieminen et al. (1996) (Patient 4), Palfi et al. (1997), Falkenberg et al. (1997) [Patient 2: Smoker], Hourigan et al. (1998), McCarthy et al. (2000), Ment & Ludman (2002) [Smoker, ↑ TC], Alhadad et al. (2010) [Protein C deficiency], Liljeqvist et al. (2008), Frogel et al. (2009).
Cardiac Hypertrophy	Nieminen et al. (1996) (Patient 2), Mark et al. (2005)
Cardiomyopathy	Ahlgrim & Guglin (2009), Bispo et al. (2009) [↑ ALT & AST].
Endocarditis	Nieminen et al. (1996), [Patient 1: ↓ HDL], Fineschi et al. (2007) (Patients 1 & 2), Frogel et al. (2009).
Atrial Fibrillation	Sullivan et al. (1999) [Tachycardia, ↑ CK].
Heart Failure	Clark & Schofield (2005) [Sinus tachycardia, ↑ALT, AST]
Subdural Haematoma	Alaraj et al. (2005), Alhadad et al. (2010) [Protein C deficiency].

BP= Blood Pressure, TC= Total Cholesterol, LDL= Low-density lipoprotein, HDL= High-density lipoprotein, Trig= Triglycerides, CK= Creatine Kinase, ALT= Alanine Transaminase AST= Aspartate Transaminase, Apo B= Apolipoprotein B. Case Studies where no cause of death is reported or ascertained are grouped under 'sudden death' whereas those case studies where death is attributed to a myocardial infarction are grouped accordingly.

2.3 Anabolic Steroid Use and Cardiovascular Disease Risk Factors

Independent risk factors for CV disease include hypertension, hyperlipidemia, diabetes, obesity and a family history of premature coronary disease. High levels of homocysteine and high sensitivity C-reactive protein [hsCRP] have also been used as surrogate markers for the development of CV disease (Ridker et al., 2002). In the absence of data from epidemiological or coronary imaging studies assessing CV disease presence or prevalence in AS users, studies have assessed the prevalence of known risk factors, with or without comparison to matched controls. The link between each of these factors and the development/progression of atherosclerosis, combined with case reports of the presence of risk factors in AS users who have had significant CV events (see Table 2.1), has prompted their assessment as a potential measure of future CV disease risk. Not surprisingly these data are somewhat contradictory and thus inconclusive (see Table 2.2).

Hypertension has been identified as an important CV disease risk factor (Dawber et al., 1957). Freed et al. (1975) and Riebe et al. (1992) reported significantly higher systolic blood pressure in bodybuilders using AS compared to non-AS using bodybuilding controls. Conversely, Sader et al. (2001) and Lane et al. (2006) reported no elevation of blood pressures in AS users. The disparity between studies may reflect significant variance in the inclusion of smokers and the lack of control of diet across the groups. Of interest is that only Riebe et al. (1992) assessed blood pressure during exercise and this was elevated in AS users. The importance of an exaggerated blood pressure response to exercise for CV risk has been postulated (Chaney and Eyman, 1988) but not fully assessed in AS users. Another factor

associated with elevated arterial blood pressure is aortic stiffness (lowered aortic compliance). Stiffer arteries tend to augment pulse-pressure and alter SBP and/or DBP. Interestingly, large artery stiffness may be increased with resistance training (Kawano et al., 2008). The impact of AS use on central or large artery stiffness has received some attention recently. For example Kasikcioglu et al. (2007) found that AS use could be associated with a reduction in aortic elastic properties (increased stiffness), which could directly increase risk of a CV event. The authors suggested that AS directly affect nitric oxide-mediated relaxation through the inhibition of guanylate cyclase, a second messenger that carries the signal for smooth muscle to relax.

Elevated total cholesterol (TC) and low-density lipoproteins (LDL) and low high-density lipoproteins (HDL) cholesterol have been shown to increase CV risk (Robinson et al., 2009). Studies of TC, LDL and HDL levels in AS users have produced contradictory outcomes. Although most studies report little difference in TC, most demonstrate a decrease in HDL with some observing an increase in LDL (Hartgens et al., 2004, Lane et al., 2006, Sader et al., 2001). Lane et al. (2006), observed little difference in total cholesterol levels between AS users, (4·0 ± 0·83 mmol/l), and non-users, (3·8 ± 0·38 mmol/l), whilst LDL (2·9 ± 0·7 vs. 2·1 ± 0·3 mmol/l), was significantly elevated and HDL (0·7 ± 0·4 vs 1·3 ± 0·3 mmol/l) significantly decreased in the AS group. A rise in LDL cholesterol often leads to increased arterial fat deposition and this change in lipoprotein profile is therefore associated with an increased atherosclerotic risk (Robinson et al., 2009). Apolipoprotein, a component of HDL cholesterol, has also been studied in AS users. Studies, by Hartgens et al. (2004) and Hartgens & Kuipers (2004) reported

conflicting data, possibly due to issues of drug dosage and comparisons of single to poly-drug regimens. Whilst the exact mechanisms for the effect of AS on lipid profiles is yet to be fully established, some mechanisms have been postulated. Applebaum-Bowden et al. (1987) suggested that after administration of stanozolol for 7 days there was a decrease in HDL levels with a concomitant increase in hepatic triglyceride lipase (HTGL). HTGL is partly responsible for the production of LDL but these results would also suggest a possible catabolic effect of HTGL on HDL, thereby leading to negatively affected lipid profiles.

High sensitivity C-reactive protein (hsCRP) is a marker of inflammation and has been associated with CV events including myocardial infarctions and stroke (Ridker et al., 2002). Measurement of hsCRP is not routinely performed by doctors in the evaluation of CV risk in the general population. As such, limited attention has been paid to hsCRP in AS users. Grace and Davies (2004) reported that hsCRP was elevated in AS-using bodybuilders compared to both non AS-using bodybuilders and sedentary controls. Although the mechanism(s) for this observation is not known, and such findings require replication, the data highlights the possibility that AS use could result in local or systemic inflammation that has the potential to damage cardiomyocytes and/or the vascular endothelium.

Elevated levels of the amino acid homocysteine (HCY) have also been associated with increased CV disease risk (Boushey et al., 1995) as a consequence of endothelial damage (McCully, 2005). As with many CV disease risk factors, the data regarding HCY and AS use are conflicting. Zmuda et al. (1997) reported no change

in HCY in AS users whereas Graham et al. (2006) observed high HCY levels even in those subjects who had abstained from AS use for 3 months. The disparity in outcomes is difficult to explain but maybe related to subject-specific issues such as diet. A possible explanation for an increase in HCY in AS users is that the AS could affect the absorption of certain B vitamins (in particular B_6 and B_{12}), a deficiency that can lead to an increase in HCY levels. Other medical conditions which cause elevated haemoglobin levels (polycythaemia) are associated with increased CV risk and this therefore may be an additional cause in AS users.

Finally, Alén (Alen, 1985) and Lane et al. (Lane et al., 2006) reported significantly elevated haematocrit (Hct)/haemoglobin (Hb) levels in AS users. In addition, Lane et al. (Lane et al., 2006) observed Hb levels were not different to controls after a 3 month abstinence from AS. In contrast, Hartgens et al. (Hartgens et al., 1995) reported that Hct and Hb remained unaffected.

In summary, whilst it has become quite apparent that AS use can have a significantly negative effect on HDL levels (Applebaum-Bowden et al., 1987, Hartgens et al., 2004, Lane et al., 2006), and therefore increase CV risk (Robinson et al., 2009), much of the research that has assessed the association of AS use with alterations in CV disease risk factors is limited, contradictory and may be multifactorial. It is , therefore, worthwhile reflecting on case study data in Table 2.1 where there are no clear patterns of measurable CV disease risk in cases with "hard" CV event end-points. Scope for on-going work reflecting long-term poly-drug users with attention paid to variations with cycle-phase is apparent. Further, when available data for CV

disease risk factors in AS users is combined with case study reports, one could surmise that factors additional to traditional CV disease risk factors may be responsible for any association between AS use and CV disease occurrence. It is for this reason that other research groups have assessed the link between AS use and cardiac electrical, structural and functional parameters as well as any impact of AS use on vascular health.

Table 2.2 Anabolic Steroids and associated risk factors.

Risk Factor	Studies	Subjects [n]	Findings
Blood Pressure	Freed et al. (1975)	ASU vs P [n=13]	ASU ↑ SBP*
	Kuipers et al. (1991)	ASU vs P [n=14]	ASU ↑DBP*
	Riebe et al. (1992)	ASU vs BC [ASU=10, BC=10]	ASU ↑ SBP* @ rest and exercise ASU ↑ DBP* during exercise.
	Sader et al. (2001)	ASU vs BC [ASU=10, BC=10]	No significant difference between groups.
	Lane et al. (2006)	ASU vs BC [ASU=10, Off-AS=8, BC=10]	No sig. Difference.
Cholesterol	Baldo-Enzi et al. (1990)	ASU vs BC & AS On vs Off treatment. [ASU=14, On/Off AS=10, BC=17]	ASU ↓TC** ASU ↓HDL*** On ↓HDL***
	Zmuda et al. (1993)	T vs TL vs T & TL (3 weeks administration)	T ↓ HDL* T & TL ↓ HDL* No significant change in LDL in all groups
	Sader et al. (2001)	ASU vs BC. [ASU=10, BC=10]	TC No sig. Difference ASU ↓HDL ***

	Hartgens et al. (2004)	ASU vs BC [ASU=19, BC=16]	TC No sig. Difference ASU ↑LDL* ASU ↓HDL*
	Lane et al. (2006)	ASU vs ASA vs BC vs SC [ASU=10, Off-AS=8, BC=10]	TC No sig. Difference ASU ↑LDL* ASU ↓HDL***
C-Reactive Protein (CRP)	Grace & Davies (2004)	ASU vs BC vs SC. [ASU=10, BC=10, SC=8]	ASU ↑ CRP * vs BC & SC
Homocysteine (HCY)	Zmuda et al. (1997)	T vs TL vs T & TL (3 weeks administration) [n=14]	No change in all groups
	Graham et al. (Graham et al., 2006)	ASU vs ASA vs BC & SC. [ASU=10, ASA=10, BC=10, SC=10]	ASU** & ASA* ↑HCY vs BC & SC.
Apolipoproteins (APO)	Hartgens et al. (Hartgens et al., 2004)		
	Study 1.	ASU vs BC [ASU=19, BC=16]	ASU ↓ APO (A-1 & B) ***.
	Study 2.	AS (200mg/week of Nandrolone Decanoate) vs P [n=16]	No significant difference between groups.
Haemoglobin (Hb)	Alen (Alen, 1985)	ASU vs BC [ASU=5, BC=6]	Tendency for Hb to be higher in ASU group.

SBP= systolic blood pressure, DBP= diastolic blood pressure, TC= total cholesterol, HDL=High-density lipoprotein, LDL= Low-density lipoprotein, T=Testosterone, TL= Testolactone, BC= bodybuilding controls, SC= sedentary controls, ASU= anabolic steroid users, ASA= abstinent from steroids for 3 months, P = placebo. * $p<0.05$, ** $p<0.01$. *** $p<0.001$

2.4 Anabolic Steroid Use and Cardiac Electrical Activity, Structure and Function

The assessment of cardiac electrical activity, structure and function are commonly used in the determination of the presence or risk of CV disease. An example is the association of increasing left ventricular (LV) mass and prognostic CV disease relevance (Savage et al., 1990, Yeboah et al., 2007).

2.4.1 Cardiac Electrical Activity

Case reports have shown a possible effect of AS use on cardiac electrical activity with some finding an association between AS use and atrial fibrillation and cardiac failure precipitated by sinus tachycardia (Sullivan et al., 1999, Clark and Schofield, 2005). Human (and animal) studies have shown an association between testosterone and an increase in the rate of cardiac repolarisation (Bidoggia et al., 2000, Fulop et al., 2006). Consequently the potential for AS use to alter cardiac electrical activity would seem a valuable line of enquiry. However, there is limited research on the effect of AS abuse on cardiac de/repolarisation or any other aspect of the ECG in humans. Whilst previous findings have suggested prolonged QT interval may be present in some athletes as a result of training (Stolt et al., 1999), one study has suggested that AS use may be associated with a shortening of the QT interval (Bigi and Aslani, 2009). It has been suggested that the androgenic component of AS impacts on cardiac electrical activity via facilitation of the expression of two potassium channel proteins, Kir2.1 and Kir4.3, which are responsible for mediation of I_{k1} and I_{to} respectively (Fulop et al., 2006). The relevance of the effect on I_{k1} becomes clear as it has been cited as an important current involved in ventricular repolarisation which may cause the shortening of the QT interval (Liu et al., 2003). To further understand the role that AS may have on cardiac electrical activity, as well as the mechanism through which they act, it is clear more research is necessary.

A number of measures of cardiac structure and function have been investigated in order to ascertain the effects of AS use on the heart (Tables 2.3-2.5). However, it is clear that the deployment of novel non-invasive imaging techniques in the study of AS users is limited to only a few recent studies. New imaging tools provide both

global and regional assessments of cardiac structure and function (Langeland et al., 2005) that may provide extra insight in regard to the impact of AS upon myocardial tissue.

2.4.2 Cardiac Structure

A number of morphological adaptations of the heart have been observed in AS users. In a group of top-level bodybuilders, Urhausen et al. (1989) reported AS users had an increased LV posterior and septal wall thickness compared to a group of non-using bodybuilders. This resulted in an increased LV wall thickness:diameter ratio as well as an increased LV muscle mass:volume ratio. These findings were supported by Sachtleben et al. (1993) who noted AS users had greater LV mass, inter-ventricular septal wall thicknesses and LV diameter in diastole compared to non-AS using bodybuilding controls. It was also observed that when the users were "on" cycle they had a greater LV mass, septal wall thickness, and LV diameter compared to when "off" cycle. Some other studies have reported differences in cardiac structure between AS using and non-AS using athletes (D'Andrea et al., 2007, Dickerman et al., 1998). Both D'Andrea et al. (2007) and Dickerman et al. (1998) reported cardiac dimensions in AS users that were either greater than sedentary controls or outside of normal ranges, despite no significant difference to non-AS using athletes. These studies would suggest that training may be as potent a stimulus for cardiac adaptation as AS use. At odds with most previous literature, Nottin et al. (2006) observed no differences in LV wall thickness but significantly greater LV cavity size and mass in AS using body-builders compared to non-using athletes. This supports an eccentric as opposed to a concentric LV remodelling in AS users. The

reason for this contradiction is unclear, however significant differences in training between groups could offer an explanation.

Marsh, et al. (Marsh et al., 1998) observed AS can directly alter cardiac muscle protein metabolism, following activation of cell surface androgen receptors, through possible modulation of gene transcription in animal and human cardiac tissue. This theory offers an explanation for how AS use could cause structural and functional changes to the cardiac tissue of regular AS users.

Table 2.3. Studies showing effects of anabolic steroids on cardiac structure.

Study	Subjects [n]	Parameters Measured	Findings
Urhausen et al. (1989)	ASU vs BC [ASU=14, BC=7]	Total Heart Vol., LVM, LVDd, LVPW, IVS, LVWT:D, LVM:LVV.	**Total Heart Vol. & LVM:** No difference **LVDd:** ASU↓** vs BC **LVPW, IVS, LVWT:D & LVM:LVV:** ASU↑** vs BC
Sachtleben et al. (1993)	ASU (On & Off Cycle) vs BC [ASU=11, BC=13]	LVM, LV and IVS wall thicknesses, LVD(s&d)	**LVM and IVS thickness:** ASU (On)↑* vs ASU (Off) **LVM, IVS & LV posterior wall thickness:** ASU (ON)↑* vs BC
Dickerman et al. (1998)	ASU vs BC [ASU=6, BC=7]	LV wall thickness,	**LV wall thickness:** 6/6 ASU >11mm 3/7 BC > 11mm
Hartgens et al. (2003)	ASU vs BC [ASU=17, BC=15]	LVEDd, LVM, LVMI, IVS, RVD, PWEDWT, LVET.	**No significant differences observed.**
Nottin et al. (2006)	ASU vs BC vs SC [ASU=6, BC=9, SC=16]	LVD(s&d), LVM, LVV(s&d), LV wall thicknesses.	**LVM & LVDd:** ASU↑* vs BC & SC **LVV(s&d):** ASU↑** vs SC **LV wall thicknesses:** No sig. difference between group
D'Andrea et al.(2007)	ASU vs BC vs SC [ASU=20, BC=25, SC=25]	LVM, LVDd	**LVM & LVDd:** No sig. difference between groups
Kasikcioglu et al. (2009)	ASU vs BC [ASU=12, BC=14]	LV and RV.	**LVM:** ASU↑* vs BC **RVD:** ASU↓* vs BC
Baggish et al. (2010)	ASU vs BC [ASU=12, BC=7]	IVS, LVPWd, LVIDd, LVM	**No significant differences observed.**

ASU= Anabolic steroid user, BC=Bodybuilding control, SC= Sedentary control, LV=Left ventricle, RV=Right ventricle, IVS=Inter-ventricular septum, LVM=Left ventricular mass, LVD=Left ventricular diameter, PWEDWT= Posterior wall end diastolic wall thickness

2.4.3 Systolic Function

Whilst gross changes in cardiac structure provide one focus for studies of AS use, it is also pertinent to assess if AS use has any impact upon LV function. Both LV systolic (Table 2.4) and diastolic (Table 2.5) function have been assessed in AS users and controls in cross-sectional studies. Earlier studies, such as those by Nottin et al. (2006) and Hartgens et al. (2003) observed no difference in ejection fraction (EF) between strength trained athletes who did and did not use AS. The assessment of global LV systolic function may mask subtle or regional changes in function. Consequently, most recent research has adopted new imaging tools that can assess tissue velocities or strain in localised segments of LV wall tissue. Nottin et al. (2006) observed no significant differences between AS users and non-users in peak systolic myocardial veolicty at the mitral annulus (S_m) in the left ventricular posterior wall (LVPW), inter-ventricular septum (IVS), inferior wall or anterior wall despite slightly elevated values seen in the AS group. D'Andrea et al. (2007) found reduced peak strain and strain rates at both the mid and basal levels in the LVPW and IVS in AS users versus non-AS users and a control group of sedentary men. Kasikcioglu et al. (2009) measured the LV septal and RV free wall S_m in AS users, bodybuilding controls and sedentary controls. No difference was observed in S_m between the two bodybuilding groups and sedentary controls in either the LV or RV. Conversely, in a recent study Baggish et al. (2010) observed a reduced EF in an AS group compared to non-AS users, that was also clinically relevant in many individuals (i.e. <55%). They also reported significantly reduced longitudinal and radial strain in AS users when compared to non-AS users. The reason for the contradictions in the research mentioned previous is not entirely clear. Baggish et al. (2010) has suggested that participant differences between studies, such as older AS users as well as the lower

level of competitive individuals used in their study, could have been the cause of the differences observed. This is because possible reductions in cardiac function with age in addition to previous studies using high level competitors which could have favoured 'healthier' AS users. A reduction in ejection fraction could possibly be caused by a reduction in myocardial elastance. As LeGros et al. (2000) have suggested, an increase in collagen cross-links within the myocardium through up-regulation of lysyl oxidase, an enzyme responsible for initiating cross-links between adjacent collagen molecules. Further in vitro studies of the direct mechanistic pathway that may be responsible for this change are required to develop our understanding of the direct effects of AS on myocardial cells.

Table 2.4. Studies showing effects of anabolic steroids on systolic cardiac function.

Study	Subjects	Parameters Measured	Findings
Hartgens et al. (2003)	ASU vs BC [ASU=17, BC=15]	EF	**No significant differences observed.**
Nottin et al. (2006)	ASU vs BC vs SC [ASU=6, BC=9, SC=16]	S_m	**No significant difference observed.**
D'Andrea et al. (2007)	ASU vs BC vs SC [ASU=20, BC=25, SC=25]	$\mathcal{E}$ and SR @ LV lateral wall and IVS, S_m	**S_m:** **No significant differences observed.** **Peak systolic LV strain rate and strain:** **IVS:** ASU↓*** vs BC & SC **LV lateral wall:** ASU↓** vs BC & SC
Kasikcioglu et al. (2009)	ASU vs BC vs SC [ASU=12, BC=14]	LV and RV.	**LV:** **S_m:** **No significant differences observed.** **RV:** **S_m:** **No significant differences observed.**
Baggish et al. (2010)	ASU vs BC [ASU=12, BC=7]	EF, Longitudinal $\mathcal{E}$, Radial $\mathcal{E}$	**EF:** ASU↓* vs BC **Longitudinal $\mathcal{E}$:** ASU↓* vs BC **Radial $\mathcal{E}$:** ASU↓* vs BC

.ASU= Anabolic steroid user, BC=Bodybuilding control, SC= Sedentary control, LV=Left ventricle, RV=Right ventricle, IVS=Inter-ventricular septum, LVM=Left ventricular mass, LVD=Left ventricular diameter, PWEDWT= Posterior wall end diastolic wall thickness, EF=Ejection Fraction, $\mathcal{E}$=peak strain, SR=Peak Strain Rate. *p<0.05, **p<0.01, ***p<0.001

2.4.4 Diastolic Function

There has, to some extent, been greater attention paid to whether AS use might alter LV diastolic function, possibly because of a potential link to AS-induced concentric hypertrophy. Dickerman et al. (1998) did not observe any differences in Doppler-derived early (E) and late (A) diastolic filling velocities as well as the E:A ratio in bodybuilders who did or did not use AS. Krieg et al. (Krieg et al., 2007) found no

significant difference in E:A ratios of AS using and non-using strength trained athletes, although they did find a significant reduction in early to late diastolic tissue velocities (E_m/A_m) in the AS group. Nottin et al. (2006) reported a greater isovolumic relaxation time and a smaller peak early diastolic (E) filling velocity in AS users, showing a reduced contribution of early relaxation to LV filling. This observation was supported by Baggish et al. (2010) who also commented that the lower E:A ratio in AS users was clinically significant in 10 out of the 12 athletes assessed. Nottin et al. (2006) also recorded tissue Doppler measurements of early (E_m) and late diastolic (Am) myocardial wall velocities at the mitral annulus (in septal, lateral, inferior and anterior wall segments) as an adjunct to diastolic filling blood flow velocities. In the AS using bodybuilders peak E_m and the peak E_m/A_m ratio were significantly smaller than the bodybuilding and sedentary controls. D'Andrea et al. (2007) and Kasikcioglu et al. (2009) also reported significantly lower E_m:A_m ratios in AS using bodybuilders compared to non-AS using bodybuilders and sedentary controls. A recent study by Baggish et al. (2010) also found a significantly lower E_m in the AS users but interestingly didn't report strain rate measurements in diastole.

A reduction in early diastolic filling parameters (whether assessed regionally or globally) suggests AS use has a negative effect on early relaxation and thus the suction that is exerted on LV inflow by the rapid decline in LV pressure compared to left atrial pressure. A reduction in early relaxation in AS users could be explained by a reduction in myocardial elastance (LeGros et al., 2000) reflecting a stiffer LV. Despite evidence of a direct effect of AS use on cardiac structure and function being,

at times, contradictory, some effort has been directed to try and ascertain potential mechanism(s) using animal models. The androgenic component of AS has received some attention with research showing that AS can affect extracellular calcium channels as well as calcium mobilisation from the endoplasmic reticulum (Lieberherr and Grosse, 1994). It has been suggested that this effect on calcium channels can, in turn, affect mitochondrial permeability which may allow the release of apoptogenic factors. This is supported by the findings of Zaugg et al. (2001) that found dose-dependent apoptotic cell death in rat myocytes.

Table 2.5 Studies showing effects of anabolic steroids on diastolic cardiac function.

Study	Subjects	Parameters Measured	Findings
Dickerman et al. (1998)	ASU vs BC [ASU=8, BC=8]	E, A, E:A ratio	**No significant differences observed.**
Hartgens et al. (2003)	ASU vs BC [ASU=17, BC=15]	E, A, E:A ratio	**No significant differences observed.**
Nottin et al. (2006)	ASU vs BC vs SC [ASU=6, BC=9, SC=16]	E_m, A_m, E_m: A_m	**E_m:** ASU↓**vs BC & SC **A_m:** **No significant difference observed.** **E_m: A_m:** ASU↓ vs BC** vs SC*
D'Andrea et al. (2007)	ASU vs BC vs SC [ASU=20, BC=25, SC=25]	E_m: A_m ratio, strain rate and strain @ LV lateral wall and IVS, S_m	**E_m: A_m ratio:** ASU↓** vs BC & SC
Krieg et al. (2007)	ASU vs BC vs SC [ASU=14, BC=11, SC=15]	E_m:A_m, E:A	**E_m: A_m ratio:** ASU↓ vs BC & SC **E:A:** **No significant differences observed.**
Kasikcioglu et al. (2009)	ASU vs BC vs SC [ASU=12, BC=14]	LV and RV.	**LV:** **E:A, E_m, A_m, & E_m:A_m:** ASU↓* vs BC **RV:** **E_m:** ASU↓* vs BC
Baggish et al. (2010)	ASU vs BC [ASU=12, BC=7]	E, A, E:A, E', A'	**E, E:A & E':** ASU↓ vs BC **A:** ASU↑* vs BC **A':** **No significant differences observed.**

ASU= Anabolic steroid user, BC=Bodybuilding control, SC= Sedentary control, E=Early diastolic filling velocities, A=Atrial diastolic filling velocities, E:A=Early:Atrial filling ratio, E'=Early diastolic tissue velocity, A'=Atrial diastolic tissue velocity.

2.5 Anabolic Steroids and Vascular Health

Limited positive effects of therapeutic doses of testosterone on vascular function have been described (Ong et al., 2000). The impact upon vascular health of AS use at higher concentrations has received less attention. This is somewhat surprising given the potential negative CV consequences of AS use reported in the literature (Grace and Davies, 2004, Hartgens and Kuipers, 2004, Baldo-Enzi et al., 1990, Appleby et al., 1994, Baggish et al., 2010). The progression from endothelial dysfunction to significant clinical atherosclerosis in coronary arteries is important in underpinning and predicting CV events.

The assessment of peripheral conduit arteries, using a number of techniques, facilitates the assessment of the CV risk and atherosclerotic burden of the circulatory system (Takase et al., 1998, Takase et al., 2005). As atherosclerosis is a progressive disease in which endothelial dysfunction is an early and integral feature, assessment of the function of endothelium may provide an "early-window" on the negative effects of AS use. In a case study by Green et al. (1993), an individual taking part in a study assessing forearm blood flow in response to methacholine (MCh) and sodium nitroprusside (SNP), potent NO-mediated vasodilators, had values that were dramatically decreased on the second testing session. The investigators ascertained that the participant had been self-administering AS for four months. He agreed to abstain from AS before the final testing session. Values had returned to the level of the first testing session following the period of abstinence. This suggests that AS have an acute effect on the NO dilator system that is somewhat reversible following a short period of abstinence.

Flow-mediated dilation (FMD) is a non-invasive method used to assess conduit artery function and abnormal FMD values can predict future CV event rates (Gokce et al., 2003). Ebenbichler et al. (2001) and Sader et al. (2001) reported that FMD and glyceryl trinitrate (GTN)-induced vasodilation of the brachial artery were reduced in AS users compared to healthy sedentary controls, indicating that some effect of AS on vascular smooth muscle function may be evident. In support of this contention, Lane et al. (2006) reported that endothelial-independent dilatation was impaired in those currently using AS, compared to those who had abstained from AS use for 3 months and a group of bodybuilding controls. Interestingly, Sader et al. (2001) reported a similar reduction in FMD in both AS-using and non-AS using bodybuilders, suggesting that prolonged and intense resistance training alone may be responsible for negative impacts on vascular health. This conclusion is endorsed by studies of the effect of resistance training on vascular function in otherwise healthy subjects (Miyachi et al., 2004, Rakobowchuk et al., 2005). Whilst not investigating the effects of AS use in strength-trained athletes, McCredie et al. (1998), examined the effect of testosterone implants on vascular reactivity in a group of female-to-male transsexuals against a group of female controls. They found that GTN values were significantly reduced in the transsexual group, whilst there was a mean difference of 1.8% in FMD scores between groups with the transsexual group having a lower mean FMD score. Whilst the authors did state that the high number of smokers within both groups is a likely confounding factor, the results imply a reduced relaxation capability within the vasculature due to high androgen exposure.

A number of mechanisms for an AS mediated reduction in vascular function as a consequence of AS use have been proposed. These include suggestions that AS may have a direct, negative effect on the NO dilator system. Kasikcioglu et al. (2007) proposed that down-regulation of the nitric-oxide dilator system could underpin an AS-related increase in arterial stiffness and reduction in FMD. AS use has been shown to have a negative effect on endothelial growth (D'Ascenzo et al., 2007). It is also possible that AS have a detrimental effect of endothelial cell turnover and renewal, implying that AS could cause stiffening within the heart and vasculature through affecting endothelial proliferation and propagation. However, no direct studies of endothelial progenitor cells currently exist. In addition, it has also been suggested that AS not only attenuate the effect of vasodilators, demonstrated in rabbits, but also enhance the effects of vasoconstrictors (Ammar et al., 2004). McCrohon et al. (1999) investigated the effect of AS on monocyte adhesion to endothelial cell in vitro and they found that androgens caused a dose-related increase in monocyte adhesion to endothelial cells. This increase in cell adhesion was mediated by an up-regulation in vascular cell adhesion molecule-1 (VCAM-1). Whilst this study was performed in vitro, it does give an indication of another possible mechanism through which AS might negatively impact atherosclerotic risk.

The effects of AS use on vascular health is inconclusive, yet significant negative effects of AS on endothelial and vascular smooth muscle function have been demonstrated (Sader et al., 2001, Ebenbichler et al., 2001). Whilst resistance training alone could cause a reduction in endothelial function, AS could further confound this effect by significantly impairing vascular smooth muscle relaxation (Sader et al., 2001). Despite conflicting evidence with regards to the effect of endothelial

dependent relaxation as a result of AS, it is noteworthy to show that a mechanism for endothelial vascular relaxation impairment has been demonstrated that could help to explain the propensity of CV events in AS users. Although this was demonstrated using very large relative doses (D'Ascenzo et al., 2007).

Summary

The current data regarding the effects of AS use/abuse on CV health is largely contradictory and inconclusive. Whilst causal relationships for negative CV effects have been suggested from case-study data, cross-sectional studies have been less definitive and there is an absence of longitudinal studies detailing effects within individuals. To date the most consistent data reflects a significant negative effect of AS use on lipid profiles and in particular a reduction in HDL levels (Lane et al., 2006, Baldo-Enzi et al., 1990). A common theme appearing in recent data is the suggestion that a decrease occurs in the relaxation properties of both the myocardium and the vascular wall. An increased stiffness within the ventricles of the heart and vasculature could mean a reduced ability of AS users to cope with physiological stressors.

Despite this, the data reviewed, mainly in the form of a burgeoning body of case study reports of CV events and disease end-points, raises significant concerns about the CV health consequences of AS use. This may be particularly relevant in specific individuals, but predicting those at greater risk among the AS using community is beyond our capacity at present. Current evidence, allied to an appreciation of many technical and design limitations when studying AS use, should prompt on-going

research into numerous aspects of CV health, risk factors as well as CV structure and function in those who choose to use AS for promoting performance and/or image enhancement.

As has been demonstrated through this literature review, it is apparent that there are significant gaps in the literature that require greater investigation. Despite research into cardiac structure and function, including the use of novel techniques such as strain and strain rate, there is a lack of diastolic data using this technique. Further to this, whilst cardiac magnetic resonance imaging provides the most comprehensive non-invasive assessment of cardiac structure and function, this technique has yet to be utilised in a group of AS users. Finally, the effects of exercise on cardiovascular function can provide a useful insight into the ability of the CV system to adapt to physiological stress. Due to the lack of data in AS users that utilise these techniques, this thesis will employ assessment of diastolic function using speckle-tracking, strain and strain rate, as well assessing CV function using cardiac MRI in addition to assessing the CV response during and after exercise in AS users.

Chapter 3. Anabolic steroid use and longitudinal, radial and circumferential cardiac motion

3.1 Introduction

Anabolic steroids (AS) are synthetic derivatives of the male hormone testosterone. It has long been established that AS increase skeletal muscular size and strength through an up-regulation of protein synthesis. These effects have led to the increasing trend of AS use for both performance and image enhancement (Bahrke and Yesalis, 2004). The exact number of current AS users in the UK and worldwide is difficult to gauge due to the legal issues surrounding their possession and use. Despite this, reports from needle and syringe programmes (NEP's) have pointed to a large and continual growth of AS use in the UK, with the number of AS-injecting individuals attending the NEP's increasing 2000% from 1991-2006 (McVeigh et al., 2003).

Due to this trend for increasing AS use, there is growing concern about the cardiovascular (CV) health consequences of AS use. This is despite the fact that empirical evidence of increased CV risk is often equivocal. Concerns about the CV health risks have been prompted by substantial case study data that has linked AS use with CV end-points such as myocardial infarction, (Lunghetti et al., 2009) stroke (Santamarina et al., 2008) and cardiomyopathies (Bispo et al., 2009). Establishing a causal relationship from case studies is difficult and thus a number of traditional and new CV risk factors, such as blood pressure, blood lipid profiles as well as homocysteine and C-reactive protein (CRP) have been studied in case-control studies of AS users (Riebe et al., 1992, Graham et al., 2006, Grace and Davies, 2004), again with equivocal results. Other tools have been used to assess CV health including

ECG, but data is limited even though a recent study linked AS use to shortened ventricular repolarisation (Bigi and Aslani, 2009).

Some studies have suggested that left ventricular (LV) morphology and function may be negatively altered with AS use but data is not consistent (De Piccoli et al., 1991, Nottin et al., 2006). With continuing developments in non-invasive imaging, recent studies have assessed LV function at a regional level as well as measuring global function (D'Andrea et al., 2007, Baggish et al., 2010). Using tissue-Doppler and cardiac strain ($\mathcal{E}$) assessment both D'Andrea et al. (2007) and Baggish et al. (2010) observed reduced LV function in AS users. D'Andrea et al. (2007) reported only longitudinal $\mathcal{E}$ and Baggish et al. (2010) presented data only for longitudinal and radial $\mathcal{E}$ in a small number of AS users. A comprehensive evaluation of cardiac $\mathcal{E}$ in all three planes of motion (longitudinal, radial and circumferential), as well as assessment of rotation and torsion, would provide a significant addition to our understanding of the CV consequences of AS use. The determination of strain rate (SR) to provide peak SR in both systole and diastole provides added subtlety to functional cardiac measures.

The aim of the current study, therefore, was to assess traditional CV risk factors as well as to develop a more comprehensive assessment of LV structure and function using state-of-the-art non-invasive imaging tools. We hypothesised that there would be a significant effect of AS use on traditional risk factors as well as LV systolic and diastolic cardiac function at global and regional levels. We also investigated the

short-term impact of a small number of case studies following brief removal from AS.

3.2 Method

Subjects

Strength training individuals (n=47, male=46, female=1(AS)) were recruited through local gyms, personal contacts and local syringe exchange programmes. Posters and information sheets were placed in various locations and also distributed by hand to individuals. The posters stated that there was a study investigating the effects of strength/resistance training, either with or without the use of AS on cardiovascular health. Inclusion criteria were; participants aged between 18 and 50 years of age; resistance training history of minimum of 3 years with 3-4 training sessions per week. Exclusion criteria for the study were the presence of known respiratory, CV or musculoskeletal disease. Specific inclusion criteria for the AS using group (AS: n=28, age=31±7 yr) included a documented self-report history of AS use for at least 2 years (including on and off-cycles). Inclusion criteria for the non-AS (NAS) group (n=19, age=28±8 yr) included self reported history of never taking AS. Prior to taking part in the study, details of each part of the testing were described to each of the participants. The study was granted ethics approval from the Liverpool John Moores Ethics Committee and participants provided written informed consent.

Design

A cross-sectional cohort design was utilised for the study with participants required to make a single visit to our laboratory. Initially, subjects completed self report

questionnaires related to general health, training status and history as well as detailed accounts of AS use. This was followed by assessment of body composition, a venous blood sample and a comprehensive CV evaluation including brachial artery blood pressure, a resting 12-lead ECG and an echocardiogram. All tests were conducted on the participants following an overnight fast, as well as a 24 hr abstention from resistance training.

Protocols

Participant History and AS use

Training history included data on years of training, the average number and length of sessions per week, as well as self-reported one repetition maximums for the bench press and squat (Table 3.1). Those in the AS group provided a detailed history of AS use including names, dosage and cycling information and none of the participants self-reported co-abuse of any other illicit substances. For simplicity we provide a list of exemplar AS used but note that all subjects used multiple AS in various stacking procedures with varied periods of abstinence or "off-cycles". The types of AS currently being used by some of the AS participants included, Trenbolone (15), Testosterone (13), Sustanon (9), Boldenone (7), Nandrolone (10), Meathandrostenolone (8), Drostanolone Propionate (3), Oxandrolone (4) and Stanozolol (3). Of those in the AS group that provided sufficient information to perform an analysis of their daily usage (n=14), we found that the mean AS dose was 232 mg/day with a standard deviation of 208 mg (range 35 – 707 mg/day). To give an indication of an AS regimen we include a case exemplar of a participants AS use, Testosterone Propionate = 800mg/wk, Testosterone Cypionate = 500 mg/wk, Stanozolol = 350 mg/wk, Nandrolone Decanoate = 600 mg/wk. This was broken

down into three injections of Testosterone Propionate (1= 400 mg, 2= 200 mg), two of Cypionate (250 mg/injection) and two of Nandrolone Decanoate (300 mg/injection) per week and 25mg of oral Stanozolol twice a day. This was repeated for 8 weeks.

Table 3.1 Body Composition Data and Training history in AS and NAS groups (data are mean ± SD).

	AS (n=28)	**NAS (n=19)**	**P value**
Height (cm)	178 ± 8	178 ± 7	0.98
Weight (kg)	96 ± 15	81 ± 9	0.01
BSA (m^2)	2.18 ± 0.21	1.99 ± 0.14	0.01
BMI	30.23 ± 4.23	25.32 ± 2.92	0.01
Lean body mass	81.1 ± 13.3 (n=22)	70.7 ± 6.5 (n=15)	0.01
Fat mass	14.4 ± 7.7 (n=22)	9.9 ± 7.7 (n=15)	0.04
Body Fat %	14.6 ± 6.5 (n=22)	11.9 ± 3.3 (n=15)	0.15
Training duration (years)	7 ± 5	11 ± 9	0.16
Sessions/Week (n)	5 ± 1	4 ± 1	0.03
Avg. Session Length (min)	75 ± 26	64 ±16	0.12
Bench Press 1RM (kg)	143 ± 36	113 ± 26	0.01

Squat 1RM (kg)	194 ± 39	145 ± 59	0.01

BSA = Body Surface Area, BMI = Body Mass Index , 1RM = One Repetition Maximum

Body-Composition

Height and body mass were recorded using standard scales allowing the calculation of BMI and BSA (Du BoisD, 1916). In a sub-sample from both groups, body-composition, including lean mass, fat mass and body fat percentage, was measured using dual energy x-ray absorptiometry (DXA, QDR Discovery A, Hologic, MA) (AS n=22, NAS=15).

Lipid Profile

Venous blood samples were taken from the antecubital vein directly into serum gel (serum) and lithium heparin vacutainers (plasma) (BD, Oxford, UK). Samples were centrifuged for 10 min at 3000 rpm, after which, plasma and serum was placed into aliquots for immediate assessment of total cholesterol (AS n=19, NAS n=9), high-density lipoprotein (HDL) (AS n=20, NAS n=12), low-density lipoprotein (LDL) (AS n=6, NAS n=4), triglycerides (AS n=15, NAS n=6) using the Daytona RS Blood Analysis machine (Randox, Co. Antrim, N. Ireland).

Blood Pressure and ECG

At the end of a 10 minute resting supine period repeated resting brachial artery blood pressures were recorded from the left arm via an automated blood pressure monitor (AS n=27, NAS n=18) (Dinamap; GE Pro 300V2, GE Healthcare). A resting 12-lead ECG was performed (Mac 1200, GE Healthcare Systems, WI, USA) after this supine

rest (AS n=28, NAS n=19). Key parameters assessed included rate, QRS complex voltages and QTc duration (Bazett correction).

Echocardiography

For assessment of global and segmental cardiac structure and function ultrasound echocardiography (Vivid Q, GE Healthcare, Norway) was used to gather images of the left ventricle (LV) in multiple planes from parasternal and apical acoustic windows (AS n=37, NAS=19). A single experienced echocardiographer performed all imaging with the subject in the left lateral decubitus position. Intra-observer reliability was assessed through intra-class correlations for 2D, Doppler, TDI, and Ɛ/SR data (Chan-Dewar et al., 2010a, 2010b) with a range of 0.693-0.993 ($P<0.05$).

Parasternal long axis views allowed the collection of M-mode images at the tips of the mitral valve leaflets perpendicular to septal and posterior walls taking care to ensure clear endocardial definition. From M-mode traces septal (IVSd) and LV posterior wall thickness (LVPWd) in diastole as well as LVchamber dimensions at end-diastole and systole (LVIDd, LVIDs) were assessed following American Society of Echoardiography guidelines (Lang et al., 2005). We estimated LV mass using a regression corrected cube formula (Devereux et al., 1986). A LV mass index was constructed by scaling allometrically for height raised to the power 2.7 as this is the technique most often used in a clinical setting. (de Simone et al., 1995). In a sub sample (n=37, AS=22, NAS=15) we scaled LV mass for individual differences in lean body mass derived from DEXA.

Apical 2 and 4-chamber views were digitised to asses LV end-diastolic (LVEDV) and end-systolic (LVESV) volumes which then allowed the estimation of stroke volume (SV) and ejection fraction (EF) using Simpsons' bi-plane method. From the 4-chamber view, colour Doppler and pulsed wave Doppler were used to assess peak flow velocities across the mitral valve. Using a 4 mm sample volume in the area of peak flow LV early (E) and late (A) diastolic in-flow velocities were recorded. In combination with LV outflow spectral Doppler envelopes we also measured the isovolumetric relaxation time (IVRT) from closure of the aortic valve to opening of the mitral valve. From the same view tissue-Doppler measures of myocardial wall velocities were recorded. Taking care to adjust filters and scale and with the septal wall parallel to the ultrasound beam we interrogated the mitral annulus at the septal wall, recording peak systolic (S') as well as early (E') and atrial (A') diastolic tissue velocities. This also allowed the production of the E/E' ratio that has been shown to estimate left atrial pressure (Nagueh et al., 1997).

Segmental and global $\mathcal{E}$ and SR data were obtained from parasternal short axis views at the base (just below mitral valve) and apex (1-2 cm above LV cavity obliteration) as well as via a 4-chamber apical view. Cine loops of LV motion were captured for off-line analysis (Echopac, GE Heathcare, Norway). Specific speckle-tracking software that tracks natural acoustic markers or "kernals" facilitated the estimation of $\mathcal{E}$ and strain rate in six wall segments in all views. In short axis views the LV was split into septal, anteroseptal, inferior, posterior, anterior and lateral wall segments. Radial, circumferential and rotational data from these segments were averaged to provide global $\mathcal{E}$, SR, rotation (Rot) and rotation rate (RotR) data. Comparison of basal and apical rotation facilitated the calculation of LV torsion (Oxborough et al.,

2010, Oxborough et al., 2009, George et al., 2009). In the long axis view the LV was split into basal, mid-wall and apical septal wall segments as well as basal, mid-wall and apical lateral wall segments. Again these were averaged to provide global measures of longitudinal $\mathcal{E}$ and SR. SR data were recorded in systole (S SR) and early diastole (ESR). Two-dimensional image optimisation was performed including maintaining frame rate between 40 and 90 fps. For all measurements images were analysed off-line by a single experienced technician with no knowledge of group allocation. Data reflect the average of 3-5 continuous cardiac cycles.

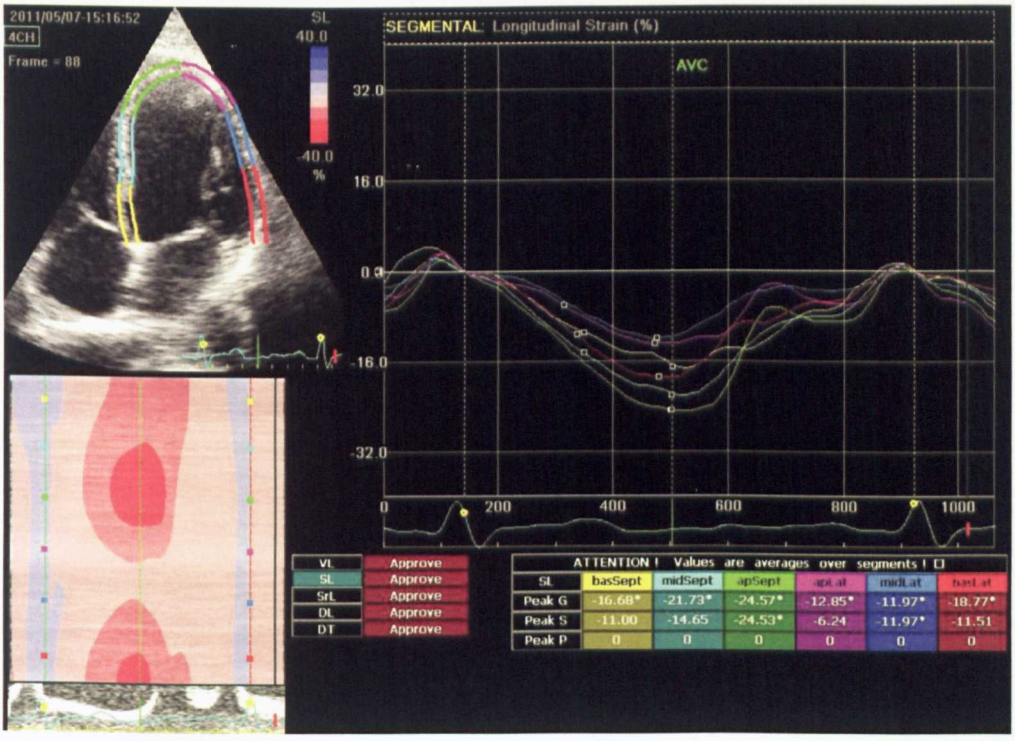

Figure 3.1 Longitudinal Ɛ and SR image derived from myocardial speckle tracking.

Case Studies of "On" and "Off" Cycle Assessments

A sub-group of AS users (n=4, male=3, female=1) were tested during both a period of AS use ('On' Cycle) and a period of abstinence ('Off' Cycle). The same experimental measures were taken at both testing points. In order to make the study

as relevant to 'real-world' scenario's, we aimed to test participants at the end of an 'On' and an 'Off' cycle. The 'On/Off' cycle durations ranged from 8-12 weeks.

Statistical Analysis

Statistical analysis of data was performed using statistical software package SPSS Version 17 (IBM inc., Armonk, NY, USA). All data were subjected to tests of normality. Differences between AS and NAS participants were analysed using paired T-Tests if normally distributed or the Mann-Whitney U-Test if not normally distributed (e.g. E/A ratio data). A P value <0.05 was considered significant.

3.3 Results

Participant History and AS use

The AS group performed on average one more training session per week than the NAS group whilst the NAS had a longer (yr) history of training. There was little difference in the average session length between the two groups. Maximal bench press and squat were also significantly higher in the AS group (Table 3.1).

Body Composition

Whilst height did not differ between groups, the AS group were significantly heavier, which resulted in a significantly elevated BSA and BMI (Table 3.1). Lean mass and fat mass (81.1 ± 13.3 vs. 70.7 ± 6.5; 14.4 ± 7.7 vs. 9.9 ± 7.7, respectively, $P<0.05$) were both significantly elevated in the AS users, whilst body fat percentage (14.6 ± 6.5 vs. 11.9 ± 3.3) was elevated in the AS group, this was not significantly different between groups.

Lipid Profile

LDL and HDL were significantly elevated and reduced, respectively, in the AS group, which led to a significantly greater total cholesterol to HDL ratio in the AS group (Table 3.2). Total cholesterol was elevated in the AS group, although this was not statistically significant. There was little difference in triglyceride levels between groups (0.84 ± 0.28 vs. 0.82 ± 0.28).

Blood Pressure and ECG

Mean systolic pressures were elevated but not significantly higher, whilst mean diastolic pressures were lower in AS users, they did not reach statistical significance (systolic blood pressure: 133±15 vs. 126±12 mmHg; P=0.11; diastolic blood pressure: 67±7 vs. 73±10 mmHg; P=0.07). Resting heart rate was significantly higher in AS users (79±12 vs. 64±13 beats.min^{-1}; P=0.01). Fourteen AS users and eight NAS met the voltage criteria for LV hypertrophy but and voltage data for R waves in V5 and V6, as well as S waves in V1 And V2, were not different between groups. R-R corrected QT interval (QTc) was also not significantly different between AS users (409±20 ms) and NAS (401±23 ms). Three members of each group had a QTc marginally below the 380 ms cut off for short QT.

Cardiac Structure and Function

Thickness of the IVSd, LVPWd, as well as LV mass were significantly higher in the AS group (Table 3.2). LV mass index, when scaled for height remained significantly higher in the AS group, however when scaled for lean body mass in a sub sample, it was no longer significant (P=0.13). There was no significant difference between

groups for LV internal dimensions or volumes in systole or diastole yet there was significantly reduced ejection fraction in the AS group. In diastole, significant reductions in E/A, E', A' and E'/A' were observed in the AS group. A was significantly elevated in the AS group. There was no significant difference between groups in isovolumetric relaxation time (103 ± 14 vs. 103 ± 15 ms) and E wave deceleration time (182 ± 37 vs. 202 ± 40 ms).

Table 3.2 Structural and functional data for the LV, and lipid profiles in AS and NAS groups (data are mean ± SD).

	AS (n=28)	**NAS (n=19)**	**P value**
IVSd (mm)	13 ± 2	12 ± 2	0.01
LVPWd (mm)	12 ± 2	11 ± 1	0.01
LVIDd (mm)	53 ± 5	52 ± 4	0.49
LV Mass (g)	280 ± 60	231 ± 44	0.01
LVMI ($g/Ht^{2.7}$)	59 ± 13	49 ± 11	0.01
LVMI ($g/ffm^{1.0}$)	3.57 ± 0.53	3.27 ± 0.66	0.13
	(n=22)	(n=15)	
EF (%)	59 ± 8	63 ± 6	0.04
E ($cm.s^{-1}$)	72 ± 14	77 ± 15	0.27
A ($cm.s^{-1}$)	54 ± 11	39 ± 12	0.01
E/A	1.36 ± 0.29	2.04 ± 0.59	0.01
S' ($cm.s^{-1}$)	9 ± 3	9 ± 2	0.79
E' ($cm.s^{-1}$)	10 ± 2	12 ± 2	0.01
A' ($cm.s^{-1}$)	9 ± 2	7 ± 2	0.01
E'/A'	1.16 ± 0.35	1.91 ± 0.56	0.01
E/E'	7.44 ± 1.67	6.05 ± 1.20	0.01

Total Cholesterol	4.78 ± 1.53	4.04 ± 0.69	0.18
(TC) (mmol/L)	(n=19)	(n=10)	
LDL (mmol/L)	3.68 ± 0.47	2.41 ± 0.49	0.01
	(n=6)	(n=4)	
HDL (mmol/L)	0.69 ± 0.26	1.21 ± 0.31	0.01
	(n=20)	(n=12)	
TC:HDL	8.21 ± 4.72	3.11 ± 0.47	0.01
	(n=19)	(n=8)	

LV = Left Ventricle, LVMI = Left Ventricular Mass Index ($g/ht^{2.7}$), LVMi=Left Ventricular Mass index ($g/ffm^{1.0}$), EF = Ejection Fraction, E = Early Diastolic Flow, A = Late Diastolic Flow, IVRT = Isovolumetric Relaxation Time, E Dec = Early Diastole Deceleration, E' = Early Diastolic Tissue Velocity, A' = Late Diastolic Tissue Velocity, LDL = Low-Density Lipoprotein, HDL = High-Density Lipoprotein.

Myocardial Ɛ and SR (longitudinal, circumferential and radial)

In the longitudinal plane peak Ɛ was significantly reduced in the AS group (Table 3.3). There was no significant difference in peak S SR, whilst peak E SR was significantly reduced and peak A SR significantly elevated, in the AS group. This led to a significantly reduced E/A SR ratio in the AS users.

Table 3.3. Longitudinal Ɛ and SR data for AS and NAS groups (data are mean ± SD unless otherwise stated).

	AS (n=25)	**NAS (n=19)**	**P value**
Ɛ (%)	-14.6 ± 2.3	-16.9 ± 2.2	0.01
Peak S SR ($.s^{-1}$)	-1.09 ± 0.23	-1.09 ± 0.18	0.97
Peak E SR ($.s^{-1}$)	1.45 ± 0.31	1.65 ± 0.23	0.02
Peak A SR ($.s^{-1}$)	1.12 ± 0.60	0.80 ± 0.25	0.04
E/A SR Ratio†	1.63	2.41	0.02

Ɛ = Peak Strain, SR = Strain Rate, † = Data given as Median.

In the circumferential plane peak $\mathcal{E}$ and S SR did not differ between groups at the basal level ($P>0.05$) whilst peak $\mathcal{E}$ was significantly reduced in the AS group at the apical level ($P<0.05$, Table 2.4). Peak E SR was significantly reduced in the AS users at the apical level and E/A SR was significantly reduced in the AS group at both basal and apical levels. At the apical level Peak E RotR was significantly elevated and peak rotation was significantly reduced in the AS group. Peak A SR was significantly elevated in the AS users at the basal level. Peak A RotR was significantly elevated in AS users at the basal level, which also lead to a significantly reduced E/A RotR. All other data were similar between groups (Table 3.4). Torsion was not different between the two groups.

Table 3.4. Circumferential $\mathcal{E}$, SR, rotation, rotation rate and torsion in AS and NAS groups (data are mean ± SD unless otherwise stated).

		AS (n=26)	NAS (n=18)	P Value
Basal Level	**$\mathcal{E}$ (%)**	-11.8 ± 7.2	-13.2 ± 8.5	0.57
	Peak S SR ($.s^{-1}$)	-1.49 ± 0.42	-1.20 ± 0.81	0.13
	Peak E SR ($.s^{-1}$)	1.52 ± 0.47	1.39 ± 0.81	0.49
	Peak A SR ($.s^{-1}$)	0.90 ± 0.41	0.59 ± 0.60	0.05
	E/A SR Ratio†	1.99	3.37	0.03
	Rotation (°)	-3.52 ± 5.42	-3.75 ± 4.12	0.88
	Peak S RotR ($.s^{-1}$)	-69.3 ± 35.2	-63.8 ± 19.6	0.55
	Peak E RotR ($.s^{-1}$)	64.5 ± 27.5	64.43 ± 28.7	0.99
	Peak A RotR ($.s^{-1}$)	47.9 ± 21.1	34.0 ± 15.3	0.02
	E/A RotR Ratio†	1.52	2.25	0.03
Apical Level	**$\mathcal{E}$ (%)**	-20.6 ± 5.8	-24.6 ± 6.0	0.04
	Peak S SR ($.s^{-1}$)	-1.82 ± 0.58	-1.62 ± 0.45	0.28
	Peak E SR ($.s^{-1}$)	1.99 ± 0.64	2.71 ± 1.04	0.01
	Peak A SR ($.s^{-1}$)	1.12 ± 0.54	0.83 ± 0.38	0.08
	E/A SR Ratio†	2.19	3.94	0.01
	Rotation (°)	3.62 ± 4.31	6.84 ± 4.57	0.04
	Peak S RotR ($.s^{-1}$)	-49.6 ± 19.3	-52.8 ± 36.7	0.74
	Peak E RotR ($.s^{-1}$)	58.3 ± 34.6	30.2 ± 32.5	0.02
	Peak A RotR ($.s^{-1}$)	34.6 ± 14.1	22.5 ± 25.0	0.08
	E/A RotR Ratio†	1.66	1.74	0.79
	Torsion	6.30 ± 5.27	9.86 ± 6.44	0.07
		(n=21)	(n=15)	

$\mathcal{E}$ = Circumferential Strain, SR = Circumferential Strain Rate, RotR = Rotation Rate, S = Systole, E = Early Diastole, A = Late Diastole. †= Data given as Median.

In the radial plane, there was no difference in peak Ɛ and S SR at the basal levels whilst peak Ɛ and S SR were significantly elevated in the AS group at the apical level (Table 3.5). Peak E SR were similar in AS and NAS groups at both levels, but A SR was significantly elevated in the AS group at the basal level which led to a significant depression in the basal E/A radial SR ratio.

Table 3.5. Radial plane Ɛ and SR in AS and NAS groups (data are mean ± SD unless otherwise stated)

		AS (n=26)	NAS (n=18)	P Value
Basal Level	**$\mathcal{E}$ (%)**	25.5 ± 11.6	30.7 ± 14.5	0.20
	Peak S SR ($.s^{-1}$)	2.13 ± 0.96	1.99 ± 1.41	0.69
	Peak E SR ($.s^{-1}$)	-1.72 ± 0.66	-1.64 ± 0.49	0.66
	Peak A SR ($.s^{-1}$)	-1.34 ± 0.57	-0.88 ± 0.54	0.01
	E/A SR Ratio†	1.48	2.99	0.01
Apical Level	**$\mathcal{E}$ (%)**	34.3 ± 14.5	22.2 ± 12.8	0.01
	Peak S SR ($.s^{-1}$)	2.00 ± 0.53	1.40 ± 0.76	0.01
	Peak E SR ($.s^{-1}$)	-2.48 ± 1.06	-2.45 ± 1.17	0.94
	Peak A SR ($.s^{-1}$)	-1.06 ± 0.57	-0.79 ± -0.48	0.14
	E/A SR Ratio†	2.78	3.23	0.22

$\mathcal{E}$ = radial strain, SR = Radial Strain Rate, S = Systole, E = Early Diastole, A = Late Diastole. † = Data given as Median.

Case Studies of AS users "On" and "Off" Cycle

Moderate changes were seen in mass from 'On' to 'Off' cycle with only a significant change seen in one subject (Table 3.6). HDL levels increased in all the three subjects when 'Off' cycle where data were available. Minimal differences in structure were

seen at the different points of the AS cycles, with the greatest changes seen in diastolic function with two subjects showing improved E/A values when 'Off' cycle and all those tested at both time points exhibiting improved E/A SR values.

Table 3.6. Cardiac structure, function and HDL Data from AS users when 'On' and 'Off' Cycle.

Subject & On/Off		Mass (g)	HR (bpm^{-1})	HDL (mmol/l)	LVMi (g/ffm^{-1})	E/A	Long $\mathcal{E}$ (%)	E/A SR
1	On	80.4	68	0.64	3.77	1.39	-14.03	0.93
	Off	79.4	63	1.05	3.58	1.22	-14.03	1.69
2	On	126	73	0.44	3.35	1.83	-12.57	0.69
	Off	118.3	56	0.58	3.60	1.85	-12.24	1.57
3	On	105.3	70	-	3.64	1.49	-18.39	1.29
	Off	106.6	70	-	3.68	1.76	-19.29	1.45
4 *	On	69.4	60	1.04	2.88	1.85	-15.72	1.60
	Off	70.3	59	1.56	3.03	2.1	-21.02	4.28

HR=Heart Rate, LVMi=Left Ventricular Mass ($g/ffm^{1.0}$), E/A=Early:Atrial Diastolic filling ratio, E'/A'= Early:Atrial Diastolic tissue velocity ratio, TC=Total Cholesterol, LDL=Low-Density Lipoprotein, HDL=High-Density Lipoprotein, $\mathcal{E}$ = Peak Strain, SR = Strain Rate, * female subject

3.4 Discussion

The unique contribution of this study is that it builds on the recent work of D'Andrea, Caso, Salerno et al. (2007) and Baggish, Weiner, Kanayama et al. (2010) by providing a comprehensive profile of cardiac $\mathcal{E}$ and SR data in multiple planes of LV motion. Specifically, the current study observed decreased longitudinal peak $\mathcal{E}$ as

well as well as some evidence of decreased diastolic SR in the circumferential (apical) and radial planes (basal). Further we provide data consistent with previous findings that AS use is associated with significant alterations in lipid profiles (Hartgens and Kuipers, 2004, Lane et al., 2006) as well as left ventricular concentric hypertrophy and diastolic impairment (Nottin et al., 2006, Kasikcioglu et al., 2009). Taken together this data suggest that an increased CV risk profile exists in AS users.

Initially we observed alterations in body composition, lipid parameters and resting heart rate in AS users. The significant increases in weight, BSA and BMI seen in the AS users were somewhat predictable (Bhasin et al., 1996) as AS have been shown to up-regulate protein synthesis, thereby increasing the amount of lean muscle that can be developed when used in conjunction with intense resistance training (Payne et al., 2004). It is unlikely that the significant differences in body size and composition can be explained solely by small differences in the training exposure of the two groups.

In addition we observed an altered lipid profile in AS users. Whilst mean total cholesterol was higher in AS users, the difference between groups was not statistically significant which supports data from Sader et al. (2001) but contradicts Baldo-Enzi et al. (Baldo-Enzi et al., 1990). It can be argued that lipid profiles are more important than overall cholesterol when determining CV and atherosclerotic risk (Robinson et al., 2009). The decrease in HDL in AS users in the present study agrees with past research (Sader et al., 2001, Baldo-Enzi et al., 1990, Hartgens et al., 2003, Lane et al., 2006). Likewise, an increase in LDL in the current study also

supports previous data (Hartgens et al., 2003). Supraphysiological doses of AS lead to high hepatic androgen exposure and high androgen levels can alter levels of lipoprotein (a), which directly effects the formation of HDL (Hartgens et al., 2004).

The AS user group had a significantly elevated resting heart rate compared to NAS users. Whilst increased heart rates in AS users have been shown in previous studies (D'Andrea et al., 2007) these differences have not been significant. Case studies have observed increased heart rates in AS users (Sullivan et al., 1999) but the present study is the first to report a significant difference in resting HR in a cohort study. An elevated resting HR in AS users could be due to a number of mechanism(s) including; a relative lack of aerobic/endurance training in AS users, an indirect effect of the increased atherosclerotic risk, through alterations in risk factors such as lipid profiles, or a direct effect of steroids on cardiac pacemaking. Whilst resting systolic and diastolic blood pressures observed in the present study did not differ significantly between the two groups, systolic BP was elevated whilst diastolic BP was reduced in the AS group (7 and 6 mmHg). Blood pressure data were highly variable between individuals and a lack of between group significance may be due to low power with respect to this data.

Larger LV wall thicknesses observed in the AS users in the present study confirms data reported in previous studies (Nottin et al., 2006, Sachtleben et al., 1993, Baggish et al., 2010). It is also important to note that these data contradict other studies. Specifically, D'Andrea et al. (2007) and Hartgens et al (2003) reported no increased wall thickness in AS users. Comparisons between AS-user studies can be

complicated by between-subject differences in training history, AS use history etc that may explain variable outcomes. The increase in LV wall thickness and LV mass may be expected as the receptor mechanisms that result in an increase in skeletal muscle protein up-regulation with AS use, are present within the myocardium (Marsh et al., 1998). In the current study the increased LV wall thickness contributed to an elevated LV mass that remained even after scaling for between-subjects differences in height (Dewey et al., 2008). When LV mass was scaled for lean mass in a sub-sample (n=37, AS=22, NAS=15), the difference between groups was no longer statistically significant (P=0.13). This suggests that some, but likely not all, of the LV hypertrophy could be explained by an increase in whole body lean tissue. Whether there is cardiac tissue hypertrophy in excess of body size, suggesting a possible increased anabolic effect of AS in cardiac tissue over skeletal muscle requires verification. Whilst we cannot exclude small between-group differences in training stimulus as a potential mediator of LV morphology, it is unlikely to underpin all of the observed variation between groups.

The increased LV morphology in AS users was also associated with significant between group differences in LV function. Notably the AS users had a reduced EF. The EF, however, remained within the normal range which contradicts a recent report (Baggish et al., 2010) but confirms other studies (Hartgens et al., 2003). The lack of difference in S' between groups also supports previous studies (Kasikcioglu et al., 2009, Nottin et al., 2006). Whilst myocardial Ɛ and S SR assessment have become increasingly useful in quantifying regional myocardial function in individuals, from both a clinical (Abraham et al., 2007) and research perspective

(Langeland et al., 2005) its application in AS-users has to this point been incomplete (Baggish et al., 2010, D'Andrea et al., 2007). We observed a significant reduction in peak longitudinal Ɛ in AS users, similar to that reported previously (D'Andrea et al., 2007, Baggish et al., 2010), as well as a significant reduction in circumferential Ɛ at the apical level. A small non-significant reduction in radial Ɛ at the basal level along with an increase in radial Ɛ at the apical level in AS users was observed in the current study, yet greater, and significant, decreases in AS users were reported by Baggish et al. (2010). The absolute values of radial strain were lower in the AS users in the current study compared with the AS users assessed by Baggish et al. (2010). Circumferential Ɛ as well as S SR in both apical and basal LV levels has not been reported in previous literature. Our observations of these parameters in AS users suggests that there is no consistent change in indices of LV motion/contraction between planes of motion and further that these data should be confirmed in on-going studies. Similarly, peak rotation and peak systolic rotation rates have not been reported previously in AS users and in the current study, excluding peak rotation at the apical level, were largely unaltered by a history of AS use. Consequently, LV torsion whilst reduced in AS users was not significantly different between groups.

Whilst evidence of impairment of global diastolic function was observed in AS users, it is interesting to note that E and IVRT were not altered in the AS group. A lengthened IVRT has been reported before in AS users (Nottin et al., 2006). Other alterations observed in the AS users were a decreased E', suggestive of early relaxation impairment and increased A and A' suggesting altered atrial contraction or LV compliance. Both E/A and E'/A' ratios were reduced in AS users, which is

suggestive of an overall reduction in "efficiency" of LV motion/filling and again these changes parallel published data (Kasikcioglu et al., 2009). The lack of significant difference in E in the current study is somewhat surprising but given a reduction in E', this may reflect pseudo-normalisation (Khouri et al., 2004) which is supported by an increase in left atrial driving pressure as estimated by E/E'. An increased E/E' has also been noted in past studies and was present in the present findings (Krieg et al., 2007, Kasikcioglu et al., 2009).

Previous studies employing Ɛ and SR analysis have not assessed diastolic function (D'Andrea et al., 2007, Baggish et al., 2010). Although not consistent in all planes, levels and segments, evidence of significant alterations in E SR, A SR, as well as E and A rotation rates provide new evidence for altered diastolic function in AS users. A reduction in E SR likely reflect alterations in early relaxation/elastic recoil of LV wall segments and any alteration in A SR or A rotation rate likely reflect changes in atrial contraction or LV compliance. Overall changes in filling or tissue movement during atrial diastole probably represent an increased reliance on atrial filling in the AS users as the LV adjusts to a reduction in early filling. The diastolic impairment observed in AS users could occur through possible impaired LV relaxation, increased LV diastolic pressure and/or elevated left atrial pressure (Little and Oh, 2009). However, the specific mechanisms underpinning altered early or atrial diastolic function cannot be directly determined from the current non-invasive study. Altered loading and rate may be considered but the heart rate difference is small and available data suggests that parameters such as E, E', Ɛ and SR would increase with heart rate, not decline. Altered preload is difficult to assess in a cross-sectional

design and whilst some differences in blood pressure were apparent it is problematic to link this to LV function in the current research design. The mechanism(s) by which AS could have a direct effect on LV diastolic relaxation and/or compliance are not fully understood but some pathways have been postulated. D'Ascenzo et al. (2007) speculated that AS negatively alter endothelial cell growth, promote apoptosis and increase collagen cross-links in LV walls. Human data to support this speculation is currently limited but worth pursuing. Whether the changes in LV function are a direct consequence of long term AS use, or an indirect effect of AS, via altered lipid profiles or blood pressure, is worthy of further study.

Despite only a small number of AS subjects being tested when both 'On' and 'Off' cycle, the results start to give an indication of changes in CV parameters that can occur after a relatively short period, 8-12 weeks, of abstinence from AS use. This data also prompts on-going studies in this area. Our data suggest some improvement in the lipid profiles of those tested, with decreases in overall total cholesterol and also LDL and increases in HDL levels thereby showing an improvement in perceived CV risk. Whilst strain and strain rate data from the different cycles was somewhat inconclusive, the increase in E/A SR in the radial plane seen in all users when 'Off' cycle, in conjunction with an increase in E/A ratios in three of the subjects, gives an indication of a potential improvement in ventricular relaxation following cessation of AS use for a relatively short period of time. It is important to note, however, that despite an improvement in ventricular filling ratios in three of the subjects, the values seen were still below the average of the NAS group, indicating some restriction in ventricular relaxation remained. Nonetheless, these values were still

well within normal clinical ranges. These findings do, however, indicate a possible short-term effect of AS use on cardiac function outside of a possible role of apoptosis or increased collagen cross-links, or at least a reversibility in any increase in these cross-links associated with AS use.

The implications of the data presented in this and other studies is that AS use is associated with multiple changes in CV structure, function, health and thus risk. This provides some potential links to case studies reporting significant CV events in AS users. Large epidemiological studies, although difficult in this type of cohort, should provide further insights into the "real" risk of AS use. What implications the current data have at an individual level is hard to discern. Overall the increase in ventricular mass, decreased cardiac function (in particular diastolic impairment) as well as marginal changes in blood pressure and alterations in blood lipid profiles point to an increased CV disease risk associated with AS use. The small sample of data from 'On' and 'Off' cycle in AS users points to a possible, if limited, reversal of some of the negative CV effects seen in AS users.

As with any study of AS use there are some inherent limitations that are important to note. Investigating AS use is complicated by the heterogeneity in subject specific details (age, training status, diet etc) that were either not controlled or not assessed in the current study. Obviously inter and intra-individual differences in AS dose (volume, drug type, veracity or authenticity), self-report details of history as well as variations in "stacking" and "cycling" approach can occur within the AS using

population. We made no a-priori attempt to control these parameters in order to reflect the reality of AS use. Further to this, the differing types of AS use which are used for differing outcome, i.e. for bulking or for 'cutting' in order to potentiate lean mass, could have a confounding effect on the results but, we did not account for this in our analysis. We present some case-studies of the impact of "cycling" of AS use but this should be addressed in a larger study.

It is also pertinent to mention that all of the LV structural and functional measures in the present study were obtained through non-invasive techniques, thereby limiting the ability of the researchers to confirm the mechanistic reasons for any changes observed. Whilst we present what we consider is a comprehensive look at AS and their CV effects, the addition of radial and circumferential data at the mid-LV level may provide additional insight into global systolic functioning. Low participant numbers in certain aspects of the data, such as aspects of the lipid profiles, we present does limit their interpretation to a wider group. An important and often overlooked area is the effect of AS on haematocrit levels. There is limited data concerning this area of research but some results have demonstrated a negative impact (Alen, 1985, Lane et al., 2006). Further research could help to explain certain CV events and changes to LV geometry and function (e.g. strain and strain rate) that have been observed in AS users. Specifically, whilst we have confirmed an increase in CV risk in AS users by adding new data in relation to cardiac function in AS users at rest it would make sense to assess cardiac functional responses to exercise stress as this may be an important adjunct to risk and the onset of events seen in case study reports in AS users. Whilst we document gross structural alterations in the LV of AS

user's limited data is available for the right ventricle and the left atria. Further study using MRI techniques may interrogate the impact of AS use on LV perfusion and/or the potential presence of fibrosis.

In conclusion, the present study has reported a range of negative CV consequence of AS use in conjunction with resistance training that is in agreement with recent findings (Baggish et al., 2010, Kasikcioglu et al., 2009). We have developed the available database by indicating an association between AS use and some evidence of impaired $\acute{E}$ and SR data, largely reflecting altered diastolic cardiac motion. Combined with altered LV mass, lipid profile changes in cardiac function with AS use likely increase the CV risk in AS using subjects.

Chapter 4: MRI determination of global left and right ventricular structure, function, perfusion and fibrosis in anabolic steroid users

4.1 Introduction

The use of anabolic steroids (AS) has been associated with significant cardiac events including acute myocardial infarction (Lunghetti et al., 2009, Wysoczanski et al., 2008) and sudden cardiac death (Fineschi et al., 2007, Luke et al., 1990). An increased cardiovascular risk is assumed with the abuse of AS but the possible mechanisms remain unclear or controversial. Previous research has shown significant effects of AS on cardiovascular risk factors (CV) such as BP (Riebe et al., 1992) and total cholesterol (Baldo-Enzi et al., 1990) however these findings were inconclusive and have been contradicted in subsequent studies (Sader et al., 2001).

Whether direct alterations in ventricular structure and function may mediate an increased cardiovascular risk in AS users is unclear. There is some echocardiographic evidence of increased cardiac muscle mass in AS users (Baggish et al., 2010, Angell et al., 2011) but this is not entirely consistent (Urhausen et al., 1989, D'Andrea et al., 2007). Recent studies, using ultrasound echocardiography, have suggested negative alterations in cardiac function are associated with AS use (Hartgens et al., 2003, Baggish et al., 2010, Angell et al., 2011). Using novel deformation assessment techniques these studies have documented, in AS users, reduced longitudinal strain (ε) (D'Andrea et al., 2007), reduced radial and longitudinal ε (Baggish et al., 2010), and alterations in diastolic strain rates (Angell et al., 2011). The consequences or causes of these functional changes are not known.

Some areas of study have received less attention in AS users. Specifically, there is very little data related to right ventricular (RV) structure and function in AS users. This is partially understandable because of the historic difficulties related to non-invasive imaging of the RV. Recent studies from Belgium give renewed impetus to study the RV as both Heidbuchel et al. (2003) and Ector et al. (2007) reported complex ventricular arrhythmias in well-trained athletes were largely of right ventricular origin and in some precipitated sudden cardiac death. Other potential mechanisms that could link cardiac structure/function and cardiovascular events in AS users may relate to atherosclerosis and perfusion defects and/or the presence of internstitial fibrosis within the myocardium that could act as an arrhythmic substrate. To the best of our knowledge no data is available describing the presence of perfusion defects or interstitial fibrosis in the myocardium of AS users.

Limitations in echocardiography, including operator variability, poor spatial resolution and the use of mathematical models to predict 3D structures or function likely limit the interpretation of global data derived using this technique (Kühl et al., 2003). Cardiac magnetic resonance imaging (cMRI) is now considered the gold-standard for non-invasive assessment of global cardiac structure and function due to its increased spatial resolution and its lack of reliance on geometric assumptions (Kerber and Pesek-Bird, 2002). Unlike echocardiography, cMRI is not limited by poor acoustic windows. This allows an accurate assessment of right ventricular structure and function. Importantly, cMRI can be used to assess cardiac perfusion defects (Cury et al., 2008) and the presence of interstitial fibrosis (Jellis et al., 2010) both of which may increase the risk of a cardiovascular event. It has been speculated, but never empirically supported, that cardiac fibrosis as a consequence of AS use

may provide a mechanistic link to a substantial number of case reports of acute myocardial infarctions in AS users (Angell et al., 2011).

To the best of our knowledge cMRI has not been employed in a comprehensive assessment of cardiac structure and function in AS users. Consequently, the aim of the current study was to assess the cardiac morphology, function, perfusion and presence of interstitial fibrosis in regular AS users using cMRI. We hypothesised that AS users would have increased RV and LV dimensions in the presence of reduced systolic and diastolic function. Further, there may be case evidence of significant perfusion defects and interstitial fibrosis.

4.2 Method

Subjects

Strength trained individuals (n=22, 1 female) were recruited through local gyms, and personal contacts. Inclusion criteria were; participants aged between 18 and 50 years of age; resistance training history of a minimum of 3 years with 3-4 training sessions per week. Exclusion criteria for the study were the presence of known respiratory, cardiovascular or musculoskeletal disease. Specific inclusion criteria for the AS using group (AS: n=14, 1 female; age=30 ± 5 yrs) included a documented self-report history of AS use for at least 2 years (including on and off-cycles). Inclusion criteria for the non-AS (NAS) group (n=8, age=28 ± 6 yrs) included self reported history of never taking AS. Participants were matched for age and training history. Prior to

taking part in the study, details of each part of the testing were described to each of the participants. The study was granted approval from the Royal Brompton and Harefield Research and Development Committee as well ethics approval from the LJMU Ethics Committee. All participants provided written informed consent.

Design

A cross-sectional cohort design was utilised for the study with participants required to make a single visit to the Royal Brompton MRI laboratory. Initially, subjects completed self-report questionnaires related to general health, training status and history as well as detailed accounts of AS use. This was followed by assessment of body composition, a venous blood sample and a comprehensive CV evaluation including brachial artery blood pressure, an echocardiogram and a cardiac MRI. All tests were conducted on the participants following an overnight fast, as well as a 24 hr abstention from caffeine consumption and resistance training.

Protocols: *Participant History and AS use*

Training history data recorded included; years of resistance/strength training, the average number and length of sessions per week, as well as self-report one repetition maximums for the bench press and squat. Participants in the AS group provided a detailed history of AS use including names, dosage and cycling information. Insufficient data regarding aerobic training was provided by the AS participants, limiting a comparison between groups.

Body-Composition

Height and body mass were recorded using standard scales allowing the calculation of BMI and BSA (Du BoisD, 1916). Assessment of body-composition was made using bio-electrical impedance (InBody 230 Body Composition Analyser, Biospace Co Ltd) to estimate total fat mass and fat free mass.

Blood Pressure

At the end of a 10 minute resting supine period repeated resting brachial artery blood pressures were recorded from the left arm via an automated blood pressure monitor (V100 Dinamap, GE Medical Systems).

CMR Image Acquisition Protocol

All participants were asked to abstain from caffeine-containing beverages or drugs for 24 hours prior to imaging. All images were acquired using a dedicated 1.5T scanner (Siemens Avanto, Siemens Healthcare, Erlangen, Germany) with an eight-element phased-array receiver coil. Using scout images for planning, expiratory breath-hold, ECG-gated, balanced steady-state free precession cine images were acquired in the vertical long axis (VLA), horizontal long axis (HLA) and left ventricular outflow tract (LVOT) planes. Using the VLA and HLA images for planning ensure imaging was parallel to the atrioventricular groove. A contiguous short-axis stack was obtained from base to apex ensuring full LV and RV ventricular myocardial coverage (Kramer et al., 2008). Global LV and RV parameters (end-diastolic volume, end-systolic volume, stroke volume, and ejection fraction) and LV mass were determined using a semi-automated threshold-based algorithm after

manual tracing of epicardial and endocardial borders using commercially available software (CMRtools, Cardiovascular Imaging Solutions, London, UK). Ventricular volumes and mass were reported as absolute data and then normalised for individual differences in fat free mass (Batterham et al., 1999).

After initial acquisition of cine images at rest, adenosine was infused at 140 mcg/kg/min through a 20G cannula in the left antecubital fossa for a minimum of 3 minutes according to response (Kramer et al., 2008). Active shimming was used to ensure magnetic field homogeneity over the regions of interest, and manual frequency adjustments were carried out to ensure maximum fat-water separation with the selected radiofrequency excitation pulse. The imaging planes were planned using end-systolic frames of the long-axis cine images to ensure that the LVOT was excluded from the basal slice and that each slide was parallel to the atrioventricular groove. At peak stress, gadolinium contrast (Gadovist, Bayer-Schering, Berlin, Germany, 0.1 mmol/kg) was injected at a rate of 3.5 ml/s followed by a 15 ml saline bolus at 7 ml/s via an 18G cannula in the right antecubital fossa using a power injector (Medrad UK, Ely, Cambridgeshire, UK). Short axis perfusion images were then acquired at the base, mid-ventricle and apex together with the arterial input, function, which is the concentration of contrast in the left ventricle as a function of time during its first pass, over 50 cardiac cycles using a breath-hold hybrid echo-planar imaging (EPI) dual sequence approach. The imaging parameters were as follows: repetition time (TR), 5.1 ms; TE for first echo, 1.02 ms; flip angle, 30° degrees; base resolution, 128x128 pixels; voxel size 2.8x2.8x8 mm; bandwidth, 1860 Hz/pixel; inversion time (TI), 90 ms; parallel imaging T-SENSE R=2; and EPI-factor of 4.

Ten minutes after contrast injection for stress perfusion imaging late gadolinium enhancement images (LGE) were obtained using an ECG-gated expiratory breath-hold segmented turbo Fast Low Angle Shot (FLASH) gradient echo sequence (Simonetti et al., 2001). Images were obtained in all long-axis planes and in the same short-axis stack as for cine images with the TI optimised to null the normal myocardium. This was then repeated in an orthogonal phase encoding direction to exclude artifact. After a minimum of 30 minutes to allow for washout of gadolinium, rest perfusion images were acquired using the same protocol used at stress.

CMR Image Analysis

All data were analysed by single experienced blinded observer. Global left and right ventricular parameters (end-diastolic volume, end-systolic volume, stroke volume, and ejection fraction) and LV mass were determined using a semi-automated threshold-based algorithm after manual tracing of epicardial and endocardial borders using commercially available software (CMRtools, Cardiovascular Imaging Solutions, London, UK). Ventricular volumes and mass were as absolute data and normalised for individual differences in fat free mass (Batterham et al., 1999). Late enhancement and perfusion images were assessed qualitatively to determine the presence or absence of LGE or inducible perfusion defects respectively.

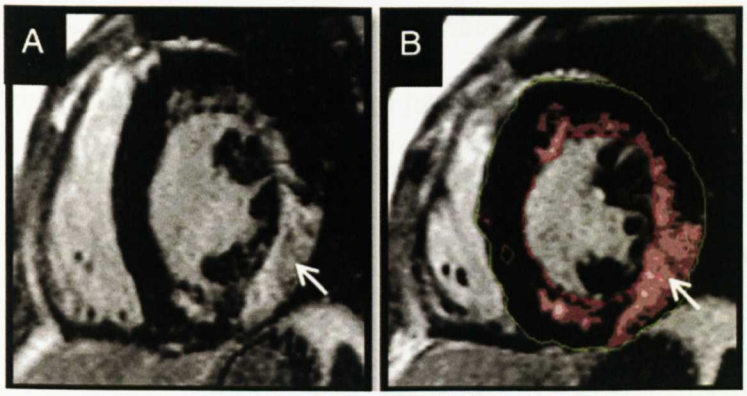

Figure 4.1 LGE images from CMRI

Echocardiography

For assessment of global diastolic filling and segmental cardiac velocity and deformation characteristics, ultrasound echocardiography (Vivid Q, GE Healthcare, Norway) was used to gather images of the left ventricle (LV) in the apical 4-chamber view. A single experienced echocardiographer performed all imaging with the subject in the left lateral decubitus position.

Doppler was used to assess early (E) and atrial (A) peak diastolic filling velocities allowing E:A filling ratio's to be equated. Tissue-Doppler imaging (TDI) was performed to determine peak systolic (S') as well as early (E') and atrial (A') diastolic tissue velocities in the basal septum. The ratios E':A' and E:E' were calculated. 2D cine loops of an apical 4 chamber view were stored for off-line assessment of segmental and global longitudinal ε and strain rate (SR) data (Echopac, GE Heathcare, Norway). Specific speckle-tracking software that tracks natural acoustic markers or "kernals" facilitated the estimation of ε and SR in six

wall segments (basal, mid-wall and apical septum as well as basal, mid-wall and apical lateral wall). These were averaged to provide global measures of longitudinal ε and SR. SR data were recorded in systole (SSR) and early (ESR) and atrial diastole (ASR). Two-dimensional image optimisation was performed including maintaining frame rate between 40 and 90 fps. All measurements were made by a single experienced technician and reflected the average of 3 to 5 continuous cardiac cycles.

Data Analysis

Statistical analysis of data was performed using statistical software package SPSS Version 17 (IBM Inc., USA). Categorical variables are presented as frequencies and percentages. All continuous data were subjected to tests of normality and are presented as mean±standard deviation (SD) for normally distributed variables or as medians with interquartile ranges for non-parametric variables. Differences between AS and NAS participants were analysed using independent T-Tests with Levene's test for uniformity of variance if normally distributed or the Mann-Whitney U-Test if not normally distributed (e.g. E/A ratio data). Two-tailed values of $p<0.05$ were considered significant Correlation analyse's were run to examine the relationship between the number of years of AS use or the amount of active AS currently being taken and LV mass, end-diastolic volume (EDV), end-systolic volume (ESV), peak wall thickness, and RV EDV and ESV, scaled for fat-free mass as well as for LV and RV ejection fraction, E:A, E':A', E:E' and longitudinal ε. A P value <0.05 was considered significant.

4.3 Results

AS Use and Training History

For simplicity we provide a list of exemplar AS used, as well as a mean total AS weekly dose, but note that all subjects used multiple AS in various stacking procedures with varied periods of abstinence or "off-cycles". The types of AS currently being used by the AS group included, Trenbolone (7), Testosterone (6), Meathandrostenolone (5), Boldenone (4), Nandrolone [Deca Durabolin] (4) Drostanolone Propionate (3), Stanozolol (3), Sustanon (3) and Oxandrolone (2). There were large individual variation in the dosages used by participants with a mean weekly dose of active ingredient being 1201 ± 698 mg (range 250 – 2000 mg). The number of different AS used in a cycle per participant also varied with a mean AS per cycle being 3 (range 1-4). An example cycle for one of the participants was 1000 mg per week of Deca-Durabolin with 1000 mg per week of Sustanon. Whilst training history and regimes did not differ between groups, the AS group had significantly greater 1 RM for both bench press and squat (see Table 4.1). Insufficient data regarding aerobic training was provided by the AS participants to make a comparison between groups. The AS group also provided limited information regarding their overall length of AS use.

Table 4.1 Training and performance data for AS and NAS groups (data are mean ± SD).

	AS	Non-AS
Years Training	9 ± 4.05	10 ± 6
Sessions/Wk	4 ± 1	4 ± 1
Avg.Session (min)	74 ± 26	69 ± 8
1RM Bench (kg)	150 ± 36	111 ± 18*
1RM Squat (kg)	189 ± 36	132 ± 52*

*= P<0.05,

Anthropometry

There was no significant difference in height, body fat percentage or fat mass between groups. The AS group were, however, significantly heavier and had significantly greater, fat free mass, BSA and BMI.

Table 4.2 Anthropometric measures for AS and NAS groups (data are mean ± SD).

	AS	Non-AS
Height (cm)	178 ± 9	180 ± 7
Weight (kg)	98.2 ± 16.2	80.3 ± 9.0*
BSA (m^2)	2.20 ± 0.22	2.00 ± 0.15*
BMI (kg/m^2)	30.9 ± 4.2	24.8 ± 1.7*
Fat Free Mass (kg)	83.68 ± 11.95	71.46 ± 7.71*
Fat Mass (kg)	14.44 ± 9.01	8.84 ± 3.10
Body Fat %†	14.1 ± 7.0	10.9 ± 3.3

BSA= Body surface area, BMI= Body mass index, 1RM= 1 repetition maximum, *= P<0.05, **= P<0.005

Blood Pressure

Whilst resting heart rate was significantly lower in the AS users both systolic and diastolic BP were not different between groups (Table 4.3).

Table 4.3 Blood pressure measures for AS and NAS groups. (data are mean ± SD).

	AS	Non-AS
Systolic BP (mm/Hg)	130 ± 16	125 ± 7
Diastolic BP (mm/Hg)	70 ± 10	66 ± 10
Resting HR(beats.min^{-1})	74 ± 8	60 ± 7**

BP= Blood pressure, HR= Heart rate. **= P<0.005

CMRI

Peak wall thickness, LVEDV and LV mass were increased in AS users but this was largely due to an increased FFM as scaled data were not different between groups (Table 4.4). A similar pattern was observed for RVEDV. LV SV and EF were similar between groups but RV EF was significantly reduced in AS users because of a significantly elevated RVESV. There were no positive perfusion defect scans or positive LGE scans for the presence of interstitial fibrosis in either group.

Table 4.4 Cardiac MRI structural and functional measures in AS and NAS groups. (data are mean ± SD).

	AS	Non-AS
Peak WT(mm)	12.8 ± 2.2	9.4 ± 1.3**
Peak WT/ffm$^{*0.33}$	0.51 ± 0.12	0.44 ± 0.10
LV EDV (ml)	201.2 ± 27.6	187.5 ± 28.4
LV EDV/FFM (ml/ffm^{-1})	2.47 ± 0.57	2.65 ± 0.47
LV ESV (ml)	79.3 ± 15.3	70.6 ± 14.7
LV ESV/FFM (ml/ffm^{-1})	0.97 ± 0.25	0.99 ± 0.22
LVM (g)	215 ± 47	163 ± 27*
LVM/FFM (g/ffm^{-1})	2.64 ± 0.76	2.32 ± 0.48
RV EDV (ml)	225.9 ± 40.8	199.8 ± 39.6
RV EDV/FFM (ml/ffm^{-1})	2.55 ± 1.04	2.81 ± 0.59
RV ESV (ml)	110.4 ± 24.7	83.1 ± 23.0*
RV ESV/FFM (ml/ffm^{-1})	1.25 ± 0.54	1.17 ± 0.35
LV SV(ml)	122 ± 13	117 ± 16
LV EF (%)	61 ± 3	63 ± 3
RV SV (ml)	115 ± 19	117 ± 18
RV EF (%)	51 ± 4	59 ± 5**
Perfusion defect	All Negative	All Negative
Late Gad	All Negative	All Negative

LV= Left ventricle, EDV= End-diastolic volume, ESV= End-systolic volume, SV= Stroke Volume, EF= Ejection Fraction, LVM= Left ventricular mass, RV= Right ventricle, WT= Wall thickness, FFM= Fat free mass, Gad= Gadolinium. *= P<0.05, **= P<0.005

Echocardiography

There was no significant difference in early (E) diastolic filling velocities between groups (Table 4.5). Atrial (A) diastolic filling velocities were significantly elevated in the AS group, leading to a significant reduction in E:A. Peak E' and E'/A' were significantly reduced and A' significantly elevated in the AS group. In addition, E:E' was significantly higher in the AS group. Longitudinal ε was significantly reduced in the AS group whilst the mean longitudinal SR data and peak S' tissue velocities were not different between groups.

Table 4.5 Echocardiographic data for AS and NAS groups (data are mean ± SD).

Measure	AS	Non-AS
E ($cm.s^{-1}$)	66 ± 0.11	77 ± 0.20
A ($cm.s^{-1}$)	52 ± 0.08	38 ± 0.61**
E:A†	1.29	1.88**
S' ($cm.s^{-1}$)	9 ± 0.01	10 ± 0.02
E' ($cm.s^{-1}$)	9 ± 0.02	13 ± 0.23**
A' ($cm.s^{-1}$)	10 ± 0.02	7 ± 0.01**
E':A'†	1.14	1.78**
E:E'†	7.32	5.66*
Longitudinal ε **(%)**	-14.15 ± 2.58	-16.58 ± 1.87*
Peak S SR ($.s^{-1}$)	-0.99 ± 0.22	-1.14 ± 0.11**
Peak E SR ($.s^{-1}$)	1.40 ± 0.36	1.65 ± 0.28
Peak A SR ($.s^{-1}$)	1.01 ± 0.34	0.72 ± 0.25
E:A SR	1.44	2.86

E= Early Diastolic Filling, A= Late Diastolic Filling, E:A= Early:Late Diastole Ratio
S'=Systolic tissue velocity, E'= Early Diastolic tissue velocity,
A'= Late Diastolic tissue velocity, E':A'= Early:Late Diastolic tissue velocity ratio. ε = Strain, SR= Strain Rate, *= $P<0.05$, **= $P<0.005$, †= Data given as Median.

Correlation analyses produced only small and non-significant associations between years of AS use or the amount of current active AS used by the AS participants with cardiac morphology or functional parameters. The ratio E:E' was moderately but significantly correlated with years of AS use.

Table 4.6 Correlation Coefficients of MRI and Echocardiography measures to years of AS use and amount of current AS use.

Measure	Years of AS Use	Current Active AS Use
LVM/FFM (g/ffm^{-1})	-.257	-.491
LV-EDV/FFM (ml/ffm^{-1})	-.306	-.362
LV-ESV/FFM (ml/ffm^{-1})	-.336	-.282
LV-EF (%)	.265	-.190
RV-EDV/FFM (ml/ffm^{-1})	-.271	-.407
RV-ESV/FFM (ml/ffm^{-1})	-.320	-.338
RV-EF (%)	.296	-.392
Peak WT (mm)	.043	-.212
E:A	-.430	-.254
E':A'	-.339	-.175
E:E'	-.586*	-.170
Longitudinal $\mathcal{E}$ (%)	-.111	-.548

LV= Left ventricle, EDV= End-diastolic volume, ESV= End-systolic volume, SV= Stroke Volume, EF= Ejection Fraction, LVM= Left ventricular mass, RV= Right ventricle, WT= Wall thickness, FFM= Fat free mass, E:A= Early:Late Diastole Ratio, E':A'= Early:Late Diastolic tissue velocity ratio, $\mathcal{E}$ = Strain. *= $P<0.05$,

4.4 Discussion

This study contributes significantly to previous research in this area by offering a unique insight into the effects of AS use/abuse on cardiac structure and function, by using gold-standard imaging techniques. To the authors knowledge this is the first time that cardiac MRI has been used in this population and the novel data produced from this study adds to the extant literature regarding the cardiac effects of anabolic steroid use/abuse. Specifically, the use of MRI demonstrated bi-ventricular enlargement in AS users (largely in line with lean mass changes), a reduction in RV EF and a decline in indices of LV tissue velocity and deformation. Despite this, we observed no perfusion defects or the presence of interstitial fibrosis in any resistance trained athlete using AS.

In the first instance, this study has confirmed the association of AS use with significant morphological changes in the LV. The significantly greater peak LV wall thickness and LV mass observed in the AS users in the present study is in agreement with previous echocardiographic findings (Urhausen et al., 1989, Sachtleben et al., 1993). It is also of interest that the quantitative differences in wall thickness and LV mass observed in the present study are comparable to the findings of previous echocardiographic studies (Sachtleben et al., 1993, Urhausen et al., 1989). Other studies have reported greater wall thickness and LV mass in AS users and controls (Baggish et al., 2010). Whilst research has shown a good correlation between echocardiographic assessment of LV mass compared to necropsy (Devereux et al., 1986), more recent data has shown significant discrepancies between echocardiography and MRI (Stewart et al., 1999). The differing geometric

assumptions and equations used in the echocardiographic assessment of LV mass also have a significant effect on the outcome values when compared to MRI, with a propensity to significantly overestimate LV mass (Missouris et al., 1996, Pluim et al., 1997). Consequently, a question may be placed over some echocardiographically-derived data reporting more substantial increases in LV mass in AS users. The increase in LV mass in AS users persisted when indexed to BSA, which is the "classical" clinical approach to scaling and is supported by professional guidelines (Lang et al., 2005). When indexing was performed by scaling to an estimate of fat free mass, as is currently supported by empirical data and reviews (Batterham et al., 1999, Dewey et al., 2008), the between group difference in LV mass largely disappeared. The AS users had a significantly greater fat free mass, which can be explained by findings that show an up-regulation of protein synthesis associated with AS use when used in conjunction with resistance training (Ferrando et al., 1998). This would suggest that cardiac morphological changes occur together, both qualitatively and quantitatively, with changes in skeletal muscle mass. There is some common sense to this as both cardiac and skeletal muscle tissue have androgen receptors to which AS may bind and promote protein anabolism (McGill et al., 1980). It is also possible that an increase in fat-free mass could have an indirect effect on cardiac morphology, due to an increased demand for blood placed on the heart through an increase in muscle mass, requiring an increase in cardiac muscle to cope with demand.

The increase in LV mass and wall thickness in AS users in the current study was also accompanied by a significant impact on function. Whilst global measures such as LV EF were not different between groups, supporting other data (Angell et al.,

2011), there was a significant reduction in longitudinal $\mathcal{E}$ as well as alterations in diastolic function in AS users. This is in agreement with the findings of Baggish et al. (2010) and D'Andrea (2007) who also reported significant reductions in longitudinal $\mathcal{E}$ in a group of AS users. In addition, we demonstrated a significant reduction in diastolic function in AS users through Doppler and Tissue Doppler measures which has been shown in previous studies (Kasikcioglu et al., 2009, Nottin et al., 2006, Krieg et al., 2007). When combined, these functional data highlight that AS use could be associated with reduced cardiac functional capacity.

We have also demonstrated, for the first time, significant effects of AS on RV structure and function. An increase in RV EDV is commensurate with a global effect of AS use on cardiac morphology and again these changes appear to be in line with group differences in estimates of fat free mass. Interestingly, and at odds with the effect on the LV, there was a significantly lower RV EF in AS users. RV EF is a global measure of systolic function and contractility in the RV and when assessed via MRI is highly accurate (REF). The lower RV EF in the AS users is a consequence of an increase in RVESV as opposed to a relative change in RV EDV. The increased RV ESV points to an intrinsic alteration in RV contractility although measures of pulmonary pressure, and thus RV afterload, are not available. The cause(s) and consequences(s) of this change in RV function are difficult to deduce from this cross-sectional, cohort descriptive study. However, a role of changes in pulmonary pressure, possibly due to polycythaemia cannot be discounted. It may also reflect the fact that the RV is more susceptible to the acute impact of exercise sessions. There is recent data (La Gerche et al., 2011) that has documented that the relative increase in afterload in the pulmonary circulation is greater than that in the

systemic circulation when exercise is imposed. La Gerche and others have speculated that there may be a link between the impact of acute, prolonged exercise on the RV and the development of an ARVC-like RV phenotype, that may also explain the increased risk, in some, of significant arrhythmias of RV origin (Heidbüchel et al., 2003, Ector et al., 2007). Most of this recent data has been described in endurance athletes performing prolonged but submaximal exercise. Whether this hypothesis transfers to intermittent resistance-type exercise, that may be further complicated by the use of AS is highly speculative but worthy of on-going research. We do know that heavy resistance exercise (MacDougall et al., 1985) can induce massive alterations in blood pressure and afterload in the systemic circulation. It would be a fair assumption that these changes also occur in the pulmonary circuit, especially in the presence of a valsalva manoeuvre, and this could be more significant in a lower pressure system.

Doppler-derived reductions in RV diastolic function have been observed in AS users (Kasikcioglu et al., 2009) but these require substantiation with newer techniques such as myocardial speckle tracking that has been used in the interrogation of RV function in an athletic setting recently (Oxborough et al., 2010). This would provide another important avenue of on-going research.

The reduced LV and RV functional data associated with AS use would imply that AS use and concomitant resistance strength/training could impair both the contractility and relaxation of the myocardium. The reasons for this impairment are still somewhat unclear, however, a number of mechanisms have been postulated.

Research by D'Ascenzo et al. (2007) suggested that AS can cause an increase in collagen cross-links across cells. This would imply that the myocardium would become more rigid and have reduced compliance, which would be in agreement with previous research which found reduced diastolic function in AS users indicated by reductions in early ventricular filling with concomitant increases in late atrial filling (Angell et al., 2011). In addition, work by Zaugg et al. (2001) found that AS induced apoptosis in rat myocardial cells, however this was seen using very high doses of AS. This suggests that AS could impair individual myocardial cell function by altering its development. Confirming such processes in a human model is very problematic. Although alterations in electrical conduction capabilities and repolarisation of myocytes have also been suggested as a possible mechanism for alterations in cardiac function, recent data has been contradictory at best (Maior et al., 2010, Bigi and Aslani, 2009).

Resting heart rates were significantly elevated in the AS group, which agrees with Angell et al. (Angell et al., 2011). Whilst it has been suggested that AS use can interfere with cardiac electrical signalling (Bigi and Aslani, 2009), it is unclear if this is a direct effect of anabolic steroids or a coincidental finding. It is also interesting to note that, in the present findings, mean systolic and diastolic blood pressure measures were higher in the AS group, although these did not reach a statistically significant level.

Despite significant changes in morphology and function of the LV and RV of AS users, as well as known alterations in lipid profiles of AS users suggestive of

significant atherosclerotic risk (Angell et al., 2011), we observed no perfusion defects in any of the AS users or the resistance-trained athletes. This would suggest that these AS using athletes were not at an elevated risk for any overt ischemic event, associated with exercise stress that could be related to a blood flow limitation. Alternatively, we hypothesised that there may be case evidence of interstitial fibrosis in some of the AS users that may provide an arrhythmic trigger and could link AS use to case studies of cardiac events such as MI or sudden cardiac death. Again we found no evidence of fibrosis in the AS users, or indeed in the non-using resistance trained athletes. In this cohort, at least, these mechanistic pathways are not apparent. Future work may wish to re-evaluate the issue of perfusion defects or fibrosis in a larger sample of resistance-trained athletes using AS. It may be pertinent to place an emphasis on those that have used the highest AS doses for the longest periods of time (and may have significant elevations in other cardiovascular risk factors such as altered lipids and elevated CRP).

The implications of the current data are that they add weight to the belief that AS use places the cardiovascular system under singificant strain and likely increases the risk of future cardiovascular events. When we add RV morphology and function to previous data from this thesis and others; for example a mal-adjustment in lipid profiles (Hartgens et al., 2004, Lane et al., 2006) concerns are raised about the long term cardiovascular health of AS users.

Whilst we feel that this study offers a unique insight in to the effect of AS on cardiac structure and function, it is limited by methodological issues that are inherent within

the area of research related to elicit AS use. Variations in drug stacks, dosages and, cycle composition lengths make a definitive statement regarding the effect of AS dose somewhat problematic. Whilst we attempted to address this through correlational analysis, only modest correlations were noted between cardiac measures and the number of years of AS use or the amount of AS currently being taken. This does, however, highlight the possible short-term, as well as reversibility, of the negative effects of AS. An important factor that is extremely difficult to control for however, is the volume of AS used throughout the users lives as well as the quality of AS used and the actual amount of active ingredient that is present in any of the substances that they have ever used. In addition, despite little difference in time spent training, it is difficult to account for the individual differences in the volume and intensity of training regimes used both between and within groups. Future research should focus on the role of the RV and the novel use of speckle tracking in the RV in this group would further develop a knowledge of the global cardiac effect of AS use. Further to this, the vast majority of research into the effects of AS use has been conducted at rest. The ability of the body to cope with the stresses of exercise is an important issue that is not well understood in AS users.

In summary, the unique application of MRI in this study confirms that AS use is associated with an LV hypertrophy and changes in LV function, primarily in diastole. Further this study presents new data on RV enlargement and a reduction in RV EF in AS users. Whilst this study documents further evidence of cardiac structural and functional adaptation to AS use we did not observe any evidence of perfusion defects or the presence of interstitial fibrosis in AS users. Based on this

and other previous data we speculate that AS results in a significant increase in the risk of CV disease progression and events.

Chapter 5. Cardiovascular responses to resistance exercise: A pilot/feasibility study of two concepts.

5.1. Introduction

Despite the complex nature of research associated with AS use, a pattern of potentially negative cardiovascular consequences, such as altered lipid profiles and changes to cardiac morphology and function are beginning to emerge (Hartgens and Kuipers, 2004). This is supported by empirical data presented in this thesis using state-of-the-art technology. A reduction in the capacity for the myocardium to relax, coupled with altered vascular function in AS users (Lane et al., 2006, Ebenbichler et al., 2001, Sader et al., 2001) suggests an increased CV risk. Despite this we found no evidence of perfusion defects or the presence of any interstitial fibrosis in AS users in the previous chapter and thus the link between AS use and a mechanism that may promote specific cardiac events, as observed in many case studies, is still lacking.

When the literature base associated with the consequence of AS use for cardiovascular health is viewed in its entirety, a striking observation is that most studies are completed at rest. Whilst the logistics of that are understandable, especially given problems associated with non-invasive imaging during exercise, this represents a weakness when linking AS use to cardiovascular risk. When anyone exercises the cardiac workload and stress are increased and it is more likely that cardiac health problems maybe "unmasked". We know that exercise *per se* increases the risk of cardiac events and sudden cardiac death in virtually all groups, from healthy through to pathology (Thompson et al., 2007). As a consequence, the focus of this exploratory chapter is cardiovascular responses to exercise and recovery in AS users.

The study of cardiovascular function during exercise and recovery is pertinent for other specific reasons. AS users engage in predominantly resistance exercise training and previous research has suggested that resistance exercise causes a marked acute increase in both systolic and diastolic pressure in both healthy (Sale et al., 1994, Greer et al., 1984, Palatini et al., 1989) and diseased patients (Hung et al., 1982). Classic work by MacDougall et al. (1985) reported significant increases in both systolic and diastolic blood pressures to a mean of 320 and 250 mmHg during double leg-press exercise to failure, with one subject recording pressures exceeding 480/350 mmHg. The authors speculated that this extremely large increase in pressures is caused by; an increase in cardiac output, sympathetic drive, intramuscular pressure and the valsalva manoeuvre (MacDougall et al., 1985). There is very limited data in AS users with respect to the blood pressure response to exercise with some saying it is augmented (Riebe et al., 1992) with others disagreeing (Sader et al., 2001). The nature of any blood pressure response to exercise in AS users should also pertain to activities commonly undertaken during training. The consequence(s) of the significant blood pressure response to resistance exercise for cardiovascular function and control during exercise have not been fully described in either users or non-users of AS. Specifically, it may be pertinent to understand the role of heavy resistance exercise (acute double leg press), with and without valsalva, on cardiac electrical activity, cardiac function and arterial blood pressure in AS users and resistance-trained controls. This provides the rationale for the first part of the exploratory data collection reported in this chapter.

Cardiovascular function during recovery is a research topic that has multiple applications. Rapid recovery of HR is often regarded as a key measure of

cardiovascular fitness and the concept of post-exertional hypotension has been assessed in endurance and resistance exercise settings as a potential process by which exercise can promote healthy blood pressure regulation (Jones et al., 2007). Of specific interest in the chapter is the concept that acute exercise may result in a transient cardiac fatigue as well as the appearance of biomarkers of cardiac cell damage (Shave et al., 2010b). There is a growing evidence base that acute but prolonged endurance exercise may be followed by a transient reduction in cardiac systolic and diastolic function during recovery (Oxborough et al., 2010).

The combination of exercise-related circumstances (mode, intensity, duration, volume etc.) that promote "cardiac fatigue" is not broadly described as most available evidence is restricted to endurance-based field studies (George et al., 2008). The impact of other exercise modes is important to determine but to date we are aware of only one study that has assessed the cardiovascular response to acute resistance training (Carranza-García et al., 2011, Stephenson et al., 2005). Stephenson et al. (2005) study suggested that cardiac function was unaltered during recovery from resistance exercise but intensity was only moderate and relatively insensitive cardiac techniques were employed. The second study proposed in this chapter aims to build on this work.

The mechanism(s) underpinning exercise-induced cardiac fatigue are largely unknown. Despite this many have promoted the idea that cardiac fatigue is consequent to small pockets of cardiomyocyte damage (Scharhag et al., 2008). This is based upon the description, in many studies, that cardiac fatigue occurs at the same

time as an elevation in serum biomarkers of cardiac cell damage (Shave et al., 2010a). The most common biomarker used is cardiac troponin (either cTnI or cTnT) that are regulatory proteins present in cardiac muscle. They are cardiac-specific and commonly used as a marker of cardiac damage following myocardial infarctions and acute coronary events (Ammann et al., 2004). Whilst cTnI and cTnT are widely reported to be elevated after endurance exercise (WHYTE et al., 2000, Shave et al., 2010a) a recent study by Shave et al. (2010a) noted that cTnI was increased after as little as 30 min of fatiguing, high intensity running exercise. The response of cTnI to high intensity resistance exercise is not known. Neither are we aware of whether the use of AS will mediate cardiac biomarker release, or indeed the development of cardiac fatigue.

NT-pro ß-natriuretic peptides (NT-proBNP) is another cardiac biomarker often reported to be elevated after prolonged exercise that is used clinically to provide evidence of chronic cardiomyocytes stretch that underpins poor LV function (Bhalla et al., 2004, Atisha et al., 2004). As with cTnI, little is known about the response of NT-proBNP to resistance exercise. This drives the specific rationale for the second exploratory trial reported in this chapter.

As this final empirical study is exploratory in nature we make no a-priori statement of hypotheses and we have recruited small numbers, in the first instance, to pilot the feasibility of this work.

5.2 Methods

Subjects

Strength trained individuals (n=12) were recruited for both studies through local gyms, personal contacts and local syringe exchange programmes. Posters and information sheets were placed in various locations and also distributed by hand to individuals. The posters stated that there was a study investigating the acute effects of resistance exercise, either with or without the use of AS, on cardiovascular function. Inclusion criteria were; participants aged between 18 and 50 years of age; resistance training history of minimum of 2 years with 3-4 training sessions per week. Exclusion criteria for the study were the presence of known respiratory, CV or musculoskeletal disease. Specific inclusion criteria for the AS using group included a documented self-report history of AS use for at least 2 years (including on and off-cycles). Inclusion criteria for the non-AS (NAS) group included self reported history of never taking AS. Prior to taking part in the study, details of each part of the testing were described to each of the participants. The study was granted ethics approval from the Liverpool John Moores Ethics Committee and participants provided written informed consent. Training history included data on years of training, the average number and length of sessions per week, as well as self-reported one repetition maximums for the bench press and squat (Table 5.2). Those in the AS group provided a detailed history of AS use including names, dosage and cycling information and none of the participants self-reported co-abuse of any other illicit substances. For simplicity we provide a list of exemplar AS used but note that all subjects used multiple AS in various stacking procedures with varied periods of abstinence or "off-cycles". The types of AS currently being used by the AS

participants included Sustanon, testosterone, deca durabolin, Boldebolin and Proviron. Of those in the AS group that provided sufficient information to perform an analysis of their daily usage (n=4), we found that the mean AS dose was 1600 ± 692 mg/day (range 800-2000 mg/day).

Design

In both instance a mixed model design was employed. A between groups analysis was utilised to compare AS users with non-AS users and repeated measures were observed as various aspects of cardiovascular function and biomarkers were recorded at different time points.

In both trials participants were required to make two visits to our laboratory and gym. Initially, subjects completed self-report questionnaires related to general health, training status and history as well as detailed accounts of AS use. After this a main testing session was conducted on the participants following an overnight fast, as well as a 24hr abstention from resistance training. Specific trial details will now be confirmed.

Trial 1

In this study participants (AS n=4, Age= 31 ± 6; NAS n=8, Age=23 ± 2) were assessed at rest, at multiple points during acute leg press exercise at 90% 1 RM (concentric, isometric and eccentric phases) as well as during recovery. Trials were

undertaken with and without the performance of a valsalva. Participants were asked to cycle at 60 rpm on a bicycle ergometer (Recline Exercise Bike 500, Technogym, Italy) for 5 min prior to testing and were then seated in a semi-recumbent leg press machine. Following a warm-up of 3-5 repetitions, at 3 different, but increasing weights, participants performed 4 leg-press repetitions (2 valsalva and 2 without valsalva). During each repetition, participants were required to take the weight and perform a leg press until the knee was at approximately 90 degrees. Each repetition lasted a total of 9 seconds with a 3 second descent, a 3 second hold at the bottom of the lift followed by a 3 second extension, returning the bar to its starting position. This was to allow for peak measurements during concentric, isometric and eccentric contraction phases. Due to limitations in data collection, the highest peak of a measure was taken irrespective of where it occurred. There was a one minute rest between each repetition.

Blood Pressure and ECG

After a standardised warm-up noted above subjects had blood pressure and a single lead ECG recorded continuously during a 10 min seated resting period. Arterial blood pressures were recorded from the right forefinger via an automated blood pressure monitor (Finometer PRO, Finapress, Amsterdam, The Netherlands). Comparisons of finger arterial blood pressure measures and intra-arterial BP have shown that finger areterial BP gives a satisfactory representation of arterial BP (Imholz et al., 1998). A single-lead ECG (V5) was also monitored and recorded continuously at 200 Hz using an analogue-to-digital converter (Powerlab/16SP ML795, ADInstruments) and displayed on a PC computer with commercially

available software (Chart version 7, ADInstruments), in order to infer stroke volume and cardiac output.

Echocardiography

A commercially available ultrasound echocardiography system (Vivid Q, GE Healthcare, Norway) was used to gather images of the left ventricle (LV) in the four-chamber long-axis apical acoustic window. Because of the repeated measures data capture during these trials over short time periods (3 s for each portion of the lift), we were limited to one acoustic view and one imaging mode. To capture LV systolic and diastolic functional indices we utilised pulsed-wave tissue-Doppler imaging (TDI) with a 2 mm sample volume placed in the mid-wall of the septum at the level of the mitral annulus. Care was taken to image the septal wall parallel to the ultrasound beam. We continuously recorded tissue velocities that included in each cardiac cycle; peak systolic (S') as well as early (E') and atrial (A') diastolic tissue velocities. A single experienced echocardiographer performed all imaging with the subject in a reclined position on the leg press machine. Intra-observer reliability for TDI data has been assessed and published within our laboratory (Chan-Dewar et al., 2010b, Chan-Dewar et al., 2010a)

As this was a feasibility or exploratory trial with low numbers, data analysis was completed descriptively.

Trial 2

In this study measurements of cardiac function, blood pressure and biomarkers of cardiac cell damage and stress (serum samples) were taken at rest and following a full body resistance exercise session (AS n=5, age=29 ± 5yr; NAS n=6, age= 25 ± 1 yr). The exercise protocol consisted of a warm-up on the stationary bike for 10 minutes, followed by the full body resistance exercise routine. The exercises performed included the back squat, bench press, bent-over row, behind-neck press, preacher curls and triceps extensions. Each exercise was performed for 8-12 reps for 3 sets with one minute rest between each set. Each participant had a 1 RM assessed *a-priori* and resistance was then assigned for each activity (Table 5.1). The total resistance exercise session duration was c. 60 minutes and repeat assessment of cardiac function, blood pressure and biomarkers were made at 15 min of recovery. Relative exercise intensity for each exercise was determined according practice trials in a small group of regular resistance trainers.

Table 5.1. Relative exercise intensity per exercise.

Exercise	**% 1RM (relative to what exercise)**
Squat	70% (Squat)
Bench Press	70% (Bench Press)
Bent-Over Row	50% (Bench Press)
Behind-Neck Press	50% (Bench Press)
Preacher Curl	20% (Bench Press)
Triceps Extension	15% (Bench Press)

Blood Pressure

Duplicate brachial artery blood pressures were recorded from the left arm via an automated blood pressure monitor (Dinamap; GE Pro 300V2, GE Healthcare).

Blood Samples

Venous blood (5 ml) was collected from the brachial antecubital vein directly into serum gel (serum) vacutainers (BD, Oxford, UK). Blood was allowed to clot (~45 min), centrifuged for 10 min at 3000 rpm and stored at -80°C for later analysis. cTnI was determined using the TnI-Ultra assay for Advia Centaur XP immunoassay system (Siemens Medical Solutions Diagnostics, Frimley, Surrey). Assay detection limit was 0.006 ug/L with a linear calibration range up to 50 ug/L (Apple et al., 2008). Assay precision in our laboratory was estimated as 10% CV at 0.045 ug/L (Collinson et al., 2009). NT-proBNP was determined using the NT-proBNP assay for Immulite 2500 (Siemens Medical Solutions Diagnostics, Frimley, Surrey). The assay detection limit was 20 pg/ml with a linear calibration range up to 35,000 pg/ml (Gardner et al., 2003).

Echocardiography

Global and segmental cardiac function were assessed using ultrasound echocardiography (Vivid Q, GE Healthcare, Norway). A single experienced echocardiographer performed all imaging with the participant in the left lateral decubitus position. In our laboratory intra-observer reliability has been assessed through intra-class correlations for 2D, Doppler, TDI, and E/SR data (Chan-Dewar et al., 2010a, Chan-Dewar et al., 2010b), with a range of 0.693-0.993 ($P<0.05$).

Specifically, parasternal long axis views allowed the collection of M-mode images of the LV at the tips of the mitral valve leaflets perpendicular to septal and posterior walls taking care to ensure clear endocardial definition. From M-mode traces septal (IVSd) and LV posterior wall thickness (LVPWd) in diastole as well as the LV chamber dimension at end-diastole and systole (LVIDd, LVIDs) were assessed following American Society of Echoardiography guidelines (Lang et al., 2005). We estimated LV mass using a regression corrected cube formula (Devereux et al., 1986). Apical 2 and 4-chamber views were digitised to asses LV end-diastolic (LVEDV) and end-systolic (LVESV) volumes which then allowed the estimation of stroke volume (SV) and ejection fraction (EF) using Simpsons' bi-plane method. From the 4-chamber colour Doppler and then pulsed wave Doppler were used to assess peak flow velocities across the mitral valve. Using a 4 mm sample volume in the area of peak flow LV early (E) and late (A) diastolic in-flow inflow velocities were recorded. From the same view tissue-Doppler measures of myocardial wall velocities were recorded. Taking care to adjust filters and scale and with the septal wall parallel to the ultrasound beam we interrogated the mitral annulus at the septal wall, recording peak systolic (S') as well as early (E') and atrial (A') diastolic tissue velocities. This also allowed the production of the E/E' ratio that has been shown to estimate left atrial pressure (Nagueh et al., 1997).

Segmental and global ε and SR data were obtained from parasternal short axis views at the base (just below mitral valve) and apex (1-2 cm above LV cavity obliteration) as well as via a 4-chamber apical view. Cine loops of LV motion were captured for off-line analysis (Echopac, GE Heathcare, Norway). Specific speckle-tracking software that tracks natural acoustic markers, or "kernals", facilitated the estimation

of $\mathcal{E}$ and strain rate (SR) in six wall segments in all views. In short axis views the LV was split into septal, anteroseptal, inferior, posterior, anterior and lateral wall segments. Radial and circumferential data from these segments were averaged to provide global $\mathcal{E}$ and SR data. SR data were compiled during systole (S SR), early diastole (E SR) and atrial diastole (A SR). In the apical 4-chamber axis view the LV was split into basal, mid-wall and apical septal wall segments as well as basal, mid-wall and apical lateral wall segments. Again these were averaged to provide global measures of longitudinal $\mathcal{E}$ and SR. Two-dimensional image optimisation was performed including maintaining frame rate between 40 and 90 fps. Data reflect the average of 3-5 continuous cardiac cycles.

Data analysis was descriptive on the basis of this being a pilot or feasibility study.

5.3 Results

Trial 1

Body Composition and Performance

There was no substantial difference in height or weight between the two groups and the weight lifted during the leg press was similar between the two groups, although a higher mean was observed in the NAS group.

Table 5.2 Anthropometric and Exercise Data

	AS	NAS
Height (cm)	180 ± 7	161 ± 38
Weight (kg)	85 ± 11	91 ± 34
Weight Lifted (kg)	210 ± 69	225 ± 36

Heart rate, stroke volume systolic and diastolic blood pressure were different following exercise compared to baseline, whilst there was no meaningful difference between the valsalva to non-valsalva exercise conditions. Systolic blood pressure was higher in the AS group at rest and had a greater peak during both exercise conditions.

Table 5.3 Peak heart rate, BP's, cardiac output and stroke volume at rest and during valsalva and non-valsalva exercise bouts (data are mean ± SD).

		AS	NAS
Heart Rate (bpm^{-1})	**Rest**	75 ± 9	65 ± 17
	Valsalva	95 ± 17	85 ± 16
	Non-Valsalva	101 ± 15	90 ± 15
Systolic BP (mmHg)	**Rest**	141 ± 22	131 ± 24
	Valsalva	205 ± 83	184 ± 28
	Non-Valsalva	200 ± 67	160 ± 29
Diastolic BP (mmHg)	**Rest**	62 ± 17	59 ± 14
	Valsalva	114 ± 14	100 ± 14
	Non-Valsalva	101 ± 18	93 ± 6
Cardiac Output (l/ min)	**Rest**	8.95 ± 1.82	10.63 ± 2.30
	Valsalva	10.37 ± 2.57	10.96 ± 2.78
	Non-Valsalva	9.66 ± 2.92	10.82 ± 2.24
Stroke Volume (ml)	**Rest**	73 ± 9	71 ± 24
	Valsalva	100 ± 13	96 ± 25
	Non-Valsalva	91 ± 16	96 ± 26

BP=Blood Pressure.

Both S' and A'measures were higher in the AS group. The difference in A' then resulted in a lower E':A' in the AS group. E' did not differ significantly between groups. In both groups E' and A', but less so for S', were increased during exercise with often higher values during valsalva.

Table 5.4 Tissue Doppler Measures at rest and during valsalva and no-valsalva exercise bouts (data are mean ± SD).

		AS	NAS
S' ($cm.s^{-1}$)	**Rest**	0.12 ± 0.02	0.09 ± 0.01
	Valsalva	0.16 ± 0.03	0.11 ± 0.01
	Non-Valsalva	0.16 ± 0.05	0.12 ± 0.01
E' ($cm.s^{-1}$)	**Rest**	0.12 ± 0.01	0.13 ± 0.01
	Valsalva	0.15 ± 0.01	0.16 ± 0.01
	Non-Valsalva	0.16 ± 0.03	0.17 ± 0.02
A' ($cm.s^{-1}$)	**Rest**	0.08 ± 0.01	0.06 ± 0.01
	Valsalva	0.12 ± 0.02	0.08 ± 0.01
	Non-Valsalva	0.12 ± 0.02	0.08 ± 0.01
E':A'	**Rest**	1.54	2.15
	Valsalva	1.31	2.03
	Non-Valsalva	1.37	2.10

S'=Systolic tissue velocity, E'=Early Diastolic tissue velocity, A'=Late Diastolic tissue velocity, E':A'=Earyl:Late Diastolic tissue velocity ratio.

Trial 2

Body Composition and Performance

There was no substantial difference between groups for height or weight. A trend in both of these data resulted in a higher BSA in the AS group. The number of training sessions per week, average session length and 1RM data for both bench press and squat were similar between groups (Table 5.5). Insufficient data regarding aerobic training was provided by the AS participants, limiting a comparison between groups.

Table 5.5 Anthropometric and Training Data (data are mean ± SD).

	AS	NAS
Height (cm)	181 ± 10	176 ± 5
Weight (kg)	96 ± 21	76 ± 12
BSA (m^2)	2.16 ± 0.27	1.91 ± 0.13
Sessions/Wk	5 ± 1	4 ± 1
Avg. Session Length (min)	75 ± 15	67 ± 23
1RM Bench (kg)	110 ± 21	105 ± 37
1RM Squat (kg)	105 ± 41	137 ± 48

BSA= Body surface area, 1RM= One repetition maximum.

Heart Rate and BP

There was no difference between groups at rest for heart rate, systolic and diastolic blood pressure. Heart rates increased in both groups, in a similar fashion, following exercise. Post-exercise systolic blood pressure was unchanged but diastolic blood pressure dropped in both groups.

Table 5.6 HR and BP Pre and Post Exercise (data are mean ± SD unless otherwise stated).

	AS		NAS	
	Pre	**Post**	**Pre**	**Post**
HR (bpm^{-1})	55± 8	81 ± 10	58± 7	84 ± 1
Systolic BP (mm/Hg)	130 ± 10	131± 14	122 ±11	125 ± 7
Diastolic BP (mm/Hg)	75 ± 11	67 ± 7	68 ±6	58 ± 2

HR= Heart Rate, BP= Blood Pressure.

Echocardiography

Cardiac structural data were generally higher in the AS group but in both groups this data did not change after exercise. LV volumes were similar between groups and in both AS and NAS both LVEDV and LVESV decreased post-exercise. As a consequence EF did not change in either group after a bout of resistance training.

Data for E, A and E:A were similar at rest in both groups. Post-exercise E declined marginally, A increased and E:A declined to a similar extent in both groups. The same pattern was obvious in the TDI data except for the fact that A' data did not alter post-exercise. E:E' data were slightly elevated in AS users only.

Table 5.7 Cardiac structural and functional measures pre and post exercise (data are mean ± SD unless otherwise stated).

	AS		NAS	
	Pre	**Post**	**Pre**	**Post**
IVS (mm)	11.2 ± 1.0	10.8 ± 2.0	9.9 ± 1.2	10.9 ± 1.1
LVPW (mm)	11.3 ± 0.9	11.3 ± 0.8	8.4 ± 1.1	9.1 ± 1.6
LVD (mm)	50.1 ± 4.2	48.2 ± 4.5	50.2 ± 2.6	49.2 ± 2.9
LVM (g)	216 ± 47	198 ± 58	158 ± 27	182 ± 17
LVEDV (ml)	120 ± 22	111 ± 25	119 ± 15	110 ± 25
LVESV (ml)	48 ± 16	45 ± 12	51 ± 9	43 ± 11
EF (%)	61 ± 6	60 ± 4	57 ± 4	63 ± 6
E ($cm.s^{-1}$)	84 ± 8	78 ± 22	81 ± 7	74 ± 11
A ($cm.s^{-1}$)	53 ± 8	65 ± 11	51 ± 3	57 ± 9
E:A	1.60 ± 0.23	1.20 ± 0.33	1.61 ± 0.12	1.32 ± 0.21
S' ($cm.s^{-1}$)	10 ± 1	11 ± 1	9 ± 1	10 ± 1
E' ($cm.s^{-1}$)	13 ± 1	11 ± 3	13 ± 2	12 ± 1
A' ($cm.s^{-1}$)	9 ± 2	8 ± 1	8 ± 1	8 ± 1
E':A'	1.63	1.21	1.71	1.58
E:E'	6.55	6.76	6.24	5.85

LV = Left Ventricle, LVD= Left Ventricular Diameter, LVM = Left Ventricular Mass, LVEDV=Left Ventriculad End-Diastolic Volume, LVESV= Left Ventricular End-Systolic Volume, EF = Ejection Fraction, E = Early diastolic Flow, A = Late diastolic Flow, E:A= Early:Late diastolic flow ratio, S'=Systolic tissue velocity, E'=Early Diastolic tissue velocity, A'=Late Diastolic tissue velocity, E':A'=Earyl:Late Diastolic tissue velocity ratio, E:E'= Early diastolic flow:Early diastolic tissue velocity ratio.

Longitudinal $\mathcal{E}$ and SR

Longitudinal $\mathcal{E}$ and peak SR did not differ markedly between groups and most data was not altered after a resistance exercise training session. The one variable to

change was an elevation in A SR in AS users only that resulted in a decline in the E:A SR ratio in AS users only.

Table 5.8 Pre-Post Longitudinal Strain (data are mean ± SD unless otherwise stated)

	AS		NAS	
	Pre	**Post**	**Pre**	**Post**
$\mathcal{E}$ (%)	-14.8 ± 1.3	-14.1 ± 2.4	-15.0 ± 4.5	-14.3 ± 5.2
SR($.s^{-1}$)	-0.93 ± 0.06	-1.22 ± 0.30	-1.11 ± 0.33	-1.13 ± 0.3
E SR($.s^{-1}$)	1.56 ± 0.27	1.56 ± 0.31	1.60 ± 0.38	1.68 ± 0.38
A SR($.s^{-1}$)	0.68 ± 0.09	0.97 ± 0.19	0.78 ± 0.20	0.82 ± 0.2
E:A SR	3.56	1.57	2.14	2.26

Circumferential and radial $\mathcal{E}$ and Strain Rate

There was no large effect of exercise or group on circumferential or radial peak $\mathcal{E}$ at basal or apical levels. In addition, no effect of group or exercise was also seen in peak basal or apical circumferential S SR. A similar pattern was apparent for E SR, A SR and E:A SR ratio. Generally E SR was slightly reduced post-exercise, A SR was slightly increased and the ratio was further reduced, often to a greater extent in the AS users.

Table 5.9 Circumferential $\mathcal{E}$ and SR, in AS and NAS groups (data are mean ± SD unless otherwise stated).

		AS		NAS	
		Pre	Post	Pre	Post
Basal Level	**$\mathcal{E}$ (%)**	-15.7 ± 3.7	-14.4 ± 6.2	-15.6 ± 2.3	-14.3 ± 6.2
	Peak S SR ($.s^{-1}$)	-1.61 ± 0.34	-1.67 ± 0.21	-1.40 ± 0.25	-1.49 ± 0.24
	Peak E SR ($.s^{-1}$)	1.92 ± 0.27	1.64 ± 0.45	1.75 ± 0.17	1.52 ± 0.46
	Peak A SR ($.s^{-1}$)	0.87 ± 0.33	0.97 ± 0.26	0.52 ± 0.25	0.73 ± 0.15
	E/A SR Ratio†	2.45	1.63	3.35	2.96
Apical Level	**$\mathcal{E}$ (%)**	-25.2 ± 4.7	-22.1 ± 5.5	-22.5 ± 5.9	-21.7 ± 9.4
	Peak S SR ($.s^{-1}$)	-2.42 ± 0.43	-2.02 ± 0.49	-1.48 ± 0.32	-4.50 ± 6.20
	Peak E SR ($.s^{-1}$)	2.69 ± 0.51	2.35 ± 1.13	2.25 ± 0.86	2.00 ± 0.98
	Peak A SR ($.s^{-1}$)	0.87 ± 0.20	1.17 ± 0.27	0.59 ± 0.07	0.97 ± 0.37
	E/A SR Ratio†	4.02	2.40	3.47	2.20

Table 5.10 Radial $\mathcal{E}$ and SR in AS and NAS groups (data are mean ± SD unless otherwise stated).

		AS		NAS	
		Pre	Post	Pre	Post
Basal Level	**$\mathcal{E}$ (%)**	27.1 ± 9.5	20.7 ± 28.5	23.7 ± 11.0	27.7 ± 12.9
	Peak S SR ($.s^{-1}$)	1.70 ± 0.49	2.23 ± 0.93	1.43 ± 0.44	2.12 ± 0.51
	Peak E SR ($.s^{-1}$)	-1.68 ± 0.54	-1.43 ± 0.68	-1.30 ± 0.50	1.30 ± 0.33
	Peak A SR ($.s^{-1}$)	-1.36 ± 1.31	-1.71 ± 1.08	-0.64 ± 0.33	-0.98 ± 0.36
	E/A SR Ratio†	0.93	0.92	1.64	1.79
Apical Level	**$\mathcal{E}$ (%)**	36.6 ± 31.6	30.1 ± 17.2	20.9 ± 7.6	22.9 ± 16.1
	Peak S SR ($.s^{-1}$)	2.32 ± 0.96	2.69 ± 0.59	1.21 ± 0.48	1.45 ± 0.77
	Peak E SR ($.s^{-1}$)	-3.28 ± 0.99	-2.31 ± 0.41	-1.88 ± 0.83	-2.39 ± 1.02
	Peak A SR ($.s^{-1}$)	-0.81 ± 0.34	-1.69 ± 0.80	-0.78 ± 0.37	-0.81 ± 0.69
	E/A SR Ratio†	4.65	1.58	2.84	2.78

Bloods

Only one detectable cTnI sample was obtained at rest (AS) and only one detectable cTnI value was observed post-exercise (AS, but different participant). Both these detectable values were low and not clinically relevant. At rest NT-proBNP was marginally higher in the AS group, but no change post-exercise was noted in either group.

Table 5.11 Pre and Post-Exercise cTnI and NT-proBNP data in both groups (data are mean ± SD; for cTnI the detection limit was 0.02).

		AS	NAS
cTnI (mg/L)	**Pre**	0.024 ± 0.001	0.02 ± 0
	Post	0.023 ± 0.007	0.02 ± 0
NT-proBNP (pg/mL)	**Pre**	33.28 ± 15.85	24.38 ± 8.92
	Post	30.45 ± 9.32	22.12 ± 3.28

cTnI= Cardiac Troponin I, NT-proBNP= N-terminal prohormone of brain natriuretic peptide.

5.4 Discussion

The key finding from this chapter is that the execution of a single resistance exercise or the completion of a standard resistance exercise programme provides an increase in stress upon the cardiovascular system and that this may be mediated by AS use. As pilot, or feasibility, studies some valuable information was garnered that will inform the progression to full studies. Specifically, the assessment of cardiovascular function was possible within both protocols although some unique challenges were encountered. In the leg press exercise we were limited to single window, single imaging mode analysis (TDI) but the body position occasionally interrupted the transducer position resulting in abandoned trials. Further, we initially were interested in assessing blood pressure and cardiac function in short discrete sections of the lift (3 s concentric lift, 3 s isometric hold, 3 s eccentric lowering). Whilst with training we could maintain these timings even with a valsalva the current limitations of data collection tools meant that the acquisition of cardiac functional data throughout these periods was problematic, hence we simply report here peak blood pressure and cardiac data. In the "training session" trial 2 the major concern was the

consistency of post-training assessment time. Past literature has not identified a single, and most relevant, time so we tried to control this at 15 min post. This provided 2 challenges; firstly that a prolonged imaging protocol meant that all scans could not be collected at the same time (scan time c. 10 min) and secondly that the blood sample could not be taken concomitantly with the echocardiogram. Despite these technical issues, we feel that advancing with these studies is feasible and potentially very interesting with minor changes in study design and set-up. In terms of the data collected, albeit in a small sample of AS and NAS participants, this feasibility study adds novel data to the area of AS research both during and immediately following high intensity resistance exercise. Specifically, in trial one a very high systolic blood pressure was recorded during single repetitions of seated double leg press that were augmented by AS use, although valsalva did not seem to alter blood pressure to any meaningful extent. The second trial provides data suggesting that resistance training can reduce aspects of diastolic function. There was some sporadic evidence that this may also be mediated by AS use but obviously requires further verification. Although exploratory, taken together the findings could indicate a possible increase in CV event risk in AS users during or immediately following exercise.

Trial 1

Heart rate and systolic blood pressure increased in both groups during the acute resistance exercise movements as would be expected (MacDougall et al., 1985). Systolic blood pressure was, however, noticeably increased in the AS group in all conditions compared to NAS. This contradicts some blood pressure data collected in

AS users at rest including data presented in Chapter 3 and 4 of this thesis. However, an elevated blood pressure in AS users has been reported before (Riebe et al., 1992, Freed et al., 1975). The greater systolic blood pressures observed in the AS participants during exercise (as well as rest) may mediate and increased CV event risk during exercises that are commonly employed in resistance training. The strain of exercise has long been associated with an increased CV event risk in those with elevated blood pressures (Kurl et al., 2001). A decreased ability to regulate haemodynamics (Kasikcioglu et al., 2007), in AS users could help to explain the association between AS and adverse CV events. It is interesting to note that the peak systolic blood pressures reported in all participants (AS or NAS) were less than those reported in the classic paper by MacDougall et al. (1985). This could be explained by the application of single repetitions in the current study compared to a cumulative effect of sets at high intensity to failure used in the MacDougall study. This may also explain the lack of difference (from rest to exercise, between groups and from non-valsalva to valsalva) in diastolic blood pressure in the current study. Likewise it seems a single leg-press with and without a valsalva manoeuvre did not alter blood pressure response. Also, it is pertinent to note that whilst we used a validated non-invasive blood pressure assessment tool, MacDougall had in-dwelling arterial pressure catheters for direct blood pressure assessment.

The mechanism(s) behind an increased systolic blood pressure response to a single leg press will likely include an increase in cardiac output, sympathetic stimulation and increased afterload (via compression of major arteries in the lower limbs) as suggested by MacDougall et al. (1985). The exact interplay and importance of each of these processes is difficult to assess in the current design. Suffice it to say that a

brief increase in intra-thoracic pressure due to a short-lived valsalva did not alter these processes. This is despite the understanding that a valsalva will lead to a short-term decrease in cardiac output from a reduction in preload, mediated by the increases in pressures normally observed, but this was not necessarily the case in the present data. The impact of AS in mediating a higher systolic blood pressure at rest and during these brief exercise trials is also difficult to fully elucidate. An increased systolic BP response in the AS users could suggest a decrease in arterial compliance/increased arterial resistance (Franklin et al., 1997). An increase in arterial resistance, amongst AS users, has been postulated from previous findings (Kasikcioglu et al., 2007, Lane et al., 2006), although this may be expected to impact both systolic and diastolic blood pressures. It could be that AS use augments the rapid increase in cardiac output and/or sympathetic stimulation as this could influence systolic pressure predominantly.

An augmented sympathetic stimulation may explain the increase in S' observed in both groups during leg press exercise, which resulted in higher S' during lifts with and without valsalva in the AS group. An increased afterload or exaggerated heart rate response (both observed here in AS users) could explain this difference in systolic tissue velocities. As has been observed in the previous chapters (3 and 4) and other recent findings (Baggish et al., 2010), AS have been associated with decreased diastolic cardiac function and ventricular compliance (Angell et al., 2011, Baggish et al., 2010, D'Andrea et al., 2007, Kasikcioglu et al., 2009). Whilst both groups had an increase in E' and A' during the exercise bouts; the increase in A', was greater in the AS users and thus the E':A' was lower in AS users at rest and during exercise, with or without valsalva. Whilst it is important to note that the

figures seen in both groups were both within the expected clinical range for the age group (Stohn et al, 1997), the results would indicate an increased reliance on atrial diastolic filling both at rest and during exercise in the AS users. In addition, this would suggest an increase in cardiac workload and stress in the AS users. When combined with increased systolic blood pressures, during exercise and at rest, these findings could indicate an increased CV event risk associated with AS use.

Clearly there are constraints and limitations to the design and data collected in Trial 1, not least the very low sample size for AS users. Added to some issues in data collection it is likely that to move forward with this trial would require some minor adjustments to data collection and study design. Specifically, we would like to determine a way of data assimilation to specific aspects of the lift (isometric components etc) as well as looking at a design that employed cumulative repetitions to failure, in line with the MacDougall study. Consequently, stopping data collection at this feasibility stage is sensible to reduce time and expense of staff and participants. Despite this the current form of this study did detail some evidence of elevated cardiovascular strain and risk in AS users and thus this is an important avenue of study to continue with.

Trial 2

Trial 2 progressed much more smoothly from the point of view of data collection thus we collected slightly more data in this feasibility trial. Despite this we still elected to describe data and outcomes here prior to movement to a more advanced

study with bigger participant numbers and multiple assessment points during recovery.

Predictably, both groups had an increase in heart rate following the full body exercise bout but there was no change in systolic or diastolic blood pressures following the exercise bout, with no difference between groups. This interestingly, is at odds with the previous trial but likely suggests a rapid blood pressure recovery before measurement at 15 min post-training (as well as the potential limitation of low numbers in both trials). An increased heart rate would be expected to augment some facets of cardiac function (Giannaki et al., 2008) whereas the lack of an increase in afterload would not complicate cardiac functional data interpretation

In both groups LVEDV and LVESV were reduced, to a similar extent, after the resistance training session. This has been observed after endurance exercise and competition (George et al., 2004) and may reflect a hypovolemia associated with sweat loss due to exercise. It cannot be explained by posture as both pre and post-training assessments were undertaken in a supine position, which theoretically maximises LVEDV at rest. With both LVEDV and LVESV decreasing post-exercise there was no exercise-related drop in EF in either group. This global systolic functional index has demonstrated a similar lack of effect in a prior study (Stephenson et al., 2005) as well as in multiple endurance exercise trials of a similar duration (Middleton et al., 2007). The lack of systolic "cardiac fatigue" is borne out by data for systolic tissue velocities and ε that were largely unchanged post-exercise in both groups. It may be that the total exercise volume and thus stress placed upon

the heart were not substantial enough in a single intermittent resistance exercise training session to produce a noticeable fatiguing effect.

Post-exercise there was evidence of an alteration in aspects of LV diastolic function. Specifically, a reduction in E:A and E':A', were also seen in both groups following exercise, with a slightly larger drop in AS users. This seems to reflect an alteration in E, A and E' post exercise. This suggests that both early diastolic filling (active relaxation and suction) and atrial contraction (atrial contractility and ventricular compliance) may be altered with such exercise. Data for diastolic SR are more sporadic but again some support exists for a reduction in global and segmental diastolic function after resistance training. Again, this maybe more apparent in AS users, although confirmation is required in a bigger trial. Of interest, an isolated diastolic cardiac fatigue has been reported after shorter duration/volume endurance exercise studies (GEORGE et al., 2004) and may reflect the fact that early diastolic function is largely dependent on a small mass of longitudinally aligned sub-endocardial LV cardio-myocytes. A smaller mass of myocytes may be more prone to fatigue after exercise imposition (the same concept may apply in the RV; (Oxborough et al., 2010)). Interestingly, Shave et al. (2007) suggested, in a meta-analysis, that diastolic functional changes after acute exercise bouts were not as influenced by exercise duration (total cardiac work) as systolic function.

The mechanism(s) underpinning a diastolic cardiac fatigue after a single resistance exercise training session are currently unknown although a few ideas have been proposed. Depressed function due to ischemia or cardiac damage has been proposed

by many (Scharhag et al., 2008) but evidence of a direct link is lacking. Here we saw no evidence of ischemia on the ECG and biomarkers of cell damage were sporadic at best. Neither would this theory explain an isolated diastolic cardiac fatigue. A desensitisation or down-regulation of ß-adrenergic receptors in the heart has been proposed as a mechanism to underpin a reduced chronotropic or ionotropic drive post prolonged exercise. Some evidence exists to support this (Hart et al., 2006) but this pertains to prolonged endurance exercise where catecholamine's are chronically elevated for hours. Also the data of Hart et al. (2006) and others did not explain changes in LV diastolic function which is what occurred in isolation here. Others have postulated metabolic reasons for a cardiac fatigue (reduced substrate availability and calcium handling issues; (Scott and Warburton, 2008)) although no direct human evidence exists to support these mechanisms. With the current data a reduced early diastolic filling likely reflects poor active relaxation and this could be influenced by poor calcium sequestration and sensitivity. An effect of AS use on cardiac myocytes has been demonstrated in vitro, highlighting a possible increase in collagen cross-links between myocytes which could offer an explanation for possible decreases in ventricular relaxation in the AS group (LeGros et al., 2000). It has also been suggested that AS use could be associated with a negative effect on nitric-oxide release from vascular endothelial cells, thereby reducing the vasodilatory capability of the vasculature which could explain the increased afterload and could have a knock on effect on cardiac function (D'Ascenzo et al., 2007). Irrespective of this speculation further work is needed to attempt to address putative mechanisms.

The appearance of biomarkers of cardiac cell damage (cTnI) or stress (NT-proBNP) were limited, small and likely of no clinical significance in the current study (Shave

et al., 2010b). Detectable cTnI was observed in one AS participant at rest and one AS user post-exercise. Although concentrations were low and not clinically relevant the combination with a lack of detectable cTnI in any NAS participant, suggests that this may be valuable to study in a bigger sample. Recent data from endurance exercise trials and even short high intensity running suggested a much bigger and more prevalent release of cTnI with acute exercise. It may be that the current study reflects the intermittent nature of resistance exercise and that the cardiac stress is insufficient to consistently activate pathways and mechanisms of cTnI release. Technically, we are limited by the assessment of one single blood draw post-exercise and thus on-going studies should develop the data collection in recovery (Middleton et al., 2007). Although baseline NT-proBNP was slightly higher in AS users the change with exercise was negligible and similar between groups. This provides more support that the cardiac stress, stretch or workload associated with a single resistance exercise training session is quite small (especially in comparison to ultra-endurance exercise trails reported in previous studies; (Scott et al., 2009)). The higher baseline NT-proBNP data in AS users are still within normal limits and thus have no clinical relevance. Despite this, again, confirmation in a bigger study would be valuable.

Overall, the study of an acute resistance exercise training session in AS and NAS was logistically easier to complete than trial 1. As a feasibility exercise the general running of the study was successful and thus slightly more participants completed successfully. Despite this there are important lessons for the development of the study and specific research questions. Most important of these is the need to collect post-exercise data (scanning and blood samples) at multiple time points during recovery.

General Limitations

It is worth noting that the present studies were based on either single high-intensity exercise bouts or a single full-body resistance exercise workout and may not reflect a 'typical' AS user's normal exercise programme. Nevertheless, the studies were designed in such a way so as to illicit the greatest CV response in participants. Further to this, whilst groups were matched for volume of training, through number of sessions per week and average length of session, significant variations in training methods could affect the findings. Future studies could focus on participants through a typical training period over a number of weeks to examine the cumulative effect of exercise, as well as AS use on CV parameters. It would be valuable to add measures of intra-thoracic pressure as well as estimates of LV afterload and wall stress in future studies. As has been highlighted in previous chapters, there are some limitations that are inherent within AS research. Individual differences between AS users and non-users, as well as differences within groups, in training status and methods can complicate analysis of findings in this area of research. Variations in AS dose (volume, drug type, authenticity) as well as differences in 'stacking' and 'cycling' procedures between users can further confound the issues surrounding AS research.

General Conclusions

Despite the limited numbers of participants and some specific design issues related to these exploratory trials, the studies themselves represent feasible propositions for on-going study. The findings themselves give some indication of 1) the elevation in systolic blood pressure with acute resistance exercise that is seemingly augmented

by AS use, and 2) the impact of an acute resistance exercise training session on a reduction in LV diastolic function during early recovery, which again may be partially mediated by AS use. On top of data assessed at rest (Chapter 3 and 4) this extends the notion that AS could increase CV risk by augmenting CV work during and after exercise.

Chapter 6. General Discussion

There are reports of increased AS use, largely in recreational users as a means of performance and image enhancement (Lenehan et al., 1996, Korkia and Stimson, 1997). Consequently, the health effects of AS come into sharp focus. The impact of AS use on the cardiovascular system represents an important public health issue. This chapter draws together empirical data contained in this thesis. A brief synopsis of findings is followed by an overarching discussion of developing issues across the thesis. This section will then reflect on the implications of issues of relevance to AS users, researchers and clinicians. Self-reflection on limitations related to the studies in this thesis will serve as a backdrop for suggestions for future research. Finally a conclusion section will reflect on the original aims and hypotheses stated at the beginning of the thesis.

6.1 Synthesis of findings

In studies one and two the focus of the data collection was around novel methods for the assessment of cardiac structure and function. We did view other CV risk data (e.g. blood pressure, lipids) but these were secondary outcome variables. In both studies we recruited self–report AS users with varying histories of both training and AS use. These participants were compared to an age and training exposure-matched group of non-AS users.

In the first study we reported that resistance training and AS use resulted in a higher lean mass and significantly greater strength compared to the matched control group. From a cardiac perspective, LV hypertrophy was apparent in AS users who also

demonstrated a reduction in diastolic function compared to controls. Diastolic function was described using a range of standard and novel imaging procedures. Left ventricular systolic function was preserved globally although there was some inter-individual heterogeneity. Secondary findings included a negative alteration in lipid profile in AS users and a significantly elevated resting heart rate. Blood pressure, whilst slightly higher was not significantly different between groups. In a small cohort of case studies from within the AS users we documented changes in cardiac, blood and body composition data during periods of withdrawal (off-cycles). Although limited by the sample size and case study approach there was some suggestion that cardiovascular risk is reduced by periods of abstinence from AS.

In the second study we reported data derived from cMRI and speckle-tracking echocardiography again in age and training-exposure matched AS users and non-users. cMRI is the "gold-standard" method for assessing cardiac structure with high resolution and no requirement for geometric model estimation. As with study one, we documented LV hypertrophy in AS users that was associated with diastolic dysfunction determined using a range of ultrasound imaging modes. Uniquely we described a RV hypertrophy that was also associated with a decrease in RV ejection fraction. Despite these changes in cardiac structure and function we found no individual evidence of perfusion defects or interstitial fibrosis that were speculated as potential links between AS use and cardiac events.

For the third study we adopted a developmental approach which focused on the cardiac response to exercise in AS users (and matched controls). This pilot study was

set up to assess the feasibility of two different approaches to assessing the impact of resistance exercise upon cardiac function in AS users. In trial one, we adopted an acute single repetition model using a double leg press, with and without valsalva, on blood pressure regulation, heart rate and cardiac function (using TDI only). In trial two we adopted the "cardiac fatigue" model of assessing cardiac function at rest and then in early recovery from a standard resistance training session. Despite small numbers due to technical issues and the nature of the feasibility approach, the AS users demonstrated a much larger systolic blood pressure response to a double leg press, with or without valsalva as well as subtle differences in the response of cardiac function, notably in diastole. After a model resistance training session LV systolic function was well maintained in both AS users and non-users. LV diastolic function was altered during recovery in both groups but the effect may be more pronounced in AS users. An elevation in cTnT above detection limits occurred only in AS users (1 pre-exercise and 1 post-exercise) but these responses are too limited to draw any conclusions. NT-proBNP was higher at rest in AS users but there was no meaningful exercise response. All cTnT and NT-proBNP values were below clinical cut-off levels.

6.2 Overarching Issues

Through the comprehensive approach taken in study one and study two as well as the feasibility trials in study three some points related to the empirical data were raised directly or in-directly on a number of occasions and this is the focus for this short reflective section.

Overall, the alterations in resting cardiac structure, function, CV risk factors as well as exercise responses would suggest that AS could increase the risk of developing occult CV disease or encountering a CV event. The magnitude of this increase in risk is almost impossible to estimate and despite the possible reversibility of the negative consequences of their use, one would assume that with continued AS use the risk would continue to rise due to a cycle of increased stress and atherosclerotic risk factors when using AS. Despite this it became apparent across studies that an increase in CV disease risk was highly variable; person-to-person, and measurement-to-measurement as well as study-to-study. Some data sets were more consistent, such as diastolic function which will be discussed later. That said, classic CV risk factors such as blood pressure were highly variable. In the resting studies these were elevated but not significantly in AS users but in the exercise trials blood pressure was elevated at rest and during an acute leg press but not before and after a resistance training session, however low numbers in the exercise trials need to be considered when comparing the conflicting results from the different studies. Whilst study one and two found little difference in BP measures at rest, study three demonstrated greater BP levels in AS users both at rest and during exercise. Elevated blood pressures and increased arterial stiffness have been associated with significant increases in cardiovascular disease/event risk (Dawber et al., 1957, Laurent et al., 2001) but this could be as much to do with resistance training as with AS use (Kawano et al., 2008). Whilst Kasikcioglu et al. (2007) reported that AS use was associated with increased aortic stiffness, suggesting a reduction in nitric-oxide mediated dilation through inhibition of second messengers to the smooth muscle surrounding the arteries, this is not a consistent finding. Whilst the importance of an exaggerated BP response to exercise for cardiovascular risk has been proposed

(Chaney and Eyman, 1988), and is partially supported by the findings in study three, this hypothesis is yet to be fully proven in AS users.

Measures on LV systolic function (EF, Ɛ) were altered in some studies but not in others. It was also apparent that no perfusion defects or interstitial fibrosis was observed by highly accurate cMRI. One simple way to evaluate these disparate findings is that an increase in CV risk is not obligatory when taking AS. It may be that some inter-individual differences in the physiological response to AS use, or quite simply the AS use/dose history may have had a impact on the development of CV risk. These facets cannot be deduced from the current study and are likely very difficult to assess in any self-report based study of AS in humans. It may be possible that some individuals are quite "resistant" to negative CV side-effects of AS use, whereas others at potentially similar dosages maybe more "susceptible". An individual approach to human studies within case series may try to unpick some of these issues.

Some "negative" CV responses to AS use were more consistent and deserve more attention. Significant alterations in lipid profiles were observed in study 1 and were also apparent in the cohort in study 2 (this data was not reported here because of a low n). Abnormal lipid profiles would suggest an increased risk of the development of arterial plaques, and has also been observed in previous findings (Hartgens et al. 1987). A mechanistic process responsible for the alteration of lipid-profiles in AS users, has been postulated by Applebaum-Bowden (1987) who reported an increase in LDL producing hepatic-triglyceride lipase (HTGL) following AS administration.

Whilst suggesting an increased production rate of LDL, these findings could also imply a catabolic effect of HTGL on HDL.

Another consistent outcome across studies was the presence of an LV (as well as RV in study 2) hypertrophy. Wall thickness and LV mass were different in absolute measures when compared with matched controls. This finding confirms and extends previous data (Sachtleben et al., 1993, Dickerman et al., 1998, Nottin et al., 2006). Despite significant cardiac structural changes in the AS groups, these differences were no longer present once they were scaled according to fat free mass. This highlights the importance of appropriate scaling (Batterham et al., 1999; Dewey et al., 2008). Further it provides indirect support for whole body protein anabolism in all muscular beds and thus suggests a mechanism for cardiac hypertrophy in AS users. Whilst an up-regulation of protein metabolism has been demonstrated in skeletal muscle beds following AS administration (Cellotti and Cesi, 1992), Marsh et al. (1998) have also demonstrated a similar increase in cardiac tissue. Interestingly, it would suggest that in the presence of normal function the development of LV mass in line with whole body fat free mass may be less insidious. However, whilst systolic function was largely normal in AS users the same cannot be said of diastolic function.

The changes seen in RV function are certainly worthy of further investigation. A drop in RV systolic function observed in study 2 as well as previous research suggesting a drop in RV diastolic function, could implicate a change in pulmonary pressure as well as reduction in RV compliance and elasticity, possibly greater than

that seen in the LV (Kasikcioglu et al., 2007). A number of mechanistic processes may be responsible for this negative effect of AS on pulmonary pressure outside of myocardial changes affecting ventricular compliance. Hematocrit levels have received limited attention in the area of AS research with the data suggesting a possible role of AS in increasing haematocrit and haemoglobin (Lane et al., 2006). By virtue of the fact that AS are indiscriminate in the androgen receptors they bind to, an up-regulation of red blood cell production from the bone marrow would be a legitimate assumption. An increase in haemotocrit levels would be indicative of an increase in blood volume and viscosity, in turn leading to an increase in blood pressure that would be most notable in the lower pressure ranges seen in the pulmonary system. Combined with a possible effect of AS on vascular compliance, it would therefore be reasonable to surmise that this possible change would have a direct effect on RV function through both systole and diastole.

A consistent finding in study one, was the persistent reduction in diastolic cardiac measures within the AS users, whether this was at rest (study 1 and 2) or during/after resistance exercise (study 3). Although a reduction in LV diastolic function has been observed by some parameters in recent studies (Baggish et al., 2010, D'Andrea et al., 2007, Kasikcioglu et al., 2009, Nottin et al., 2006, Krieg et al., 2007), this study extended this with new regional and global measures of LV diastolic function (notably strain data in the longitudinal, circumferential and radial plane). Although peak longitudinal and radial strain data has previously been shown to be negatively affected with AS use (Baggish et al., 2010, D'Andrea et al., 2007), this study was the first to demonstrate a negative association of AS use with depressed diastolic strain rates. The fact that this depression in function may persist or extend when in an

exercise setting provides further cause for concern. It may be that the major impact of AS use is upon early diastolic relaxation (that is compensated for with augmented atrial or late diastolic function). Again, the causes are speculative but a number of mechanisms for a reduction in cardiac relaxation have been postulated. LeGros et al. (2000) suggested in increase in collagen cross-links between myocytes with AS. In addition to this an effect of AS on calcium cross-channels (Lieberherr and Grosse, 1994) as well as an increased rate of apoptosis (Zaugg et al., 2001) have also been suggested as possible mechanisms for a reduction in cardiac diastolic relaxation capacity.

It is also important to consider the possible reversibility of some of the negative consequences of AS use following a relatively short period of abstinence, observed in study 1. Whilst the data regarding 'On/Off' cycle comparisons is on a small number of users, each of them showed improvements in measures of either cardiovascular risk or cardiac function. The key areas of change observed were increases in HDL as well increases in E:A ratios. Despite difficulty in extrapolating these findings to a wider group, they could suggest that any proposed increase in CV risk may be exaggerated when the findings occur when users are 'On' cycle. It is, however, difficult to assess the long-term effects of repeatedly increasing CV stress through negative effects on atherosclerotic risk factors as well as decreases in diastolic function. The acute changes seen following cessation of AS use may be precipitated by an acute drop in enzymes such as hepatic triglyceride lipase as well as an increase in lysyl oxidase inhibitors, causing down-regulation in LDL and collagen cross-link formation respectively. Whilst the reversibility of the effects of AS use on cardiac function has received some attention, they contradict the present

findings somewhat as they suggest that function may not completely recover following cessation for several years (Urhausen et al., 2004).

6.3 Implications

The findings from the studies included in this thesis can have a number of implications for AS users and researchers/clinicians. Whilst noting the discussion above there is some clear evidence that AS use has the potential for negative CV health side effects and in some this could be fatal. It is important that this message is delivered clearly and honestly to this sub-culture, alongside the data that an increase in CV risk factors or specific facets of proposed mechanisms of CV events are not obligatory in response to AS use that mirrors the specific cohorts recruited within this thesis. Such honesty of effect may actually have a more profound effect on AS users who are often immune to the "tub-thumping" medic or public health specialist.

It is important to note that the present findings were all performed in young to middle aged AS users who demonstrated a broad range of AS dose and use history (years, cycle-specific data). There was no correlation between amount and time of use and major cardiac variables in study 2 that limits conclusions about the accumulative effect of AS use in this cohort. Whether decades of use would produce different results is not known but is pertinent for clinicians as we are now approaching 40yr of AS availability to resistance athletes. Another point for clinicians to consider is that the secretive nature of AS use often means that users are

reluctant to divulge their use to a medical practitioner and so it can be difficult for practitioners to be aware of any increased risk that patients might be under.

Whilst the findings could not be said to be completely conclusive, they do suggest that AS use can be associated with a number of negative CV side effects that may not be apparent to the user or clinician/scientist for some time. Of particular interest to the present AS user is the effect of AS on lipid profiles that were observed. The role of AS in negatively altering lipid profiles has developed to become one of the few quite well established consequence of AS use (Angell et al., 2011). This is an important consideration for any current or potential user to consider, as the role of LDL and HDL levels in CV disease progression is well established one and although perfusion defects were not noted in the current cohort some manifestation of coronary artery disease or coronary artery spasm is likely implicated in many case studies of significant CV events.

From a scientific/research perspective, the present findings illustrate the need for more comprehensive and detailed research studies that can apply the latest technological advancements to improve accuracy within the area of CV assessment. Whilst not being definitive, the findings from this thesis do highlight themes (such as negative alterations of lipid profiles; diastolic dysfunction) that are starting to become an established consequence of continual AS use and a greater understanding of the role AS play in causing this effect is required.

6.4 Limitations

O'Sullivan et al. (2000) stated that limitations of, and barriers to, research in the field of AS use are substantial. Because of issues of legality and the consequent clandestine use of AS, large population-based epidemiological data detailing CV disease presence in AS users are unavailable. Open self-reporting on a large scale over a long time period is hard to obtain. Simply gaining access to this group is a difficult task. Many users are suspicious of 'outsiders' asking questions and this can lead to a failure to reach many imbedded in the culture. That being said, different methods have been employed to achieve greater access to users. Employing a cross-departmental/profession approach between those working in clinical sciences and public health (e.g. needle-exchange settings) can greatly improve the access of data. The immersion of a researcher into the culture, in order to gain the trust of AS users is still likely to be the most effective way to get open and honest accounts of AS use.

Randomised-control (placebo) trials of AS use are "few and far between" and are limited by ethical concerns in respect of the AS dose used. In "real-life", the dose of AS is often large and complex. Multiple steroids (stacking) are taken together, often with other substances, in increasing and decreasing dosage profiles. Another consideration is that the "true" dose of AS is also difficult to verify, even in honest self-report cases, because of the black-market system of purchase and the widespread distribution of counterfeit drugs (Thevis et al., 2008). Whilst we made some attempts to verify data and we could not account for all drug-related limitations and we did not even contemplate a randomised control trial for ethical reasons.

It is quite clear that the use of AS may be suspended (off-cycle) for short periods before resumption (on-cycle) but all of these practices differ significantly between individuals. We made a brief, case-by-case attempt to assess the impact of AS withdrawal in study 1. This was limited by current practice, was highly variable between athletes and as such conclusions were significantly constrained.

Limitations to the direct impact of AS use on cardiovascular disease presence or end-point are apparent. As a consequence of these limitations, the direct CV health impacts of AS use are still to be fully determined. What data exists in humans has largely been derived from case studies or case-series/case-control studies (Evans-Brown et al., 2008). Whilst we employed case series data with variables that attempted to reflect CV disease and event risk, these approaches are open to error of association. Despite some attempts to assess homogenous and matched groups we acknowledge; 1) the use of diverse subject groups with variable training exposure and dietary practices, 2) the lack of urine assessment to verify drug load, 3) the wide spectrum of AS dosage (quantitatively), drug type or source (e.g. veterinary), drug quality (e.g potency and 'homemade' AS), and stacking or poly-drug regimens that are often associated with the use of AS, and 4) the limitations of accuracy in self-report AS use.

6.5 Future Research

On the basis of (de)limitations noted above and in the specific empirical chapters we would make the following suggestions as to productive lines of on-going enquiry related to AS use.

In the first instance, the small subject numbers in most cross-sectional studies is a limiting factor to this area of research alongside the inter and intra-individual drug dosages. More longitudinal studies of "real-life" AS use, from as early in the AS use history, as is reasonably possible, would be illuminating. This could also factor in multiple withdrawals from AS use (off cycles) along with the differing cycles that are used for 'bulking' or 'cutting'. The specific issue of reversibility of any CV effects of AS is also an issue that requires attention. This could assess short-term withdrawal as well as assessing long-term users who have ceased use.

Of what limited data there is regarding AS use, a vast majority of the focus has been of their effects in men. Despite the majority of non-medicinal users being male, there are still a number of competitive female body builders and athletes that use AS. It could also be argued that due to the very nature of AS being derived from male hormones, the risk posed to women could potentially be greater than those seen in men. In order to further understand the potential negative consequences of AS use, the inclusion of female participants in any longitudinal studies is necessary.

Integral to future studies, alongside longitudinal designs (etc), is the need to utilise state-of-the-art techniques to assess CV health and generate new data and insights. For example, with a greater spatial resolution and the ability to tissue track as well as assess fibrosis and perfusion, using cardiac magnetic resonance imaging would allow for a much more comprehensive assessment of cardiac structure and function as well as determine if perfusion defects, inflammation and/or fibrosis existed within the cardiac muscle of AS users. Of specific interest would be to develop the database associated with RV adaptations to AS use. Whilst we demonstrated a RV hypertrophy and a reduced RV EF we did not assess RV diastolic function or indeed assess regional and global deformation using speckle-tracking echocardiography.

The cellular mechanisms responsible for any negative CV changes that occur from AS use/abuse also require further attention. Whilst previous research has examined the effects of AS on hepatic (Welder et al., 1995b) and endothelial cells (Welder et al., 1995a), work on myocardial cells has been limited to animal studies (Zaugg et al., 2001). Future animal in vitro work should aim to replicate doses to that which occurs in human users with a particular focus on proteomics and the area of sub-cellular enquiry.

6.6 Conclusions

This thesis has provided a significant insight in to the effect of AS use on CV function, as well as markers of CV disease/event risk. The results provide a greater understanding of the possible effects of continued AS use and suggests possible

explanations for the reasons behind these changes. Due to the limitations inherent within the field of research, however, it does limit the scope of conclusions possible from the findings. In addition, the clinical implications of such findings should be interpreted cautiously. Nonetheless, the findings do highlight the need for a wider scope of research to further understand the mechanisms/processes that are responsible for any change as well as the magnitude of possible negative effects associated with AS use.

In study one, we were able to accept part of our hypothesis due to the observation of significant changes in cardiac structural measures as well as systolic and diastolic measures in the AS group. Further to this, we also observed a significant effect of AS on CV risk factors, and more specifically, lipid profiles. Whilst these differences did not necessarily persist across all measures, they were consistent enough to suggest a negative effect of AS use on CV risk factors and cardiac function.

Similarly, study two also found significant cardiac structural and functional differences in fat free mass, meaning only a partial acceptance of the original hypothesis is possible. The decrease in RV systolic function as well as significant reductions in diastolic LV function support the hypothesis of a significant negative effect of AS on cardiac function.

Studies 3 and 4 had no hypotheses attached to them but were instead used to ascertain the feasibility of CV testing during resistance exercise. Both studies

demonstrated potential issues regarding limitations in consistency of data capture, and more specifically cardiac data capture, during and after high-intensity resistance exercise. However, despite these issues, the findings from both demonstrate that further understanding the acute response to, and recovery from, exercise in AS users and non-users is both worthwhile and feasible.

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Appendices

Participant Consent forms

Health profiles of weight/strength trainers. Consent form

You will be given a copy of this sheet to take with you.

Please tick:

☐I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without my medical care or legal rights being affected.

☐ I understand that any personal information collected during the study will be anonymized and remain confidential.

☐ I understand that I can choose not to answer any questions which I feel uncomfortable answering.

☐ I agree that quotes from my questionnaire/interview may be reported in published documents but that this will be anonymous and no-one will be able to identify me from this.

☐ I agree to complete the body composition scan (DEXA scan).

☐ I agree to provide blood, saliva and urine for sample testing.

☐ I agree to that the DNA from my blood can be used to test for the length of the androgen receptor gene.

☐ I agree to allow my blood sample to be securely stored for future research.

☐ I agree to complete the ECHO (echocardiogram), ECG (electrocardiogram) and blood pressure testing.

☐ I agree to complete the arterial structure and function testing.

Signed: ..... (participant)
Date: .....

I, ... (investigator's full name) certify that the details of this study have been fully explained and described in writing to the participant named above and have been understood by him/her.

Signed: ..... (investigator)
Date: .....

Cardiac Magnetic Resonance Imaging in Strength Trainers: Consent Form

You will be given a copy of this sheet to take with you.

Please Tick:

- ☐ I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and had these answered satisfactorily.
- ☐ I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without my medical care or legal rights being affected.
- ☐ I understand that any personal information collected during the study will be anonymized and remain confidential.
- ☐ I agree that quotes from my questionnaire may be reported in published documents but that this will be anonymous and no-one will be able to identify me from this.
- ☐ I agree to complete the Cardiac MRI scan.
- ☐ I agree to provide blood and urine for sample testing.
- ☐ I agree to complete an echocardiogram, ECG (electrocardiogram) and blood pressure testing.
- ☐ I agree to complete the body composition assessment using bioelectrical impedance.

Participant Name:..... Date: / /

Signature:.....

I, (investigators full name) certify that the details of this study have been fully explained and described in writing to the participant named above and have been understood by him/her.

Signed:..... Date: / /

LIVERPOOL JOHN MOORES UNIVERSITY

CONSENT FORM

Cardiovascular Responses to Acute Competitive Sporting Activity

Principal Investigator: Mr. Peter Angell, Research Institute for Sport and Exercise Sciences

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐

3. I understand that any personal information collected during the study will be anonymised and remain confidential ☐

4. I agree to take part in the above study and agree to my tissue (blood samples) being used as part of the study. ☐

Name of Participant Date Signature

Name of Researcher Date Signature

Peter Angell

Name of Person taking consent Date Signature

(if different from researcher)

Note: When completed 1 copy for participant and 1 copy for researcher

Participant Information Sheets

Health profiles of weight/strength trainers

Participant Information Sheet (Group B)

Title of study

Health profiles of weight/strength trainers

Study location:

School of Sport and Exercise Sciences, Liverpool John Moores University.

Study co-ordinator:

Mr Peter Angell

School of Sport and Exercise Sciences

Liverpool John Moores University

15-21 Webster Street

Liverpool

L3 2ET

Telephone: 0796 842 2497

Email: p.angell@2004.ljmu.ac.uk

Please contact Peter Angell (details above) if there is anything you do not understand or if you would like more information. Take time to decide whether or not you wish to take part.

What is the study about?

The effects of anabolic steroids on health when used in weight/strength training are poorly understood. The purpose of this study is to look at the effect of these drugs on the body over 1 steroid cycle (and how these compare to weight/strength trainers who have never used anabolic steroids). Some of the key areas we will be looking at will be body composition and the structure and function of the heart and blood vessels. The results of this study will allow

us to better understand the effects of anabolic steroids on the body, and develop health care and related services for anabolic steroid users.

What does the study involve?

Along with providing general information on your health, lifestyle, training and steroid use (and related drug use), we would like to follow your health over a steroid cycle (i.e. on/off cycle). For this we would like you to keep a daily diary (which will be provided, and will take approximately 15 minutes per day to complete) that records steroid use, training regimen, diet, general health and whether you experience any side effects. We will also need you to attend an assessment at our School of Sport and Exercise Sciences on 2 separate occasions over the course of the cycle. The procedures that will be performed *on each visit* are outlined below, and, during your first visit to School of Sport and Exercise Sciences, you will be fully familiarised with these procedures and given the opportunity to ask any questions that you may have.

Questionnaires: You will be asked to complete a number of questionnaires on your health, lifestyle, training and steroid use (and related drug use). We would also like to discuss your views and perceptions on steroid use and what health services you feel steroid users need.

Body composition: A procedure known as Dual Energy X-ray Absorptiometry (DEXA) will be used to assess the amount and distribution of your body fat, lean tissue as well as bone mineral density. The procedure is non-invasive and takes approximately 5 minutes. It involves lying stationary on your back on a moving table whilst low-dose x-ray (equivalent to the dose of radiation experienced during a 2 hour aircraft flight) are passed through the body. To prevent interference with the procedure, you will be required to remove any metal or items of jewellery. Prior to DEXA we will measure your height and weight.

Blood and urine sampling: Blood samples will be collected from a vein in your arm. A small amount (25ml) of blood will be taken. This amount is negligible and will not cause any adverse effects. Your blood will be used to measure a number of different health markers — including lipids, glucose, hormones and clotting factors. We will also look at the composition of part of your androgen receptor gene by DNA analysis. A full list of tests that will be performed on your blood is available upon request.

We will also ask you to provide a small urine sample (90ml), which will be used to measure glucose, protein as well as any anabolic steroids and related substances present in your urine. A full list of tests that will be performed on your urine is available upon request.

Anabolic steroid testing: We would like test some of the anabolic steroids (and any other substances) that you are using in your training for research purposes. This will allow us to examine things such as the dose and the drugs present in the preparations. If you are happy for us to do this please let us know as soon as possible so that we can make the necessary arrangements with you.

Blood pressure: Automated blood pressure measures will be taken alternately on your left and right arm whilst at rest (either in a seated position or lying on your back on a bed). All measurements will be taken twice. Although this procedure is non-invasive, it may cause some mild discomfort in your arm, which will subside when the cuff pressure is released.

Electrocardiography (ECG): An ECG will be performed where 10 electrodes are placed on your chest wall that will record the electrical activity of your heart. During this examination you will only be required to lie on your back on a bed in a resting state.

Echocardiography: An echocardiographic examination of the heart will be performed. This uses ultrasound technology to enable a non-invasive and painless assessment of heart structure and function. During this examination you will only be required to lie on your back on a bed in a resting state.

Arterial structure and function: The blood vessels of both your legs and upper arms will be scanned (using ultrasound technology) during a number of procedures that will measure your arteries ability to dilate (i.e. it assesses their function) whilst seated or lying on your back on a bed. Ultrasound is a completely safe, non-invasive measurement technique. One of the procedures will involve inflating a cuff, which will be placed around your upper arm (i.e. above the elbow joint) to occlude the circulation for five minutes while the cuff is inflated. Although these procedures are non-invasive, they are likely to cause mild discomfort in your arms, which will subside when the cuff pressure is released. During another one of the procedures, the blood vessels will be scanned before and after a small dose of a drug called glyceryl trinitrate (GTN) is sprayed under your tongue with the acute changes to the artery being measured. When sprayed under your tongue in this way, GTN causes your arteries to relax, allowing a short-term increase in blood flow. Rare side effects could include a headache or a slight lowering of blood pressure, both of which only last for a short period of time. Although GTN has been used in this way for many decades and is considered to be very safe in the doses we will use, you should inform us if you are taking *any forms* of medication or drugs.

Are side-effects associated with any of the procedures?

The procedures we will be using are all well-established techniques which have been used in numerous research studies (including with athletes).

- In the arterial structure and function procedures, use of glyceryl trinitrate (GTN) — which causes your arteries to relax, allowing a short-term increase in blood flow — could cause a headache or a slight lowering of blood pressure in some individuals, both of which only last for a short period of time. Although GTN has been used in this way for many decades and is considered to be very safe in the doses we will use, you should inform us if you are taking *any forms* of medication or drugs.

How long will it take?

Completing the diary will take you approximately 15 minutes per day. Each visit to the School of Sport and Exercise Sciences will take about 3-4 hours (~8 hours in total), typically between 9am and 1pm on a weekday.

What will I gain from taking part?

You will have information on your body fat and lean tissue (muscle) mass as well as your bone mineral density. From the electrocardiography, echocardiography, arterial structure and function you will find out about the structure and function of your heart and blood vessels, both before and after exercise (following the data analysis, a report will be provided on this). You will also help inform the development of health care and related services for steroid users.

Will my data be kept confidential?

All the information collected about you during the course of the study will be kept strictly confidential. Any identifying information about you will not be disclosed to anyone outside of the research group. You do not have to give us your full name. At the end of the study we may ask your permission to give you a letter to take to your GP (General Practitioner) so that they can discuss the results in more detail with you if they are important to your health. However, we will only provide you with a letter after you have given us permission. If you do not have a GP, we will provide you with details of where you can register with one.

It is expected that the results of this study will be published in a scientific journal and to inform health policy, but no identifying reference will be made to those individuals who took part. Liverpool John Moores University is registered under the Data Protection Act and has an Information Security Policy to safeguard the collection, processing and storage of confidential data.

However, should you suggest, imply or state that you are involved in specific serious criminal activities (i.e. acts of terrorism, offences against children) then we will inform the necessary authorities.

What else do I need to know?

- You must be aged 18 or over to participate in this study and planning to undertake anabolic steroid cycles in the near future.
- We will pay travel/parking expenses up to a maximum of £15 per session attended. Please remember to keep any receipts/tickets and bring them with you.
- If you agree to participate it is essential that you do not smoke, or eat any food or caffeine* for 12 hours before an assessment session. Restraint from exercise and alcohol for the 24 hours prior to any assessment sessions is also mandatory.
- A qualified practitioner will take your blood sample. Blood* and urine will only be used for this study, and, following analysis, any that remains will be disposed of. *If you decide that you would like to participate in the University of Southampton's growth hormone study, part of the blood sample that you give will be sent to this research team. On the consent forms for the Southampton study you can decide if you wish to allow them to keep your blood sample, securely, for use future research (please see separate sheet for details).

- Please note that, unfortunately, this study will not be able to provide you with the results of the: 1. questionnaires/discussions; 2. the blood and urine tests; and, 3. the anabolic steroid (and related drugs) testing.

- We will provide you with a list of health services that are available to you upon request.

- If you are interested in receiving the final results of this study then please get in contact with Peter Angell. However, please bear in mind that data such as these often take many months to analyse and prepare for publication.

- ***You have the right to withdraw from the study at any time without giving a reason, and without prejudice to access of services that are already being provided or may subsequently be provided to you. However, completion of all the procedures will greatly assist this research.***

* *Please note that caffeine is found in many common food and drinks such as chocolate, tea, coffee, soft drinks (such as cola and energy drinks), over-the-counter medications (such as pain-relief products) and some nutritional supplements.*

How do I take part in the study?

If you would like to take part, please contact Peter Angell (contact details listed above) for further details.

What if I wish to complain about the way in which this study has been conducted?

In the first instance if you have any complaints or concerns please contact Peter Angell (contact details listed above). Alternatively, you can contact Keith George, Professor of Exercise & Cardiovascular Physiology on 0151 231 4088 or email k.george@ljmu.ac.uk.

Cardiac Magnetic Resonance Imaging in weight/strength trainers

Participant Information Sheet

Title of study

Health profiles of weight/strength trainers

Study location:

Royal Brompton Hospital

Sydney Street

London

SW3 6NP

Study co-ordinator:

Mr Peter Angell

School of Sport and Exercise Sciences

Liverpool John Moores University

15-21 Webster Street

Liverpool

L3 2ET

Telephone: 0796 842 2497

Email: p.angell@2004.ljmu.ac.uk

Please contact Peter Angell (details above) if there is anything you do not understand or if you would like more information. Take time to decide whether or not you wish to take part.

What is the study about?

We require weight/strength trainers who are using, or have never used, anabolic steroids. Some of the key areas we will be looking at are the differences between body composition and the structure and function of the heart and blood vessels. The results of this study will allow us to better understand the effects of anabolic steroids on the body, and develop health care and related services for anabolic steroid users.

What does the study involve?

We would like you to attend an assessment at The Royal Brompton Hospital ,Chelsea, on one occasion. The procedures that will be performed on this visit are outlined below, and, during your first visit you will be fully familiarised with these procedures and given the opportunity to ask any questions that you may have.

Cardiac MRI: Magnetic resonance imaging (MRI) is a non-invasive medical test that helps physicians diagnose and treat medical conditions.

MR imaging uses a powerful magnetic field, radio frequency pulses and a computer to produce detailed pictures of organs, soft tissues, bone and virtually all other internal body structures. The images can then be examined on a computer monitor, printed or copied to CD. MRI does not use ionizing radiation (x-rays).

Detailed MR images allow physicians to better evaluate various parts of the body and certain diseases that may not be assessed adequately with other imaging methods such as x-ray, ultrasound or computed tomography (also called CT or CAT scanning).

Cardiac MRI (cMRI) is based on the same basic principles of MRI but with the visual optimisation for use in the cardiovascular system. Using these techniques allows us to assess the structure and function of the heart and its valves, major vessels and its surrounding structure. It also allows for diagnosis of cardiovascular problems through assessment of the thickness and size of heart walls and chambers.

Prior to the examination, cannular lines will be inserted in to veins in your arms. This allows infusion of Adenosine and Gadolinium. Adenosine will be infused during part of the MRI examination in order to measure how your heart reacts to a period of stress. The Gadolinium is used as a contrast agent to measure the rate of perfusion within the heart, which is used as a gauge of the health the myocardiam.

Each examination is usually completed in around 60 minutes.

Questionnaires: You will be asked to complete a number of questionnaires on your health, lifestyle and training. We would also like to discuss your views and perceptions on topics such as your health, lifestyle and training.

Body composition: A procedure known as bio-electrical impedance will be used to assess the amount and distribution of your body fat and lean tissue. The procedure is non-invasive and takes approximately 5 minutes. It involves standing on a small machine, similar to a set

of scales and holding 2 points of contact with your hands. Prior to bio-electrical impedance we will measure your height and weight.

Blood sampling: Blood samples will be collected from a vein in your arm. A small amount (25ml) of blood will be taken. This amount is negligible and will not cause any adverse effects. Your blood will be used to measure a number of different health markers — including lipids, glucose, hormones and clotting factors. A full list of tests that will be performed on your blood is available upon request.

Blood pressure: Automated blood pressure measures will be taken alternately on your left and right arm whilst at rest (either in a seated position or lying on your back on a bed). All measurements will be taken twice. Although this procedure is non-invasive, it may cause some mild discomfort in your arm, which will subside when the cuff pressure is released.

Electrocardiography (ECG): An ECG will be performed where 10 electrodes are placed on your chest wall that will record the electrical activity of your heart. During this examination you will only be required to lie on your back on a bed in a resting state.

Echocardiography: An echocardiographic examination of the heart will be performed. This uses ultrasound technology to enable a non-invasive and painless assessment of heart structure and function. During this examination you will only be required to lie on your back on a bed in a resting state.

Are side-effects associated with any of the procedures?

The procedures we will be using are all well-established techniques which have been used in numerous research studies (including with athletes).

How long will it take?

Your visit to the Royal Brompton Hospital will take about 3-4 hours,

What will I gain from taking part?

You will have information on your body fat and lean tissue (muscle) mass. From the electrocardiography, echocardiography, and cardiac MRI you will find out about the structure and function of your heart and major blood vessels leaving the heart (following the data analysis, a report will be provided on this). You will also help inform the development of health care and related services for steroid users.

Will my data be kept confidential?

All the information collected about you during the course of the study will be kept strictly confidential. Any identifying information about you will not be disclosed to anyone outside of the research group. You do not have to give us your full name. At the end of the study we may ask your permission to give you a letter to take to your GP (General Practitioner) so that they can discuss the results in more detail with you if they are important to your health.

However, we will only provide you with a letter after you have given us permission. If you do not have a GP, we will provide you with details of where you can register with one.

It is expected that the results of this study will be published in a scientific journal and to inform health policy, but no identifying reference will be made to those individuals who took part. Liverpool John Moores University is registered under the Data Protection Act and has an Information Security Policy to safeguard the collection, processing and storage of confidential data.

However, should you suggest, imply or state that you are involved in specific serious criminal activities (i.e. acts of terrorism, offences against children) then we will inform the necessary authorities.

What else do I need to know?

- You must be aged 18 or over to participate in this study and have never taken anabolic steroids (or related drugs).
- If you agree to participate it is essential that you do not smoke, or eat any food or caffeine* for 12 hours before an assessment session. Restraint from exercise and alcohol for the 24 hours prior to any assessment sessions is also mandatory.
- A qualified practitioner will take your blood sample. Blood will only be used for this study, and, following analysis, any that remains will be disposed of.
- Please note that, unfortunately, this study will not be able to provide you with the results of the: 1. questionnaires/discussions.
- We will provide you with a list of health services that are available to you upon request.
- If you are interested in receiving the final results of this study then please get in contact with Peter Angell. However, please bear in mind that data such as these often take many months to analyse and prepare for publication.
- ***You have the right to withdraw from the study at any time without giving a reason, and without prejudice to access of services that are already being provided or may subsequently be provided to you. However, completion of all the procedures will greatly assist this research.***

** Please note that caffeine is found in many common food and drinks such as chocolate, tea, coffee, soft drinks (such as cola and energy drinks), over-the-counter medications (such as pain-relief products) and some nutritional supplements.*

How do I take part in the study?

If you would like to take part, please contact Peter Angell (contact details listed above) for further details.

What if I wish to complain about the way in which this study has been conducted?

In the first instance if you have any complaints or concerns please contact Peter Angell (contact details listed above). Alternatively, you can contact Keith George, Professor of Exercise & Cardiovascular Physiology on 0151 231 4088 or email k.george@ljmu.ac.uk.

LIVERPOOL JOHN MOORES UNIVERSITY

PARTICIPANT INFORMATION SHEET

Cardiovascular Responses to Resistance Exercise

Principal Investigator: Peter Angell, Research Institute for Sport and Exercise Sciences

You are being invited to take part in a research study about how the cardiovascular system responds to strenuous intermittent exercise. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part or not.

1. What is the purpose of the study?

The purpose of the study is to assess the effects of an acute bout of resistance exercise on the heart's electrical activity (ECG), heart structure and function (echocardiogram scan), arterial blood flow (vascular scan of the leg), brain blood flow, blood pressure, and the appearance of cardiac biomarkers in the blood. Research has suggested that resistance exercise can result in transient alterations to blood pressure, the function of the heart as well as the release into the circulation of biomarkers of cardiac damage. It has also been suggested that this response it exaggerated in anabolic steroid (AS) use. These issues require further exploration in resistance trained athletes who do and do not use AS. This data should inform athletes, coaches, scientists and medics by providing in-depth feedback regarding cardiovascular structure and function following resistance exercise.

2. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do you will be given this information sheet and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw will not affect your rights or any future service you receive.

3. What will happen to me if I take part?

Before performing the testing session, you will be required to attend a screening/familiarisation session to check your health and suitability to take part in the study as well as to attain your 1 rep maximums (1RM's).

The study will involve you participating in some tests before and after a high intensity, full body, series of resistance exercises. This exercise session will include Squats, Bench Press, Bench Pulls, Overhead Press and Upright Rows for 3 sets of 5 reps at 80% of 1RM, with a recovery period of 1 ½/2 minutes between sets. We will perform all the tests at the LJMU CETL building. Prior to all the testing sessions you will be required to undertake 6 hour fast , refrain from caffeine, chocolate and alcohol for 8 hrs and strenuous physical activity for 24 hrs.

We will perform a number of measurements (described below) prior to your bout of exercise and then again at 10 and 60 minutes following the exercise session.

Testing session 1, before and at 10 and 60 minutes following the exercise programme:

a) We will ask you to complete a health and training questionnaire which will take c.5 min.

b) We will measure your body weight and height using electronic scales and a stadiometer. We will ask you to remove all outer clothing and shoes and will take c. 2 min.

c) We will take a recording of the electrical activity of your heart by placing 10 adhesive electrodes on the surface of your skin in the chest region. This is non-invasive, painless and will take c. 5 min whilst you are lying down.

d) We will take pictures of the structure and function of your heart using an ultrasound scanning machine. This uses 4 adhesive electrodes, a transducer placed on your chest wall with some gel. This is non-invasive, painless and will take c. 15 min whilst you are lying down.

e) We will measure the blood flow in your femoral and carotid artery using an ultrasound scanning machine. This is non-invasive, painless and will take c.10 minutes.

f) We will also measure brain blood flow in your cerebral artery using an ultrasound scanning machine. The probe will be fixed in position using a headframe. This is non-invasive, painless and will take c. 5 min.

g) We will measure your blood pressure by placing a clip over your middle finger. This is non-invasive, painless and will take c. 2 min whilst you are lying down.

h) We will take a small (5 ml) blood sample from a vein (near your elbow) in your arm. This will be taken whilst you are seated by a trained technician or doctor.

The second testing session will comprise of a single set of high intensity resistance exercise on the leg press machine, where you will be asked to perform 5 repetitions at 90% of your 1RM. During this exercise bout, ultrasound images will be continuously taken of your heart and of you femoral (upper leg) and carotid (neck) arteries. Your brain blood flow and blood pressure will also be measured throughout the exercise set. These parameters will then be measured 5 minutes following completion of the exercise bout.

Testing session 2:

a) We will measure your body weight and height on electronic scales and stadiometer. We will ask to remove all outer clothing and shoes and will take c. 2 min.

b) We will take a recording of the electrical activity of your heart before and 5 minutes post exercise, by placing 10 adhesive electrodes on the surface of your skin in the chest region. This is non-invasive, painless and will take c. 5 min whilst you are lying down.

c) We will take pictures of the structure and function of your heart using an ultrasound scanning machine. This uses 4 adhesive electrodes, a transducer placed on your chest wall with some gel. This is non-invasive, painless and will be performed continuously throughout the exercise bout and for 5 minutes after.

d) We will measure the blood flow in your femoral and carotid artery using an ultrasound scanning machine. This is non-invasive, painless and will be performed continuously throughout the exercise bout and for 5 minutes after.

e) We will also measure brain blood flow in your cerebral artery using an ultrasound scanning machine. The probe will be fixed in position using a headframe. This is non-invasive, painless and will be measured continuously throughout the exercise bout and for 5 minutes after.

f) We will measure your blood pressure throughout the exercise bout using a portapres which clips on to the end of your finger. This is non-invasive, painless and will be performed continuously throughout the exercise bout and for 5 minutes after.

4. Are there any risks / benefits involved?

There is a small risk of infection due to a needle being used to take the blood sample from your arm. This may also result in bruising at the site of needle insertion. Your sporting activity may result in a level of muscle damage/soreness if you are unaccustomed to the intensity and/or duration of the exercise. This can last between 24 and 72 hours.

The benefit of being involved is that you will have a thorough assessment of cardiac electrical activity, structure and function provided by expert clinicians and scientists.

5. Will my taking part in the study be kept confidential?

All of the data collected will be treated as confidential. When stored data will be anonymised so that your identity will not be revealed. All data will be stored on a password protected computer system.

Contact Details of Researcher

If you have any questions relating to any of the techniques used during the research study please feel free to contact me to discuss this further. Participation in this research study is voluntary and you are free to withdraw at any time without prior explanation.

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Note: A copy of the participant information sheet should be retained by the participant with a copy of the signed consent form.

Data Collection Sheet

Data Sheet

Name:.....

Subject Code:.....

Date of Birth: .../.../.....

Contact Details: Telephone Number:.....

e-mail:.....

Height:.....cm

Weight:.....kg

BP: 1.....

2.....

3.....

Training:

1. How many times do you train, on average, per week?

2. How long, on average, would each training session take?
   If large variations between sessions, please give details:

3. What is your one repetition maximum for the bench press and squat?
   (please state whether weights are in kg's or lbs)
   Bench Press: Squat:

History (Delete as appropriate):

Have you recently (within last 3 months), or are you currently using anabolic steroids or other performance-enhancing drugs? Yes/No

If Yes, please give details of types, quantities used, duration of usage and planned total length of usage.

Have you ever used anabolic steroids or other performance-enhancing drugs? Yes/No

If Yes, please give details of types, quantities used and duration of usage.