Cardiovascular Health in Adolescent Females with Anorexia Nervosa: Echocardiographic Abnormalities, Biophysical Properties of the Aorta, and Ventricular Function during Exercise

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ABSTRACT

Anorexia nervosa (AN) is a common disorder affecting the adolescent female population. In order to investigate the structure and function of the cardiovascular system in adolescents with AN, we performed case-control studies using echocardiography at rest to assess cardiac dimensions, systolic ventricular function, diastolic ventricular function, and vascular stiffness as well as echocardiography during exercise to investigate cardiac responses to hemodynamic stress. We found that female adolescents with AN have decreased left ventricular (LV) dimensions, LV wall thickness, LV mass, and abnormalities of diastolic ventricular function when compared to controls. Our patients demonstrated increased pulse wave velocity of the aorta, indicating increased aortic stiffness and potential increased future cardiovascular risk. During exercise, patients with AN demonstrated normal patterns of cardiovascular response with progressive exercise, although cardiac responses at peak exercise were decreased compared to controls. These findings demonstrate abnormalities in cardiac and vascular structure and function in adolescent females with AN.

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List of Abbreviations

2D – Two dimensional

β-index - Arterial wall stiffness index

σPS – Meridonal wall stress at peak systole

AN – Anorexia nervosa

AN-AR – Anorexia nervosa restrictive subtype

AN-BP – Anorexia nervosa binge/purging subtype

AOCSA - Aortic cross sectional area

AOd – Aortic diameter in diastole

AOflow - Peak aortic flow

AOs – Aortic diameter in systole

ApoA1 – Apolipoprotein A1

ApoB – Apolipoprotein B

avO_2 – Arteriovenous oxygen difference

BMI – Body mass index

BP – Blood pressure

bpm – Beats per minute

BSA – Body surface area

CHF – Congestive heart failure

CI – Cardiac index

cm – Centimeter

CO – Cardiac output

CO_2 – Carbon dioxide

CSA – Cross sectional area

CV - Cardiovascular

dBP – Diastolic blood pressure

DSM-IV - Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

DT – Deceleration time

ECG - Electrocardiogram

EF – Ejection fraction

Ep - Elastic pressure-strain modulus

ET – Ejection time

ETc – Ejection time corrected for heart rate

fb – Breathing frequency

FS – Fractional shortening

HDL – High density lipoprotein

hsCRP – High sensitivity C-reactive protein

IL-6 – Interleukin 6

IVRT – Isovolumic relaxation time

IVS – Interventricular septum

IVSD – Interventricular septum diameter

kg – Kilogram

L – Liter

LA – Left atria/atrial

LDL – Low density lipoprotein

LV – Left ventricle/ventricular

LVEDD – Left ventricle end-diastolic diameter

LVESD – Left ventricle end-systolic diameter

LVM – Left ventricular mass

LVMI – Left ventricular mass indexed

LVOT – Left ventricular outflow tract

LVOTd – Left ventricular outflow tract diameter

LVPWD – Left ventricular posterior wall diameter

LVPWDd – Left ventricular posterior wall diameter in diastole

LVPWDs – Left ventricular posterior wall diameter in systole

m – Meter

MHz – Megahertz

MI – Myocardial infarction

min – Minute

mL – Milliliter

mmHg – Millimeters of mercury

MPI – Myocardial performance index

MRI – Magnetic resonance imaging

MV – Mitral valve

MVCFc - Mean velocity of circumferential fiber shortening

O_2 – Oxygen

PAoV – Peak aortic velocity

PP – Pulse pressure

PV – Pulmonary venous

PWV - Pulse wave velocity

RER – Respiratory exchange ratio

RV- Right ventricle/ventricular

s – Second

sBP- Systolic blood pressure

SPSS – Statistical Package of the Social Sciences

SSCE – Semi-supine cycle ergometry

SV – Stroke volume

SVI – Stroke volume index

SVR – Systemic vascular resistance

SVRI – Systemic vascular resistance indexed

T_1 - Time from onset of QRS to onset of ascending aortic Doppler envelope

T_2 - Time from onset of QRS to onset of descending aortic Doppler envelope

TAC – Total arterial compliance

TAC – Total arterial compliance indexed

TDI – Tissue Doppler imaging

TG - Triglycerides

TT – Transit time

VCO_2 – Carbon dioxide production

VE – Minute ventilation

VE/VCO_2 – Ventilatory equivalent for carbon dioxide

VE/O_2 – Ventilatory equivalent for oxygen

VO_2 – Maximal oxygen uptake

VT – Tidal volume

VTI - Velocity-time integral

W – Watts

Zc - Characteristic impedance

Zi - Input impedance

List of Appendices

Chapter 1: Introduction, Overview, and Literature Review

Introduction

Anorexia nervosa (AN) is an eating disorder characterized by a restriction of energy intake, low body weight, fear of gaining weight, and a distorted body image [1]. AN has an approximate lifetime prevalence between 1-4% and carries the highest mortality rate of any other psychiatric disorder [2]. This disorder is most commonly diagnosed in females with a peak incidence between 15-19 years [2]. The diagnosis is based on meeting the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, text revision (DSM-IV-TR) which includes:

1. Refusal to maintain body weight at or above a minimally normal weight for age and height: Weight loss leading to maintenance of body weight <85% of that expected or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected.
2. Intense fear of gaining weight or becoming fat, even though under weight.
3. Disturbance in the way one's body weight or shape are experienced, undue influence of body weight or shape on self evaluation, or denial of the seriousness of the current low body weight.
4. Amenorrhea (at least three consecutive cycles) in postmenarchal girls and women. Amenorrhea is defined as periods occurring only following hormone (e.g., estrogen) administration [3].

AN has effects on multiple organ systems secondary to the patient being in a state of starvation. Cardiac involvement is among the most common complications of AN and affects almost 80% of patients [4]. Electrophysiologic abnormalities seen in AN include prolongation of the QT interval and increased QT dispersion, bradycardia, first degree heart block, premature ventricular contractions, and other arrhythmias [5-8]. AN has been associated with structural, echocardiographic, and functional cardiac anomalies including decreased cardiac mass, mitral valve prolapse, pericardial effusions, reduced left ventricular (LV) dimensions, and decreased cardiac output (CO) [5, 9-11]. Cardiac complications, particularly abnormalities of the QT interval, contribute to the risk of sudden death in patients with AN [5, 8].

In addition to the risk of sudden death, it has also been suggested in the literature that patients with AN may be predisposed to future cardiovascular (CV) risk [12-14]. Arterial stiffness is being increasingly recognized as a marker of risk for future CV events, including stroke and myocardial infarction (MI), in both healthy and diseased populations [15-17]. Pulse wave velocity (PWV) is a validated measure of arterial stiffness and is defined as the speed of travel of the pressure pulse along an arterial segment [17, 18]. Increased arterial stiffness creates a faster propagation of the pressure pulse which causes PWV to increase. PWV is an independent, early, and sensitive predictor of CV risk and can be calculated using Doppler echocardiography [15-17, 19, 20]. Arterial stiffness as a marker for vascular health and CV risk in AN has not been previously studied.

Despite seemingly normal cardiac function in the resting state, physiological stresses can unmask cardiac dysfunction. One common method of unmasking cardiac

pathology is to assess patients during the hemodynamic stress of cardiopulmonary exercise. A few studies in patients with AN have suggested possible subclinical cardiac dysfunction, however, further investigation in order to determine if exercise can unmask echocardiographic ventricular dysfunction has not been previously performed in this population [21, 22]. Although patients with AN have been shown to have decreased exercise endurance, decreased maximal oxygen uptake (VO_2), and blunted heart rate (HR) and blood pressure (BP) response to exercise, assessment of cardiac responses to incremental exercise is limited [23-26].

Purpose of the Study:

The purpose of our study was to assess the cardiovascular system and its function in adolescent females with AN. We began by performing a complete echocardiographic study at rest in patients and controls in order to characterize the echocardiographic parameters of left atrial (LA) and LV cardiac dimensions, systolic ventricular function, and diastolic ventricular function at rest in adolescent females with AN. To measure their vascular health and the potential for future CV risk, we characterized the biophysical properties of the aorta, including the PWV, using Doppler echocardiography. Finally, in order to try to unmask subtle changes in cardiac function in adolescent females with AN, we assessed their exercise capacity and echocardiographic ventricular function during exercise, as this represents a state of hemodynamic stress. The goal of our research was to comprehensively assess the cardiovascular health of this patient population.

Thesis Presentation Format:

The following thesis will be presented in manuscript format. It consists of three manuscripts which will divide the content into echocardiographic abnormalities at rest, the biophysical properties of the aorta, and exercise capacity and echocardiographic ventricular function during exercise in adolescent females with AN.

Literature Review:

Structural, functional, and echocardiographic abnormalities in AN

Studies regarding cardiac abnormalities in AN have looked at several structural, functional, and echocardiographic abnormalities in young women with this disorder. These studies are described below. For ease of interpretation, the cardiac abnormalities described in the studies below are summarized in Appendix 1.

St John Sutton *et al*. (1985) studied 17 females with AN (mean age of 26) and compared them to 10 normal weight controls [26]. They showed a decreased systolic blood pressure (sBP) and diastolic blood pressure (dBP) in the AN group as compared to controls, but no difference in resting HR [26]. LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), LV posterior wall diameter (LVPWD), end-diastolic and end-systolic volume indexes, LV mass (LVM), and LV mass indexed (LVMI) were decreased in the AN group as compared to controls [26]. There were no changes in the LV chamber shape, the end-diastolic relative wall thickness, or the LV mass-to-volume ratio, indicating a normal LV architecture [26]. End-systolic meridonal and circumferential wall stresses, fractional shortening (FS), ejection fraction (EF), and velocity of circumferential fiber shortening (MVCFc) showed no difference between groups, indicating normal LV afterload and systolic ventricular function at rest [26].

De Simone *et al.* (1994) studied 21 white women with AN with a mean age of 22 years [9]. The control groups consisted of 19 normal weight women (BMI 20-27.3) and 22 constitutionally thin women (BMI <20) [9]. The constitutionally thin control group did not meet criteria for AN and had normal scores on a food questionnaire [9]. When compared to normal weight and constitutionally thin women, the AN group were found to have lower HR, sBP, and dBP [9]. The incidence of mitral valve motion abnormalities was higher in women with AN, but this was not functionally significant as there was no important mitral regurgitation in any of the groups [9]. LVEDD and LVM were smaller in the AN group than controls and these differences persisted after indexing for height and controlling for body weight, height, BP, and HR [9]. The authors believed that LV mass was decreased due to both a reduction in afterload and preload of the LV [9]. LV relative wall thickness was elevated in the AN group after controlling for height and weight [9]. There were no changes in end-systolic stress or FS, although the percent predicted FS was decreased in AN patients, which the authors felt may indicate mild LV dysfunction [9]. CO and cardiac index (CI) were lower in AN patients [9]. Systemic vascular resistance (SVR) was increased in AN. Women with AN had a smaller LA diameter [9]. There were no differences between groups in the transmitral peak E wave velocity (which represents early diastolic LV filling), however, the AN group had a significantly smaller peak A wave velocity (which represents the contribution of atrial contraction to LV filling in late diastole) and increased E/A ratio [9]. These abnormalities were reported to indicate impaired LV filling [9].

Romano *et al.* (2004) studied 91 young females with AN with a mean age of 20.5 years compared to 62 normal weight controls [11]. Their results were very similar to

those of de Simone *et al*. (1994), including females with AN having statistically significant decreases in HR, LVEDD, LVM, LVMI, stroke volume (SV), and CO as well as increased SVR [9, 11]. Their results differed from those of de Simone *et al.* (1994) in terms of cardiac contractility as they found a statistically significant decrease in EF in women with AN as compared to controls [9, 11]. Additionally, Romano *et al.* (2004) found that stroke work and the percent predicted LVM were significantly decreased in the AN group [11]. Their results showed that a greater percentage of subjects in the AN group (19 vs 5%) had an inadequate LVM when compared to the control group [11]. The authors concluded that AN is a condition of low hemodynamic load giving cardiac hypotrophy [11].

Vazquez *et al*. (2003) studied 30 adolescent females with AN and compared them to 30 age matched controls of normal weight [4]. The authors found that the AN group had decreased HR and LV dimensions including decreased LVEDD, LVESD, interventricular septum diameter (IVSD), LVM, and LVMI [4]. There were no differences in LVPWD or in LV function as measured by FS and EF [4].

Eidem *et al.* (2001) retrospectively studied 13 adolescents with AN and used 13 normal weight pediatric controls for comparison [21]. They investigated the use of the myocardial performance index (MPI), which is a ratio of isovolumic contraction time and isovolumic relaxation time (IVRT) to ejection time. The MPI is used as a measure of global ventricular performance including both systolic and diastolic function [21]. Similar to the previous studies, LVM was found to be decreased. Similar to de Simone *et al.* (1994) and Vazquez *et al*. (2003), there was no difference in clinical systolic ventricular function [4, 9, 21]. No difference between groups was seen in diastolic function using the

usual measurements of mitral E/A ratio and deceleration time, which is in contrast to the study by de Simone *et al*. (1994) in which the AN group had an elevated E/A ratio [9, 21]. Despite not finding differences between groups in the variables commonly used clinically to assess systolic and diastolic function, the AN group has a significantly elevated LV MPI as compared to controls, indicating worsened ventricular function [21]. There was no difference in the right ventricular (RV) MPI [21]. This study concluded that the increase in MPI suggests subclinical LV dysfunction in their population of adolescent females with AN [21].

Galetta *et al*. (2003) studied 25 females with AN compared to 25 age matched controls of normal weight (BMI >20 kg/m^2) and 25 thin females (BMI <20 kg/m^2) without AN [27]. Their results agreed with the above studies in showing that the AN group had a decreased HR, BP, LVEDD, LVESD, LVM, LVMI, FS, EF, and mitral valve peak A velocity with an increased mitral valve E/A ratio and no difference in the mitral valve peak E velocity [27]. The findings for the mitral valve peak E velocity, peak A velocity, and E/A ratio indicated abnormal LV diastolic filling and were comparable to those reported by de Simone *et al.* (1994) [9, 27]. They additionally investigated the IVRT, which is also an indicator of diastolic function, and found no differences between groups [27]. They demonstrated no difference in IVSD, in contrast to Vazquez *et al.* (2003), and no difference in LVPWD [4, 27].

In a later study, Galetta *et al*. (2005) investigated 20 young females with AN compared to 20 thin and 20 normal weight controls as in their previous study [22]. They showed the same differences between the AN and control groups as seen in their previous study, with the exception of showing a decrease in the IVSD and LVPWD in the AN

group which was not demonstrated in the previous study [22]. They additionally studied tissue Doppler imaging in AN as an early marker of cardiac dysfunction by looking at the myocardial systolic wave (S'), the early diastolic wave (E'), and the diastolic wave during atrial contraction (A') [22]. The AN group had a lower S' peak in the lateral LV wall and interventricular septum (IVS), indicating impaired systolic LV function [22]. The peak S' correlated with LVM [22]. There was no difference in the peak E', A', or E'/A' ratio, indicating that the LV diastolic function, as measured by tissue Doppler, was not impaired [22].

The largest study of echocardiographic abnormalities in adolescent females with AN was performed by Kastner *et al.* (2012), although they investigated fewer echocardiographic parameters than the majority of previous studies [28]. They studied 173 adolescent females with AN compared to 41 normal weight controls [28]. Similar to the previous studies, they showed decreased LVEDD and LVESD in the AN group and no difference in FS, IVSD, or LVPWD [28]. They also showed an increased incidence of pericardial effusions in the AN group (34.7% vs 0%) although these were all small to moderate in size and were clinically silent [28]. There were no risk factors to predict the occurrence of an effusion in the AN group [28].

A cross-sectional study by DiVasta *et al*. (2010) compared 38 female adolescents and young women who were admitted for medical complications of AN to published normal values [29]. They found that approximately 1/3 of patients with AN had a lower than predicted LVMI, LVPWD, and IVSD [29]. LV end-systolic stress was lower than average in 70% of subjects and MVCFc was better than predicted. The EF and FS for the AN subjects was within normal limits [29].

Several studies have also demonstrated reversibility of echocardiographic changes with weight restoration in AN. St John Sutton *et al.* (1985) found increases in LVMI and LV end-diastolic volume indexed after weight gain, but no change in FS, EF, and wall stress [26]. Mont *et al.* (2003) looked at 31 patients with AN (80% female) and showed a significant increase in HR, LVEDD, LVESD, LA size, LVM, LVMI, CO, CI, transmitral A wave velocity, and transmitral E wave velocity with weight restoration [30]. There were no significant changes with weight restoration in IVSD, LVPWD, EF, mitral deceleration time, E/A ratio, or IVRT [30]. Olivares *et al*. (2005) studied 40 adolescent females with AN compared to 40 normal weight controls [10]. They found improvements in HR, LVEDD, LVESD, LVM, LVMI, and CO with weight restoration [10]. There were no changes in LVPWD, IVSD, FS, or EF [10]. Mitral valve prolapse was found in 8 patients in the AN group (of no hemodynamic significance) and persisted in 3 after weight restoration [10]. Ulger *et al*. (2006) investigated 11 adolescent females with AN and showed that 1 year after refeeding, there was recovery of HR, sBP, dBP, LVEDD, IVSD, LVPWD, LVM, and LVMI [31]. Kastner *et al.* (2012) demonstrated an improvement in LVESD with weight restoration, however there was no improvement in LVEDD as was demonstrated in prior studies [28]. Kastner *et al.* (2012) also showed no change in IVSD, LVPWD, or FS [28]. DiVasta *et al*. (2010) had follow-up echocardiographic data on 17 subjects with AN and showed decreased IVSD, decreased MVCFc, and no change in absolute LVM with weight restoration, which is in contrast to previous studies [29]. Overall, the majority of studies show that the many of functional and echocardiographic abnormalities in patients with AN are reversible with weight restoration.

In summary, echocardiographic structural and functional cardiac abnormalities including mitral valve prolapse, pericardial effusions, reduced LV dimensions, decreased cardiac mass, and decreased CO have been well documented in the literature. The majority of studies suggest that systolic ventricular function as assessed by the conventional echocardiographic measures of EF and FS is normal in patients with AN, although studies using tissue Doppler and the MPI suggest that subclinical cardiac dysfunction may be present in this patient population. There is disagreement on the presence of diastolic ventricular function in AN as transmitral velocities have demonstrated abnormalities, while other measures of diastolic function have not shown differences. Many of the demonstrated cardiac abnormalities in AN have been shown to be at least partially reversible with nutritional rehabilitation. The majority of studies performed have been in adult populations and have investigated small numbers of patients.

Future Cardiovascular Risk in AN

Although women with AN are at increased risk of sudden death related to suicide and arrhythmias, they may also be at risk of atherosclerotic CV disease with increasing age [13, 14]. Several studies have documented the presence of biomarkers which are associated with increased future CV risk in females with AN. However, whether or not these risk factors which are associated with MI and stroke in other populations also confer increased risk in AN is unknown. One such risk factor is dyslipoproteinemia, which is a known risk factor in other populations for future CV events including MI and stroke. Elevation in total serum cholesterol, low-density lipoprotein (LDL), and triglycerides

(TG) as well as a decrease in high density lipoprotein (HDL) are associated with an increase in CV risk. Apolipoprotein B (ApoB) is a structural protein of LDL, with high levels of ApoB indicating increased CV risk [32]. Apolipoprotein A1 (ApoA1) is a structural protein of HDL, with high ApoA1 being protective of CV events [32]. Other reported risk factors for CV disease are inflammatory markers including elevated levels of high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) [33]. The following paragraphs will summarize the current literature on biomarkers associated with CV risk in the presence of AN.

The presence of elevated cholesterol levels in patients with AN was first documented by Klinefelter in 1965 [34, 35]. Since that time, multiple recent studies have confirmed the finding of hypercholesterolemia, with more variable results for LDL and HDL levels. Case *et al*. (1999) examined 9 young adult females with AN of the binge-purge subtype and found elevated total cholesterol, LDL, apoB, apoA1, triglycerides (TG), and intermediate-density lipoprotein-apoB levels compared to 10 controls [36]. Boland *et al*. (2001) studied 101 females with AN and found increased total and LDL cholesterol levels with no difference in HDL or TG as compared to 95 age matched controls [37]. Zak *et al*. (2005) compared 16 women with AN to 25 healthy controls and documented increased levels of total cholesterol, TG, and HDL [38]. Ohwada and colleagues (2006) found elevated total cholesterol, LDL, HDL, ApoA1, and ApoB in 39 women with AN as compared to 24 controls [35]. Rigaud *et al.* (2009) studied 120 adult women with AN compared to 120 healthy women and found elevated total cholesterol (which was higher in the AN binge-purge subtype than in the restrictive AN subtype), LDL, HDL, ApoA1, ApoB, and TG [39]. Matzkin and colleagues found in 2 separate

studies that total cholesterol and LDL were elevated in women with AN [40, 41]. Nova *et al.* (2007) studied 14 women with AN and found elevated total cholesterol and LDL, decreased HDL, and no difference in TG [42]. There remains, however, some controversy in the literature with some reports of normal cholesterol levels in AN. Arden *et al*. (1990) studied 16 females with AN compared to 16 athletic normal weight controls, and found no difference in cholesterol values or LDL at diagnosis, but the authors acknowledge that the study may have been underpowered to detect a difference in cholesterol values between groups [43].

Fewer studies have been performed investigating inflammatory and ultrasound markers associated with CV risk in AN. Lawson *et al.* (2007) studied 181 women with AN compared to 41 healthy controls [12]. Women with AN had lower hsCRP levels than healthy controls, however, 20% of women with AN and on oral contraceptive pills had high-risk hsCRP levels, which may place this subset of women at increased CV risk [12]. Birmingham *et al.* (2003) investigated the intima-medial thickness of the carotid artery using ultrasound, which is a surrogate measure of coronary atherosclerosis, in 18 women with AN compared to an unspecified number of age-matched controls [44]. There was no difference in the intima-medial thickness of the carotid artery between the groups [44].

The majority of the studies listed above have been performed on adult women with AN. There are few reports on biomarkers associated with future CV risk in adolescents with AN. Misra *et al.* (2006) studied 23 adolescent girls with AN and compared them to 20 healthy adolescent female controls [13]. Girls with AN had lower TG, increased HDL, and unchanged total and LDL cholesterol levels as compared to controls [13]. They also found increased Apo-B and IL-6 levels but decreased hsCRP

[13]. The only change in these biomarkers with weight gain was a decrease in the IL-6 levels [13]. These authors reported an uncoupling of CV risk factors in this adolescent cohort, with the decreased hsCRP, low TG, and elevated HDL levels suggesting decreased CV risk whereas the increased IL-6, ApoB, and ratios of ApoB/HDL and ApoB/LDL indicate increased CV risk [13].

Although the actual risk of MI and the contribution of the above mentioned risk factors in identifying women with AN with risk of future CV events is unknown, there are reports of MI in young women with AN. Garcia-Rubira *et al.* (1994) reported a case of a 39 year old woman with AN since the age of 17 who developed an inferior MI during hyperalimentation therapy [45]. Abuzeid and Glover (2011) reported a case of a 39 year old woman with AN since the age of 13 who presented with an acute anterior wall MI with 100% occlusion of the proximal left anterior descending coronary artery [46]. Rigaud *et al.* (2012) reported 2 cases of MI in their study of 41 severely malnourished AN patients [47]. The clinical characteristics of these 2 cases were not described; however, the oldest that these women could have been was 45 years as this was the maximum age of the cohort [47]. Rigaud *et al.* (2009) also reported 2 cases of MI in women with AN aged 37 and 41 years, although it is unclear if these are the same patients reported in the Rigaud *et al.* (2012) study [39, 47]. The incidence of MI in patients with AN is unknown and it is likely that fatal cases of MI are likely under-recognized in the setting of AN. Although studies specifically regarding atherosclerotic risk in AN have not been performed, these cases of premature MI in women with AN raise the possibility that AN may be associated with increased atherosclerotic CV risk.

The majority of published studies demonstrate an improvement in dyslipidemia with weight restoration. Ohwada *et al*. (2006) showed decreases or normalization in total cholesterol, TG, HDL, and LDL with weight gain [35]. Rigaud and colleagues (2009) demonstrated that 95% of the AN patients who achieved a normal BMI had normalization of all lipoprotein levels [39]. Jauregui-Garrido *et al.* (2012) studied 102 women with AN and showed a decrease in total cholesterol, LDL, TG, LDL/HDL ratio, and total cholesterol/HDL and an increase in HDL with weight restoration [48]. Some studies, however, have shown persistence of dyslipoproteinemia despite weight restoration. Arden *et al.* (1990) demonstrated that HDL and ApoA1 increased with weight restoration, however, ApoB remained elevated [43]. Nova *et al* (2007) showed no difference in total cholesterol, LDL, or TG with weight gain, although there was an increase in the HDL level [42].

In summary, females with AN may be at risk for CV disease in the future as indicated by case reports of MI in young women with AN, and the presence of biomarkers associated with CV risk including dyslipidemia and elevated IL-6 and hsCRP. However, there remains controversy in the literature as some studies report an absence of known risk factors for atherosclerotic disease in the AN population, which may be related to these studies being underpowered to detect differences between groups. The effect of weight restoration on these risk factors is unclear as there is also controversy in the literature regarding the effect of nutritional rehabilitation on cholesterol and lipoproteins. As well, there is no literature on the effect of weight restoration in AN on the inflammatory markers associated with CV risk. Further study to determine the risk of atherosclerotic CV disease in patients with AN is necessary.

Arterial stiffness, pulse wave velocity, and future cardiovascular risk

Large arteries are distensible structures which play a critical role in accommodating the SV during ventricular systole and providing adequate distribution of blood flow during diastole [18]. One of the earliest detectable manifestations of adverse structural and functional changes in the vessel wall is arterial stiffness [18]. Increased arterial stiffness is associated with CV risk including coronary artery disease and stroke [15, 18].

The most validated method of noninvasively measuring arterial stiffness is using pulse wave velocity (PWV) [18]. PWV is calculated by measuring the transit time of the pressure pulse at 2 different points in a vascular segment, and dividing the distance travelled by the transit time. PWV is inversely related to vascular compliance, such that a stiffer vessel will result in an elevated PWV. It is an accurate, simple, and reproducible measurement and is considered the gold standard index of arterial stiffness [18, 49-51]. PWV has been shown in many studies to be an independent predictor of coronary artery disease and stroke in healthy adults and in adults with diseases known to be associated with CV risk [18]. Mattace-Raso *et al*. (2006) studied 2835 apparently healthy subjects as part of the Rotterdam Study [15]. They found that the risk of coronary heart disease increased with increasing aortic PWV and that PWV was an independent predictor of coronary heart disease and stroke [15]. PWV has also been associated with increased fatal stroke, all-cause mortality, and CV mortality in hypertensive adults [52, 53]. Mitchell *et al.* (2010) reported that increased PWV in subjects of the Framingham Heart Study was associated with increased risk for a first CV event, which consisted of MI, unstable

angina, heart failure, or stroke [54]. Cruickshank *et al.* (2002) found that in adults with diabetes and impaired glucose tolerance, PWV was an independent predictor of all cause and CV mortality [55].

Pulse wave velocity has also been studied in pediatric populations. Despite their younger age, pediatric patients with disease states thought to be associated with increased arterial stiffness have shown abnormalities in aortic PWV. Sandor *et al.* (2003) demonstrated elevated PWV in children with Marfan syndrome and inflammatory connective tissue disease, with the results in Marfan syndrome being confirmed in a larger study by Bradley *et al.* (2005) [56, 57]. Bradley *et al.* (2010) demonstrated elevated PWV in children aged 8-13 with a history of being born small for gestational age [58]. Harris *et al*. (2012) demonstrated increased PWV in obese children as compared to controls [59]. Al Huzaimi *et al.* (2013) demonstrated increased PWV in children with a history of Kawasaki disease as compared to controls [60]. There have been no studies assessing arterial stiffness or PWV in either adolescents or adult patients with AN as a predictor of potential future CV risk.

In summary, arterial stiffness is one of the earliest manifestations of vascular disease and is associated with increased CV risk. PWV is the most validated method of measuring arterial stiffness and has been shown in many studies to be associated with the risk of CV events. PWV has been assessed in many adult and pediatric populations, however, there is no literature regarding arterial stiffness in patients with AN despite suggestions that these patients may be at increased risk for future CV disease.

Exercise Capacity and Cardiac Function During Exercise in Anorexia Nervosa

Exercise places physiologic stresses on the body and hemodynamic stresses on the heart. As many patients with AN have abnormalities in cardiac size and structure, it is conceivable that maladaptive responses to exercise and the associated hemodynamic stresses may occur in this population. Many adolescent patients with AN exercise excessively during the acute phase of the disorder, with rates as high as 80% being reported in the literature [61]. In addition, adequately dosed exercise may be beneficial for patients with AN, although there remains controversy regarding the role of exercise in AN treatment [62-65]. As such, it is important to determine how the CV system responds to exercise in AN.

Subnormal exercise tolerance, maximal oxygen uptake (VO_2), HR response, and BP response during exercise in patients with AN have been reported in the literature. Fohlin (1977) studied 31 adolescents (19 girls and 12 boys) with AN and found a blunted maximal HR response and decreased VO_2 out for proportion to the decrease in body weight [66]. Fohlin *et al.* (1978) studied 17 female and 11 male adolescent patients with AN and found a blunted peak HR response and decreased VO_2 [67]. Gottdiener *et al.* (1978) studied 11 adult females with AN with a treadmill exercise test and found subnormal BP and HR responses to exercise [23]. Davies *et al.* (1978) studied 15 adolescents (5 boys and 10 girls) with AN and found that VO_2 was decreased 20-40% below normal in the female AN patients, which was greater than could be attributed to their change in body size and composition [68]. St John Sutton *et al.* (1985) performed exercise testing in 17 females with AN and found decreased exercise duration, blunted HR and BP responses, and decreased VO_2 [26]. Roche *et al.* (2004) investigated 14

patients with AN and 8 patients with significant weight loss but not meeting criteria for AN [25]. Exercise testing was performed using a cycle ergometer, which demonstrated chronotropic incompetence in 59% of all subjects, and this group had a lower BMI than patients without chronotropic incompetence [25]. AN patients with a lower BMI had more severe limitation to maximal exercise, lower peak HR, and lower peak VO_2 [25]. They also found that BMI correlated to peak VO_2 and maximal HR [25].

Nudel *et al*. (1984) compared 20 adolescent patients with AN to 15 controls using bicycle exercise testing [24]. The AN patients had decreased HR, sBP, VO_2, plasma norepinephrine, and plasma dopamine levels [24]. Twenty-five percent of the patients developed significant ST depression with exercise, which may represent myocardial dysfunction with exercise [24]. These authors concluded that patients with AN have abnormal working capacity, CV response, and sympathetic responses to exercise [24]. They also theorized that part of the blunted HR and BP response was related to a decreased sympathetic response, as evidenced by lower norepinephrine levels [24]. Lands *et al.* (1992) examined 9 adolescent girls with AN and 10 adolescent controls during progressive and steady-state exercise using a cycle ergometer [69]. Subjects with AN performed less work than expected, with the maximum work achieved correlating with BMI percentile [69]. Cardiac output measured by the indirect (CO_2 rebreathing) Fick method was comparable between AN patients and controls [69]. The normalized CO was achieved in the AN patients by a high HR compensating for a low SV [69]. However, the result of a high HR in AN patients is surprising as the majority of studies suggest a blunted HR response in this group of patients. They concluded that exercise capacity in

AN is limited by diminished muscle mass or muscular dysfunction rather than by a diminished cardiac or ventilatory response [69].

Studies of echocardiographic function during exercise in patients with AN has been limited. Rowland *et al*. (2003) studied 8 adolescent females with AN compared to 24 healthy controls using upright cycle exercise and Doppler measurement of aortic diameter and aortic flow from the suprasternal notch to determine the SV, peak aortic velocity, and CO [70]. Patients with AN had decreased resting and maximal HR [70]. Maximal relative VO_2 was lower in patients than controls, indicating decreased aerobic fitness [70]. They demonstrated increased SV index in patients compared to controls, with a normal pattern of increase in SV index with progressive exercise [70]. There was no difference in maximal CI between groups, as the increased SV compensated for the decreased HR in AN patients, which is opposite to what was found Lands *et al.* (1992) [69, 70]. There were no dysrhythmias during testing, although one patient demonstrated "scooping" of the ST segment with exercise [70]. They concluded that aerobic fitness was lower in patients with AN but there was no evidence of abnormal myocardial performance since SV, peak aortic velocity, and rate of acceleration of blood flow continued to increase with progressive exercise [70]. Of note, the control subjects were younger than the patients in this study, which may have affected the results the study.

The effects of weight restoration on exercise performance in patients with AN has also been investigated. Fohlin (1978) studied 3 male and 5 female adolescents with AN after refeeding and found an increase in maximal HR response and maximal VO_2 [71]. Rigaud *et al.* (1997) studied 15 patients with AN compared to 15 normal weight controls with serial upright bicycle exercise testing during refeeding [72]. Prior to weight

restoration, AN patients had a 49% lower workload reached with exercise [72]. The workload reached correlated with body weight, fat-free mass, and leg circumference [72]. After refeeding, the workload reached improved to normal values in the AN patients despite VO_2 and body weight remaining decreased from normal after 45 days of nutritional rehabilitation [72]. They concluded that muscle performance was restored by refeeding and this occurs before normal nutritional status is achieved [72]. Waller *et* al. (1996) studied 10 patients with AN using cycle ergometry and found that VO_2 increased linearly with increases in body weight [73]. Mont *et al.* (2003) studied 31 patients with AN before and after weight restoration using Bruce protocol treadmill exercise testing [30]. They found increases in exercise duration, maximal HR, and workload after weight restoration [30]. DiVasta *et al*. (2010) studied 38 females with AN after nutritional rehabilitation using Bruce protocol treadmill exercise testing [29]. They concluded that the exercise test was normal, however did not state what criteria this was based on [29]. They found no relation between the degree nor amount of pre-admission exercise and the stress test performance [29].

Overall these studies demonstrate that patients with AN have decreased exercise tolerance, peak HR, peak BP, and peak VO_2. Limited echocardiographic data during exercise suggests normal SV and CO responses to progressive exercise, although ST changes with exercise have been described. Weight restoration improves exercise endurance and VO_2 as well as HR and BP response. Studies of exercise in adolescents with AN have been limited and have included small numbers of patients. As well, more detailed assessment of echocardiographic LV myocardial function with exercise has not been previously performed in this population.

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Chapter 2: An Echocardiographic Study of Cardiac Size and Function in Adolescent Females with Anorexia Nervosa

Abstract

Background: Cardiac involvement occurs in the majority of patients with anorexia nervosa (AN), however, controversy still exists regarding systolic and diastolic function in females with AN. The purpose of our study was to investigate echocardiographic indices of cardiac dimensions, output, and systolic and diastolic function in adolescent females with AN.

Methods: We performed a retrospective case-control study of adolescent females with AN compared to healthy adolescent controls. A complete echocardiogram including a detailed left ventricular (LV) function protocol was performed for controls and patients with AN during their acute phase of illness. LV dimensions, LV mass (LVM), LV mass indexed for body surface area (LVMI), left atrial (LA) size, stroke volume (SV), cardiac output (CO), and cardiac index (CI) were measured. LV systolic function was measured using fractional shortening (FS), mean velocity of circumferential fiber shortening (MVCFc), and systolic tissue Doppler imaging (TDI) at the LV lateral wall ($LV_{lateral}$), interventricular septum (IVS), and right ventricular free wall (RV). Diastolic function was measured using transmitral E and A wave velocity, pulmonary venous velocities, and diastolic TDI E' and A' wave velocities. BMI percentiles were calculated for patients and controls and patients were divided into those of BMI $\leq 10^{th}$ percentile ($AN \leq 10^{th}$) and $>10^{th}$ percentile ($AN > 10^{th}$).

Results: We studied 95 adolescent AN patients (AN_{total}) and 58 healthy adolescent female controls. There were 70 patients in the AN≤10^{th} group. AN_{total} and AN≤10^{th} groups had reduced LV dimensions, LA size, LVM, LVMI, SV, CO, and CI compared to controls, with no differences between AN>10^{th} and controls. There were no differences between controls and all AN groups in FS, MVCFc, or systolic TDI. Pulmonary venous A wave velocity was significantly decreased in AN_{total} and AN≤10^{th} as compared to controls with no difference in transmitral E or A wave velocity or E/A. $LV_{lateral}$ E' and A', IVS E' and A', and RV A' were significantly decreased in AN_{total} and AN≤10^{th}, with only a decrease in RV A' in AN>10^{th} as compared to controls.

Conclusions: Adolescent females with AN have downsized LV dimensions, LVMI, and CI, but systolic ventricular function remains preserved. Diastolic TDI is decreased in AN patients. Patients with BMI ≤10^{th} percentile demonstrate changes in LV dimensions and diastolic function and warrant more careful monitoring.

Introduction

Anorexia nervosa (AN) carries one of the highest mortality rates of the psychiatric disorders, with cardiac complications contributing to the risk of sudden death in these patients [1-5]. Cardiac involvement, including both electrophysiologic and structural abnormalities, occurs in approximately 80% of patients with AN [2, 6, 7]. Echocardiographic structural and functional cardiac abnormalities including decreased cardiac mass, mitral valve prolapse, pericardial effusion, reduced left ventricular (LV) dimensions, and decreased cardiac output (CO) have been well documented in the literature [2, 6, 8-11].

Despite reduced LV dimensions and mass, ventricular systolic function at rest in females with AN, as measured by the standard echocardiographic indices of ejection fraction (EF) and fractional shortening (FS), has consistently been shown not to differ when compared to female controls [7-9, 11-14]. However, studies of systolic tissue Doppler indices and the myocardial performance index (MPI) have suggested that there may be subtle decreases in systolic function at rest in females with AN [12, 13]. Fewer studies have investigated diastolic function in AN, and there have also been conflicting results. While transmitral A wave velocities and E/A ratio have been shown to be different in patients with AN, other measures of diastolic function including tissue Doppler have not shown differences [8, 13, 14]. The majority of these studies have, however, been performed on relatively small numbers of patients and in adult populations [7, 8, 10-14].

The purpose of our study was to investigate echocardiographic indices of cardiac dimensions, output, and systolic and diastolic function in a large number of adolescent females with AN and to compare these parameters to control subjects. To our knowledge, this is one of the largest series of echocardiographic changes in adolescent females with AN that has been published in the literature and the largest tissue Doppler imaging (TDI) study in AN patients.

Methods

Subjects

We retrospectively studied female adolescents with AN who were referred by the Eating Disorders Program for routine cardiac assessment at the Children's Heart Centre at

the British Columbia Children's Hospital between October 2003 and August 2010. The diagnosis of AN was made clinically by a multidisciplinary team including psychiatry and adolescent medicine and was based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria [15]. Patients were studied during the acute phase of presentation and treatment for AN and consisted of both hospitalized patients and those seen as outpatients. If patients had more than one assessment, only the data from the complete initial assessment were used. Patients with evidence of structural heart disease were excluded. The control group was composed of healthy female adolescents from our established healthy control Doppler echocardiography database. The controls were of normal weight and did not have a history of AN. Control patients were volunteers recruited from friends and family of hospital employees.

Echocardiographic data were recorded as well as height (cm), weight (kg), body mass index (BMI, kg/m^2), body surface area (BSA, m^2), heart rate (beats/min) and blood pressure (BP, mmHg) at the time of study. Ethics approval for this study was obtained from the Children's and Women's Hospitals' Research Ethics Board and the University of British Columbia's Clinical Research Ethics Board.

Echocardiography

All subjects underwent a complete echocardiogram by an experienced echocardiographer using our lab's detailed LV function protocol. The examination was performed in the left lateral decubitus position.

Measurements of LV dimensions and mass were performed. Standard assessment of LV internal diameters were measured at end-systole (LVESD) and end-diastole

(LVEDD) with the interventricular septal wall diameter (IVSD) and LV posterior wall diameter (LVPWDd) measured at end-diastole using 2D-targeted M-mode, according to the American Society of Echocardiography [16]. The LV posterior wall diameter in systole (LVPWDs) was also measured. LV wall mass (LVM) was calculated as $1.05[(LVEDD + 2*LVPWDd)^3 - LVEDD^3]$. Left atrial (LA) diameter was measured using M-mode in the parasternal short axis at the level of the aortic valve.

Indices of LV systolic function were calculated using echocardiographically-derived measurements. FS was calculated to measure LV contractility using the formula 100*(LVEDD-LVESD)/LVEDD. The diameter of the LV outflow tract (LVOTd) was measured in the parasternal long axis view and was used to calculate the cross-sectional area (CSA) using the equation $CSA = LVOTd^2 * \pi/4$. Outflow velocity of the LV outflow tract (LVOT) was measured by pulse wave Doppler interrogation in the long axis apical view, as recommended by the American Society of Echocardiography [17]. The velocity-time integral (VTI) and ejection time (ET) were obtained from this pulsed wave Doppler measurement. ET was corrected for heart rate (ETc) using the equation $ETc = ET/\sqrt{RR}$. Stroke volume (SV, mL) was calculated as the product of the VTI and the CSA. Cardiac output (L/min) was calculated as the product of heart rate and SV divided by 1000. Meridonal wall stress (the tension within the wall of the LV) at peak systole (σPS) was calculated as 0.34*systolicBP*LVESD/LVPWDs*(1 + (LVPWDs/LVESD)) [18]. The mean velocity of circumferential fiber shortening (MVCFc) was derived from the equation (LVEDD-LVESD)/(ETc/1000*LVEDD). MVCFc is an indicator of myocardial performance that is sensitive to changes in contractile state, but it is highly dependent on loading conditions [19]. Therefore, we examined the relationship of MVCFc to σPS as a

preload independent, afterload-adjusted measure of cardiac contractility. To determine the relationship between σPS and MVCFc, the mean values for these variables for both the control and AN groups were plotted against published normal pediatric values [18].

Diastolic function was measured using pulse Doppler transmitral velocities, pulmonary venous velocities, and tissue Doppler measurements as recommended by the American Society of Echocardiography [20]. Mitral valve inflow was measured in the apical four chamber view with pulsed wave Doppler interrogation at the level of the mitral valve leaflets. The measurements made of mitral inflow included the peak early filling (E wave) and late diastolic filling (A wave) velocities, the deceleration time of the early filling velocity (DT), and the A wave duration. The inflow velocity E/A ratio was derived. Mitral valve propagation velocity was determined from the slope of the first aliasing velocity of the mitral valve inflow using colour M-mode at the center of the LV inflow blood column in the apical four chamber view. Pulsed wave Doppler interrogation of pulmonary venous flow was performed in the apical four chamber view with slight superior angulation using a sample volume placed 1-2 cm into the right upper pulmonary vein. The peak velocities of the systolic (S wave), early diastolic (D wave), and late diastolic (A wave) waves were measured as well as the A wave duration.

TDI was acquired in the apical four chamber view with pulsed wave Doppler interrogation at the level of the cardiac base. The systolic (S'), early diastolic (E'), and late diastolic (A') annular velocities were measured in the lateral LV wall, the interventricular septum (IVS), and the right ventricular (RV) free wall. These myocardial velocities measure the shortening or contraction (S') and lengthening or relaxation (E' and A') of myocardial segments in the longitudinal plane [21, 22]. The ratio of mitral

valve inflow E wave velocity to tissue Doppler E' (E/E' ratio) was calculated, which has been shown to correlate with LV filling pressures [21].

Body Size Indexing

Body surface area was calculated using the DuBois formula BSA (m^2) = $0.20247(height)^{0.725}(weight)^{0.425}$, with height measured in meters and weight in kilograms [23]. Standardization for body surface area (BSA) was performed by dividing the LVM, SV, and CO by the BSA to give the LVM index (LVMI), SV index (SVI), and cardiac index (CI), respectively. BMI percentiles were calculated for all subjects using data from the Centers for Disease Control growth data [24].

Statistical Analysis

We compared the mean parameters of the total group of AN patients to the control group using one way ANOVA. In order to determine the effect of the degree of patient malnutrition on echocardiographic parameters, we performed an additional subgroup analysis by dividing the AN group into those with a BMI less than or equal to the 10^{th} percentile (lowest weight AN group) and those with a BMI greater than the 10^{th} percentile (higher weight AN group). These subgroups were compared to the control group using a one way ANOVA with post-hoc analysis performed using Tukey's test. Pearson's product-moment correlations were performed to investigate the relationship between BMI percentile and the echocardiographic variables. Since the range of normal BMI varies across the age distribution of our subjects, BMI percentile for age was used instead of the absolute BMI value to determine the relationship of body composition to the different

echocardiographic parameters. Multivariable linear regression was performed to determine the relative contribution of age, systolic BP (sBP), heart rate, LVM, and BMI percentile on the E/A and E/E' ratios. We also subdivided the AN group in those of the AN binge/purging subtype (AN-BP) and the AN restrictive subtype (AN-R) and compared these subtypes to control subjects using a one way ANOVA with post-hoc analysis performed using Tukey's test. Due to the number of comparisons performed in our study, we considered $p \leq 0.005$ to indicate a statistically significant difference, p values of $0.005 < p \leq 0.05$ to be marginally statistically significant, and p values >0.05 to be non-significant.

Results

Clinical Characteristics

Ninety-seven patients with AN were identified from our echocardiographic database during the study period. Two patients were excluded from the analysis, one for absent height and BMI data and the other for evidence of LV non-compaction on the echocardiogram. There were a total of 95 young females in the AN group and 58 healthy young female controls. Table 1 outlines the baseline characteristics of the AN and control groups. The mean ages and heights of the AN and control groups were not significantly different. Twenty-two of the patients were AN-BP type and 73 were the AN-R type. The total AN group had a significantly lower weight, BMI, BSA, mean heart rate, sBP, and diastolic BP (dBP) as compared to control subjects. The distributions of BMI and BMI percentile for the AN and control groups are demonstrated in Figure 1.1A and 1.1B, respectively. Nine patients had mild myxomatous changes of the mitral valve or mitral

valve prolapse. Fifteen patients had mild to moderate pericardial effusions of no hemodynamic significance with the majority of these being trace or small effusions.

The lowest weight AN subgroup was composed of 70 patients with 25 patients in the higher weight AN group. On subgroup analysis, the lowest weight AN group had significantly decreased weight, BSA, BMI, BMI percentile, sBP, and dBP as compared to the control group. The only significant differences between the higher weight AN group and controls were decreased BMI percentile, sBP, and dBP in the AN patients. Seventeen AN patients had more than one echocardiogram available for analysis. The low number of repeat assessments and the variability in the timing of the repeat assessments precluded further analysis of these repeat echocardiograms.

Chamber Dimensions

Table 1.2 summarizes the comparison of LV dimensions and LA size in patients with AN compared to controls. LV dimensions including LVEDD, LVESD, IVSD, and LVPWDd were significantly smaller in patients with AN than in controls. BMI percentile correlated with LVEDD ($r=0.356$, $p<0.001$), LVESD ($r=0.316$, $p<0.001$), IVSD ($r=0.299$, $p<0.001$), and LVPWd ($r=0.404$, $p<0.001$). LVM was significantly reduced in the AN group as compared to the control group, and remained reduced when LVM was indexed for BSA. BMI percentile correlated with LVMI ($r=0.340$, $p<0.001$). LA size was also decreased in patients with AN and correlated with BMI percentile ($r=0.419$, $p<0.001$) and LVMI ($r=0.368$, $p<0.001$).

On subgroup analysis, the AN patients of lowest weight showed significant decreases in LV dimensions, LVM, LVMI, and LA size when compared to controls. In

contrast, the AN patients with a BMI >10^{th} percentile showed no significant differences in these parameters when compared to controls.

Left Ventricular Systolic Function

The comparisons between the AN and control groups for LV function are outlined in Table 1.2. Systolic ventricular function at rest, as measured by FS, was similar in patients with AN when compared to controls. Myocardial performance as measured by MVCFc and σPS showed no significant differences between the AN group and controls. As demonstrated in Figure 1.2, the relationship between MVCFc and σPS was normal for both the AN and control groups. SV was reduced in patients with AN compared to controls, however this difference was no longer statistically significant when SV was indexed for BSA. Both CO and CI were decreased in the AN group. The differences seen between the total AN group and controls were unchanged in the subgroup analysis comparing AN patients with a BMI ≤ 10^{th} percentile to controls. Additionally, the lowest weight AN group had a significantly decreased wall stress as compared to controls. The subgroup analysis comparing the AN patients with a BMI > 10^{th} percentile demonstrated no differences compared to controls in any of the parameters outlined in Table 1.2. The relationship between MVCFc and σPS remained normal for both AN subgroups, as demonstrated in Figure 1.2. BMI percentile correlated with SV ($r = 0.366$, $p<0.001$), CO ($r = 0.436$, $p<0.001$), and CI ($r=0.250$, $p=0.002$). There were no significant correlations between BMI percentile and FS, MVCFc, σPS, and SVI.

Left Ventricular Doppler Filling Pattern and Diastolic Function

As summarized in Table 1.3, transmitral E wave velocity, A wave velocity and duration, DT, and propagation velocity were similar between groups. The E/A ratio was not different from controls for the total AN group, however, was significantly increased in the subgroup analysis when the AN patients of lowest weight were compared to controls. E/A ratio inversely correlated with BMI percentile ($r = -0.229$, $p = 0.005$) but not and LVMI. Transmitral A wave velocity correlated with BMI percentile ($r = 0.208$, $p=0.012$) but not LVMI. There were no significant correlations between transmitral E wave velocity, DT, or propagation velocity with LVMI or BMI percentile. Multiple linear regression analysis was performed to assess the relative contribution of age, sBP, heart rate, BMI percentile, and LVM on the E/A ratio. The model was statistically significant and explained a moderate amount of the variance in E/A, $F(5,137)=11.035$, $p<0.001$, adj $R^2=0.287$. Heart rate was the only independent predictor of the E/A ratio ($\beta=-0.494$, $p<0.001$).

Pulmonary venous Doppler indices for the different groups are provided in Table 1.3. Pulmonary venous S wave velocity, D wave velocity, and A wave duration showed no difference between patients with AN and controls. Pulmonary venous A wave velocity was significantly decreased in AN patients and the AN patients of lowest weight when compared to controls. Pulmonary venous A wave velocity correlated with BMI percentile ($r=0.270$, $p=0.003$) but not LVMI. There were no significant relationships between other pulmonary venous Doppler indices and BMI percentile or LVMI.

Pulsed Tissue Doppler Imaging

Mean TDI indices for all groups are given in Table 1.4. The S' wave of the base of the LV lateral wall, IVS, and RV free wall were similar between groups, indicating no difference in longitudinal systolic function as measured by TDI. The LV lateral wall and IVS peak E' and A' as well as the RV A' velocities were significantly decreased in the AN group as compared to the control group. The LV lateral wall E/E' ratio was significantly increased in the AN group as compared to controls. On subgroup analysis, LV lateral wall peak E' and A', IVS peak E' and A', and RV A' velocities remained decreased in the lowest weight AN group when compared to controls. The AN patients with a BMI > 10^{th} percentile had a significantly decreased RV A' velocity compared to controls, with no other significant differences between these groups. The E/E' ratio correlated inversely with BMI percentile ($r=-0.230$, $p=0.006$) with no significant correlation to LVMI. We performed multiple linear regression analysis using age, sBP, heart rate, BMI percentile, and LVM to predict the E/E' ratio. The model was statistically significant but explained only a small amount of the variance in E/E', $F(5,135)=2.285$, $p=0.050$, adj $R^2=0.044$. None of the variables in the model were independent predictors of the E/E' ratio.

Comparison of AN Subtypes

The AN-R (n=73) patients showed no difference from the control group in age or height, but demonstrated significant decreases in weight (40.5 vs 51.2 kg, $p<0.001$), BSA (1.37 vs 1.52 m^2, $p<0.001$), BMI (15.5 vs 19.7kg/m^2, $p<0.001$), BMI percentile (7.3 vs 45.5, $p<0.001$), sBP (95.4 vs 108.9mmHg, $p<0.001$), dBP (58.7 vs 65.3mmHg, $p<0.001$),

and heart rate (61.4 vs 69.4 beats/min, p=0.003). The AN-R patients showed reductions in LV dimensions compared to controls including LVEDD (4.28 vs 4.49 cm, p=0.011), LVESD (2.68 vs 2.82cm, p=0.037), IVSD (0.70 vs 0.76 cm, p=0.016), LVPWDd (0.67 vs .074 cm, p=0.002), LVM (103.4 vs 133.1 g, <0.001), LVMI (76.1 vs 85.9g/m^2, p=0.012), and LA diameter (2.80 vs 3.06 cm, p=0.001). The hemodynamic parameters of SV (49.8 vs 56.8mL, p=0.001), CO (2.96 vs 3.83 L/min, p<0.001), and CI (2.15 vs 2.55 L/min/m^2, p<0.001) were decreased in the AN-R group compared to controls, with no differences in SVI, FS, MVCFc, or σPS. Pulmonary venous A wave velocity was significantly decreased in the AN-R patients as compared to controls (0.15 vs 0.17, p=0.007) with no significant differences in other pulmonary venous or transmitral Doppler parameters. Analysis of the TDI results showed significant reductions in AN-R patients as compared to controls in LV lateral wall E' (16.2 vs 18.1 m/s, p=0.006), IVS E' (13.5 vs 15.1 m/s, p=0.001) and A' (4.7 vs 5.9 m/s, p<0.001), and RV free wall A' (6.6 vs 9.0 m/s, p<0.001) and an elevation of E/E' in AN-R patients (5.8 vs 5.0, p = 0.004).

AN-BP patients were significantly older than control patients (16.5 vs 15.1 yrs, p=0.017) with decreased BMI (17.8 vs 19.7kg/m^2, p=0.005), BMI percentile (17.0 vs 45.5, p<0.001), sBP (96.0 vs 108.9 mmHg, p<0.001), dBP (58.7 vs 65.3 mmHg, p<0.001) and heart rate (60.4 vs 69.4 beats/min, p = 0.025). There were no significant differences in LA diameter, LV dimensions, or wall thickness other than a marginally significant reductions in LVPWDd (0.97 vs 0.74, p = 0.044), LVM (110.8 vs 133.1g, p=0.029), and LVMI (74.5 vs 85.9 g/m^2, p=0.049). AN-BP patients demonstrated no differences from controls in FS, SV, SVI, CO, MVCFc, or σPS but had a marginally significant decrease in CI (2.20 vs 2.55L/min/m^2, p=0.040). There were no significant differences between AN-

BP patients and controls in pulmonary venous or transmitral Doppler indices. There were no significant differences between the AN-BP and control groups in TDI parameters aside from a decreased RV A' velocity in the AN-BP patients (6.0 vs 9.0m/s, $p<0.001$).

Compared to AN-R patients, AN-BP patients were significantly older (16.5 vs 15.2 yrs, $p=0.034$), and had a significantly increased BMI (17.8 vs 15.5 kg/m^2, $p=0.001$) but no difference in BMI percentile (17 vs 7.3, $p = 0.118$).

Discussion

Our study demonstrates that in a large sample of adolescent females with AN, there are multiple differences in the structure and diastolic function of the heart as compared to female controls of normal weight. These differences include diminished chamber dimensions, disproportionately decreased LVM, decreased CI, and decreased diastolic TDI velocities. When the AN patient group is subdivided into patients with a BMI $\leq 10^{th}$ percentile and $> 10^{th}$ percentile, it is the patient group with the greatest degree of malnutrition that shows significant differences in cardiac structure and function when compared to control subjects.

Patients with AN had significantly reduced resting heart rates when compared to the control group. This has been documented in multiple studies in both adolescents and young adult females [7, 8, 10, 13, 14, 25]. The reduced heart rate is likely related to an increase in vagal tone [14, 26, 27]. Galetta *et al.* (2002) demonstrated a decreased T3 level in patients with AN, which is also seen in other patients with chronic malnutrition, and may be a contributing cause of bradycardia [28]. Other proposed contributors to the

presence of bradycardia have included electrolyte losses, adverse drug effects, reduced glycogen content of the myocardium, and myofibrillar atrophy [7].

LV dimensions, LV wall thickness, LVM, and LA diameter were significantly decreased in patients with AN as compared to controls. These findings are in keeping with those of other studies investigating cardiac dimensions in AN [7-14]. In our study, mean LVM was reduced by approximately 20-25% in the total and low weight AN groups as compared to the control group. LVM remained significantly decreased despite indexing for BSA, indicating a disproportionately greater fall than would be expected based solely on the smaller body habitus of the females in the AN group. The correlation between BMI percentile and LVMI suggests that weight loss is accompanied by a reduction in the LVM. The etiology of the cardiac hypotrophy seen in AN has not been clearly elucidated. Possible causes include decreased afterload from relative hypotension leading to down-regulation of the LVM, reduced preload leading to ventricular remodeling, and a direct effect of malnutrition causing muscular atrophy as occurs in the skeletal musculature [8, 11, 26].

Despite the decreased LVM and LV dimensions, resting systolic function as assessed by FS and MVCFc was similar between the AN group and controls. These results are in keeping with the majority of published studies concerning systolic function at rest in AN, which have demonstrated no differences in EF, FS, or MVCFc [7-9, 11-14, 26]. We found that patients with AN and controls had similar MVCFc, however, MVCFc is affected by LV afterload (or the BP) and patients with AN have been shown to have a significantly decreased BP compared to controls [8, 10, 11, 13, 14]. We therefore investigated the relationship between MVCFc and σPS, which is reported to be a preload

and afterload independent indicator of systolic function in the absence of ventricular outlet obstruction [18]. Our study demonstrates a normal MVCFc-σPS relationship in the total AN group and the AN subgroups, which suggests normal contractility at rest in patients with AN. Our study also demonstrates that σPS is similar between the AN and control groups, although the AN patients of lowest weight have a significantly decreased σPS as compared to controls. According to LaPlace's law, LV wall stress is influenced by both LV afterload and wall thickness. As BP, LV dimensions, and wall thickness are decreased in our patients with AN, the net effect is that σPS remains relatively normal, although this compensation is not complete in the most malnourished AN patients. End-systolic wall stress (which is closely related to σPS) in patients with AN has been investigated previously and has demonstrated conflicting results, with being unchanged when compared to controls by St. John Sutton *et al.* (1985) and lower than predicted for age by DiVasta *et al*. (2010) [11, 18, 26]. In our study, all TDI S' wave velocities, which are a reflection of longitudinal systolic function, were unchanged between the AN groups and control subjects. Overall our results demonstrate no statistically significant decrease in systolic function at rest in adolescents with AN. There have, however, been some reports in the literature of subclinical impairment of LV systolic function in patients with AN. Eidem *et* al. (2001) found that adolescent females with AN had a significantly increased MPI, indicating a subclinical impairment in the active phase of LV function [12]. Galetta *et al.* (2005) demonstrated decreased LV S' TDI velocities in young adult females with AN as compared to controls, which would indicate subclinical systolic impairment of the LV in patients with AN [13]. These findings by Galetta *et al.* (2005) are in disagreement with the findings in our study [13].

SV was decreased in our patients with AN compared to controls, however, this difference was no longer present once SV was indexed to BSA. Therefore, in relation to the BSA, there was no difference in the amount of blood ejected by the heart per beat. As a result of the relative bradycardia in our patients with AN, the amount of blood ejected per minute, or the CO, was decreased in the AN group and this difference was maintained when CO was indexed to the BSA. The decreased SV, CO, and CI are corroborated by other studies in patients with AN [8, 10]. As also shown by Romano *et al*. (2004), the combination of decreased BP and decreased SV demonstrated in our study is consistent with a low cardiac workload state which may contribute to the decrease in LVM in patients with AN [10].

Abnormal LV filling patterns in AN patients have been previously documented with studies showing decreased transmitral peak A velocity and increased E/A ratio, indicating that the majority of LV filling occurs in early diastole [8, 13, 14]. Some authors have suggested that this diastolic pattern indicates impaired LV filling in patients with AN [8]. Other authors have felt that this filling pattern may be a physiologic adaptation to bradycardia as a decreased heart rate favours early diastolic filling, with a similar filling pattern also being seen in bradycardic athletes [14, 29]. The transmitral A wave velocity, which represents the contribution to LV filling from atrial contraction in late diastole, may be reduced due to more complete left atrial emptying occurring during the prolonged diastolic phase associated with bradycardia [14]. These previous studies which have assessed the transmitral Doppler patterns in AN have been performed in adult women and we are not aware of studies regarding these indices in pediatric patients. We found that E/A ratio was significantly increased in our adolescent patients with AN and a BMI $\leq 10^{th}$

percentile, however, we did not find a decrease in the peak A wave velocity. The difference in transmitral Doppler findings in our study compared to previous studies may be due to our patients being less bradycardic than the adult patients in these previous cohorts, which could account for less complete left atrial emptying in early diastole. To our knowledge, pulmonary venous Doppler velocities have not been previously studied in patients with AN. We found that the pulmonary venous A wave velocity was decreased in the total AN group and those with a BMI $\leq 10^{th}$ percentile. Pulmonary venous A wave velocity reflects the velocity of blood flow reversal in the pulmonary veins as a result of atrial contraction. It is, therefore, conceivable that more complete LA emptying in prolonged diastole (similar to the rationale for decreased transmitral A wave velocity explained above) could lead to a decreased pulmonary venous A wave velocity in patients with AN and bradycardia. The potential importance of heart rate leading to abnormalities in the transmitral, and possibly pulmonary venous, Doppler velocities is strengthened by the fact that our multiple linear regression analysis demonstrated that the only independent predictor of E/A ratio was heart rate, with E/A being inversely related to heart rate. The increased E/A ratio in AN patients with a BMI $\leq 10^{th}$ percentile demonstrated in our study is suggestive of a restrictive pattern, but may not signify restrictive physiology. The transmitral and pulmonary venous Doppler indices may be difficult to interpret in AN as these indices can be altered by disturbances in preload, which is felt to be decreased in patients with AN [8]. This state of depressed preload is supported by our study as we found that patients with AN have decreased LA size, CO, CI, and SV as compared to control subjects. Due to the difficulty in assessing Doppler

filling patterns in the presence of decreased preload, any abnormalities in the transmitral or pulmonary venous Doppler velocities should be interpreted with caution.

In contrast to the results of transmitral and pulmonary venous velocity, our TDI results are more suggestive of disordered diastology in adolescent patients with AN. TDI velocities are measures of the longitudinal contraction and relaxation of the myocardium and are less preload dependent than transmitral indices [21, 22]. TDI measurements are helpful in differentiating people with diastolic dysfunction from normal individuals [30]. Patients in our study with AN had significantly decreased E' velocities of the lateral LV wall and IVS and decreased A' velocities of the lateral LV wall, IVS, and RV free wall. These findings are in contrast to those of Galetta *et al*. (2005) who found no difference in the E' and A' velocities between their patients with AN and controls, although these authors acknowledged that their results were inconsistent with the evidence that diastolic dysfunction usually precedes systolic dysfunction [13]. We also found that the E/E' ratio at the lateral wall, which correlates with LV filling pressure, was increased in patients with AN. This finding suggests that the LV filling pressures in patients with AN may be increased as compared to control subjects. Alterations in diastolic function and LV compliance would not be surprising in the setting of AN. In histologic studies of severely malnourished animals and humans, abnormalities of the myocardium have included gross edema, myofibrillar atrophy, interstitial edema, fibrosis, and cellular infiltration, which can result in reduced LV compliance [31-36]. Congestive heart failure (CHF) is an uncommon but reported complication seen in patients with AN that appears to be associated with the period of refeeding [35, 37, 38]. The mechanisms of heart failure in AN are unclear and are reported to include electrolyte deficiency, micronutrient

deficiency, and increased metabolic demand [37-39]. Although the clinical impact of this abnormal diastolic pattern is unknown, it is conceivable that in cases of severe AN diastolic dysfunction could contribute to the development of CHF in the setting of precipitously increased preload with inappropriate nutrient repletion and volume expansion [35].

We performed a subgroup analysis in order to determine the effect of the severity of malnutrition in AN on echocardiographic findings. In discussion with our adolescent medicine colleagues, we chose to use the 10^{th} percentile of BMI as the cutoff value in our subgroup analysis. Determination of a cutoff value of BMI or BMI percentile that confers risk of cardiac abnormalities in AN would require a larger study and was outside of the scope of our investigation. Our subgroup analysis demonstrated that the patients with AN and a BMI $\leq 10^{th}$ percentile showed the same differences from controls as the total AN group, as well as an additional significant increase in the E/A ratio and decrease in σPS as compared to controls. In contrast, the patients with AN and a BMI $> 10^{th}$ percentile showed no differences from the controls aside from decreased mean BMI percentile, sBP, dBP, and TDI RV free wall A' velocity. These results suggest that the abnormalities seen in cardiac dimensions and ventricular function in AN are related to the degree of malnutrition as the most severely malnourished patient group with AN demonstrated these differences. We are unaware of previous studies that have related BMI percentile to echocardiographic findings in pediatric cohorts of AN patients.

We also investigated the echocardiographic changes in AN by subtype. We found that the differences in LV dimensions and diastolic function between the AN-R group and controls were similar to those found between the AN patients of BMI $\leq 10^{th}$ percentile

and controls. In contrast, the patients with AN-BP had fewer differences from controls despite having lower BMI, BMI percentile, BP, and heart rate as compared to the control group. The AN-BP patients had a marginally significant reduction in LVM, LVMI, and CI with no other substantial differences in cardiac dimensions or diastolic function. AN-BP patients had a higher mean BMI percentile than the AN-R patients, however, this difference was not statistically significant. Although part of the increased number of differences from controls among the AN-R patients could potentially be explained by a greater degree of malnutrition in this group, it is possible that differences in the physiology or the disease process between the AN-R and AN-BP patients may also partially explain the differences seen in the degree of cardiac changes.

Limitations of our study include the retrospective case-control nature of the study and the potential biases associated with this design, including selection bias (as this study was performed at a tertiary care center and may have included more severely affected patients than the general AN population) and misclassification/information bias (via the misclassification of AN patients and their subtypes). We used a normal weight control group and did not compare the AN group to constitutionally thin females as other studies have done, however, the use of the thin weight control group did not substantially change the results of these studies compared to using the normal weight control group only [8, 13, 14]. The measures of echocardiographic ventricular function in our study were only taken in the resting state and we were thus unable to determine the impact of hemodynamic stress on ventricular function. The retrospective nature of our study did not allow us to determine the MPI in our patients as simultaneous Doppler interrogation of ventricular inflow and outflow is not included in our lab's detailed LV function protocol.

The number of controls in our study was fewer than the number of patients due to the difficulty of recruiting healthy adolescent female volunteers to undergo echocardiography. It is possible that an increased number of controls could have demonstrated additional significant differences between the patient and control groups. Although our results show no substantial differences in cardiac size and function between the AN patients with a BMI > 10^{th} percentile and controls, we may have been underpowered to detect differences between these groups as the minority of patients in our cohort had a BMI percentile in this range. We may have also been underpowered to detect differences between the AN-BP group and controls as the majority of the patients in our study were of the AN-R subtype. Lastly, although our study demonstrates differences in echocardiographic parameters between patients with AN and controls, the clinical importance of such findings in unclear. DiVasta *et al*. (2010) found few truly pathologic cardiac findings in a cohort of adolescents hospitalized with AN and felt that less intensive cardiac monitoring may be reasonable in this patient population [26].

Further study is required to determine echocardiographic systolic and diastolic ventricular function in adolescent patients with AN during times of increased metabolic requirements. Despite our findings of normal systolic function and some changes in diastolic function at rest, it is possible that during times of hemodynamic stress that systolic or diastolic function could worsen in patients with AN and lead to deterioration. In addition, it is unclear if the abnormalities that we documented in our study are related to adaptation to the altered physiologic state in AN or if these are truly pathologic changes associated with AN. In order to help answer this question, future study on the reversibility of these findings with weight restoration is required. Although several

studies have investigated changes in systolic function and cardiac structure with weight restoration, little research has focused on the reversibility of diastolic abnormalities with weight restoration [6, 9, 11, 26, 40, 41]. It is possible that some of the histologic changes seen in starvation models, such as edema and cellular infiltration, are reversible while others, such as fibrosis, are not reversible with nutritional repletion. Our study is the first to document diastolic changes by TDI in patients with AN and are in contrast to the findings of Galetta *et al.* (2005) [13]. The difference in findings between our TDI analysis and that performed by Galetta *et al.* (2005) may be partially explained by the difference in mean age of our study subjects or by potential differences in disease severity and/or duration [13]. Due to these contrasting results, it is important for the findings of our TDI analysis to be repeated in a future study prior to drawing conclusions from these results.

In conclusion, the present study is one of the largest comprehensive echocardiographic studies of chamber dimensions and LV systolic and diastolic function which has been performed in adolescent females with AN. LV dimensions, wall thickness, and mass were decreased in the AN group, with a disproportionate decrease in LVM compared to the decrease in BSA. Systolic function was preserved at rest in our patient population. The TDI results are suggestive of a disturbance in diastolic function as compared to our control subjects. Our subgroup analysis demonstrated that the identified changes in cardiac size and function were present in the most malnourished patients with a BMI $\leq 10^{th}$ percentile, with no substantial differences in the AN patients with less severe malnutrition and a BMI $> 10^{th}$ percentile. The AN patients of AN-R subtype demonstrated similar differences from controls as the AN patients of BMI $\leq 10^{th}$ percentile, whereas those of AN-BP subtype demonstrated fewer changes in the

echocardiographic parameters studied. Further investigation is needed to fully assess the clinical relevance of the differences shown by our study in order to guide clinical follow up.

Table 1.1: Baseline Characteristics of all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=58)	Total AN Group* (n=95)	AN Subgroups[§]	
			BMI $\leq 10^{th}$ percentile (n=70)	BMI $>10^{th}$ percentile (n=25)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	15.2 (2.6)	15.5 (1.7) p = 0.360	15.4 (1.8) p = 0.702	16.0 (1.3) p = 0.178
Height (cm)	161.1 (9.1)	161.6 (8.4) p = 0.695	161.4 (9.1) p = 0.820	162.3 (6.2) p = 0.663
Weight (kg)	51.7 (9.9)	42.2 (8.3) p < 0.001	39.2 (6.7) p < 0.001	50.2 (6.6) p = 0.874
BSA (m^2)	1.53 (0.18)	1.40 (0.16) p < 0.001	1.36 (0.15) p < 0.001	1.52 (0.12) p = 0.998
BMI (kg/m^2)	19.8 (2.7)	16.0 (2.4) p < 0.001	15.0 (1.7) p < 0.001	19.0 (1.5) p = 0.341
BMI percentile	45.4 (25.9)	9.5 (15.5) p < 0.001	2.2 (3.1) p < 0.001	30.1 (17.7) p = 0.001
Systolic BP (mmHg)	108.8 (9.6)	95.5 (9.3) p < 0.001	94.0 (9.4) p < 0.001	99.6 (7.9) p < 0.001
Diastolic BP (mmHg)	65.0 (9.1)	58.2 (6.7) p < 0.001	57.8 (6.4) p < 0.001	59.5 (7.6) p = 0.007
Heart rate (bpm)	69.4 (14.6)	61.2 (12.9) p < 0.001	60.5 (13.3) p = 0.001	63.1 (11.9) p = 0.131

AN= Anorexia nervosa, SD = standard deviation, BP = blood pressure, BMI = body mass index

*p values determined using one- way ANOVA comparing AN group to controls

[§] p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 1.2: Cardiac Dimensions and Left Ventricular Systolic Function in all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=58)	Total AN Group* (n=95)	AN Subgroups§	
			BMI ≤10th percentile (n=70)	BMI>10th percentile (n=25)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
LVEDD (cm)	4.49 (0.46)	4.31 (0.38) p = 0.009	4.23 (0.36) p = 0.001	4.54 (0.32) p = 0.862
LVESD (cm)	2.82 (0.37)	2.69 (0.29) p = 0.018	2.62 (0.27) p = 0.001	2.90 (0.25) p = 0.545
LVPWDd (cm)	0.74 (0.15)	0.67 (0.10) p < 0.001	0.65 (0.10) p < 0.001	0.71 (0.10) p = 0.546
IVSD (cm)	0.76 (0.13)	0.70 (0.12) p = 0.005	0.69 (0.11) p = 0.002	0.75 (0.11) p = 0.911
LA diameter (cm)	3.06 (0.34)	2.83 (0.39) p = 0.001	2.77 (0.34) p < 0.001	3.01 (0.47) p = 0.851
LV mass (g)	133.1 (41.8)	105.1 (28.9) p < 0.001	97.8 (26.3) p < 0.001	125.7 (26.0) p = 0.623
LV mass index (g/m^2)	85.9 (21.5)	75.8 (17.5) p = 0.002	73.1 (16.3) p = 0.001	83.3 (18.9) p = 0.830
Fractional Shortening (%)	37.3 (4.1)	37.4 (3.7) p = 0.759	37.9 (3.8) p = 0.575	36.1 (3.2) p = 0.417
SV (mL)	56.8 (12.7)	50.1 (12.0) p = 0.001	48.4 (11.0) p < 0.001	54.6 (13.6) p = 0.714
SVI (mL/m^2)	37.3 (6.2)	35.6 (7.2) p = 0.141	35.5 (6.8) p = 0.308	35.9 (8.4) p = 0.674
CO (L/min)	3.83 (0.93)	3.04 (0.91) p < 0.001	2.90 (0.83) p < 0.001	3.43 (1.03) p = 0.156
CI ($L/min/m^2$)	2.55 (0.57)	2.16 (0.58) p < 0.001	2.13 (0.56) p < 0.001	2.26 (0.65) p = 0.088
MVCFc (circumferences/s)	1.15 (0.16)	1.20 (0.17) p = 0.082	1.22 (0.18) p = 0.054	1.14 (0.13) p = 0.983
Stress at peak systole ($dynes/cm^2$)	60.1 (16.8)	56.0 (12.9) p = 0.096	53.6 (12.4) p = 0.032	62.8 (12.1) p = 0.712

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index, LVEDD = left ventricular end-diastolic diameter, LVESD = left ventricular end-systolic diameter,

LVPWD = left ventricular posterior wall diameter, IVSD = interventricular septal wall diameter, LA = left atrium, LV = left ventricle, SV = stroke volume, SVI = stroke volume index, CO = cardiac output, CI = cardiac index, MVCFc = mean velocity of circumferential fiber shortening

*p values determined using one- way ANOVA comparing AN group to controls

§ p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 1.3: Transmitral and Pulmonary Venous Indices in all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=51)	Total AN Group* (n=95)	AN Subgroups[§]	
			BMI $\leq 10^{th}$ percentile (n=70)	BMI $>10^{th}$ percentile (n=25)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
MV E wave velocity (m/s)	0.87 (0.16)	0.90 (0.17) p = 0.232	0.92 (0.17) p = 0.180	0.85 (0.15) p = 0.878
MV A wave velocity (m/s)	0.37 (0.13)	0.35 (0.11) p = 0.239	0.34 (0.10) p = 0.261	0.37 (0.11) p = 0.993
E/A ratio	2.54 (0.79)	2.85 (1.05) p = 0.062	3.01 (1.11) p = 0.022	2.43 (0.73) p = 0.880
MV E wave deceleration time (ms)	168.0 (46.3)	168.1 (40.4) p = 0.994	164.5 (41.3) p = 0.894	178.1 (36.5) p = 0.593
MV A wave duration (ms)	114.3 (18.3)	113.4 (16.5) p = 0.778	111.5 (17.0) p = 0.652	118.8 (13.7) p = 0.518
MV propagation velocity (m/s)	74.9 (23.1)	71.3 (29.3) p = 0.503	70.2 (25.3) p = 0.680	74.4 (38.5) p = 0.998
PV S wave (m/s)	0.43 (0.12)	0.45 (0.13) p = 0.554	0.45 (0.13) p = 0.800	0.44 (0.13) p = 0.973
PV D wave (m/s)	0.61 (0.13)	0.63 (0.14) p = 0.333	0.63 (0.14) p = 0.569	0.62 (0.11) p = 0.905
PV A wave (m/s)	0.17 (0.04)	0.15 (0.04) p = 0.015	0.14 (0.04) p = 0.005	0.17 (0.05) p = 0.994
PV A wave duration (msec)	90.1 (18.2)	94.2 (21.3) p = 0.289	92.3 (22.5) p = 0.851	99.4 (17.0) p = 0.221

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index, MV = mitral valve, PV = pulmonary venous

*p values determined using one- way ANOVA comparing AN group to controls

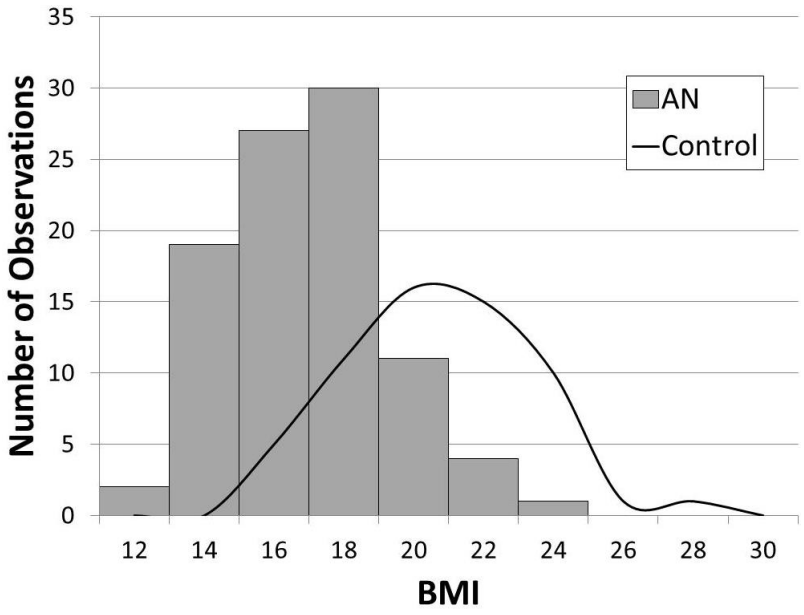
[§] p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 1.4: Tissue Doppler Indices in all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=50)	Total AN Group* (n=95)	AN Subgroups[§]	
			BMI ≤10th percentile (n=70)	BMI>10th percentile (n=25)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
LV lateral base S' (m/s)	10.4 (2.0)	10.4 (2.8) p = 0.965	10.7 (2.7) p = 0.846	9.6 (2.8) p = 0.411
LV lateral base E' (cm/s)	18.1 (3.0)	16.5 (3.4) p = 0.006	16.3 (3.6) p = 0.013	16.9 (2.7) p = 0.304
LV lateral base A' (cm/s)	5.5 (1.5)	4.8 (1.9) p = 0.034	4.7 (1.9) p = 0.030	5.3 (2.1) p = 0.912
IVS base S' (cm/s)	8.1 (1.1)	7.9 (1.5) p = 0.519	8.0 (1.6) p = 0.932	7.7 (1.3) p = 0.581
IVS base E' (cm/s)	15.1 (2.0)	13.7 (2.6) p = 0.001	13.6 (2.6) p = 0.004	13.7 (2.4) p = 0.055
IVS base A' (cm/s)	5.9 (1.1)	4.8 (1.5) p < 0.001	4.6 (1.5) p < 0.001	5.4 (1.4) p = 0.295
RV base S' (cm/s)	13.1 (1.6)	12.6 (2.3) p = 0.115	12.7 (2.3) p = 0.580	12.0 (2.3) p = 0.077
RV base E' (cm/s)	16.0 (2.9)	15.4 (3.7) p = 0.329	15.7 (3.7) p = 0.923	14.4 (3.4) p = 0.156
RV base A' (cm/s)	9.0 (2.6)	6.4 (2.5) p < 0.001	6.1 (2.3) p < 0.001	7.4 (2.8) p = 0.030
MV E wave/LV lateral base E' (cm/s)	5.0 (1.1)	5.7 (1.5) p = 0.004	5.9 (1.6) p = 0.001	5.2 (1.2) p = 0.840

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index, LV = left ventricle, IVS = interventricular septum, RV = right ventricle, MV = mitral valve
*p values determined using one- way ANOVA comparing AN group to controls
[§] p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

A.



B.

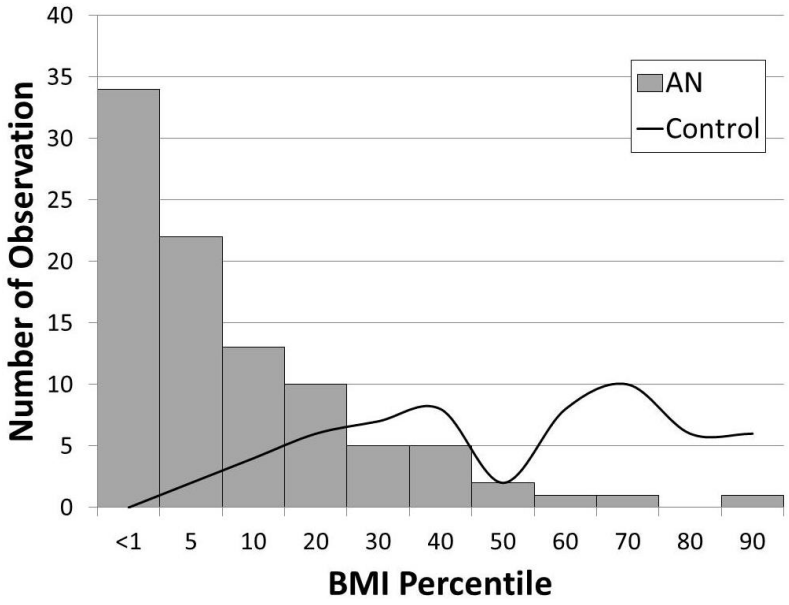


Figure 1.1. Frequency distributions of (A.) BMI and (B.) BMI percentile for the anorexia nervosa (AN) and control groups. BMI = body mass index.

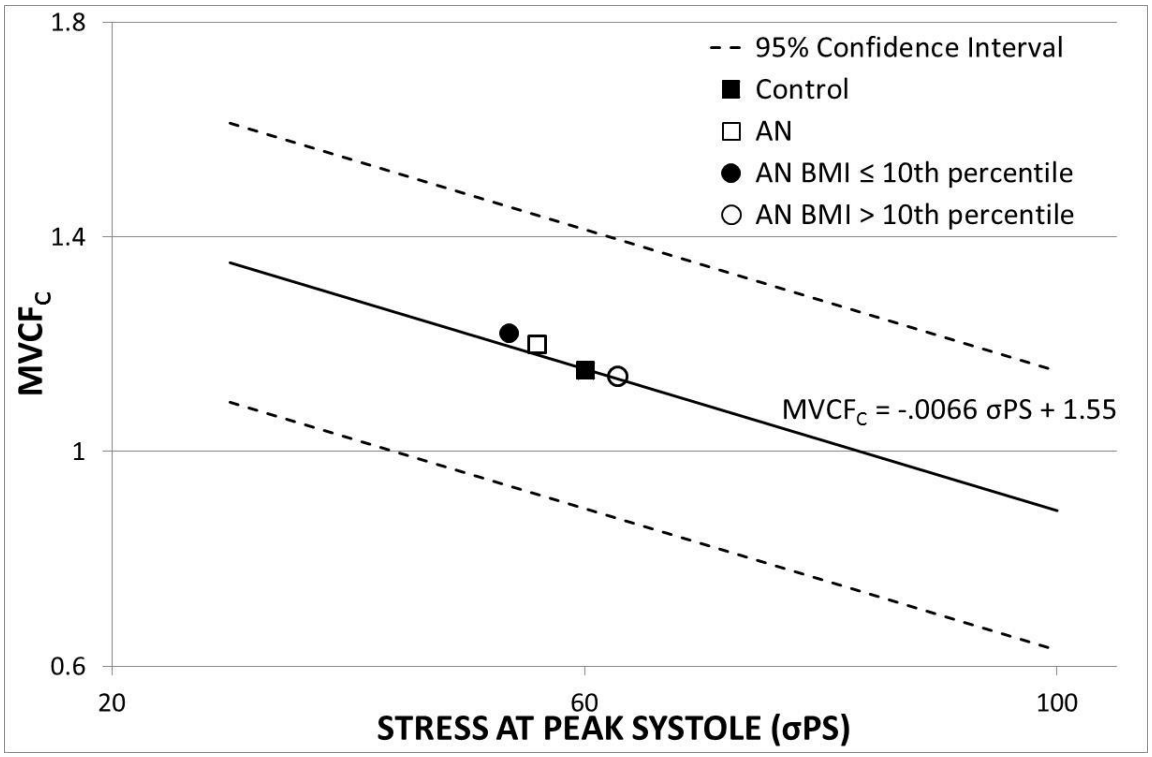


Figure 1.2. Comparison of the relationship of meridional wall stress at peak systole (σPS) to the mean velocity of circumferential fiber shortening (MVCFc) in adolescent patients with anorexia nervosa (AN), control subjects, and the subgroups of AN patients with a BMI ≤ 10th percentile and those with a BMI > 10th percentile. The normal relationship between MVCFc and σPS is provided by the solid line and the equation MVCFc = -0.0066σPS + 1.55, as determined by Sandor *et al*. (1992) [18]. The 95% confidence interval for this relationship is provided by the dashed lines. Permission to adapt this graph from the publication by Sandor *et al.* (1992) was obtained [18].

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Chapter 3: Biophysical Properties of the Aorta in Adolescent Females with Anorexia Nervosa

Abstract

Background/Hypothesis: Anorexia nervosa (AN) is associated with cardiovascular complications and abnormalities of biomarkers associated with cardiovascular risk. Arterial stiffness, which is a risk factor for cardiovascular disease, has not been previously studied in patients with AN. This study aimed to determine the biophysical properties of the aorta in adolescent females with AN as compared to control subjects using Doppler echocardiography.

Methods: This was a retrospective case-control study. Aortic diameter and pulse wave transit time were measured with echo-Doppler. Blood pressure was recorded at the time of the echocardiogram. Pulse wave velocity (PWV), aortic input impedance (Zi), characteristic impedance (Zc), arterial pressure-strain elastic modulus (Ep), and arterial wall stiffness index (β-index) were calculated. Patients were divided into those with BMI ≤ or > 10th percentile to assess the effect of patient malnutrition on the biophysical properties of the aorta.

Results: There were 94 adolescent females with AN and 60 adolescent female controls. There was no difference in age between AN patients and controls (15.5±1.7 vs 15.1±2.6 years, p=0.220). BMI (16.0±2.4vs 19.7 ± 2.7, p<0.001) and BMI percentile (9.4±15.6.vs 45.5 ± 26.2, p<0.001) were significantly lower for AN patients than for controls. PWV (443±106 vs 383±77cm/s, p<0.001), Zc (179±55 vs 149±37, p<0.001), and β-index

(3.07±1.09 vs 2.66±0.75, p=0.013) were significantly higher in AN patients than controls. There was no significant difference in Zi (198±53 vs 197±36, p=0.869) while Ep was decreased (232±84 vs 267±79, p=0.014) in AN patients compared to controls. PWV remained elevated compared to controls when AN patients were divided into those with BMI ≤ or > 10th percentile. Using multiple linear regression, the only significant predictor of PWV was the presence of AN.

Conclusions: The increased PWV, which is the most sensitive indicator of vascular dysfunction, indicates increased aortic stiffness in adolescent females with AN compared to controls. Increased PWV was not related to the degree of patient malnutrition. Our study suggests that patients with AN may be at increased risk of future cardiovascular disease. Further studies are required to determine if these changes persist with treatment and to determine long term outcomes.

Introduction

Anorexia nervosa (AN) is associated with many cardiovascular complications including electrophysiologic abnormalities, decreased left ventricular (LV) dimensions and wall mass, pericardial effusions, mitral valve prolapse, and reduced cardiac output [1-4]. AN also has the highest mortality rate of any psychiatric disorder, with some of these deaths attributed to cardiac complications such as arrhythmias [1, 5, 6]. There have also been case reports of women with AN who experience myocardial infarction (MI) secondary to atherosclerosis at a much earlier age than anticipated [7-9]. Several studies have shown that women with AN have risk factors for future atherosclerotic cardiovascular disease including dyslipoproteinemia, elevated high sensitivity C-reactive

protein (hsCRP), and increased interleukin-6 (IL-6) [10-19]. The majority of studies regarding risk factors associated with future cardiovascular disease have been performed in adult populations [10-14, 16-19].

Arterial stiffness is associated with cardiovascular disease including coronary artery disease and stroke [20, 21]. The most validated method of noninvasively measuring arterial stiffness is by measuring pulse wave velocity (PWV), which is defined as the speed with which a pulsatile blood wave travels along the length of an artery [20, 22]. PWV is a load-independent measure which is an independent predictor of stroke, coronary artery disease, and cardiovascular mortality in healthy and diseased adult populations [20, 21, 23-26]. PWV is considered the most sensitive and earliest indicator of cardiovascular risk [21, 27, 28]. PWV can be measured non-invasively using a simple Doppler echocardiography technique described by our laboratory [29]. Using this technique, the biophysical properties of the aorta including the PWV, characteristic impedance (Zc), input impedance (Zi), elastic pressure-strain modulus (Ep), and arterial wall stiffness index (β-index) can be assessed. Doppler echocardiography has been used to study the biophysical properties of the aorta in several pediatric and adult populations [29-37] and has been shown to be comparable with MRI-derived PWV measurements [38].

To our knowledge, arterial stiffness as an indicator of cardiovascular risk has not been previously studied in patients with AN. The aim of our study was to assess the biophysical properties of the aorta in adolescent females with AN. Our hypothesis was that adolescent females with AN would demonstrate abnormalities of the biophysical properties of the aorta as compared to healthy adolescent female controls.

Methods

Subjects

We performed a retrospective case-control study of female adolescents with AN compared to female adolescent controls. The AN patients were referred by the Eating Disorders Program for routine cardiac assessment at the Children's Heart Centre at British Columbia Children's Hospital between October 2003 and August 2010. The diagnosis of AN was made clinically by a multidisciplinary team including psychiatry and adolescent medicine and was based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria. Patients were studied during the acute phase of presentation and treatment for AN.

The control group was composed of healthy female adolescents from our established healthy control PWV database. The controls were of normal weight and did not have a history of AN. The controls were volunteers recruited from friends and family of hospital employees.

We recorded the echocardiographic data as well as height (cm), weight (kg), body mass index (BMI, kg/m^2), body surface area (BSA, m^2), heart rate (beats/min or bpm), systolic blood pressure (sBP, mmHg), diastolic blood pressure (dBP, mmHg), and pulse pressure (PP, mmHg) at the time of study. BSA was calculated using the DuBois formula $BSA\ (m^2) = 0.20247(height)^{0.725}(weight)^{0.425}$, with height measured in meters and weight in kilograms [39]. BMI percentiles were calculated for all subjects using data from the Centers for Disease Control growth data [40]. We determined the length of illness as the

duration of time between the diagnosis of AN and the time of the echocardiographic study. Ethics approval for this study was obtained from the Children's and Women's Hospitals' Research Ethics Board and the University of British Columbia's Clinical Research Ethics Board.

Doppler Echocardiography

All subjects underwent a complete echocardiogram including our lab's PWV protocol performed by an experienced echocardiographer. The biophysical properties of the aorta including the aortic stiffness and impedance indices were determined using Doppler echocardiography as previously described by our laboratory [29]. If patients had multiple cardiac assessments, only the data from the initial assessment were used. The diameter of the aortic annulus was measured at the level of the valve leaflets using two-dimensional (2D) echocardiography in the parasternal long-axis view. An M-mode recording of the ascending aorta was made from a high left or right parasternal view with the ascending aorta being kept as close to a right angle to the Doppler beam as possible. The maximum aortic diameter in systole (AOs) and the end-diastolic aortic diameter (AOd) were measured from this M-mode recording using the inner edge method. The aortic diameter measurements were averaged over three consecutive cardiac cycles.

In a suprasternal long axis view, a pulsed wave Doppler recording of the ascending aorta was obtained by placing a sample volume in the center of the aorta at the level of the valve leaflets. The peak aortic velocity was measured from this Doppler tracing. A pulsed wave Doppler recording of the descending aorta was then immediately obtained by placing the sample volume as distally as possible in the center of the

descending aorta. The ascending and descending aorta pulsed Doppler tracings were recorded at a speed of 100mm/s. These two tracings were recorded directly after one another to eliminate the possibility of a change in heart rate confounding the results. The time from the onset of the QRS to the onset of the ascending (T_1) and descending (T_2) aortic Doppler envelopes were measured over at least 3 cardiac cycles and were averaged. The transit time was calculated as the difference between these two times. Using the same suprasternal long axis view, the length of the aortic arch was measured along the central axis of the aorta using electronic calipers between the two points where the pulsed Doppler tracings were taken. Pulse wave Doppler interrogation in the long axis apical view was performed and the velocity-time integral (VTI) was obtained from this tracing.

Calculations

We calculated hemodynamic parameters and biophysical properties of the aorta using the equations given in Table 2.1.

Statistical Analysis

Our primary outcome of interest was the aortic PWV. Secondary outcomes included the Zi, Zc, Ep, and β index.

Univariate analysis was performed on all continuous variables. We compared the mean parameters of the total group of AN patients to the control group using a one way ANOVA. In order to determine the effect of the degree of patient malnutrition on the biophysical properties of the aorta, we performed an additional subgroup analysis by dividing the AN group into those with a BMI less than or equal to the 10^{th} percentile and

those with a BMI greater than the 10th percentile. These subgroups were compared to the control group using a one way ANOVA with post-hoc analysis performed using Tukey's test. We also subdivided the AN group in those of the AN binge/purging subtype (AN-BP) and the AN restrictive subtype (AN-R). We compared these subtypes to control subjects and each other using a one way ANOVA with post-hoc analysis performed using Tukey's test. We omitted the values of Ep and β-index from the analysis for one of the AN patients as these were outliers that distorted the statistical analysis. On review of the source data from this patient, the sampled plane of the aorta varied during the cardiac cycle, making the measurements of AOs and AOd inaccurate for this patient.

We performed Pearson product moment correlations to investigate the associations between BMI percentile, BSA, age, heart rate, and AN duration on the biophysical properties of the aorta. Since the range of normal BMI varies across the age distribution of our subjects, BMI percentile for age was used instead of the absolute BMI value to determine the relationship of body composition to the biophysical properties of the aorta. Multivariable linear regression was performed to determine the relative contribution of presence of AN, BMI percentile, heart rate, sBP, and age on PWV. As total arterial compliance (TAC) is inversely related to heart rate and directly related to body size, we performed a multiple linear regression analysis to control for these variables and determine if presence of AN is an independent predictor of TAC [41]. Inter-rater reliability for PWV was quantified using the intra-class correlation coefficient to assess absolute agreement using a two-way mixed model in a subset of 21 control patients. Due to the number of comparisons performed in our study, we considered $p \leq 0.005$ to indicate a statistically significant difference, p values of $0.005 < p \leq 0.05$ to be

marginally statistically significant, and p values >0.05 to be non-significant. All statistical analyses were performed using SPSS Statistics, version 17.0 (SPSS Statistics for Windows, Version 17.0, SPSS Inc., Chicago).

Results

Ninety-seven patients with AN were identified from our echocardiographic database during the study period. Three patients were excluded from the analysis: one for absent height and BMI data, another for evidence of LV non-compaction on the echocardiogram, and one for not having the PWV protocol performed. There were 94 adolescent females in the AN group and 60 healthy adolescent female controls. Table 2.2 summarizes the baseline characteristics of the AN and control groups. There were no significant differences in the mean age or height of the AN and control groups. The total AN group had a significantly lower mean weight, BMI, BMI percentile, BSA, mean heart rate, sBP, dBP, and PP as compared to control subjects. The lowest weight AN subgroup consisted of 70 patients with 24 patients in the higher weight AN group. On subgroup analysis, the AN patients with a BMI $\leq 10^{th}$ percentile had significantly decreased mean weight, BSA, BMI, BMI percentile, heart rate, sBP, dBP, and PP as compared to the control group. The only significant differences between the AN patients with a BMI $>10^{th}$ percentile and controls were decreased BMI percentile, sBP, and dBP. The mean time from diagnosis of AN to the time of study was 97 days (range 8-1009 days).

Hemodynamic parameters for all groups are provided in Table 2.3. When compared to controls, the total AN group had significantly decreased peak aortic velocity, peak aortic flow (AOflow), stroke volume (SV), and cardiac output (CO). AN patients

with a BMI $\leq 10^{th}$ percentile had significantly decreased peak aortic velocity, SV, and CO compared to controls, while there were no differences between the controls and AN patients with a BMI > 10^{th} in any of the parameters outlined in Table 2.3. There were no differences in aortic annulus diameter, aortic cross sectional area (AOCSA), or total arterial compliance (TAC) between controls and the total AN group or the AN patients with BMI $\leq 10^{th}$ percentile and > 10^{th} percentile. Multiple linear regression analysis was performed using TAC as the dependent variable and presence of AN, height, weight, and heart rate as independent variables. The model was statistically significant and explained a small amount of the variance in TAC, $F(4,139)=10.420$, $p<0.001$, adj $R^2=0.209$. Presence of AN was not an independent predictor of TAC ($p=0.116$) although heart rate ($\beta= -0.326$, $p<0.001$) and weight ($\beta= 0.258$, $p=0.034$) were independent predictors.

The biophysical properties of the aorta for the total AN group as compared to controls are summarized in Table 2.4. The PWV was significantly increased in AN patients as compared to controls. In addition, Zc and β-index were significantly increased and Ep was significantly decreased in AN patients as compared to controls. Zi was similar between the AN and control groups. On subgroup analysis, PWV and Zc remained significantly increased for both the BMI $\leq 10^{th}$ percentile and BMI >10^{th} percentile subgroups as compared to controls. Ep remained significantly decreased and β-index trended towards being significantly increased in the AN patients with a BMI $\leq 10^{th}$ percentile, with no significant changes in AN patients with a BMI >10^{th} percentile.

There was no correlation between PWV or β–index and BMI percentile, BSA, heart rate, or age. Zi correlated with heart rate ($r=0.176$, $p=0.031$) and inversely correlated with BSA ($r=-0.311$, $p<0.001$) and age ($r=-0.354$, $p<0.001$). Zc inversely correlated with

BMI percentile (r=-0.212, p=0.008), BSA (r= -0.371, p<0.001), and age (r= -0.213, p=0.008). Ep correlated with heart rate (r=0.287, p=0.001), BMI percentile (r=0.226, p=0.006), BSA (r=0.333, p<0.001), and age (r= 0.233, p=0.005). There was no correlation between the time since diagnosis of AN at the time of study and the biophysical properties of the aorta. Multiple linear regression analysis using PWV as the dependent variable and presence of AN, heart rate, BMI percentile, sBP, and age as independent variables produced a statistically significant model which explained a small amount of the variability in PWV, $F(5,144)=2.506$, $p=0.005$, adj $R^2=0.078$. The only significant predictor of PWV was the presence of AN ($\beta=0.344$, $p=0.003$) with an unstandardized $\beta=71.479$.

Twenty-two of the AN patients were of the AN-BP subtype and 72 were the AN-R subtype. As shown in Table 2.5, patients with AN-R had similar age and height as controls with decreased weight, BSA, BMI, BMI percentile, sBP, dBP, heart rate, and PP compared to controls. In comparison to control patients, patients with AN-BP were older and had similar height, weight, BSA, and PP. AN-BP patients had decreased BMI, BMI percentile, sBP, dBP, and heart rate compared to controls. When compared to AN-BP patients, AN-R patients were younger (p=0.028) and had decreased weight (p=0.002), BSA (p=0.009), and BMI (p<0.001) with no significant differences in height (p=0.420), BMI percentile (p=0.112), sBP (p=0.966), dBP (p=0.582), heart rate (p=0.957), or PP (p=0.404). The hemodynamic parameters and biophysical properties of the aorta in the AN-R and AN-BP patients as compared to controls are shown in Table 2.5. Patients with AN-R had decreased peak aortic velocity, peak aortic flow, SV, and CO as compared to controls. AN-R patients had increased PWV and Zc as compared to controls, while the

differences in Ep and β index trended towards significance. The only significant difference in hemodynamic parameters and biophysical properties of the aorta between the AN-BP patients and controls was an increased PWV. There were no significant differences in hemodynamic parameters or biophysical properties of the aorta between AN-R and AN-BP patients.

Our inter-rater reliability for determining PWV was good with an intra-class correlation coefficient of 0.856 ($p<0.001$).

Discussion

To our knowledge, our study is the first to demonstrate increased arterial stiffness in patients with AN. In a large sample of adolescent females with AN, we found that PWV was significantly increased in comparison to controls. PWV is considered the gold standard index of aortic stiffness and an elevation in PWV has been demonstrated to be an independent, sensitive, and early indicator of cardiovascular risk in healthy adults as well as adults with hypertension, diabetes, and renal disease [21, 27, 28]. There are different methods available to assess PWV, including the carotid-femoral method, Doppler echocardiography, and MRI, with the carotid-femoral method being the most commonly used method in adult patients [22, 29, 38]. Our study used Doppler echocardiography, which has also been used to show elevations in aortic PWV in other pediatric populations including patients with a history of Marfan syndrome, inflammatory connective tissue disease, Kawasaki disease, systemic lupus erythematosus as well as obese children and those born small for gestational age [29, 31-34, 36, 42, 43].

Our study indicates that adolescent females with AN have a stiffer thoracic aorta than healthy controls. There are multiple mechanisms that have been associated with increased arterial stiffness including structural changes in collagen and elastin caused by inflammation, increased luminal pressure, and abnormalities of remodeling pathways, as well as changes in vascular smooth muscle tone which can be caused by abnormalities in angiotensin II, endothelin, oxidative stress, and nitric oxide [44]. The pathological processes that lead to a stiffer aorta in the setting of AN are unclear. One possible mechanism of increased aortic stiffness is endothelial dysfunction. It has been suggested that nitric oxide and its association with endothelial function may play a role in the stiffness of large arteries [45]. Patients with AN are nutritionally deficient in L-arginine, an aminoacid which helps maintain endogenous nitric oxide activity, and this deficiency may potentiate arterial stiffness and cardiovascular risk in this population [46]. Patients with AN also have abnormalities in the renin-angiotensin-aldosterone system, and it is possible that these abnormalities may play a role in arterial stiffness [47, 48]. Aortic stiffness impedes the ejection of blood from the LV, thus increasing the work of the heart which subsequently promotes cardiac hypertrophy. Despite the increase in arterial stiffness in the setting of AN, the heart may be able to continue normal function and circumvent a hypertrophic response as the relative hypotension associated with AN decreases the LV afterload and may counteract the effect of a stiffer aorta. Although we were able to document increased arterial stiffness in patients with AN, it is unknown if these changes are permanent or reversible. Studies have demonstrated that other cardiovascular changes including decreased LV mass, decreased LV dimensions, bradycardia, dyslipidemia, and cardiac output can be improved with weight restoration in

patients with AN, however, the effect of weight restoration on vascular properties has not been documented [3, 49, 50].

The finding of increased arterial stiffness in patients with AN may indicate that these patients have increased future cardiovascular risk including hypertension, coronary artery disease and stroke. Our findings, suggestive of potentially increased cardiovascular risk in patients with AN, are supported by literature indicating that patients with AN have abnormalities in biomarkers which are associated with cardiovascular risk. Multiple studies have shown that patients with AN have elevated cholesterol and dyslipoproteinemia when compared to controls [10, 11, 13, 14, 16-19, 51]. Abnormalities in inflammatory markers including hsCRP and IL-6 have also been documented in women with AN. Lawson *et al.* (2007) found that 20% of women with AN who were taking oral contraceptive pills had high-risk hsCRP levels [12]. In a study of 23 adolescent girls with AN, Misra *et al*. (2006) reported an "uncoupling" of cardiovascular risk factors [15]. They found decreased hsCRP, low triglycerides, and elevated high density lipoprotein (HDL) levels which would suggest decreased cardiovascular risk, however, they also found increased IL-6, apolipoprotein B (ApoB), and increased ratios of ApoB/HDL and ApoB/low density lipoprotein (LDL) which indicate increased cardiovascular risk.

There remains some controversy in the literature regarding cardiovascular risk in females with AN, as some studies have suggested an absence of risk factors associated with future cardiovascular disease. Arden *et al.* (1990) showed no difference in cholesterol values or LDL between patients with AN and controls at admission, although the authors acknowledged that the study may have been underpowered to detect a

difference between the groups [52]. Birmingham *et al*. (2003) found no difference in the intima-media thickness of the carotid artery, which correlates with coronary atherosclerosis, between AN patients with a history of chest pain and healthy controls [53, 54]. Although the actual risk of myocardial infarction (MI) and the contribution of the aforementioned risk factors to future cardiovascular events are unknown, there are case reports of MIs secondary to atherosclerosis in women aged 37-41 years [7-9]. Despite some negative studies regarding cardiovascular risk factors in AN, the literature supporting increased rates of dyslipoproteinemia and inflammatory biomarkers as well as the case reports of MI in patients with AN suggest that this patient population may be at increased cardiovascular risk. Although increased arterial stiffness can occur in the absence of abnormalities of biomarkers, our findings of elevated PWV in AN further strengthens the hypothesis that these patients may be at risk for future cardiovascular disease.

PWV did not correlate with BMI percentile and remained elevated in comparison to controls when patients were divided into those with BMI $\leq$ and $> 10^{th}$ percentile. Our study was thus not able to document a relationship between arterial stiffness and the degree of malnutrition. PWV also did not correlate with the time since diagnosis of AN in our patient population, although we only studied the PWV at the first available echocardiogram in the acute phase of treatment of AN. Investigation of patients with longer durations of illness would be needed to determine if there is a relationship between disease duration and arterial stiffness. We did not find a correlation between PWV and heart rate, however, we did not manipulate patient heart rate in our study to investigate its relationship with PWV. The relationship between PWV and heart rate has been shown

elegantly in studies of patients with pacemakers where an increase in the patient's heart rate confers an increase in their PWV [55-57]. As such, relative bradycardia in our AN patients as compared to controls may be masking the degree of arterial stiffness in our patients with AN. The mechanism of PWV increase with tachycardia is poorly defined, although it has been suggested that there may be a reduction in the time for elastic recoil of the arterial wall due to a shortened cardiac cycle [55-57]. The results of our multiple linear regression analysis demonstrated that in a model using presence of AN, heart rate, BMI percentile, sBP, and age, the only independent predictor of PWV was presence of AN, and that the presence of AN increases the PWV by a mean of 71.5cm/s when the other variables in the model are held constant. The R^2 value of our model was low indicating that there may be other factors which were not measured in our study that influence the degree of arterial stiffness in AN.

Analysis of the other measures of aortic stiffness demonstrates variable results in AN patients. In contrast to PWV, many of these indices are load-dependent indicators of aortic stiffness. Normal pressure and flow pulses of the arterial system consist of a set of sinusoidal waves or harmonics [58]. Impedance is the ratio of a pressure harmonic to the flow harmonic at the same frequency [58]. The aortic impedance spectrum represents the ratio of pressure and flow harmonics existing in the aortic root and is analogous to vascular resistance [33, 58]. The physical properties of the aorta, including the viscoelasticity, inertia, and aortic dimensions, determine the characteristic impedance (Zc) [33, 58]. Input impedance (Zi) represents the resistance to ejection of the LV in a pulsatile flow system and is influenced by reflected pressure and flow waves generated in the distal arterial tree [33, 58]. In addition to heart rate, height, and arterial branching

affecting the reflected waves, increased arterial stiffness also influences the timing of wave reflections to alter the aortic input impedance. Zc was significantly increased in the AN group due to the inclusion of PWV in the formula to calculate Zc. The elevation in Zc is consistent with increased aortic stiffness in AN patients as compared to controls. There was no difference in Zi between the AN groups and controls due to a significant decrease in both the peak aortic flow and PP in the AN group as compared to controls, which are both components of the equation to calculate Zi. The Ep and β index measures are attempts at applying Hooke's law of stress and strain. Ep expresses the ratio of stress, or PP, to the strain, or the percent change in the arterial diameter during the cardiac cycle. The AN patients in our study showed a decrease in Ep as compared to controls, which would signify decreased arterial stiffness, but this decrease in Ep was likely caused by the significantly decreased PP in AN patients as compared to controls. β index is reported to be less load-dependent within the physiologic range of intravascular pressure than impedance values and Ep [59]. The elevation in β index in the AN group is consistent with increased aortic stiffness in AN patients as compared to controls.

Total arterial compliance (TAC) characterizes the entire arterial tree and is the inverse of arterial stiffness [60]. TAC is used as a surrogate index of arterial stiffness such that increased stiffness results in TAC becoming decreased, and it is an independent predictor of cardiovascular events and mortality in patients with hypertension [60-62]. The AN patients in our study showed no difference in TAC as compared to control subjects. TAC is inversely related to heart rate and directly related to body size, but despite controlling for these factors, the presence of AN was not an independent predictor of TAC in our study [41]. TAC was estimated in our study by the ratio of PP to SV. This

method of estimation is based on an ejection phenomenon, and has been shown to correlate with Liu's method of estimating TAC based on diastolic decay of the aortic pressure [63, 64]. However, the SV/PP method was derived in the setting of normal individuals based on their pattern of diastolic decay and may not be applicable in the AN population.

Our study demonstrated mixed results when investigating the additional measures of the biophysical properties of the aorta. The increased Zc and β index in AN patients suggests increased arterial stiffness in this population, while the results of Zi, TAC, and Ep suggest either unchanged or decreased arterial stiffness. These additional measures of the biophysical properties of the aorta have their limitations and may be of questionable utility in the setting of our AN population due to the significant differences in the hemodynamic properties of the cardiovascular system in AN patients as compared to controls, particularly the relative hypotension and decreased PP, SV, CO, and peak aortic flow. Since PP, BP, SV, and peak aortic flow are used to calculate these other measures of arterial stiffness, the significant abnormalities in the hemodynamic parameters in AN patients may account for our mixed results. The measures of Zi, Ep, β index, and TAC are also limited due to their load-dependence. PWV, in contrast, is not calculated using these hemodynamic parameters and is load-independent. Zc is heavily influenced by the value of PWV as this is a part of the equation used to calculate Zc. Therefore, the best indicator of arterial stiffness remains PWV which demonstrated increased arterial stiffness in our adolescent AN population. The increased PWV in AN was not related to the degree of patient malnutrition as assessed by BMI percentile; however, the patients with a BMI $\leq 10^{th}$ percentile were more hemodynamically abnormal than those with a

BMI >10th percentile, and therefore had greater aberrations in the additional indices of aortic stiffness.

There have been indications of differences in the serum biomarkers associated with cardiovascular risk between AN subtypes. Case *et al*. (1999) compared fasting lipid levels in patients with bulimia nervosa (BN), AN-BP, and controls [51]. They found that the AN-BP patients had significantly higher cholesterol, ApoB, apolipoprotein A1, and LDL-cholesterol levels than BN patients and controls, and elevated triglycerides and intermediate-density lipoprotein-apoB levels when compared to controls [51]. Rigaud *et al*. (2009) found that total cholesterol and LDL-cholesterol levels were greater in AN patients with the binge-purge subtype than in those with the restrictive subtype [18]. Our study showed that PWV remained increased in comparison to controls for both the AN-R and AN-BP groups, and that there was no significant difference in aortic stiffness between the AN-R and AN-BP groups.

Limitations of our study include the retrospective case-control nature of the study and the potential biases associated with this design including selection bias (as this study was performed at a tertiary care center and may have included more severely affected patients than the general AN population) and misclassification/information bias (via the misclassification of AN patients and their subtypes). The method of measuring PWV using Doppler echocardiography has its limitations including difficulties in obtaining accurate aortic dimensions in the setting of poor echocardiographic windows and the potential for measurement error due to the small time differences between the onset of Doppler flow in the ascending and descending aorta. Although these limitations could potentially lead to making large differences in a single patient's PWV due to small

measurement errors, the coefficient of variation for interobserver, intersessional, and inter-reviewer assessments has been reported to range between 2-16% [33], and our study demonstrated good inter-rater reliability in the calculation of PWV. We did not have the results of cholesterol or other serum biomarkers associated with cardiovascular risk and thus were not able to relate these risk factors to PWV. TAC and the biophysical properties of the aorta other than PWV have their limitations as they are calculated using hemodynamic variables, as discussed previously. The validity of using these calculated values is unclear in the face of the significant differences in multiple hemodynamic parameters seen in AN patients as compared to controls. We also did not assess the wave reflection of the arterial tree in the AN patients. The number of control patients in our study was fewer than the number of patients due to the difficulty of recruiting healthy adolescent female volunteers to undergo echocardiography. It is possible that an increased number of control patients could have demonstrated further differences between the patient and control groups. As well, due to the minority of patients having a BMI >10th percentile or the AN-BP subtype, we may have been underpowered to detect differences between these groups and controls.

Future studies are required in order to determine the reversibility of the arterial changes in patients with AN following weight restoration. The impact of increased arterial stiffness or any of the serum biomarkers associated with cardiovascular risk on the incidence of MI in AN is also unknown. The effect of hemodynamic stress or exercise on the biophysical properties of the aorta in patients with AN would also be an area of future investigation. As there is no other published literature on arterial stiffness in AN, it is important for the results of the present investigation to be repeated in future studies.

In conclusion, this is the first study to investigate arterial stiffness as indicated by PWV in adolescent patients with AN using Doppler echocardiography. PWV was significantly elevated in adolescent patients with AN. Increased PWV in AN was not related to the degree of patient malnutrition as assessed by BMI percentile. Our study suggests that patients with AN may be at increased risk for future cardiovascular disease.

Table 2.1 Formulae for Calculating the Biophysical Properties of the Aorta

Parameter	Formula
AOCSA (cm^2)	$(\pi/4)$ (aortic annulus diameter)^2
AOflow (cm^3/s)	(peak aortic velocity) · AOCSA
SV (mL)	VTI · AOCSA
CO (L/min)	(Heart rate) · SV/1000
PP (mmHg)	sBP-dBP
TAC (mL/mmHg)	SV/PP
TT (s)	$T_2 - T_1$ (T_2 = time from onset of QRS to onset of descending aortic Doppler envelope; T_1 = time from onset of QRS to onset of ascending aortic Doppler envelope)
PWV (cm/s)	aortic length/TT
Ep (mmHg)	PP/[(AOs – AOd)/AOd]
β index	ln(sBP/dBPd)/[(AOs-AOd)/AOd]
Zi ($dyne \cdot s/cm^5$)	PP/AOflow
Zc ($dyne \cdot s/cm^5$)	PWV·ρ/AOCSA (ρ is the density of blood = 1.06 g/cm^3)

AOCSA = aortic cross-sectional area, AOflow = peak aortic flow, SV = stroke volume, VTI = velocity time integral, CO = cardiac output, TT = transit time, PWV = pulse wave velocity, Ep = Elastic pressure-strain modulus, sBP = systolic blood pressure, dBP = diastolic blood pressure, AOs = maximum aortic diameter, AOs = end-diastolic aortic diameter, β-index = Arterial wall stiffness index, PP = pulse pressure, Zi = Input impedance, Zc = Characteristic impedance, 1 mmHg = 1,333 $dyne/cm^2$, ρ = blood density = 1.06 g/cm^3

Table 2.2: Baseline Characteristics of all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=60)	Total AN Group* (n=94)	AN Subgroups[§]	
			BMI ≤10th percentile (n=70)	BMI>10th percentile (n=24)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	15.1 (2.6)	15.5 (1.7) p = 0.220	15.4 (1.8) p = 0.702	15.9 (1.2) p = 0.249
Height (cm)	160.5 (9.8)	161.6 (8.5) p = 0.446	161.4 (9.1) p = 0.821	162.1 (6.3) p = 0.723
Weight (kg)	51.2 (10.3)	42.0 (8.2) p < 0.001	39.2 (6.7) p < 0.001	50.1 (6.8) p = 0.856
BSA (m^2)	1.52 (0.19)	1.40 (0.16) p = 0.002	1.36 (0.15) p < 0.001	1.52 (0.12) p = 1.000
BMI (kg/m^2)	19.7 (2.7)	16.0 (2.4) p < 0.001	15.0 (1.7) p < 0.001	19.0 (1.6) p = 0.359
BMI percentile	45.5 (26.2)	9.4 (15.6) p < 0.001	2.2 (3.1) p < 0.001	30.6 (17.9) p = 0.002
Systolic BP (mmHg)	108.9 (9.5)	95.5 (9.3) p < 0.001	94.0 (9.4) p < 0.001	99.6 (7.9) p < 0.001
Diastolic BP (mmHg)	65.3 (9.1)	58.2 (6.7) p < 0.001	57.8 (6.4) p < 0.001	59.5 (7.6) p = 0.007
Heart rate (bpm)	69.4 (14.6)	61.1 (13.0) p < 0.001	60.5 (13.3) p = 0.001	62.9 (12.1) p = 0.126
Pulse pressure (mmHg)	43.6 (7.8)	37.3 (7.5) p < 0.001	36.3 (7.4) p < 0.001	40.1 (7.1) p = 0.133

AN= Anorexia nervosa, SD = standard deviation, BP = blood pressure, BMI = body mass index

*p values determined using one- way ANOVA comparing AN group to controls

[§] p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 2.3: Hemodynamic Parameters in all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=60)	Total AN Group* (n=94)	AN Subgroups[§]	
			BMI ≤10th percentile (n=70)	BMI>10th percentile (n=24)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Aortic Annulus Diameter (cm)	1.86 (0.17)	1.85 (0.17) p = 0.740	1.85 (0.17) p = 0.855	1.87 (0.16) p = 0.968
AOCSA (cm^2)	2.74 (0.49)	2.72 (0.49) p = 0.743	2.70 (0.50) p = 0.863	2.77 (0.45) p = 0.974
Peak aortic velocity (m/s)	1.08 (0.16)	0.99 (0.18) p = 0.002	0.98 (0.19) p = 0.004	1.01 (0.16) p = 0.247
AOflow (cm^3/s)	2.91 (0.58)	2.68 (0.68) p = 0.039	2.64 (0.69) p = 0.057	2.80 (0.63) p = 0.765
AOd (cm)	2.05 (0.25)	2.04 (0.27) p = 0.776	2.03 (0.30) p = 0.846	2.08 (0.19) p = 0.914
AOs (cm)	2.40 (0.24)	2.39 (0.27) p = 0.760	2.37 (0.30) p = 0.799	2.43 (0.19) p = 0.855
AOs-AOd/AOd	0.174 (0.047)	0.174 (0.056) p = 0.934	0.175 (0.057) p = 0.994	0.174 (0.052) p = 1.000
SV (mL)	56.8 (12.7)	50.1 (12.0) p = 0.001	48.4 (11.0) p < 0.001	55.0 (13.7) p = 0.807
CO (L/min)	3.83 (0.93)	3.04 (0.92) p < 0.001	2.90 (0.83) p < 0.001	3.45 (1.04) p = 0.197
TAC (mL/mmHg)	1.29 (0.34)	1.39 (0.41) p = 0.153	1.38 (0.40) p = 0.452	1.41 (0.44) p = 0.394

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index
AOCSA = aortic cross-sectional area, AOflow = peak aortic flow, AOd = diastolic diameter of the ascending aorta, AOs = systolic diameter of the ascending aorta, SV = stroke volume, CO = cardiac output, TAC = total arterial compliance
*p values determined using one- way ANOVA comparing AN group to controls
[§] p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 2.4: Biophysical Properties of the Aorta in all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=60)	Total AN Group* (n=94)	AN Subgroups[§]	
			BMI $\leq 10^{th}$ percentile (n=70)	BMI $>10^{th}$ percentile (n=24)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
PWV (cm/s)	383 (77)	443 (106) $p < 0.001$	439 (107) $p = 0.003$	454 (106) $p = 0.007$
Zi (dyne·s/cm^5)	197 (36)	198 (53) $p = 0.869$	198 (53) $p = 0.995$	200 (56) $p = 0.971$
Zc (dyne·s/cm^5)	149 (37)	179 (55) $p < 0.001$	178 (56) $p = 0.002$	179 (54) $p = 0.027$
Ep (mmHg)	267 (79)	232 (84) $p = 0.014$	230 (89) $p = 0.040$	238 (73) $p = 0.347$
β index	2.66 (0.75)	3.07 (1.09) $p = 0.013$	3.06 (1.12) $p = 0.058$	3.08 (1.01) $p = 0.190$

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index, PWV = pulse wave velocity, Zi = Input impedance, Zc = Characteristic impedance , Ep = Elastic pressure-strain modulus, β-index = Arterial wall stiffness index

*p values determined using one- way ANOVA comparing AN group to controls

[§] p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 2.5 Hemodynamic Parameters and Biophysical Properties of the Aorta in Patients with AN Divided by Subtype Compared to Controls

	Control Group (n=60)	AN Restrictive Subtype* (n=72)	AN Binge-Purge Subtype* (n=22)
	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	15.1 (2.6)	15.2 (1.7) p = 0.938	16.5 (1.1) p = 0.017
Height (cm)	160.5 (9.8)	160.9 (9.1) p = 0.948	163.7 (5.4) p = 0.318
Weight (kg)	51.2 (10.3)	40.3 (8.1) p < 0.001	47.6 (6.1) p = 0.236
BSA (m^2)	1.52 (0.19)	1.37 (0.16) p < 0.001	1.49 (0.10) p = 0.850
BMI (kg/m^2)	19.7 (2.7)	15.4 (2.3) p < 0.001	17.8 (2.1) p = 0.005
BMI percentile	45.5 (26.2)	7.1 (12.5) p < 0.001	17.0 (21.5) p < 0.001
Systolic BP (mmHg)	108.9 (9.5)	95.4 (8.6) p < 0.001	96.0 (11.7) p < 0.001
Diastolic BP (mmHg)	65.3 (9.1)	58.7 (6.7) p < 0.001	56.8 (6.9) p < 0.001
Heart rate (bpm)	69.4 (14.6)	61.4 (13.7) p = 0.003	60.4 (10.6) p = 0.026
Pulse pressure (mmHg)	43.6 (7.8)	36.8 (7.2) p < 0.001	39.2 (8.4) p = 0.058
Aortic Annulus Diameter (cm)	1.86 (0.17)	1.84 (0.18) p = 0.822	1.88 (0.12) p = 0.902
AOCSA (cm^2)	2.74 (0.49)	2.70 (0.52) p = 0.842	2.79 (0.36) p = 0.932
Peak aortic velocity (m/s)	1.08 (0.16)	0.9 (0.18) p = 0.002	1.04 (0.19) p = 0.588
AOflow (cm^3/s)	2.91 (0.58)	2.63 (0.69) p = 0.036	2.88 (0.59) p = 0.970
AOd (cm)	2.05 (0.25)	2.04 (0.29) p = 0.944	2.05 (0.20) p = 0.998
AOs (cm)	2.40 (0.24)	2.38 (0.29) p = 0.883	2.41 (0.21) p = 0.972

AOs-AOd/AOd	0.174 (0.047)	0.172 (0.058) p = 0.987	0.181 (0.049) p = 0.824
SV (mL)	56.8 (12.7)	48.8 (11.6) p = 0.001	54.3 (12.7) p = 0.683
CO (L/min)	3.83 (0.93)	2.96 (0.89) p < 0.001	3.29 (0.97) p = 0.053
TAC (mL/mmHg)	1.29 (0.34)	1.37 (0.40) p = 0.503	1.44 (0.45) p = 0.279
PWV (cm/s)	383 (77)	439 (105) p = 0.003	456 (113) p = 0.007
Zi (dyne·s/cm^5)	197 (36)	201 (56) p = 0.843	187 (40) p = 0.685
Zc (dyne·s/cm^5)	149 (37)	179 (58) p = 0.001	176 (46) p = 0.066
Ep (mmHg)	267 (79)	235 (91) p = 0.080	224 (61) p = 0.117
β index	2.66 (0.75)	3.07 (1.15) p = 0.051	3.07 (0.87) p = 0.244

AN= Anorexia nervosa, SD = standard deviation, BP = blood pressure, BMI = body mass index, AOCSA = aortic cross-sectional area, AOflow = peak aortic flow, AOd = diastolic diameter of the ascending aorta, AOs = systolic diameter of the ascending aorta, SV = stroke volume, CO = cardiac output, TAC = total arterial compliance, PWV = pulse wave velocity, Zi = Input impedance, Zc = Characteristic impedance , Ep = Elastic pressure-strain modulus, β-index = Arterial wall stiffness index

* p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subtypes to controls

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Chapter 4: Exercise Capacity and Ventricular Function during Semisupine Stress Cycle Ergometry in Adolescent Females with Anorexia Nervosa

Abstract

Background: Patients with anorexia nervosa (AN) have altered physiological responses to exercise. We investigated exercise capacity and echocardiographic ventricular function with exercise in adolescent patients with AN.

Methods: This was a retrospective case-control study. Sixty-six adolescent females with AN and 21 adolescent female controls exercised on a semi-recumbent ergometer to volitional fatigue in 3 minute states with 20 watt increments. Heart rate (HR), blood pressure (BP), and echo-doppler indices were measured at rest and at each stage. Fractional shortening (FS), mean velocity of circumferential fiber shortening (MVCFc), cardiac output (CO), cardiac index (CI), total arterial compliance (TAC), and systemic vascular resistance (SVR) were calculated at rest and each stage of exercise. Peak oxygen consumption (VO_2), minute ventilation (VE), and respiratory exchange ratio (RER) were measured using open-circuit spirometry.

Results: Patients with AN had a significantly lower BMI (16.7 vs 19.7kg/m^2, $p<0.001$), total work (1125 vs 1914J/kg, $p<0.001$), and test duration (13.8 vs 20.8min, $p<0.001$) when compared to controls. Multivariable linear regression demonstrated that BMI percentile, age, peak VO_2, and peak CO were significant independent predictors of exercise duration. Total work, peak VE, and peak VO_2 were significantly decreased in

AN patients. HR, systolic BP, CO, CI, FS, and MVCFc demonstrated similar patterns of increase with progressive exercise between groups, but were decreased at peak exercise in AN patients. At peak exercise, SVR was similar between groups and TAC was increased in patients with AN, with similar patterns of response to progressive exercise.
Conclusions: Adolescent patients with AN have reduced exercise capacity compared to controls but have normal patterns of cardiovascular response to progressive exercise. Exercise endurance is independently predicted by BMI percentile.

Introduction

Cardiac involvement occurs in approximately 80% of patients with anorexia nervosa (AN) [1-3]. These cardiovascular changes include reductions in heart rate (HR), blood pressure (BP), myocardial mass, left ventricular (LV) dimensions, and resting cardiac output (CO) [1-7]. Multiple studies have demonstrated that standard measures of systolic ventricular function at rest are unchanged in patients with AN [3-5, 7, 8]. There are, however, some studies that suggest possible subclinical cardiac dysfunction in patients with AN as abnormalities in the myocardial performance index and systolic tissue Doppler velocities have been described in this patient population [8, 9].

Exercise imposes physiologic and hemodynamic stresses on the cardiovascular system. Normal exercise endurance necessitates adequate myocardial performance to maintain stroke volume (SV) and CO. In the setting of abnormalities of the cardiac system and suggestions of subclinical systolic ventricular dysfunction in AN, it is important to determine myocardial performance with exercise in this patient population.

Patients with AN have been reported to have reduced exercise endurance, decreased maximal oxygen uptake (VO_2), and blunted HR and BP responses with exercise [7, 10-12]. Information regarding myocardial performance and contractility with exercise and the pattern of cardiovascular response to staged physical activity in patients with AN is limited. Rowland *et al*. (2003) studied 8 adolescent females with AN compared to healthy controls using an upright cycle ergometer and found decreased VO_2, increased SV index (SVI), and no change in the cardiac index (CI) in patients with AN compared to controls [13]. To our knowledge, there are no studies investigating myocardial function during exercise using echocardiography in patients with AN. In addition, the few studies that have been performed investigating the cardiovascular responses to exercise in AN have been limited by small sample sizes.

Myocardial function can be studied using echocardiographic measurements taken while a patient is exercising using semi-supine cycle ergometry (SSCE) stress echocardiography. This methodology allows for measurement of SV, CO, cardiac contractility, and load-independent myocardial function in addition to the standard indices of exercise tolerance, BP, and HR response. These measurements can be followed during incremental exercise to determine the pattern of cardiac response to progressive exercise. This methodology has been utilized to determine the cardiovascular responses to progressive exercise in other pediatric populations including healthy children and patients with cardiac transplant, myocardial dysfunction, and oncology patients with previous anthracycline use [14-17].

This study was designed to assess cardiovascular responses to progressive and maximal exercise in adolescent females with AN as compared to controls. To achieve

this, we used SSCE stress echocardiography and measured metabolic responses as well as indicators of myocardial function which have not been previously investigated in adolescent AN patients. To our knowledge, this is also the largest study of cardiovascular responses to exercise in adolescent females with AN.

Methods

Subjects:

We performed a retrospective case-control study. The patient group consisted of female adolescents with AN who were referred by the Eating Disorders Program for stress echocardiography at the Children's Heart Centre at the British Columbia Children's Hospital between February 2004 and March 2012. The diagnosis of AN was made clinically by a multidisciplinary team including psychiatry and adolescent medicine and was based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria [18]. Patients were studied during the acute phase of presentation and treatment for AN. If patients had more than one assessment, only the data from the complete initial assessment were used. The control group was composed of healthy adolescent female volunteers who were recruited from friends and family of hospital employees. The control subjects were physically active and most were involved in local team sports. The controls were of normal weight and did not have a history of AN. No control subjects were taking medications that would affect exercise performance. Height (cm), weight (kg), body mass index (BMI, kg/m^2), and body surface area (BSA, m^2) were recorded at the time of study. Ethics approval for this study was obtained from the

Children's and Women's Hospitals' Research Ethics Board and the University of British Columbia's Clinical Research Ethics Board.

SSCE Stress Echocardiography

Exercise testing was performed on an electronically braked, semi-supine cycle ergometer (Angio Ergometer and Echo Cardiac Stress Table, Lode BV, Groningen, Netherlands). Subjects were positioned with a torso angle of 45 degrees, with the heart approximately 15 cm above the level of the crank axis of the ergometer. Three minute stages of exercise were performed with initial and incremental workloads of 20 watts (W) per stage. Subjects were encouraged to exercise until volitional fatigue. The test was terminated when subjects were unable to sustain an appropriate pedaling rate to reach the target workload.

Simultaneous HR, BP and echocardiography measurements were performed pre-exercise and 90 seconds into each stage. Each echocardiographic data set took 60-90 seconds to acquire. Continuous 12-lead electrocardiogram (ECG) monitoring was performed throughout testing and HR was determined by the R-R interval on the ECG (Case 8000 Stress System, GE Medical Systems, Milwaukee, WI). Systolic (sBP) and diastolic (dBP) blood pressure was obtained in the right arm by auscultation. Muffling of the Korotkoff sounds defined the diastolic pressure. Peak exercise was defined as the final stage of exercise during which echocardiographic measurements were taken.

Echocardiography Protocol

Stress echocardiography was performed by an experienced pediatric echocardiographer using a Vivid 5 or Vivid 7 system (GE Vingmed Ultrasound, Horten, Norway). A 3.5MHz transducer was used for all studies. In the parasternal short-axis view, standard assessment of LV internal diameter was made at end-systole (LVESD) and end-diastole (LVEDD), with the LV posterior wall diameter measured at end-systole (LVPWDs) and end-diastole (LVPWDd) using two-dimensional (2D) targeted M-mode as per the American Society of Echocardiography [19]. The diameter of the aortic annulus was measured at the level of the valve leaflets using 2D echocardiography in the parasternal long-axis view. In a suprasternal long axis or apical four-chamber view, a pulsed wave Doppler recording of the ascending aorta was obtained by placing a sample volume in the center of the aorta at the level of the valve leaflets. The peak aortic velocity (PAoV), velocity-time integral (VTI), and ejection time (ET) were measured from this Doppler tracing. Measurements were made by the echocardiographer performing the study and a pediatric cardiologist.

Calculations

The amount of work (Joules) per stage was calculated as the product of workload (Watts) and the duration of exercise in a given stage (seconds) divided by the weight (kg). The total work was calculated as the summation of work performed per stage by the subject. The formulae for the calculation of the following variables are provided in Table 3.1. We determined the meridonal wall stress at peak systole (σPS), or the tension within the wall of the LV during exercise, in both groups. LV contractility was calculated using

the fractional shortening (FS) and the rate corrected mean velocity of circumferential fiber shortening (MVCFc), which is an indicator of myocardial performance that is sensitive to changes in contractile state [20]. Total arterial compliance (TAC) and systemic vascular resistance (SVR) were also calculated with exercise as these are major determinants of cardiac afterload. Left ventricular mass (LVM) was calculated for patients and controls.

VO_2 methods

In the latter half of the AN patients studied (n=39) and in all the controls, metabolic gas exchange parameters were measured using open-circuit spirometry by a metabolic cart (Moxus Metabolic Cart, AEI Technologies, Pittsburgh, PA). The metabolic cart was calibrated prior to each test using ambient air (20.93% O_2 and 0.03% CO_2) and calibration gas (15.0% O_2 and 5.0% CO_2). The calibration of the turbine flow meter of the volume sensor was performed with a standard 3L syringe. A calibration report was generated prior to each test.

A small flexible rubber mouthpiece was attached to a one-way, non-rebreathing valve (Style 2700B, Hans Rudolph, Inc, Kansas City, MO) to the metabolic cart. Breath-by-breath data were collected and averaged over 15-second intervals during the test. Tidal volume (VT), breathing frequency (fb), minute ventilation (VE, L/min), oxygen consumption (VO_2, L/min, mL/min/kg), carbon dioxide production (VCO_2, L/min, mL/min/kg), ventilatory equivalents for oxygen and carbon dioxide (VE/VO_2 and VE/VCO_2) and respiratory exchange ratio (RER) were measured. Subjects were

encouraged to exercise until volitional fatigue. Arteriovenous oxygen difference (avO_2) was calculated as (peak VO_2)/(peak CO in deciliters/min).

Body Size Indexing

Body surface area was calculated using the DuBois formula BSA (m^2) = $0.20247(\text{height})^{0.725}(\text{weight})^{0.425}$, with height measured in meters and weight in kilograms [21]. Standardization for body surface area (BSA) was performed by dividing the SV, CO, TAC, and LVM by the BSA to give the SVI, CI, TAC indexed (TACI), and LVM indexed (LVMI). CI was used in the formula to calculate SVR in order to obtain SVR indexed (SVRI). BMI percentiles were calculated for all subjects using data from the Centers for Disease Control growth data [22].

Statistical Analysis

Univariate analyses were performed on all continuous variables, and summary statistics are expressed as mean (SD). We compared the anthropometric parameters of AN and control groups using one-way ANOVA. The measured hemodynamic variables were compared within groups and between groups at resting and maximal exercise using one way ANOVA. In order to determine if degree of patient malnutrition affected exercise function, we divided the AN group into those with a BMI less than or equal to the 10th percentile (lowest BMI AN group) and those with a BMI greater than the 10th percentile (higher BMI AN group). These subgroups were compared to the control group using a one way ANOVA with post-hoc analysis performed using Tukey's test. Pearson's product-moment correlations were performed to investigate the relationship between

LVM and BMI percentile with exercise endurance. Multivariable linear regression was performed to determine if BMI percentile, age, VO_2, peak CO, and LVM were independent predictors of exercise endurance in patients with AN.

We considered $p \leq 0.005$ to indicate a statistically significant difference, p values of $0.005 < p \leq 0.05$ to be marginally statistically significant, and p values >0.05 to be non-significant. All statistical analyses were performed using SPSS Statistics, version 17.0 (SPSS Statistics for Windows, Version 17.0, SPSS Inc., Chicago).

Graphical Presentation of Results

The mean values of HR, BP, SVI, CI, FS, MVCFc, σPS, SVRI, and TACI for the AN and control groups were graphed for each progressive stage of exercise. Hemodynamic and echocardiographic measures of cardiac function were only graphed for stages with > 20% of subjects remaining in order to avoid misrepresentation of responses to exercise due a low proportion of subjects remaining in the later exercise stages. Box plots comparing pre-exercise and peak exercise values for HR, BP, SVI, CI, FS, SVRI, and TACI were created to compare the AN and control groups. As the relationship of σPS and MVCFc represents a load independent measure of cardiac contractility [20, 23], the mean values for these variables at pre- and peak exercise for both the control and AN groups were plotted.

Results

Clinical Characteristics

There were a total of 66 adolescent females in the AN group and 21 healthy adolescent female controls. Twelve of the AN patients were classified as the binge-purge subtype and 54 were the restrictive subtype. The baseline characteristics for the AN and control groups are provided in Table 3.2. The patients with AN were significantly older than control patients (15.6 vs 14.4 years) with no difference in height between groups. AN patients had a significantly decreased weight, BSA, BMI, BMI percentile, LVM, and LVMI compared to control subjects.

The AN subgroups with BMI ≤ 10th percentile and >10th percentile were composed of 39 patients with 27 patients respectively. Neither group had significant differences in age or height when compared to the control group. The BMI ≤ 10th percentile group had significantly decreased weight, BSA, BMI, BMI percentile, LVM, and LVMI compared to controls. The only significant differences between the BMI >10th percentile AN group and controls were decreased weight, BMI percentile, and LVM.

Exercise Performance

Figure 3.1 demonstrates the number and percentage of subjects remaining in each stage for the AN and control groups. Table 3.3 outlines the exercise performance of the groups. Patients with AN had decreased exercise duration compared to controls, and AN patients with a BMI ≤ 10th percentile had a decreased exercise capacity compared to those with a BMI > 10th percentile (12.7 vs 15.3 min, $p = 0.043$). Exercise duration correlated with BMI percentile ($r=0.524$, $p<0.001$), LVMI ($r=0.580$, $p<0.001$), peak VO_2 ($r=0.745$, $p<0.001$), and peak CI ($r=0.451$, $p<0.001$), but not age ($p=0.156$). Multiple linear regression was performed using test duration as the dependent variable with age,

BMI percentile, peak CO, peak VO_2, and LVM as independent variables to determine which variables were independent predictors of exercise duration in patients with AN. The assumptions of linearity, independence of errors, homoscedasticity, unusual points and normality of residuals were met. The model was statistically significant and explained a large amount of the variability in exercise test duration, $F(5,41)=49.374$, $p<0.001$, adj $R^2=0.840$. BMI percentile, age, peak VO_2, and peak CO were significant independent predictors of exercise duration. Regression coefficients are provided in Table 3.4.

Total work, peak VE, and peak VO_2 were significantly decreased in AN patients and in the AN subgroups compared to controls. Mean peak RER was greater than 1.0 for all groups, indicating that these groups achieved a maximal test. RER was significantly greater for the total AN and BMI $\leq 10^{th}$ percentile groups than in controls. There were no differences between patients with AN and controls in avO_2 difference.

All subjects were asymptomatic during exercise with no episodes of chest pain, unusual breathlessness, or dizziness. There were no arrhythmias or ischemic changes in the ECG with exercise.

Cardiovascular Responses to Exercise

Table 3.5 summarizes the hemodynamic parameters at pre- and peak exercise for patients with AN and controls. Figure 3.2 demonstrates the response of HR, sBP, and dBP to progressive exercise in patients with AN and controls and provides a graphical representation of the pre- and peak exercise values for these groups. HR was similar between groups while sBP and dBP were lower in patients with AN compared to controls

at pre-exercise. HR increased similarly in both groups with progressive exercise, however, was decreased in AN patients at peak exercise. sBP and dBP were decreased in AN patients at pre- and peak exercise, although the pattern of increase with progressive exercise in AN patients was parallel to controls. In both groups, sBP increased steadily with exercise while dBP demonstrated a minimal, although statistically significant, increase. The differences in pre- and peak exercise values for HR, sBP, and dBP remained present when the AN patients were stratified into those with a BMI $\leq$ and > 10th percentile.

Table 3.6 summarizes the echocardiographic measurements and measures of ventricular function at pre- and peak exercise for the AN and control groups. LVEDD and LVESD were significantly smaller in AN patients at pre-exercise with no difference at peak exercise, although these differences were no longer apparent once LVEDD and LVESD were indexed to BSA (Table 3.6). After stratification according to BMI percentile, only patients with a BMI $\leq 10^{th}$ percentile had a decreased LVEDD at pre-exercise compared to controls.

As demonstrated in Table 3.4, there were no differences in SV or SVI between AN and control groups at either pre- or peak exercise, although the SV trended towards being significantly decreased at peak exercise in AN patients. AN patients had similar CO and CI pre-exercise and significantly decreased CO and CI at peak exercise when compared to controls. These differences remained after stratification for BMI in both the lower and higher BMI AN patient subgroups. As seen in Figure 3.3, SVI initially increased and subsequently plateaued in the higher exercise stages for both the AN and control groups, with a virtually identical pattern of response to progressive exercise in

both groups. CI increased in a linear fashion with increasing exercise stage in both AN and control groups with a similar pattern of response between the groups.

Figure 3.4 demonstrates a similar pattern of increase in FS and MVCFc with progressive exercise between AN and control groups. Both FS and MVCFc were similar between groups pre-exercise but were significantly decreased in AN patients at peak exercise, with this difference persisting in AN patients with BMI $\leq 10^{th}$ percentile after group stratification by BMI percentile (Table 3.6). The patterns of increase in FS and MVCFc with progressive exercise were similar between the AN and control groups, as seen in Figure 3.4. Table 3.6 demonstrates a significant decrease in σPS in AN patients compared to controls at pre-exercise, which was also seen in both the lower and higher weight AN patients after stratification by BMI percentile. The total group of AN patients had a lower σPS than controls at peak exercise, which did not reach statistical significance in either the AN subgroups of BMI $\leq$ or $> 10^{th}$ percentile (Table 3.6). There was an appropriate decrease in σPS with progressive exercise in the control group while σPS showed little change with progressive exercise in the AN group (Figure 3.4). Figure 3.5 demonstrates a similar and appropriate response in the MVCFc-σPS relationship from pre- to peak exercise in the AN and control groups.

As outlined in Table 3.5, SVR and SVRI were lower in the AN group at pre-exercise with no difference at peak exercise when compared to controls. After stratifying for BMI percentile, both the BMI $\leq$ and $> 10^{th}$ percentile AN groups has a decrease in SVRI at pre-exercise with no other differences from controls in SVR or SVRI. Figure 3.6 demonstrates that SVRI decreases initially with exercise and begins to plateau in the latter exercise stages in both the AN and control groups. Mean TAC values for the AN group

were similar at pre-exercise and significantly increased at peak exercise when compared to controls (Table 3.5). After indexing for BSA, TACI was significantly increased at pre- and peak exercise compared to controls for the total AN group and AN patients $\leq 10^{th}$ percentile. These differences between the AN and controls groups in TAC trended towards significance for the AN patents with BMI > 10^{th} percentile. As seen in Figure 3.6, TACI decreased with progressive exercise in both AN and control groups.

Discussion

To our knowledge, this is the largest study investigating exercise performance in adolescent females with AN. This study also investigates echocardiographic measures of cardiac function with exercise which have not been previously performed in adolescent females with AN. Our study demonstrates that adolescent females with AN have decreased exercise capacity compared to controls. Although the hemodynamic and echocardiographic function values at peak exercise were decreased in AN patients for the majority of measured indices, the patterns of response to progressive exercise for these variables were similar to controls.

Our study demonstrated that the responses of HR, SVI, CI, FS, and MVCFc to progressive exercise in patients with AN mirrors those of controls. However, the values of HR, CI, FS, and MVCFc at peak exercise were significantly decreased in patients with AN. The parallel patterns of response to progressive exercise in patients and controls suggests that myocardial function is not likely the primary limiting factor to exercise performance in females with AN. One possible explanation for the decrease in the peak exercise values for cardiac hemodynamic responses and cardiac contractility in patients

with AN may be due to patients not being able to sustain exercise and achieve the same workload as controls. Despite the difference in the pattern of response to progressive exercise for σPS between the patients with AN and controls, we found a similar pattern of response to exercise in both the control and AN patients in the MVCFc-σPS relationship, which is reported to be a preload-independent and afterload adjusted indicator of systolic function in the absence of ventricular outlet obstruction [20, 23]. The normal response of the MVCFc-σPS relationship with exercise further suggests normal cardiac contractility with exercise in patients with AN. We also assessed the effect of progressive exercise on left ventricular afterload as reflected by SVR and TAC, which both decrease with progressive exercise in normal subjects [15, 24], and found similar decreases in TACI and SVRI with progressive exercise in both groups. Responses of hemodynamic variables to progressive exercise in AN were also investigated by Rowland *et al.* (2003) [13]. We confirmed their results of similar pattern of increase in SVI with exercise in AN and control patients, although they reported an increased peak SVI in patients with AN, which differs from the results of our study [13]. Our results do not suggest the presence of myocardial dysfunction during exercise in patients with AN as the patterns we documented differ from the reported pattern of response to progressive exercise in pediatric patients with known myocardial dysfunction, who show divergences from control values in stroke volume with progressive exercise [16].

Exercise endurance was significantly decreased in the patients with AN compared to controls, and was lower in AN patients with BMI $\leq 10^{th}$ percentile than those with a BMI $> 10^{th}$ percentile. These findings are suggestive that increased levels of malnourishment are associated with decreased exercise performance. The finding of

increased level of malnourishment predicting decreased exercise duration was strengthened by our multiple linear regression analysis which demonstrated that BMI percentile is an independent predictor of exercise duration in patients with AN. Decreased exercise endurance or decreased work has also been reported in multiple studies of patients with AN [7, 11, 12, 25]. Previous studies have also demonstrated a correlation between a subject's BMI or percent of ideal body weight with maximum work or exercise duration [25, 26]. The decrease in exercise capacity in the setting of more severe malnourishment may be secondary to increased deconditioning, decreased skeletal muscle mass, partial atrophy of selected muscular fibers, or to muscular dysfunction from decreased energy stores available for utilization by skeletal muscle during exercise, alterations in intracellular micronutrients, defects in ion channel function, or slowing of muscular relaxation rate [26, 27]. Skeletal muscle mass and function have been shown to be decreased in the setting of AN. Muscle biopsies in patients with AN have demonstrated atrophy of muscle fibers with type II fibers being the most affected [28-31]. Total muscle mass and muscle mass per kg of body weight is lower in patients with AN than controls [32]. Slowed muscle relaxation, increased muscle fatigue, and decreased maximum voluntary contraction forces have all been demonstrated in patients AN, with some improvements in muscle function after refeeding [27, 30]. With nutritional repletion, the majority of the weight regained is represented by fat, with gains in muscle mass lagging behind [32]. Despite the lag in regaining muscle mass, significant improvements in leg muscle performance and exercise endurance after refeeding have been demonstrated in patients with AN, which suggests that skeletal muscle dysfunction associated with malnutrition is a significant contributor of decreased exercise

performance in AN [26]. Cardiac muscle mass is also decreased in the setting of AN, which was confirmed in our study where the LVM and LVMI were decreased in AN patients compared to controls [3, 4, 6, 8, 9, 33]. Our multiple linear regression model demonstrated that LVM was not an independent predictor of exercise duration, suggesting that the decrease in cardiac mass seen in patients with AN does not significantly contribute to the decreased exercise capacity in this population. The multiple linear regression model explained a large amount of the variability in exercise duration, with the remainder of the variability possibly explained by other factors such as pre-existing level of physical activity, which was not quantified by our study,

Our AN patients had decreased aerobic fitness compared to controls as evidenced by decreased peak $VO_{2,}$ which reached 79% of the peak value for controls. Despite the fact that VO_2 is indexed for weight, which should have conferred an advantage to the AN population as their weight was significantly lower than that of controls, the VO_2 remained decreased in the AN group. Several other studies have demonstrated a decreased peak VO_2 in the AN population [7, 12, 13, 26, 34-38]. We found a peak VO_2 value of 31.3 mL/kg/min in the AN group, which is comparable to previously reported values for females with AN [11, 13, 34-36]. VO_2 is the maximal oxygen consumption which is determined by the product of CO and avO_2 difference, or the oxygen extraction at the level of the muscle. As there was no difference in the peak avO2 between the AN and control groups, a decrease in the peak CO is responsible for the decreased VO_2 in the AN population. CO is determined by the product of HR and SV. Our study demonstrated no difference in the SV at peak exercise between the two groups, but demonstrated a

significant decrease in the peak HR, making a decreased peak HR the major contributor of decreased CO and subsequently decreased peak VO_2 in our study subjects.

Although CI followed the same pattern with progressive exercise in patients with AN and controls, peak CO and CI were decreased in AN patients at peak exercise. Studies of exercise in patients with AN have demonstrated conflicting results regarding peak CO as well as the relative contribution of HR and SV to CO. Lands *et al.* (1992) demonstrated that although patients with AN were not able to perform as much work as controls with steady state exercise, their CO was appropriate for the amount of work performed, which was achieved by relatively faster HR but lower SV than predicted [25]. However, these patients were exercising at 50% of their peak workload and this may not be generalizable to CO at peak workloads. In contrast, in a study comparing 8 adolescents with AN and controls, Rowland *et al.* (2003) demonstrated similar CO between the groups which was caused by an increased peak SVI and decreased HR in the AN patients [13].

The decreased peak HR demonstrated by our study is consistent with findings in AN patients with exercise testing [7, 10-13, 34]. Nudel *et al.* (1984) found a depressed peak HR in adolescents with AN and an associated decrease in norepinephrine and dopamine levels [11]. These authors also found that AN patients with increased norepinephrine levels had significantly higher peak HR than AN patients with lower norepinephrine levels, although the peak HR was still less than that of controls [11]. Nudel *et al*. (1984) speculated that decreased cathecholamine levels partially explain the decreased peak HR in AN, but is not the only factor as the peak HR remains depressed in the setting of normal catecholamine levels [11]. Our study demonstrated that at a given

workload, the HR of AN patients and controls were similar. These findings are in contrast to those of St John Sutton *et al*. (1985) who demonstrated decreased HR in patients with AN compared to controls for a given workload [7]. Other authors have suggested that although the absolute peak HR is decreased in AN patients, the HR expressed as a percent expected for work reveals an appropriate HR response or a relative tachycardia in patients with AN, which is consistent with our findings [25].

Although we have demonstrated that females with AN have decreased VO_2 and exercise capacity, our findings of parallel indices of myocardial function suggests that myocardial function in response to exercise is normal in this patient population. Other studies have also suggested that myocardial performance is not the primary limiting factor in patients with AN to sustain exercise. Rowland *et al*. (2003) concluded that myocardial performance in patients with AN during exercise was normal, as measured by peak aortic velocity and average flow acceleration in the aorta when related to HR [13]. Lands *et al*. (1992) concluded that the diminished exercise capacity demonstrated in AN was likely caused by diminished skeletal muscle mass or muscle dysfunction rather than by cardiac insufficiency [25]. Gottdiener *et al.* (1978) demonstrated an appropriate increase in ejection fraction with supine bicycle exercise measured by radionuclide cineangiograms [10]. Our study is in agreement with the literature supporting normal patterns of myocardial response to exercise in patients with AN.

There were no adverse events associated with exercise testing in our patients or controls and no evidence of ischemia on the ECGs taken during exercise testing. Previous studies have, however, demonstrated ECG changes during exercise in patients with AN. Nudel *et* al. (1984) demonstrated significant ST depression in 25% of the AN patients

who had exercise testing performed [11]. Rowland *et al.* (2003) reported "scooping" of the ST segments in one AN patient who also had decreased FS on the resting echocardiogram [13]. Rigaud *et al* (1997) terminated an exercise test in one patient at the first exercise test after hospital admission due to an arrhythmia [26]. Our study suggests that exercise was safe in the patients that were studied, however, there exists a potential for electrocardiographic changes in the setting of AN during exercise as evidenced by previous studies. It is possible that the patients in our study population had lesser disease severity than those patients demonstrating ECG changes in previous studies.

Limitations of our study include the retrospective nature of the study and the inherent biases associated with this design. Patients were studied during the acute phase of treatment for AN and were likely at variable stages of their nutritional rehabilitation at the time of study, which may have influenced the results obtained as it has been shown that refeeding improves exercise performance [26]. Patients in our study were exercised in a semi-supine position on a cycle ergometer, which requires sufficient leg strength to overcome the resistance provided by the ergometer. Deconditioning and loss of muscle mass in the AN patients may have led to insufficient strength to overcome resistance on the ergometer and led to a decrease in total work. Performing the exercise test in the upright position on a treadmill may have allowed the AN patients to attain higher peak exercise values including heart rate and VO_2, however, would not have allowed for the echocardiographic measurements obtained by our study to be taken. Use of a 20 Watt incremental workload in our study protocol yielded an exercise test of appropriate duration in the majority of our AN patients, but resulted in long exercise times in our control subjects, making these assessments tests of endurance. Excessive exercise is

known to prevalent in patients with AN and there may be different cardiovascular responses between patients who exercise excessively and those who do not [39]. We were not able to quantify the amount of exercise performed by patients with AN prior to their diagnosis and thus were not able to assess the influence of this factor in our study. Our study only indexed the variables to BSA but it is unclear if indexing values using BSA is the most appropriate means to account for the effect of body dimension in patients with AN [13]. However, it has been shown by Rowland *et al*. (2003) that indexing by either BSA or lean body mass did not alter the conclusions of the study [13]. The number of controls in our study was fewer than the number of patients due to the difficulty of recruiting healthy adolescent female volunteers to undergo echocardiography. It is possible that an increased number of controls could have demonstrated additional significant differences between the patient and control groups. Lastly, we were unable to determine ventricular diastolic function with exercise and cannot exclude the possibility of diastolic dysfunction occurring with exercise in this patient population.

Further study is required to determine the response of the cardiac hemodynamic variables and echocardiographic indices of left ventricular function with weight restoration in patients with AN. Several studies have demonstrated an improvement in peak VO_2, exercise duration, maximal HR, and workload achieved with refeeding in AN [26, 36, 37, 40, 41], but no studies have demonstrated if there are changes in the response of FS, MVCFc, σPS, TAC, or SVR with exercise after refeeding. The mechanism of decreased exercise capacity in patients with AN and the relative contributions of deconditioning, deceased muscle mass, impaired muscular function, and impaired cathecholamine response has not been clarified and would require further investigation.

This study was not designed to establish the earliest it is safe to exercise in the most severely affected patients with AN, and this has yet to be determined.

In conclusion, adolescent patients with AN appear to have normal patterns of cardiovascular response to progressive exercise although values at pre- and peak exercise may differ from controls. Despite these normal responses to progressive exercise, exercise capacity is reduced. After controlling for age, exercise endurance is independently predicted by BMI percentile. Our study suggests that systolic ventricular function in adolescents with AN is not likely to be the limiting factor in their ability to exercise.

Table 3.1 Formulae for calculating the echocardiographic parameters

Parameter	Formula
AoCSA (cm^2)	$(\pi/4)$ (aortic annulus diameter)^2
SV (mL)	VTI · AoCSA
CO (L/min)	HR · SV/1000
PP (mmHg)	sBP-dBP
MAP (mmHg)	(sBP + 2*dBP)/3
FS (%)	100*(LVEDD-LVESD)/LVEDD
TAC (mL/mmHg)	SV/PP
SVR (Wood units)	MAP/CO
ETc (s)	ET/√RR
MVCFc (circ/s)	(LVEDD-LVESD)/(ETc/1000*LVEDD)
σPS (g/cm^2)	0.34*sBP*LVESD/LVPWDs*(1+(LVPWDs/LVESD))
LVM (g)	1.05[(LVEDD + 2*LVPWDd)^3-LVEDD^3]

AoCSA = aortic cross-sectional area, SV = stroke volume, VTI = velocity time integral, CO = cardiac output, HR = heart rate, PP = pulse pressure, sBP = systolic blood pressure, dBP = diastolic blood pressure, MAP = mean aortic pressure, FS = fractional shortening, LVEDD = left ventricular end-diastolic diameter, LVESD = left ventricular end-systolic diameter, TAC = total arterial compliance, SVR = systemic vascular resistance, ETc = envelope time corrected, ET = envelope time, RR = R-R interval, MVCFc = rate corrected mean velocity of circumferential fiber shortening, σPS = stress at peak systole, LVPWDs = left ventricular posterior wall diameter in systole, LVM = left ventricular mass, LVPWDd = left ventricular posterior wall diameter in diastole

Table 3.2: Baseline Characteristics of all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n = 21)	Total AN Group* (n = 66)	AN Subgroups[§]	
			BMI ≤10th percentile (n=39)	BMI>10th percentile (n=27)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age (years)	14.4 (1.9)	15.6 (1.9) p = 0.015	15.6 (1.8) p = 0.051	15.5 (2.1) p = 0.118
Height (cm)	162.5 (7.9)	161.0 (9.1) p = 0.482	162.7 (6.9) p = 0.996	158.4 (11.3) p = 0.240
Weight (kg)	52.3 (9.0)	43.4 (7.3) p < 0.001	41.0 (5.6) p < 0.001	46.9 (7.9) p = 0.037
BSA (m^2)	1.54 (0.15)	1.42 (0.15) p = 0.001	1.40 (0.12) p = 0.001	1.45 (0.18) p = 0.080
BMI (kg/m^2)	19.7 (2.6)	16.7 (2.1) p < 0.001	15.4 (1.4) p < 0.001	18.6 (1.3) p = 0.061
BMI percentile	48.1 (27.4)	12.6 (15.5) p < 0.001	1.9 (2.1) p < 0.001	28.1 (13.2) p < 0.001
LVM (g)	140.4 (33.0)	105.5 (29.3) p < 0.001	98.8 (21.1) p < 0.001	72.0 (13.5) p = 0.005
LVMI (g/m^2)	87.6 (17.3)	76.5 (17.6) p = 0.017	116.1 (37.2) p = 0.032	83.6 (21.1) p = 0.732

AN= Anorexia nervosa, SD = standard deviation, BSA = body surface area, BMI = body mass index, LVM = left ventricular mass, LVMI = left ventricular mass indexed
*p values determined using one- way ANOVA comparing AN group to controls
§ p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 3.3: Exercise Performance Characteristics of all Patients with AN and AN Subgroups Compared to Control Subjects

	Control Group (n=21)	Total AN Group* (n=66)	AN Subgroups[§]	
			BMI ≤10th percentile (n=39)	BMI>10th percentile (n=27)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Test duration (min)	20.8 (4.1)	13.8 (4.5) p <0.001	12.7 (4.4) p <0.001	15.3 (4.1) p <0.001
Total work (J/kg)	1914 (498)	1125 (558) p <0.001	1046 (589) p <0.001	1241 (496) p <0.001
Peak VE (L/min)	72.0 (14.4)	47.4 (18.3) p <0.001	47.0 (21.1) p <0.001	48.0 (14.8) p <0.001
Peak VO_2 (mLO_2/kg/min)	39.7 (6.8)	31.3 (8.3) p = 0.001	31.4 (9.2) p = 0.003	31.1 (7.3) p = 0.004
Peak RER	1.06 (0.07)	1.14 (0.10) p = 0.002	1.16 (0.10) p = 0.002	1.12 (0.09) p = 0.091
Peak avO_2 (mLO_2/100mL of blood)	0.204 (0.053)	0.185 (0.065) p = 0.262	0.172 (0.053) p = 0.217	0.201 (0.077) p = 0.987

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index, VE = minute ventilation, VO_2 = maximal oxygen consumption, RER = respiratory exchange ratio, avO_2 = arteriovenous oxygen difference

*p values determined using one- way ANOVA comparing AN group to controls

[§] p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 3.4 Summary of Multiple Linear Regression Analysis for Patients with AN (n=47)

Predictor Variable	B (95% CI)	β	t	p value
BMI percentile	4.4 (2.5-6.3)	0.354	4.736	<0.001
Age	45.7 (18.8-72.6)	0.252	3.431	0.001
Peak VO_2	25.2 (19.4-30.9)	0.620	8.850	<0.001
Peak CO	27.9 (19.4-30.9)	0.244	2.385	0.022
LVM	0.3 (-1.7 – 2.3)	0.027	0.280	0.781

B = unstandardized coefficient, β = standardized coefficient, t = t statistic, BMI = body mass index, VO_2 = oxygen consumption, CO = cardiac output, LVM = left ventricular mass

Table 3.5: Hemodynamic Variables at Pre- and Peak Exercise for all Patients with AN and AN Subgroups Compared to Control Subjects

		Control Group (n=21)	Total AN Group* (n=66)	AN Subgroups§	
				BMI ≤10th percentile (n=39)	BMI>10th percentile (n=27)
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
HR (beats/min)	Pre-exercise	73.4 (11.2)	74.0 (14.1) p = 0.859	72.5 (12.3) p = 0.966	76.2 (16.4) p = 0.757
	Peak exercise	186.8 (7.6)	156.7 (19.5) p < 0.001	154.0 (19.7) p < 0.001	160.3 (19.0) p < 0.001
sBP (mmHg)	Pre-exercise	119.0 (9.4)	97.4 (10.2) p < 0.001	95.3 (9.9) p < 0.001	100.6 (10.0) p < 0.001
	Peak exercise	195.0 (17.6)	144.0 (20.8) p < 0.001	138.2 (19.4) p < 0.001	152.4 (20.2) p < 0.001
dBP (mmHg)	Pre-exercise	73.0 (6.1)	59.1 (7.1) p < 0.001	56.9 (6.4) p < 0.001	62.3 (7.1) p < 0.001
	Peak exercise	81.3 (9.7)	65.4 (10.6) p < 0.001	64.0 (11.5) p < 0.001	67.5 (9.1) p < 0.001
SV (mL)	Pre-exercise	47.1 (11.3)	44.6 (11.3) p = 0.387	44.4 (10.6) p = 0.658	45.0 (12.3) p = 0.796
	Peak exercise	57.0 (13.3)	50.3 (13.7) p = 0.053	50.1 (11.9) p = 0.157	50.6 (16.1) p = 0.247
SVI (mL/m^2)	Pre-exercise	30.3 (6.5)	31.4 (6.9) p = 0.536	31.8 (7.2) p = 0.694	30.7 (6.5) p = 0.977
	Peak exercise	36.8 (7.0)	35.3 (8.2) p = 0.477	36.9 (7.9) p = 0.911	34.6 (8.7) p = 0.619
CO (L/min)	Pre-exercise	3.44 (0.93)	3.24 (0.78) p = 0.331	3.19 (0.80) p = 0.492	3.32 (0.77) p = 0.863
	Peak exercise	10.61 (2.46)	7.80 (2.12) p < 0.001	7.64 (1.93) p < 0.001	8.03 (2.39) p < 0.001
CI ($L/min/m^2$)	Pre-exercise	2.23 (0.56)	2.30 (0.54) p = 0.640	2.29 (0.55) p = 0.923	2.31 (0.54) p = 0.886
	Peak exercise	6.86 (1.34)	5.50 (1.32) p < 0.001	5.49 (1.28) p = 0.001	5.51 (1.41) p = 0.002
SVR (Wood	Pre-exercise	27.4 (7.8)	23.5 (6.5) p = 0.026	23.1 (5.8) p = 0.058	24.2 (7.6) p = 0.237

units)	Peak exercise	8.7 (1.9)	9.9 (2.8) p = 0.076	9.6 (2.6) p = 0.406	10.3 (3.2) p = 0.111
SVRI (Wood units·m^2)	Pre-exercise	42.2 (12.5)	33.3 (9.5) p = 0.001	32.2 (8.3) p = 0.002	34.8 (11.0) p = 0.041
	Peak exercise	18.1 (4.1)	17.6 (4.8) p = 0.660	16.9 (4.6) p = 0.625	18.5 (4.9) p = 0.952
TAC (mL/mmHg)	Pre-exercise	1.05 (0.31)	1.21 (0.40) p = 0.085	1.19 (0.34) p = 0.331	1.24 (0.49) p = 0.200
	Peak exercise	0.51 (0.13)	0.68 (0.27) p = 0.006	0.71 (0.24) p = 0.010	0.64 (0.32) p = 0.156
TACI (mL/mmHg /m^2)	Pre-exercise	0.68 (0.21)	0.85 (0.26) p = 0.008	0.86 (0.25) p = 0.028	0.84 (0.27) p = 0.075
	Peak exercise	0.33 (0.08)	0.48 (0.19) p = 0.001	0.51 (0.18) p < 0.001	0.44 (0.20) p = 0.074

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index, HR = heart rate, sBP = systolic blood pressure, dBP = diastolic blood pressure, SV = stroke volume, SVI = stroke volume index, CO = cardiac output, CI = cardiac index, SVR = systemic vascular resistance, SVRI = systemic vascular resistance indexed, TAC = total arterial compliance, TACI = total arterial compliance indexed

*p values determined using one- way ANOVA comparing AN group to controls

§ p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

Table 3.6: Echocardiographic Dimensions and Myocardial Performance at Pre- and Peak Exercise for all Patients with AN and AN Subgroups Compared to Control Subjects

		Control Group (n=21)	Total AN Group* (n=66)	AN Subgroups§	
				BMI ≤10th percentile (n=39)	BMI>10th percentile (n=27)
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
LVEDD (cm)	Pre-exercise	4.64 (0.36)	4.37 (0.40) p = 0.007	4.33 (0.37) p = 0.011	4.43 (0.45) p = 0.162
	Peak exercise	4.47 (0.40)	4.31 (0.42) p = 0.143	4.27 (0.42) p = 0.186	4.38 (0.41) p = 0.729
LVESD (cm)	Pre-exercise	2.93 (0.28)	2.75 (0.33) p = 0.030	2.73 (0.29) p = 0.062	2.79 (0.38) p = 0.284
	Peak exercise	2.15 (0.29)	2.22 (0.28) p = 0.364	2.23 (0.25) p = 0.555	2.19 (0.32) p = 0.857
LVEDD indexed (cm/m^2)	Pre-exercise	3.02 (0.25)	3.11 (0.34) p = 0.295	3.13 (0.35) p = 0.467	3.08 (0.33) p = 0.814
	Peak exercise	2.94 (0.29)	3.07 (0.35) p = 0.130	3.08 (0.35) p = 0.283	3.05 (0.37) p = 0.481
LVESD indexed (cm/m^2)	Pre-exercise	1.91 (0.18)	1.96 (0.24) p = 0.381	1.97 (0.24) p = 0.529	1.93 (0.23) p = 0.920
	Peak exercise	1.41 (0.21)	1.58 (0.22) p = 0.005	1.61 (0.20) p = 0.004	1.53 (0.24) p = 0.173
LVPWDs (cm)	Pre-exercise	1.11 (0.08)	1.04 (0.09) p = 0.004	1.03 (0.09) p = 0.009	1.05 (0.09) p = 0.088
	Peak exercise	1.35 (0.08)	1.22 (0.10) p < 0.001	1.19 (0.10) p < 0.001	1.26 (0.09) p = 0.004
FS (%)	Pre-exercise	36.8 (3.6)	36.9 (4.1) p = 0.897	36.8 (4.2) p = 0.999	37.1 (3.9) p = 0.971
	Peak exercise	51.9 (4.1)	48.7 (4.0) p = 0.002	47.7 (3.7) p = 0.001	50.1 (4.0) p = 0.256
MVCFc (circ/s)	Pre-exercise	1.22 (0.12)	1.17 (0.15) p = 0.234	1.18 (0.18) p = 0.654	1.16 (0.10) p = 0.383
	Peak exercise	1.62 (0.19)	1.50 (0.23) p = 0.030	1.46 (0.20) p = 0.022	1.55 (0.26) p = 0.509
σPS (g/cm^2)	Pre-exercise	78.5 (12.9)	64.5 (14.5) p < 0.001	62.9 (14.0) p < 0.001	66.8 (15.1) p = 0.016

	Peak exercise	64.3 (9.5)	57.4 (12.9) p = 0.030	57.4 (13.5) p = 0.115	57.4 (12.3) p = 0.142

AN= Anorexia nervosa, SD = standard deviation, BMI = body mass index, LVEDD = left ventricular end-diastolic diameter, LVESD = left ventricular end-systolic diameter, FS = fractional shortening, MVCFc = rate corrected mean velocity of circumferential fiber shortening, σPS = stress at peak systole,

*p values determined using one- way ANOVA comparing AN group to controls

§ p values determined using Tukey's test for post-hoc ANOVA analysis comparing AN subgroups to controls

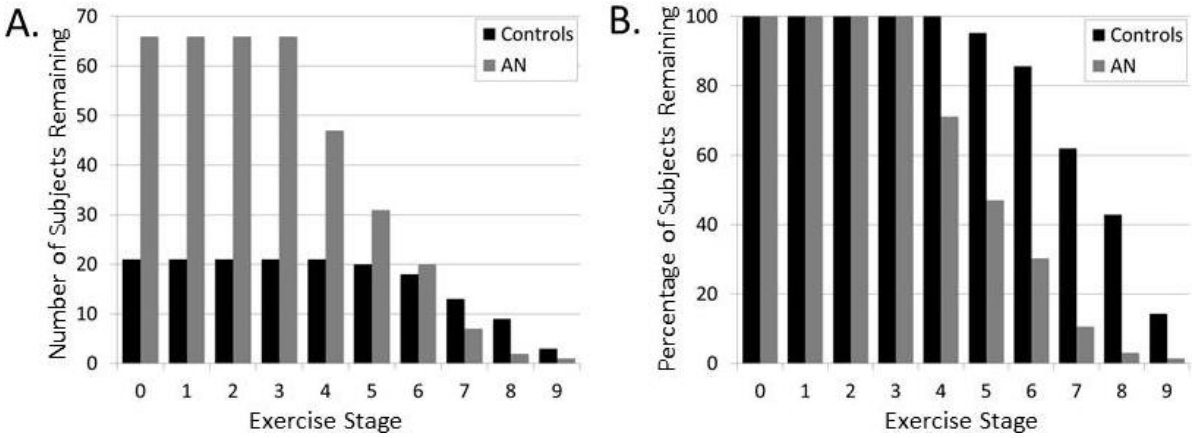

Figure 3.1: The remaining (A.) number and (B.) percentage of anorexia nervosa (AN) and control subjects per stage of exercise. Stage 0 is the pre-exercise stage, with each incremental increase in stage number representing a 20 Watt increase in workload.

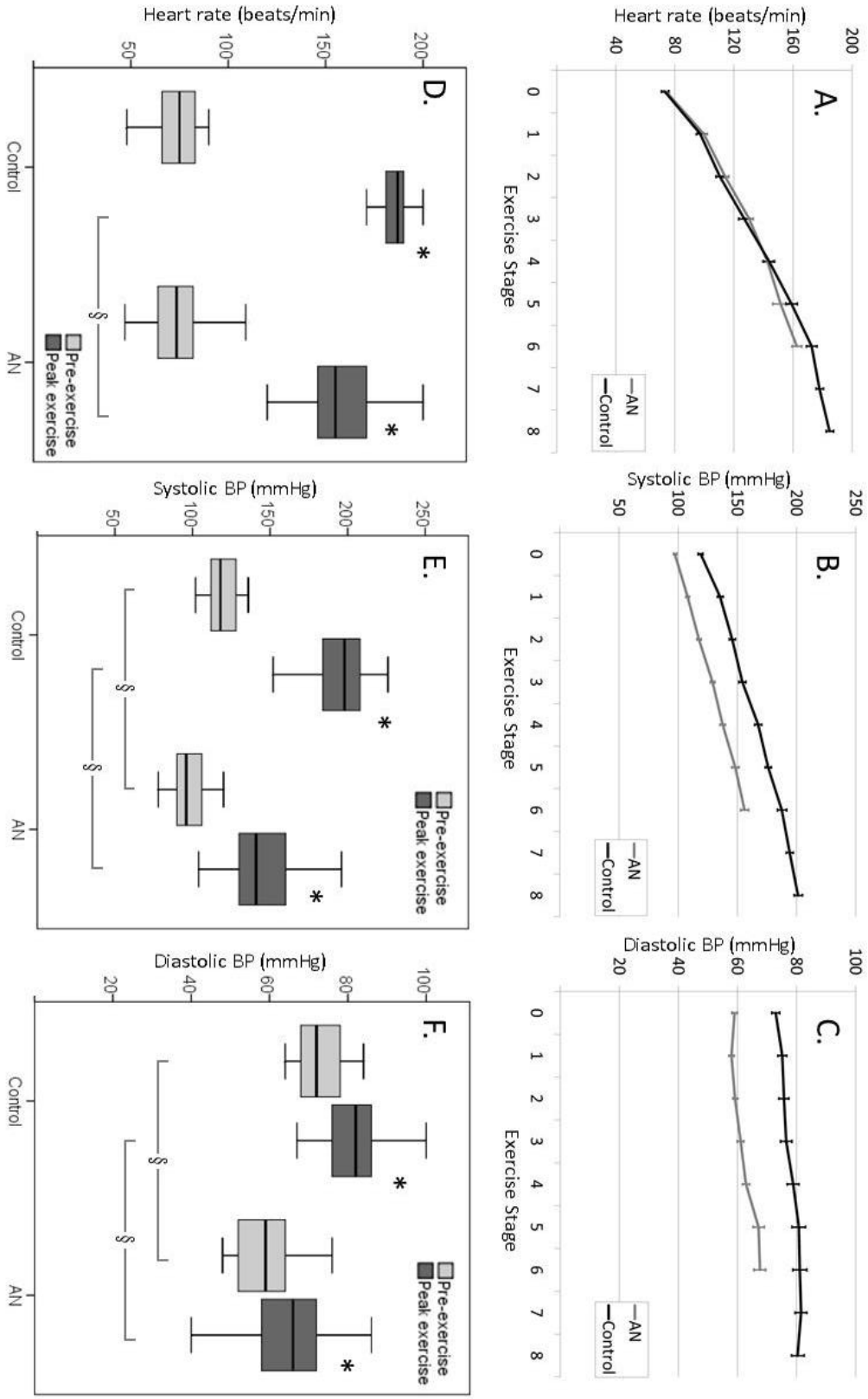
A.
Heart rate (beats/min)
Exercise Stage
AN
Control
B.
Systolic BP (mmHg)
Exercise Stage
AN
Control
C.
Diastolic BP (mmHg)
Exercise Stage
AN
Control
D.
Heart rate (beats/min)
Control
AN
Pre-exercise
Peak exercise
E.
Systolic BP (mmHg)
Control
AN
Pre-exercise
Peak exercise
F.
Diastolic BP (mmHg)
Control
AN
Pre-exercise
Peak exercise

Figure 3.2: Response of (A.) heart rate, (B.) systolic blood pressure (BP), and (C.) diastolic BP to progressive exercise in patients with anorexia nervosa (AN) and controls. The error bars indicate the standard error of the mean. Stage 0 is the pre-exercise stage, with each incremental increase in stage number representing a 20 Watt increase in workload. Box plots comparing the mean values for (D.) heart rate, (E.) systolic BP, and (F.) diastolic BP at pre- and peak exercise for the AN and control groups are provided. Significant differences ($p<0.05$) between values at pre-exercise and peak exercise for either the AN or control groups are indicated by the * symbol. Significant differences ($p<0.05$) between the control and AN groups for either pre-exercise or peak exercise values are indicated by the § symbol.

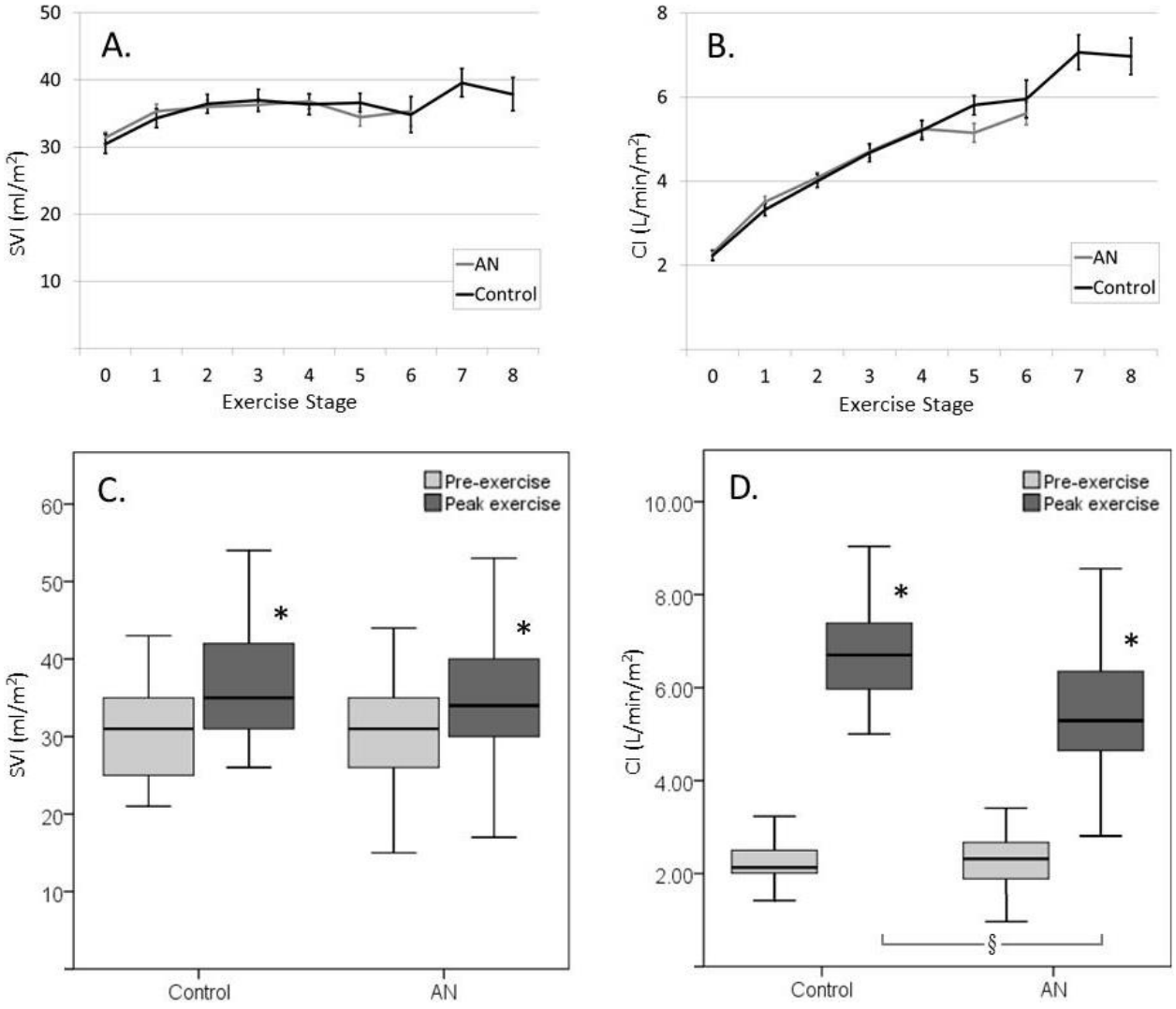

Figure 3.3: Response of (A.) stroke volume indexed (SVI) and (B.) cardiac index (CI) to progressive exercise in patients with anorexia nervosa (AN) and controls. The error bars indicate the standard error of the mean. Stage 0 is the pre-exercise stage, with each incremental increase in stage number representing a 20 Watt increase in workload. Box plots comparing the mean values for (C.) SVI and (D.) CI at pre- and peak exercise for the AN and control groups are provided. Significant differences ($p<0.05$) between values at pre-exercise and peak exercise for either the AN or control groups are indicated by the

* symbol. Significant differences ($p<0.05$) between the control and AN groups for either pre-exercise or peak exercise values are indicated by the § symbol.

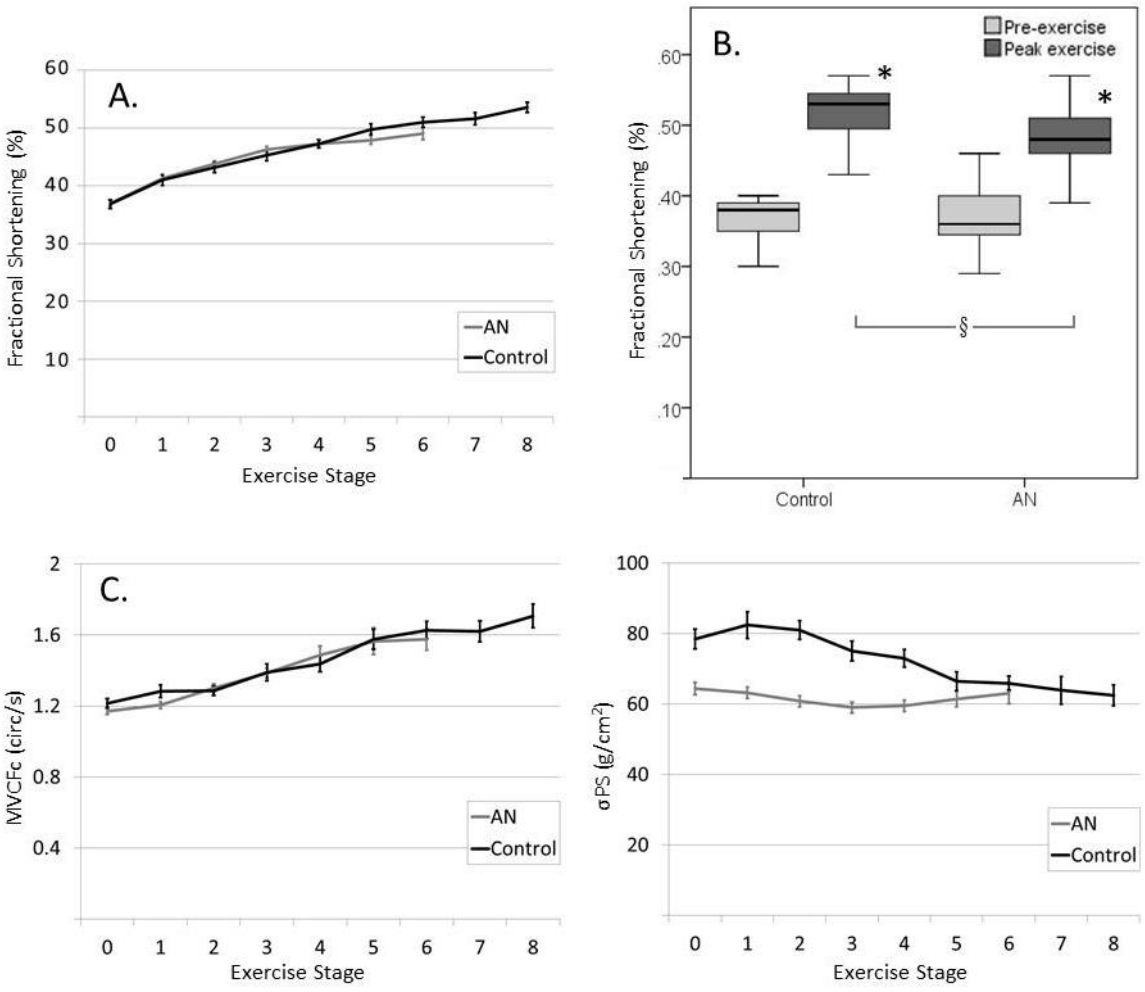

Figure 3.4: Response of (A.) fractional shortening, (C.) mean velocity of circumferential fiber shortening (MVCFc), and (D.) stress at peak systole (σPS) to progressive exercise in patients with anorexia nervosa (AN) and controls. The error bars indicate the standard error of the mean. Stage 0 is the pre-exercise stage, with each incremental increase in stage number representing a 20 Watt increase in workload. A box plot comparing the mean values for (B.) fractional shortening at pre- and peak exercise for the AN and control groups is provided. Significant differences ($p<0.05$) between values at pre-exercise and peak exercise for either the AN or control groups are indicated by the *

symbol. Significant differences ($p<0.05$) between the control and AN groups for either pre-exercise or peak exercise values are indicated by the § symbol.

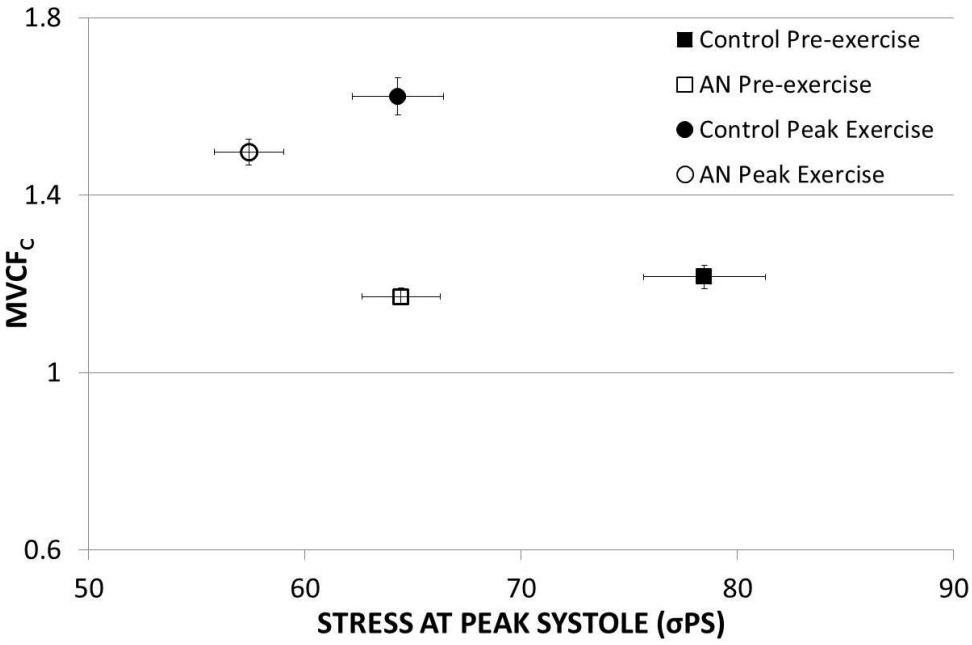

Figure 3.5: The relationship between mean velocity of circumferential fiber shortening (MVCFc) and stress at peak systole (σPS) at pre- and peak exercise for patients with anorexia nervosa (AN) and controls. The error bars indicate the standard error of the mean.

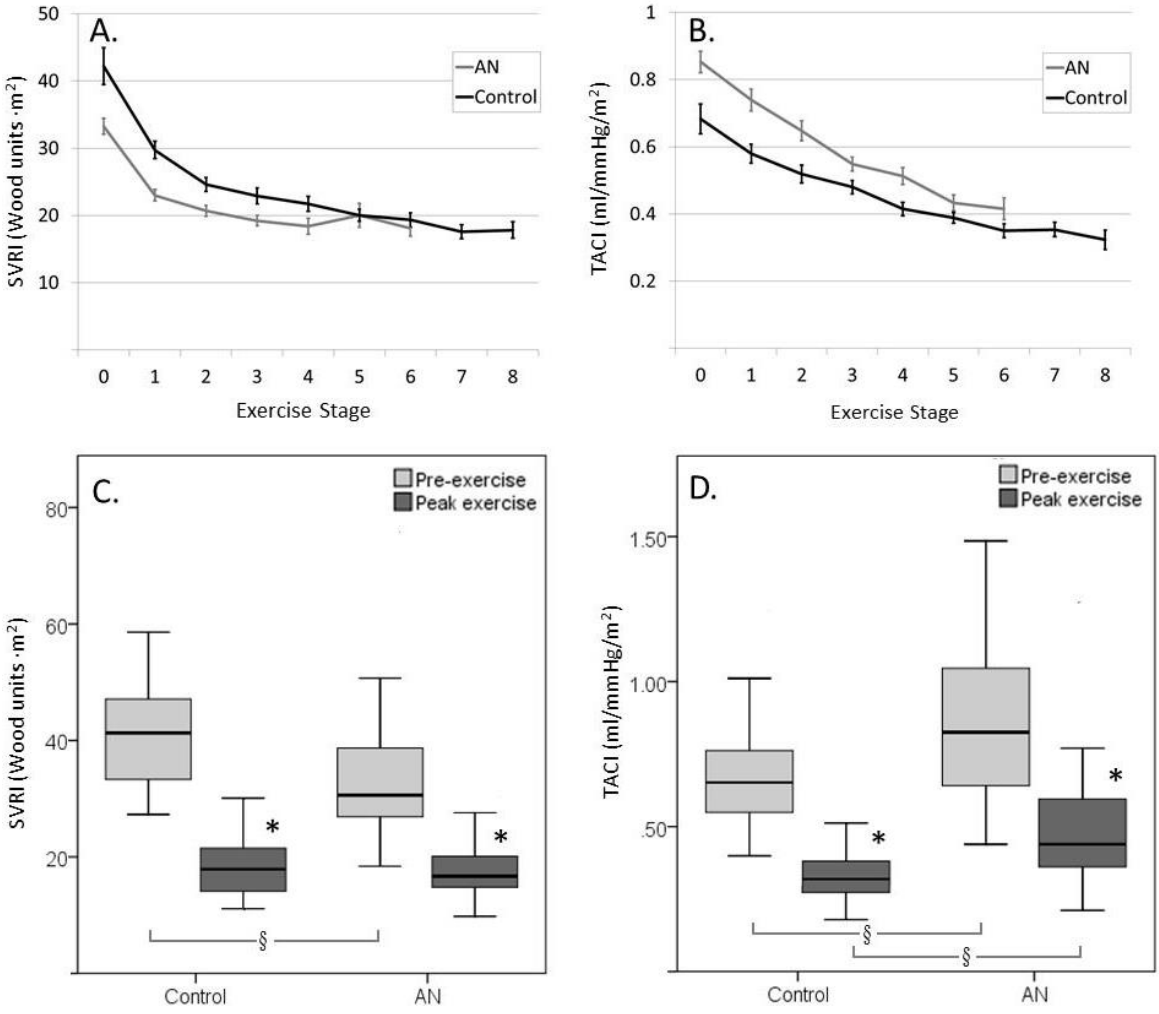

Figure 3.6: Response of (A.) systemic vascular resistance indexed (SVRI) and (B.) total arterial compliance indexed (TACI) to progressive exercise in patients with anorexia nervosa (AN) and controls. The error bars indicate the standard error of the mean. Stage 0 is the pre-exercise stage, with each incremental increase in stage number representing a 20 Watt increase in workload. Box plots comparing the mean values for (C.) SVRI and (D.) TACI at pre- and peak exercise for the AN and control groups are provided. Significant differences ($p<0.05$) between values at pre-exercise and peak exercise for either the AN or control groups are indicated by the * symbol. Significant differences

($p<0.05$) between the control and AN groups for either pre-exercise or peak exercise values are indicated by the § symbol.

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Chapter 5: Summary

To our knowledge, this study presents the largest comprehensive assessment of cardiovascular health in adolescent females with AN. We found that in a large cohort of 95 female adolescents with AN, there was a reduction in cardiac dimensions with a decrease in LA and LV size. Wall thickness and LVM were decreased in the patient group, with the decrease in wall mass being out of proportion to the decrease in body size. On all measures of systolic function performed, there were no differences at rest between the patient group and controls. However, patients with AN demonstrated decreases in diastolic tissue Doppler measurements, which are a measure diastolic ventricular function. These results suggest that although there is a downregulation in the size and thickness of the heart in adolescent patients with AN as well as subclinical abnormalities of diastolic function, systolic function at rest is preserved.

In order to determine if abnormalities in ventricular function could be unmasked during times of hemodynamic stress, we investigated ventricular function during progressive exercise in patients with AN as compared to controls. We found that the patients with AN had decreased exercise capacity and decreased measures of systolic function at peak exercise, although their cardiac responses and ventricular function with progressive exercise were similar to that of controls. Our results suggest that the decreased exercise capacity in this patient population was unlikely to be caused by cardiac systolic dysfunction due to the normal pattern of cardiac response to progressive exercise. However, we were unable to examine diastolic function during exercise and

cannot exclude the possibility of diastolic function worsening with exercise in this patient population.

Normal function of the cardiac system is dependent of the integrity of the vascular system. In addition, abnormal vascular stiffness is a risk factor for future cardiovascular events. Thus, we endeavoured to determine the biophysical properties of the aorta as a measure of vascular function in patients with AN due to the close interrelation between the heart and the vasculature. We found that patients with AN had elevated PWV of the aorta, indicating increased aortic stiffness in this patient population. This result suggests that patients with AN may be at risk for increased future cardiovascular events, although we do not know if the increased vascular stiffness is reversible with nutritional rehabilitation.

Our study also investigated the effect of degree of malnutrition, as measured by BMI percentile, on the various measures of cardiovascular function. The most malnourished patients, which we defined as those with a BMI less than or equal to the 10^{th} percentile, showed decreased LA and LV size, decreased LVM, and changes in diastolic function, while the patients with a lesser degree of malnutrition did not demonstrate these changes. Exercise endurance was independently predicted by BMI percentile. In contrast, increased arterial stiffness was not related to the degree of patient malnutrition. These results suggest that it is the most malnourished adolescent patients with AN that are likely to demonstrate changes in cardiac structure and diastolic function, however, increased vascular stiffness in AN cannot be predicted by the severity of malnutrition. In summary, our study demonstrates that patients with AN have abnormalities of their cardiovascular system including decreased cardiac size, decreased cardiac mass,

subclinical diastolic dysfunction, and increased aortic stiffness. Systolic function remains preserved in this patient population both at rest and with progressive exercise. Further study is required in order to determine if these changes are reversible with weight gain, particularly the changes in diastolic function and aortic stiffness.

Significance of the Study:

To our knowledge, this study represents the largest published comprehensive assessment of cardiovascular health in adolescent females with AN. Individually, the manuscripts in Chapters 2-4 investigate novel measures of cardiovascular structure and function and are amongst the largest studies of their kind in adolescents with AN.

Chapter 2 is one of the largest studies investigating cardiac structure and function in adolescent females with AN. This study confirms the findings of previous studies demonstrating decreased cardiac dimensions, decreased wall thickness, decreased LV mass, and similar systolic function in patients with AN compared to controls [1-4]. This is also the largest study investigating tissue Doppler in patients with AN. However, our tissue Doppler findings contrast those reported by Galetta *et al.* (2005) and bear repeating prior to drawing conclusions regarding the diastolic function in patients with AN [5]. Our study is also one of the first studies to use BMI percentile to suggest that the identified cardiac changes occur in the most malnourished patients (BMI ≤ 10^{th} percentile) with few substantial differences in cardiac parameters in less malnourished patients (BMI > 10^{th}

percentile). These finding suggest that the most malnourished patients are at higher risk of cardiac changes, which may have implications for follow-up protocols for these patients.

Chapter 3 investigates a novel topic in adolescent females with AN as, to our knowledge, arterial stiffness has not been previously evaluated in this patient population. In addition, Chapter 3 contributes to the literature supporting the premise that these patients demonstrate abnormalities in markers associated with cardiovascular risk [6-16] which may have implications for follow-up of these patients.

The role of excessive exercise in producing weight loss in AN is well documented while the role of exercise as part of therapy for AN is new and its safety is controversial [17-21]. Chapter 4 is the largest study investigating exercise function in patients with AN as well as the largest study investigating echocardiographic systolic ventricular function with progressive exercise in this patient population. This study supports the findings of subnormal exercise tolerance, decreased VO_2, blunted HR response, and decreased BP response at peak exercise in patients with AN [3, 22-28]. Chapter 4 also represents one of the only studies that demonstrates that although the parameters of cardiac function at peak exercise are different from those of controls, adolescents with AN have normal cardiac responses to progressive exercise. Our findings suggest that it was safe to exercise under the conditions of testing and is encouraging, but care should be taken in expanding this to all forms of supervised or unsupervised exercise.

Limitations

The studies performed were single-center retrospective case-control studies. This study design has several potential limitations. One limitation is the possibility of

misclassification/information bias. The classification of patients as having AN, as opposed to an alternative eating disorder, as well as the subtype of AN (AN-R vs AN-BP) was based on chart review and, therefore, there is the potential that patients were misclassified due to inadequate or inaccurate information contained in the medical record. Several potentially important baseline parameters were also not universally available in the patient records including prior level of physical activity and duration of illness prior to presentation. There was also potential for selection bias as this study was performed at a tertiary care center, which may have led to our studies investigating a more severely affected cohort of patients with AN which is not representative of the general adolescent female AN population. Due to the retrospective design, we were not able to control for the timing of the resting or exercise echocardiograms and these tests were performed at variable times in the disease course and at variable stages of nutritional rehabilitation.

Future Directions:

Our study demonstrated statistically significant differences in several measured variables; however the clinical significance of these findings is unclear at present. It is possible that the demonstrated differences in patients with AN may remain subclinical and have little impact on their clinical course, however, the potential for clinical impact cannot be ruled out in the absence of long term surveillance. The findings of our studies demonstrate that patients who are the most malnourished (BMI < 10^{th} percentile) demonstrate changes in their cardiac dimensions and diastolic function, which suggests that these patients warrant more careful monitoring. However, several factors remain unknown including the BMI percentile cutoff associated with cardiac changes, if clinical

variables other than BMI percentile are important for determining the risk of cardiac changes, and the clinical implications of these changes. The findings of our study also suggest that patients with AN may be at risk of future cardiovascular disease and may warrant long term monitoring for the possible development of premature cardiovascular disease. However, the reversibility of the increased aortic stiffness, the subset of patients at highest risk for this potential complication, the factors which worsen or lessen this risk, and the long-term clinical implication of increased aortic stiffness in the AN population are unknown. Further studies to answer these clinical questions are required. Long term studies investigating cardiac function and aortic stiffness with serial assessments in larger cohorts of patients and controls would be valuable. Such studies could potentially help answer the questions posed above, determine the impact of disease severity and duration on the cardiovascular system, as well as determine the long term impact of these changes on cardiovascular health and outcomes.

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Appendix

Appendix 1: Tabular summary of results of echocardiography in the published literature comparing females with anorexia nervosa to control groups

	de Simone *et al.* (1994)	Romano *et al.* (2003)	Vazquez *et al.* (2003)	Eidem *et al.* (2001)	Galetta *et al.* (2003)	Galetta *et al.* (2005)	Kastner *et al.* (2012)	St John Sutton *et al.* (1985)
Number of subjects	21	91	30	13	25	20	173	17
Mean/ median subject age (years)	22	20.5	15.5	16.5	17.5	22.4	15.3	26
Mean BMI	15.2	15.6	15.3	-	15.3	15.0	14.5	-
Control groups	Normal BMI and thin females	Normal weight	Normal weight age-matched	Normal weight age, gender, and heart rate matched	Normal BMI and thin females	Normal BMI and thin females	Normal weight	Normal weight
Hemodynamic parameters								
Heart rate (bpm)	Decreased (59 vs 73/76)	Decreased (55.6 vs 72.4)	Decreased (56.9 vs 82.9)	No difference (matched)	Decreased (50.6 vs 70.3/68.7)	Decreased (45.7 vs 63.7/64.2)	-	No difference
Systolic BP (mmHg)	Decreased (101 vs 118/109)	Decreased (90.8 vs 110.9)	-	-	Decreased (92.4 vs 117.3/ 112.6)	Decreased (98.8 vs 111.4/ 108.2)	-	Decreased (93 vs 110)
Diastolic BP	Decreased	Decreased			Decreased	Decreased		Decreased

(mmHg)	(66 vs 78/72)	(62.1 vs 70.3)	-	-	(50.6 vs 68.5/66.4)	55.2 vs 65.6/64.7)	-	(65 vs 74)
Cardiac Chamber Dimensions								
LA size (cm)	Decreased (2.55 vs 3.09)	-	-	-	-	-	-	-
LVEDD (cm)	Decreased (3.96 vs 4.72/4.41)	Decreased (4.26 vs 4.57)	Decreased (4.19 vs 4.49)	-	Decreased (4.08 vs 4.49/4.48)	Decreased (3.9 vs 4.5/4.4)	Decreased (4.1 vs 4.7)	Decreased (3.9 vs 4.4)
LVESD (cm)	-	-	Decreased (2.53 vs 2.78)	-	Decreased (2.14 vs 2.88/2.81)	-	Decreased (2.5 vs 2.9)	Decreased (2.6 vs 2.9)
IVSD (mm)	-	-	Decreased (6.1 vs 6.9)	-	No difference	Decreased (6.5 vs 8.0/7.9)	No difference	-
LVPWD (mm)	-	-	No difference	-	No difference	Decreased (5.9 vs 7.5/7.5)	No difference	Decreased (0.6 vs 0.7)
LV mass (g)	Decreased (73.3 vs 124/103)	Decreased (71.2 vs 96.9)	Decreased (76.2 vs 98.3)	Decreased (79.4 vs 106)	Decreased (82.7 vs 126.1/ 119.5)	Decreased (66.2 vs 109.5/ 106.8)	-	-
LV mass/BSA (g/m^2)	Decreased (54.9 vs 75.2/66.9)	Decreased (52.2 vs 61.3)	Decreased (53.9 vs 64.0)	-	-	-	-	Decreased (53 vs 79)
LV mass/height ($g/m^{2.7}$)	Decreased (21.1 vs 33.2/26.5)	Decreased (20 vs 26)	-	-	Decreased (21.2 vs 31.4/29.2)	Decreased (20.3 vs 30.1/29.5)	-	-
Relative wall thickness	No difference	-	-	-	-	-	-	No difference

Measures of Cardiac Function								
Fractional shortening	No difference	-	No difference	-	No difference	No difference	No difference	No difference
Ejection fraction	-	Decreased (62.7 vs 65.03)	No difference	No difference	No difference	No difference	-	No difference
Peak E velocity (m/s)	No difference	-	-	-	No difference	No difference	-	-
Peak A velocity (m/s)	Decreased (0.43 vs 0.56/0.52)	-	-	-	Decreased (0.359 vs 0.466/ 0.452)	Decreased (0.337 vs 0.453/ 0.443)	-	-
Peak E/A ratio	Increased (1.96 vs 1.52/1.51)	-	-	No difference	Increased (2.8 vs 1.9/1.8)	Increased (2.8 vs 1.95/1.96)	-	-
Mitral inflow deceleration time	-	-	-	No difference	No difference	-	-	-
LV MPI	-	-	-	Increased (0.49 vs 0.35)	-	-	-	-
RV MPI	-	-	-	No difference	-	-	-	-
IVRT	-	-	-	-	No difference	-	-	-
End systolic stress (dyn/cm^2)	No difference	-	-	-	-	-	-	No difference
Velocity of circumferential fiber shortening	-	-	-	-	-	-	-	No difference

Echocardiographic Hemodynamic Variables								
Cardiac output (L/min)	Decreased (2.69 vs 5.57/4.39)	Decreased (2.8 vs 4.5)	-	-	-	-	-	-
Cardiac index (L/min/m^2)	Decreased (2.01 vs 3.4/2.87)	-	-	-	-	-	-	-
Stroke volume (ml/beat)	Decreased (46.2 vs 77.6/58.4)	Decreased (50.7 vs 62.1)	-	-	-	-	-	-
Stroke index (ml/beat/m^2)	Decreased (29.1 vs 47.7/35.3)	-	-	-	-	-	-	-
SVR (dyn•s•cm^{-5})	Increased (2791 vs 1527/1636)	Increased (2191 vs 1590)	-	-	-	-	-	-
Tissue Doppler								
S' lateral	-	-	-	-	-	Decreased	-	-
S' septal	-	-	-	-	-	Decreased	-	-
E' lateral	-	-	-	-	-	No difference	-	-
E' septal	-	-	-	-	-	No difference	-	-
A' lateral	-	-	-	-	-	No difference	-	-
A' septal	-	-	-	-	-	No difference	-	-
E/E' lateral	-	-	-	-	-	Increased	-	-
E/E' septal	-	-	-	-	-	Increased	-	-
E'/A' lateral	-	-	-	-	-	No	-	-

						difference		
E'/A' septal	-	-	-	-	-	No difference	-	-
Other								
MV abnormal motion (% of subjects)	Increased (62 vs 5/9)	-	-	-	-	-	-	-

BMI = body mass index, bpm = beats per minute, BP = blood pressure, LA = left atrial, LVEDD = left ventricular end-diastolic diameter, LVESD = left ventricular end-systolic diameter, IVSD = interventicular septal diameter, LVPWD = left ventricular posterior wall diameter, LV = left ventricular, BSA = body surface area, MPI = myocardial performance index, RV = right ventricular, IVRT = isovolumic relaxation time, SVR = systemic vascular resistance, MV = mitral valve