

ABSTRACT

Title of Dissertation: OBESITY, METABOLIC SYNDROME, AND
CARDIOVASCULAR OUTCOMES IN
PEDIATRIC KIDNEY TRANSPLANT
RECIPIENTS

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Cardiovascular (CV) disease is a leading cause of morbidity amongst children after kidney transplant. The contribution of abdominal obesity and metabolic syndrome (MS) to CV risk is not well defined in this population. A prospective controlled longitudinal cohort study was conducted to investigate contributions of obesity and MS to CV morbidity in a multiracial pediatric kidney transplant population. Aims of the study were to 1) identify prevalence of CV and metabolic abnormalities 2) evaluate effects of obesity and MS on adverse CV outcomes, defined by left ventricular hypertrophy (LVH), impaired myocardial strain and increased carotid intima-media thickness (CIMT) and 3) identify the anthropometric measure of obesity, Body Mass Index (BMI), Waist-to-Height ratio (WHr), or Waist

Circumference (WC), that best predicts CV risk. Transplant recipients had standard echocardiographic measures of left ventricular size and function, strain by speckle tracking echocardiography, and CIMT measured at 1, 18, and 30 months post-transplant. 35 pre-transplant echocardiograms were analyzed retrospectively. Multivariate longitudinal regression was used to determine associations of obesity and MS with CV outcomes. Results indicated obesity and MS are prevalent among pediatric kidney transplant recipients. WHr is a more sensitive indicator of obesity-associated adverse CV outcomes compared with BMI or WC, due in part to the prevalence of short stature in this population. Obesity, MS, and hypertension are associated with post-transplant LVH. Significant predictors of impaired longitudinal strain include obesity, hypertension, and a combination of MS with elevated LDL-cholesterol, whereas higher estimated glomerular filtration rate confers a protective effect. African American pediatric kidney transplant recipients have increased CIMT, which is negatively impacted by MS, whereas the CIMT of non-African American children appears unaffected after transplant. In conclusion, obesity and MS adversely affect CV outcomes in pediatric kidney transplant recipients, highlighting the importance of efforts to maintain healthy weight, blood pressure, and lipid profile after transplant. Further studies are needed to investigate the etiology and consequences of increased CIMT in African American transplant recipients. Imaging techniques such as speckle tracking echocardiography and CIMT may provide a means of detecting subclinical myocardial dysfunction and provide opportunity for early intervention in this population.

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IN PEDIATRIC KIDNEY TRANSPLANT RECIPIENTS

by

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Dedication

This is dedicated to my close friends and family who made it all possible.

A special thanks to my niece Ella for her help.

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I would like to acknowledge the invaluable guidance and support of all of my dissertation committee members, from whom I learned so much along this journey. I would also like to thank the research sonographer for her excellent work in performing the imaging for this study. Finally, I would like to acknowledge all of the children who gave their time to participate in this study in order to benefit children with kidney disease in the future.

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List of Abbreviations

A-A	African American
APOL1	Apolipoprotein L1
BMI	Body Mass Index
BP	Blood Pressure
CKD	Chronic kidney disease
CKiD	Chronic kidney disease in children study
CMR	Cardiac magnetic resonance imaging
CNI	Calcineurin inhibitors
CV	Cardiovascular
DBP	Diastolic blood pressure
Echo	Echocardiogram
EF	Ejection fraction
eGFR	estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ESRD	End stage renal disease
FS	Fractional shortening
GEE	Generalized estimating equation
GWAS	Genome wide association studies
HDL-C	High density lipoprotein cholesterol
iPTH	Intact parathyroid hormone
IRB	Institutional Review Board
LDL-C	low density lipoprotein cholesterol
LV	Left ventricular
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
MS	Metabolic Syndrome
MSAT	Metabolic Syndrome after transplantation
mTOR	Mechanistic target of rapamycin
NHANES	National health and Nutrition Examination Study
NHLBI	National Heart, Lung, and Blood Institute
NODAT	New onset diabetes after transplantation
OGTT	Oral glucose tolerance test
PWV	Pulse wave velocity
QIC	Qasi-likelihood under Independence model Criterion
RAAS	Renin-angiotensin-aldosterone system
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Standard deviation score
SEM	Standard error of the mean

TG	Triglycerides
Tx	Transplant
VLDL-C	Very low density lipoprotein
WC	Waist Circumference
WHR	Waist to Height ratio

Chapter 1: Introduction

Objective and Rationale

The objective of this prospective controlled longitudinal cohort study was to investigate the contribution of obesity and metabolic syndrome (MS) to cardiovascular (CV) outcomes in pediatric kidney transplant recipients.

CV disease is the most common cause of death among young adults with childhood-onset kidney disease (1), and the second leading cause of death among pediatric kidney transplant recipients (2). Kidney disease is a life-long chronic illness, and the subclinical risks to CV health begin in early childhood. Autopsy studies have identified fatty streaks and atherosclerotic lesions, attributable to obesity and metabolic syndrome risk factors, in the arteries of children and young adults who died of accidental causes (3, 4). However, because the major CV events (such as myocardial infarction, stroke, or death) often don't occur until young adulthood or later, it has historically been difficult to prospectively study risk factors for severe CV outcomes in pediatric kidney transplant recipients. Therefore, evidence-based longitudinal research and clinical guidelines to identify the children at highest CV risk in this vulnerable population are lacking. The recent emergence of newer non-invasive technology, such as carotid intima media thickness (CIMT) and speckle tracking echocardiography with strain imaging, may provide the opportunity to accurately identify subclinical CV disease early in the course, allowing a window for early intervention. The identification of the metabolic and CV risk factors that best predict subclinical damage (identified by CIMT and strain), are important for accurate CV risk stratification in this population, with the ultimate goal of delaying or avoiding the occurrence of major CV events, and prolonging the life expectancy of children after kidney transplantation.

Study Aims

Primary Aims

- 1) To identify the prevalence of metabolic and CV abnormalities in a multiracial pediatric kidney transplant population.
- 2) To compare CV outcomes (left ventricular hypertrophy (LVH), longitudinal and circumferential strain, and CIMT) of transplant recipients to healthy controls.
- 3) To investigate obesity, MS, and individual components of MS as determinants of CV outcomes in the transplant recipients.

Secondary Aim

- 1) To identify the anthropometric measure of obesity, BMI, Waist-to-height ratio (WHr), or waist circumference (WC), that best predicts adverse CV outcomes in pediatric kidney transplant recipients.

Hypotheses

Hypothesis 1:

CV abnormalities including LVH, increased myocardial strain and increased CIMT will be prevalent among pediatric kidney transplant recipients.

Hypothesis 2:

CV outcomes of transplant recipients will be worse compared with controls.

Hypothesis 3:

Obesity and MS will contribute to risk of adverse CV outcomes in transplant recipients.

Hypothesis Secondary Aim:

WC for-age-percentiles may underestimate the prevalence of abdominal obesity in children of short stature. Impaired growth is common in children with chronic kidney disease. Therefore WHr may be the most sensitive method to identify pediatric kidney transplant recipients at risk for CV morbidity.

Chapter 2: Literature Review

Metabolic Syndrome after Transplantation (MSAT)

MS is the name coined for a constellation of risk factors that increase future risk of CV disease, stroke, and diabetes. MS is traditionally defined as the presence of at least 3 of the following morbidities: abdominal obesity, impaired glucose tolerance (reflecting insulin resistance), hypertension, hypertriglyceridemia, and low HDL-C cholesterol (5). The basic theory underlying MS is that while each individual component is an independent predictor of CV disease, the combination of multiple factors creates a synergistic CV risk profile that is greater than the sum of its parts. In the general population, studies have shown that childhood MS is a significant predictor of CV disease in adulthood (6). The traditional paradigm of MS is altered by a myriad of factors associated with chronic kidney disease (CKD) and transplantation. In kidney transplant recipients, typical risk factors for MS, such as excess intake of processed foods and physical inactivity, converge with transplant medication-induced effects of dyslipidemia, glucose intolerance, and hypertension to create a hybridized version of MS unique to this population. MSAT has been associated with more rapid decline in allograft function over time (7-9) and increased risk of atherosclerotic events (10). As such, the exact definition of MS has not been consistent across the transplant literature (see Table 1). A retrospective cohort study of pediatric kidney transplant recipients (n=234) reported that the rate of MS significantly increased from 18% pre-transplant to 37% at one year post-transplant, using BMI rather than abdominal adiposity to define obesity (11). A key finding of this study was that MS was significantly associated with presence of LVH at one year post-transplant (OR 2.6, 95% CI 1.2-5.9). Another study that investigated MS in 32 children after renal transplant did assess abdominal obesity, but did not *require* obesity to define MS. Of 8 children determined to have MS in this study, only 3

were obese. The fact that the majority of children determined to have MS in this study were lean suggests the involvement of individual transplant-related risk factors *other than obesity* at play in recipients (12). Therefore, MSAT differs from the classic model of MS, and the interplay of its individual components in pediatric transplant recipients is unclear.

Table 1: Summary of studies on metabolic syndrome in pediatric kidney transplant

recipients

Author	Year	MS Definition	Prevalence	Results/Associations
Ramirez-Cortez et al.(12)	2009	≥ 3 criteria: <ul style="list-style-type: none"> • WC>75cm • BP>95thile • HDL-C-C$\leq 10^{\text{th}}$ile • TG$\geq 90^{\text{th}}$ile or on statin • Glucose>140 mg/dl (3h OGTT) 	25% (8/32)	<ul style="list-style-type: none"> • Higher proportion of deceased donor grafts • Increased frequency of acute rejections and use of steroid pulses • BMI pre-transplant
Wilson et al.(11)	2010	≥ 3 criteria: <ul style="list-style-type: none"> • BMI>97thile • BP>95thile or on BP med • HDL-C<5thile or on statin • TG>95thile or on statin • Fasting glucose>100 mg/dl or on insulin 	18.8% at tx (34/181) 37% at 1 year post-tx (67/181)	<ul style="list-style-type: none"> • Higher odds of LVH (OR 2.6) • Higher odds of Eccentric hypertrophy (OR 3.0)
Maduram et al(8).	2010	≥ 3 criteria: <ul style="list-style-type: none"> • BMI>97thile • BP>95thile or on BP med • HDL-C-C<5thile • TG>95thile • Fasting 	68% in steroid group (17/25) 15% in steroid withdraw group (5/33)	<ul style="list-style-type: none"> • Lower eGFR in children at 1 year post-transplant (65) versus those without MS (88) mL/min/1.73 m²

		glucose>100 mg/dl		
Tainio et al.(7)	2014	≥ 3 criteria: <ul style="list-style-type: none"> • >120% median weight • BP>95thile or on BP med • HDL-C<40mg/dl • TG>150mg/dl • Fasting glucose>100 mg/dl 	19% at 1.5 yr post-tx (28/147) 14.2% at 5 yr post-tx (18/127)	<ul style="list-style-type: none"> • Lower eGFR at 1.5 yrs, but no difference at 5+ years post-tx

Abbreviations: BP: blood pressure, TG: triglycerides, OGTT: oral glucose tolerance test

Individual Components of Metabolic Syndrome and kidney transplant recipients

The following section will discuss the individual components of MS and how they manifest in pediatric kidney transplant recipients.

Obesity

Childhood obesity is a significant issue affecting the general pediatric population, with recent estimates indicating that 16.9% of US children are obese (13). A cross-sectional analysis of national data representing US children ages 6-17 years old revealed that obese children are at higher risk for dyslipidemia, glucose intolerance, and hypertension compared with healthy-weight children (14). It is projected that by 2025, approximately 268 million children will be overweight, including 91 million obese world-wide. These children are expected to have obesity-related comorbidities, including impaired glucose tolerance, type 2 diabetes, and hypertension (15). Causes of obesity include poor diet and physical inactivity, as well as genetic factors; genome-wide analysis studies have recently identified more than 90 susceptibility loci for BMI (16).

Obesity is known to increase CV risk in both children and adults. The pathology of obesity-related CV risk is related to the secretion of inflammatory cytokines, such as tumor necrosis

factor-alpha (TNF- α), from adipose tissue. The inflammatory cytokines induce a variety of unfavorable effects including endothelial dysfunction, glucose intolerance, vasoconstriction, and vascular dysfunction, all of which increase CV risk (17).

BMI (kg/m²) percentile-for-age is the most common method used to diagnose overweight (85th-95th percentile) and obesity (\geq 95th percentile) in children (18). However, abdominal obesity, measured by WC or WHr, is more strongly associated with high metabolic and CV risk than is high BMI in the general pediatric population (19-21). However, each of these anthropometric methods has limitations, particularly in reference to the assessment of children with CKD.

Studies have shown that BMI does not accurately reflect body composition in children with CKD, due to their altered body habitus characterized by reduced lean mass and high fat mass, as revealed by Dual X-ray Absorptiometry (22, 23). However, waist circumference-for-age percentiles are likely to underestimate abdominal obesity in children with CKD, since impaired growth and short stature are common. Therefore more sensitive anthropometric methods are needed to accurately diagnose obesity in this population.

Obesity trends in children with CKD mirror those in the general pediatric population. In a recent analysis of 799 children who participated in the Chronic Kidney Disease in Children (CKiD) study, 15% were overweight and an additional 18% were obese (24). In this CKiD cohort, median height and weight SDS were -0.55 and 0.03, respectively, and 12% had severe short stature (SDS <-1.88). The combination of short stature with preserved or above average weight compound the risk of obesity in this population (24).

Obesity is even more common in the pediatric transplant population, and studies show that the prevalence of obesity doubles (from about 15-30%) during the first year after transplantation (25). Factors contributing to post-transplant weight gain include increased appetite and

improved taste sensation with the resolution of uremia, liberalization of renal diet restrictions, as well as sedentary lifestyle and poor overall physical fitness, which are pervasive in this population (25). Obesity in pediatric transplant recipients has been associated with decreased allograft survival and increased mortality. Nutrition education and counseling to promote a heart-healthy diet and regular physical activity, with a goal of at least 60 minutes of active play daily, are recommended to promote maintaining healthy weight and decreasing CV risk after transplant (26).

Hypertension

Hypertension is a major cause of end organ damage and CV morbidity and mortality in the general pediatric population as well as in children with renal disease. A recent evaluation of trends in pediatric hypertension based on a large sample of NHANES data indicates that the prevalence is increasing and is associated with the childhood obesity epidemic across the United States (27). The strong link between obesity and hypertension is substantiated physiologically. The release of angiotensinogen by adipose tissue promotes increase in blood pressure via stimulation of the renin-angiotensin-aldosterone system (RAAS) and increased sodium reabsorption in obese individuals (28). In pediatric kidney transplant recipients, hypertension risk is further compounded by effects of immunosuppressive medications. Corticosteroids are known to increase sodium and water reabsorption and increase in renal vascular resistance, while calcineurin inhibitors (CNI) induce hypertension via afferent arteriolar constriction, stimulation of RAAS, and secretion of inflammatory cytokines leading to fibrosis of the allograft over time (29).

As such, the prevalence of hypertension in the pediatric kidney transplant population is strikingly high. In a study of 74 children, 77% had hypertension prior to transplant, and 82.4%, 71.7%, and

61% had hypertension at 1, 5, and 10 years post-transplant, respectively (30). Hypertensive children at 10 years post-transplant had 8.1 times higher risk of graft loss compared to normotensive children (30). Hypertension has been associated with increased CIMT and myocardial strain in otherwise healthy children (31, 32), and in pediatric kidney transplant recipients (33). As hypertension is a key component of MSAT, frequent blood pressure monitoring and aggressive treatment of hypertension are critical to mitigating CV risk in pediatric transplant recipients.

Dyslipidemia

Dyslipidemia is a strong risk factor for CV disease, and compelling evidence from autopsy and cohort studies in the general population indicate that atherosclerotic lesions silently begin to develop during early childhood (4, 34, 35). Individuals with MS typically exhibit a highly atherogenic lipid profile characterized by hypertriglyceridemia and low HDL-C cholesterol, which frequently occur together in association with obesity and physical inactivity. Dyslipidemia is common in children with CKD and after kidney transplantation. In a study of 366 children with CKD, 32% had hypertriglyceridemia, 18.3% had low HDL-C cholesterol, and hypertriglyceridemia independently predicted increased CIMT, and indicator of increased risk for CV disease (36).

After transplantation, the risk of dyslipidemia is compounded by the effects of commonly used immunosuppressive agents, including corticosteroids, CNI and mTOR inhibitors. A recent registry study of 386 pediatric kidney transplant recipients reported hypertriglyceridemia in 71% of children at 3 months post-transplant, 59% at 1 year post-transplant, and hypertriglyceridemia was associated with lower GFR(37). Corticosteroids alter lipoprotein metabolism and promote dyslipidemia by stimulating hepatic synthesis of VLDL-C and down-regulating LDL-C receptors

(38). A dose-dependent effect of CNI on increased lipid levels has also been demonstrated in adults, however use of tacrolimus has generally been associated with a more favorable lipid profile in comparison to cyclosporine (39). In pediatric patients, the use of immunosuppressive regimens containing cyclosporine, mTOR inhibitor, and steroids was associated with a 25-fold increased risk of dyslipidemia compared with a regimen of tacrolimus, mycophenolate, and steroids (37). The overall high CV morbidity in the pediatric transplant population places them at high risk for early-onset CV disease, as categorized by the National Heart, Lung, and Blood Institute (NHLBI) expert panel, warranting close monitoring of lipid levels, lifestyle and dietary interventions. (40). Obese children without kidney disease are also included in the NHLBI high CV risk category, therefore compounding the projected CV risk among obese children with MSAT. The first line of management for dyslipidemia characterized by high triglycerides with low HDL-C should focus on weight management, including limited intake of saturated fats and simple sugars, and increased physical activity(26). Although there are no current pharmacological treatments for lowering triglycerides in children, limited data on the beneficial effects of omega-3 fatty acids may hold promise as a future therapy (26). In addition, adjustment of the immunosuppression regimen may be considered judiciously.

Impaired Glucose Tolerance

Impaired glucose tolerance can lead to vascular endothelial dysfunction, dyslipidemia, hypertension, and vascular inflammation and hence promote the development of CV disease. In a study of moderately obese adults with varying degrees of insulin sensitivity, those with the highest insulin resistance were found to have the highest CV morbidity (41). After transplantation, glucocorticoids induce peripheral insulin insensitivity while CNI cause an acquired defect in insulin synthesis and secretion from pancreatic beta cells (42), setting the

stage for impaired glucose tolerance. In children, it has been reported that 26.2% have impaired glucose tolerance and 8.1% develop new onset diabetes after transplantation (NODAT) by 6 months post-transplant (43). NODAT is associated with abdominal obesity and MSAT, and has also been linked with increased risk of CV events after transplant (42, 44-46).

The KDOQI pediatric guidelines recommend that patients should be counseled on a diet low in simple sugars, avoiding juices, soda, and other sweetened beverages to minimize the risks of excess weight gain and hyperglycemia post-transplant (26).

Racial differences in MS and CV risks

Individuals of African descent generally carry higher risk for MS, CV disease, and kidney disease than other races (13, 47-49). The reasons for these disparities are not completely understood, and are likely multifactorial in nature. Genetic factors are known to play a role, due to the strong association of the Apolipoprotein L1 gene (APOL1) with risk of CKD in individuals of African ancestry. Relationships of APOL1 renal risk variants with increased risk of CV disease among those of African descent are also starting to emerge (50). In the Women's Health Initiative study of 749 postmenopausal A-A women, those with 2 APOL1 alleles had a lower GFR and higher risk for incident CVD compared to those with 0 APOL1 alleles (OR 1.98, $p= 8.37 \times 10^{-3}$)(51). New insights into the role of genetic factors and obesity are also being uncovered as genome wide association studies (GWAS) delve into this area. Recently, the first GWAS for BMI in individuals of African descent identified a novel BMI locus known as "SEMA4D", which appears to promote obesity through regulation of a transcription start site, and may explain some of the increased burden of obesity among this population (52). Beyond genetics, other variables contributing to differences in cardio-metabolic risk amongst ethnic

groups may include environmental exposures, cultural beliefs, psychosocial factors, and access to healthy whole foods and health care.

A-A adults, including young adults, have higher rates of adverse CV events and CV death compared with other racial groups (53, 54). Evidence shows that A-A have higher LV mass compared with other racial groups (55). Looking at subclinical markers of CV disease, studies have shown that CIMT is higher among healthy adults and children of African ancestry compared to other ethnicities (56, 57). A recent cross-sectional study by Lefferts et al. examined racial differences in CIMT and aortic stiffness, measured by pulse wave velocity (PWV) in healthy children (58). This study reported higher PWV and CIMT in A-A children compared with Caucasian children after adjustment for age, sex, blood pressure, and socioeconomic status. The etiology of increased CIMT in this population is unknown. It remains to be seen whether it may be related to environmental exposures, genetic polymorphisms, or other causes. One study suggests that higher CIMT among individuals of African ancestry may be related to physiological differences in the size of CV structures and lean body mass that vary among different ethnic groups (59), while another study concluded that a blunted nocturnal cortisol rise, caused by psychosocial stress, may account for increased risk for atherosclerosis and CIMT in obese A-A youth (60).

Few studies have investigated ethnic differences in myocardial strain. The Multi-Ethnic Study of Atherosclerosis, a prospective, observational cohort study of 6814 healthy adults representing four ethnic groups (Caucasian, A-A, Hispanic and Chinese-American), investigated racial and ethnic differences in subclinical myocardial function using cardiac magnetic resonance imaging (CMR). A-A were found to have the least favorable systolic strain, even after correcting for hypertension and LV mass (55). Another study assessed LV strain by speckle tracking echo in a

tri-ethnic (A-A, Hispanic, and Caucasian) healthy population with normal EF, and similarly found that A-A had the greatest degree of subclinical LV systolic dysfunction detected by longitudinal strain (61).

Recent evidence suggests that the relationship and degree of synergy between individual metabolic risk factors vary greatly among different ethnic groups(62). Little is known about race-specific factors impacting MSAT in pediatric kidney transplant recipients. Given the high rate of metabolic derangement, CV morbidity, and end stage renal disease (ESRD) among those of African ancestry, future studies should investigate how the effects of transplant-related factors on cardiometabolic risk differ amongst pediatric kidney transplant recipients of different ethnicities. These differences add another layer of complexity and should be further investigated in order to establish race-specific guidelines for defining MSAT and evaluating CV risk in a diverse population of pediatric transplant recipients.

Evaluation of CV Disease

Traditional evaluation by standard echocardiography

The detection of overt abnormalities in standard measures of systolic LV function by echocardiography, such as ejection fraction (EF) or fractional shortening (FS), are rare in the pediatric population (25). Even among children with obesity and MS, EF is typically found to be normal (63). The presence of abnormal EF or FS in a child would indicate that advanced CV disease is already present. Evaluation of LV mass by traditional echocardiography is also of limited use in detecting CV dysfunction in children with CKD, due to its lack of accuracy in young children, underestimation of LVH in overweight patients, and lack of reliability in fluid overloaded patients. Recent evidence is emerging to suggest that subclinical CV abnormalities do develop early in children with CKD and may portend adverse outcomes. Thus, there is a need

for newer, more sensitive tools such as speckle tracking echocardiography and CIMT for early detection of CV dysfunction in pediatric kidney transplant recipients.

Left Ventricular Hypertrophy (LVH)

LVH has traditionally been considered important in the identification of CV risk. LVH can develop early in the course of CKD, and often persists after kidney transplant, particularly in association with MSAT. A retrospective study of 234 pediatric transplant recipients reported a 40% prevalence of LVH and 2.6 times greater risk of LVH in recipients with MS than in those without MS (11). However, there is no consensus on the best way to define LVH in young children, due to the significant changes in the relationship of their height to body and heart size with rapid growth, nor in the pediatric kidney transplant population, due in part to their abnormal body composition and short stature (64). LV mass normalized to height^{2.7} is a commonly used method to evaluate LVH, as it describes the relationship between heart and body size without obscuring effects of obesity. In children ≥ 10 years of age, LVH is defined as $>40\text{g/m}^{2.7}$ in girls and $>45\text{ g/m}^{2.7}$ in boys. However, this method is not reliable in children under the age of 10 years, and in addition may underestimate relative LVM in thin children and overestimate LVM in overweight children (65-67). To address this limitation, Khoury et al. developed normal age-specific percentiles for LVM/height^{2.7}, using LVM/height^{2.7} $>95^{\text{th}}$ percentile for age to define LVH (66). Subsequently, Foster and Khoury et al. developed new LV mass reference percentiles expressing LV mass relative to lean body mass, which is the strongest determinant of LV mass (68). Thus, while some studies have reported improvement in LVH after transplant, findings have been inconsistent across the pediatric literature, due in part to the limitations discussed.

Table 2. Summary of studies on LVH by echo in pediatric kidney transplant recipients

Author Year	Design	Definition of LVH	Prevalence LVH	Results/Associations
------------------------	---------------	------------------------------	---------------------------	-----------------------------

Bullington et al.(69) 2006	Cohort	LVM/ht ($m^{2.7}$) >95 th ile for age and gender	54% at 14 months post-tx*; no change at 33 and 49 mo. (n=47)	SBP and BMI predicted LVH. LVH prevalence remained unchanged, but LVMI decreased between 14 and 49 mo. post-tx.
Guizar-Mendoza et al.(70) 2006	Cohort	LVM/ ht ($m^{2.7}$) >95 th ile for age and gender	77.5% pre-tx 52.5% post-tx (n=40)	LVH decreased after tx, and was related to baseline LVM and living-donor type.
Silverstein et al.(71) 2007	Cross-sectional	Not specified (n=45)	18.2% (n=45)	Authors noted lower prevalence of LVH compared with other studies. Insufficient data to compare LVH pre-tx vs post-tx.
Becker-Cohen et al.(72) 2008	Cohort	LVM/ht ($m^{2.7}$) > 2 SD above mean for age	54% pre-tx, 23% 3 months post-tx, 8% yr 2 post-tx (n=13) vs 50% in dialysis group(n=12)	LVM and prevalence of LVH improved after tx, but not in those who remained on dialysis. LVM correlated with BP.
Wilson et al.(11) 2010	Cross-sectional	LVM/ht ($m^{2.7}$) >95 th ile for age and gender	18.8% at tx 37% at 1 yr post-tx (n=181) 40% among overweight (n=55) 74.5% among obese (n=51)	Prevalence of LVH increased from 18.8% to 37% in first year post-tx. Metabolic syndrome strongly associated with LVH.
McLaughlin et al.(73) 2014	Cohort	3 methods: 1)LVM/ht ($m^{2.7}$) >95 th ile for age and gender 2)Children>9 yrs as above; for children≤9 yrs, >40g/ $m^{2.7}$ girls and >45g/ $m^{2.7}$ boys 3)LVM/ht ($m^{2.7}$) >51 g/ m^2	24-33% pre-tx 0-25% post-tx Varied by cut-point method (n=27)	Prevalence of LVH improved after tx but individual trends highly variable. Prevalence also varied by definition of LVH used.
Weaver et al.(74) 2016	Cohort	LVM/ht ($m^{2.7}$) z-score > 1.645	Improved from 48% to 35% from 1 to 2 yrs post-transplant	LVM improved in those on steroid avoidance vs steroids SBP and BMI independently predicted LVH.

			in steroid avoidance group (n=70 per group)	
Alparslan et al. (75) 2016	Cohort	LVM/ ht ($m^{2.7}$) >95 th ile for age and gender	37.5% pre-transplant 29.2% 6 months post-transplant (n=24)	No significant change in LVM pre vs post-transplant.
Arnold, et al. (76) 2016	Cross-sectional Echo vs CMR	Echo: LVM>95 th ile for age and height	Echo: 32% CMR:8% (n=25, 11 CKD and 14 transplant)	Echo overestimates LVH compared to CMR. CMR-LVM is correlated with future eGFR decline, echo-LVM is not.
Rumman et al.(77) 2017	Cohort	LVM adjusted for BSA, height ($m^{2.7}$), and converted to z-scores	Not reported (n=48)	No change in LVM pre vs. post-transplant. LVM of transplant patients greater vs. controls.

*Transplant is abbreviated as tx

Novel imaging tools for early detection of CV morbidity

Carotid intima media thickness (CIMT)

CIMT has emerged as a reproducible surrogate marker for early atherosclerosis (78). Non-invasive imaging of the carotid arteries is used to demonstrate the status of the intima-media thickness of the vessel. Prospective studies have demonstrated that an increase in CIMT is associated with an increase in the relative risk for stroke and myocardial infarction in the general adult population (79, 80). Increased CIMT has been found to be associated with MS and its components (hyperlipidemia, hypertension, obesity, type I diabetes) in the general pediatric population (81). Woo et al. conducted a study investigating the effect of diet and exercise on noninvasive markers of atherosclerosis in otherwise healthy obese children, and found a significant improvement in CIMT and percent body fat after one year of intervention, suggesting

that a significant change in CIMT can be detected after a 1 year period (82). Studies of CIMT in pediatric kidney transplant recipients are summarized in Table 1. Two recent studies investigating CIMT in pediatric renal transplant recipients found that CIMT was significantly greater compared to healthy controls, and CIMT correlated with duration of dialysis prior to transplantation (83, 84). Litwin et al. reported that CIMT of children with CKD or on dialysis increased by 0.7 SD over a period of one year, while CIMT improved by an average of 0.6 SD within one year in those who received kidney transplants (85). The CIMT of pediatric transplant recipients of African ancestry has not been previously reported. Only two of the nine previous studies summarized below included a small number of A-A patients, the CIMT of the A-A recipients was combined with other races in the analysis.

Table 2. Summary of studies on CIMT in pediatric kidney transplant recipients

Author	Year	Design	Population (Location)	A-A (n)	Results	Associations
Mitsnefes et al.(25)	2004	Cross-sectional	31 tx*/31 control (Cincinnati,OH)	7	CIMT tx higher vs controls	SBP, number of BP meds
Litwin et al.(86)	2005	Cross-sectional	34 tx/55 CKD / 37dialysis/270 control (Germany, Poland)	0	CIMT higher in all patient groups vs control	Higher Calcium x phosphorus, dialysis
Bilginer et al.(87)	2007	Cross-sectional	24 tx/20 control (Turkey)	0	CIMT higher in tx vs controls	Calcium x phosphorus; duration dialysis
Litwin et al.(85)	2008	Cohort, 12 months duration	32 ESRD; 19 underwent tx during study (Germany,Poland)	0	CIMT tx decreased over time by 0.7 SD	Phosphorus, duration dialysis, BP
Krmar et al. (88)	2008	Cohort, mean 4.1 yr duration	31 tx/21 control (Sweden)	0	CIMT tx stable over time, higher vs controls	No association between BP and CIMT

Delucchi et al. (84)	2008	Cross-sectional	12 tx/8 dialysis/20 control (Chile)	0	CIMT dialysis and tx higher vs controls	Duration of dialysis
Siirtola et al.(89)	2010	Cross-sectional	13 tx/26 control (Finland)	0	CIMT tx higher vs controls	GFR <60, triglycerides
Basiratnia et al.(90)	2010	Cross-sectional	66 tx/66 control (Iran)	0	CIMT tx higher vs controls	Calcitriol dose
Tawadrous et al. (91)	2012	Cross-sectional	14 tx/15 dialysis/15 control (Brooklyn,NY)	6	CIMT dialysis higher vs tx and controls	None identified
Borchert-Morlins et al. (92)	2017	Cross-sectional	109 tx (Germany)	0	CIMT elevated in 58% of tx	None identified

*Tx indicates an abbreviation for transplant recipients.

Myocardial strain by speckle tracking echocardiography

Assessment of myocardial strain by speckle tracking echocardiography utilizes new technology to analyze myocardial motion by tracking natural acoustic markers (or speckles) as they move during myocardial contraction (93). Strain is emerging as an important, non-invasive tool for the assessment of LV systolic function. Its key advantage is the ability to detect early signs of LV dysfunction that are not evident by standard echo. In addition, strain analysis is load-independent, making it well-suited for evaluation of myocardial function in children with kidney disease. Recent evidence indicates that subclinical myocardial dysfunction, detected by impaired myocardial strain but not by standard echocardiography, is present in otherwise healthy children and young adults with obesity, hypertension, and type 1 diabetes (63, 94-98). Impaired strain has also been shown to be indicative of early myocardial dysfunction in septic shock in children (99) of prognostic value for myocardial recovery after myocardial infarction (100), and an accurate predictor of cardiac events and CV mortality, superior to EF (101). Longitudinal strain

was a strong independent predictor of mortality in a recent study of adult hemodialysis patients with preserved EF, further supporting the utility of speckle tracking echocardiography in identifying early subclinical CV risk (102). Little is known about the myocardial strain of pediatric kidney transplant recipients. To date, three studies investigating myocardial strain in children with CKD have been published in the literature. The Cardiovascular Comorbidity in Children with CKD (4C) study investigated myocardial strain in 272 children with CKD in 14 European countries compared with 61 healthy controls (103). They found that despite having a normal EF, the myocardial strain of children with CKD was impaired in the radial and circumferential directions. In addition, LVH was more common in the children with CKD compared with controls (55% versus 7%, $p=0.001$), and the LVH geometry was preferentially concentric. There were no differences noted in longitudinal strain between the CKD and control groups. The authors hypothesize that the concentric LVH may have occurred as a response to impaired circumferential function, possibly suggestive of intrinsic structural abnormalities of the heart muscle in children with CKD. Another recent cross-sectional study investigated myocardial strain in children with ESRD (19 dialysis patients and 17 transplant patients) compared with 33 healthy controls (104). This study similarly found no differences in EF between patients and controls, but significantly increased LV wall thickness and impaired myocardial strain in the dialysis and transplant patients compared with controls. The myocardial dysfunction in the dialysis and transplant groups was characterized by impaired longitudinal strain, while circumferential and radial strain did not differ from controls. This is in contrast to the findings of circumferential dysfunction in the CKD population reported by the 4C study, and may reflect different LV mechanics during different stages along the continuum of renal disease. Looking closer at this continuum, Rumman et al. conducted a retrospective analysis of myocardial strain

in 48 children at 3 different time points: CKD, dialysis, and 1 year post-transplant compared with 192 healthy controls (77). Results of this study indicated that ejection fraction of children was similar to controls, and remained normal throughout dialysis and transplantation. Longitudinal and circumferential strain parameters were similar to controls during CKD. Longitudinal strain worsened during dialysis ($\beta=2.0$, 95% CI 0.4-3.6), but the association was not significant after adjustment for blood pressure and CKD. Following transplantation, longitudinal strain improved back to CKD levels.

The existing data on myocardial strain is limited, but suggests that children with CKD have subclinical myocardial dysfunction that develops during the course of CKD, worsens during dialysis, and may persist after kidney transplantation. In children, the signs of myocardial dysfunction are typically not apparent by standard echocardiography, but subclinical abnormalities are detectable by speckle tracking echocardiography. The investigation of strain in the pediatric kidney transplant population is of particular importance, as it may provide an opportunity to identify those children at highest risk and provide an opportunity for early intervention. The studies discussed above were limited primarily to Caucasian populations. Pediatric kidney transplant recipients of African ancestry are an underrepresented group that should be included in future studies.

Chapter 3: Methods

Study Design

A prospective, controlled, longitudinal cohort study was conducted. An overview of the study design is presented in Figure 1.

IRB Approval and Informed Consent

The study was conducted at Children's National, a pediatric hospital and kidney transplant center, located at 111 Michigan Avenue NW, Washington DC, 20010. Approval was obtained from the Institutional Review Board (IRB) at Children's National and from the IRB at University of Maryland College Park. All investigators completed the Collaborative Institutional Training Initiative Social and Behavioral Research Basic course, an IRB required ethics course. Informed consent was obtained from all participants over the age of 18 years and parents of those participants under the age of 18 years. Informed assent was obtained from all participants between the ages of 7 to 18 years, in accordance with the policy of Children's National IRB. Participants did not receive any financial compensation for participating in this study.

Study Participants

Transplant Study Group

Children who received a kidney transplant at Children's National, age 3-20 years, were eligible to participate in the study, with a goal to enroll a minimum of 24 transplant patients (10 obese, 14 lean), with an allowance for additional enrollment to account for the possibility of loss to follow-up due to the longitudinal nature of the study. Transplant recipients were eligible to enroll at any time between 0 and 18 months post-transplant and were then followed until 30 months post-transplant.

Inclusion criteria

- Age 3-20 years
- Kidney transplant recipient at Children's National

Exclusion criteria

- Diagnosed with diabetes prior to transplant
- Multi-organ transplant
- Underlying heart disease (to be excluded from standard echo and strain analysis only)
- Estimated Glomerular filtration rate (eGFR) <60 mL/min/1.73 m²
- Recurrent Focal segmental glomerular sclerosis with nephrotic range proteinuria post-transplant

Healthy Control Group

In addition, 24 healthy children were recruited to serve as controls.

Inclusion criteria

- Healthy non-obese children
- Age 3-20 years
- Normotensive

Exclusion criteria

- History of heart, kidney, or other chronic disease
- Obesity
- Hypertension

B. Evaluation of anthropometric parameters

Anthropometric measurement

Weight, height, and waist circumference were measured at 1 month, 18 months, and 30 months post-transplant.

Weight and Height

Weight and height of each participant were measured by a trained pediatric nurse according to standard procedure in the Heart and Kidney Clinic at Children's National at each study visit.

Weight was measured to the nearest 0.1 kg using a digital scale (Scale-tronix, Dynamic Scales, Inc., Terre Haute, IN), which was routinely calibrated according to Children's National standards. Height was measured to the nearest 0.1 cm using a stadiometer with sliding perpendicular head piece affixed to the wall (The Standard Stadiometer, Perspective Enterprises, Portage, MI). The stadiometer was routinely calibrated according to Children's National standards.

The participant was instructed to remove shoes, and touch posterior heel, buttocks, shoulder blades and head to the vertical board of the stadiometer. Frankfurt plane positioning of head was ensured.

Waist Circumference

Waist circumference was measured to the nearest 0.1 cm by a trained registered dietitian (Kristen Sgambat, MS, RD) at each study visit using a Gulick IIR fiberglass tape measure (Country Technology, Gays Mills, WI). Waist circumference was measured at the upper-most lateral border of the right ileum, at the end of an expiration, according to the NHANES procedure manual guidelines (105).

Anthropometric classification

Presence of obesity was then assessed by three different methods: BMI, waist circumference (WC), and waist:height ratio (WHR). Patients in the transplant group were stratified to the obese or non-obese group, based on the following criteria:

- **BMI** was calculated as weight (kg) divided by height (m²) and was converted to age-and-sex specific percentiles and z-scores based on the 2000 Centers for Disease Control (CDC) growth charts, with BMI \geq 95th percentile defined as obese (18).
- **WC** waist circumference percentiles were determined according to CDC age-and-sex specific tables and obesity defined as waist circumference \geq 95th percentile (106).
- **WHR** was calculated as a simple ratio of weight in cm divided by height in cm. A WHR cut point of \geq 0.539 was used to classify obese, based on NHANES III data according to the method of Kahn et al. (19).

C. Evaluation of metabolic and CV parameters

Blood Pressure and Hypertension

All transplant patients had systolic and diastolic morning blood pressure measured at 1, 18, and 30 months post-transplant, as shown in Figure 1. Blood pressure was measured by a trained pediatric nurse using an automated mobile blood pressure device with age- and-size-appropriate cuff (Mobile Aneroid model 767, Welch Allyn, Skaneateles Falls, NY). Seated blood pressure measurements were taken on the right arm, with the participant's arm extended at heart level. Systolic and diastolic blood pressures were recorded to the nearest 1 mmHg.

Prevalence of "hypertension" and "uncontrolled hypertension" were determined in the transplant group. Hypertension was defined as a patient requiring anti-hypertensive medication on a chronic basis. Uncontrolled hypertension was defined as having a systolic or diastolic blood

pressure $\geq 95^{\text{th}}$ percentile for age, sex, and height for those 2-18 years old (107) and $\geq 130/85$ mm Hg for those >18 years old (irrespective of taking blood pressure medication).

Dyslipidemia

All transplant patients had a fasting lipid panel, which included total cholesterol, direct LDL-C (not calculated), HDL-C, and triglycerides measured at 1, 18, and 30 months post-transplant (Figure 1). Patients were instructed to fast, with nothing to eat or drink except water, for 12 hours prior to the 9 am morning lab draw. For this test, participants had 1 mL of blood drawn at Children's National Laboratory. Blood was drawn in a red top tube and centrifuged to obtain a minimum of 0.5 mL plasma. The samples were analyzed using a Siemens Dimension EXL Chemistry Analyzer by enzymatic method.

Prevalence of dyslipidemia was determined in the transplant group. Abnormal lipid levels were defined according to the following:

HDL-C and LDL-C

- Ages 3-19 years: HDL-C ≤ 40 mg/dL or LDL-C >130 mg/dL based on 2011 Expert Panel Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, or on lipid lowering therapy (40).

Pediatric Triglycerides

- Ages 3-9 years: Triglycerides ≥ 100 mg/dL (40).
- Ages 10-19 years: Triglycerides ≥ 130 mg/dL (40)

Adult

- >19 years: Triglycerides ≥ 150 mg/dl, LDL-C > 100 mg/dl, HDL-C ≤ 40 (males) or ≤ 50 (women) based on Adult Treatment Panel guidelines (108).

Impaired glucose tolerance

Fasting plasma glucose level and HbA1c% (glycosylated hemoglobin) were measured at 1, 18, and 30 month time points, as shown in Figure 1.

Biochemical analysis of HbA1c% was performed in Children's National laboratory using a DCA Vantage Analyzer (Siemens Medical Solutions USA, Inc, Malvern PA). Participants had 1 mL of blood collected in an EDTA lavender top tube for point-of-care testing. The blood was then drawn into a capillary holder (0.1 mL) and placed in the buffer tray of the analyzer to activate the chemical reaction. This chemical reaction involves formation of a Schiff base compound that exists in equilibrium with glucose and hemoglobin A. Some of the Schiff base then undergoes an Amadori rearrangement to form HbA1c, which reflects the concentration of glucose present in the body over a period of time, based on the 60 day half-life of erythrocytes. The percent HbA1c of the sample is displayed on the analyzer, and may range from 2.5% to 14.0%.

Prevalence of impaired glucose tolerance was determined in the obese and non-obese transplant groups, according to the following American Diabetes Association definitions: fasting glucose >100 mg/dL, HbA1c >5.6%, or requiring insulin or oral hypoglycemic medication (109).

Metabolic Syndrome

Transplant patients with MS were defined as those who met ≥ 3 of the following 5 criteria: abdominal obesity, glucose intolerance, hypertension, low HDL-C (or on lipid-lowering medication), elevated triglycerides (or on lipid-lowering medication).

Myocardial Function and Strain

An echocardiogram was obtained at 1, 18 and 30 months post-transplant. The echocardiograms were performed by a single pediatric Diagnostic Medical Research Sonographer and measurements performed by a pediatric cardiologist, according to American Society of Echocardiography standards (110). The same instrument was used for all study participants:

iE33 xMatrix DS Ultrasound System (Philips North America Corporation, Andover, MA). Echocardiogram were analyzed using a Syngo Velocity Vector Imaging (Siemens, Germany) by a pediatric cardiologist blinded to the subject's clinical information. Standard echo parameters included LV mass indexed to height^{2.7}, FS by standard M-mode method, and EF by Simpson's method. In addition to these standard echo measures of LV size and function, measures of myocardial strain were assessed using speckle tracking analysis. Endocardial tracings of the left ventricle were performed of both the parasternal short-axis and apical four-chamber views for each subject. Parasternal short-axis tracings were conducted at the level of the papillary muscles. Myocardial strain (%) was assessed in the circumferential (short-axis measurement) and longitudinal (long-axis measurement) directions. Strain was calculated by measuring the end systolic distance between two speckles of tracked endocardium minus the original distance divided by the original distance. Because the myocardium contracts in the longitudinal and circumferential directions during systole, these values are negative percentages, and more negative values indicate better cardiac contractility. Measures of longitudinal and circumferential strain were compared between the obese and non-obese transplant groups and a healthy control group. In study participants who had a pre-transplant echo performed for clinical purposes within one year prior to transplant, echo and strain parameters were retrospectively analyzed and included for comparison. Changes in echo and strain parameters over time were assessed longitudinally, and determinants of LVH and strain were analyzed by multivariate Generalized Estimating Equation (GEE) regression analyses.

CIMT

CIMT was obtained at 1, 18 and 30 months post-transplant. B-mode ultrasound imaging of the arterial far wall segments of the right and left common carotid arteries and carotid bulbs was

performed according to a standard protocol by a single pediatric Diagnostic Medical Research Sonographer. The same instrument was used for all study participants: iE33 xMatrix DS Ultrasound System (Philips North America Corporation, Andover, MA). The CIMT protocol was as follows:

- 1) Patient is placed in the supine position with his/her neck slightly hyperextended, and rotated 30-45 degrees to the contralateral side.
- 2) Label screen Right Carotid. Scan the right carotid bifurcation in short axis.
- 3) Scan right carotid bifurcation in long axis. All efforts should be made to identify the optimal angle of orientation, visualizing both the internal and external arteries simultaneously.
- 4) Repeat long axis imaging of the carotid bifurcation in color flow to demonstrate any areas of plaque or flow disturbance.
- 5) Identify the angle of optimal image and thickest intimal interfaces on the far walls.
- 6) M-mode of common carotid artery 1 cm proximal to the bulb, (label and store image).
- 7) PW Doppler internal carotid artery to confirm correct vessel identification, (label and store image).
- 8) Image common carotid artery distal far wall, (label and store image real time and freeze frame).
- 9) Image bulb far wall, (label and store image real time and freeze frame)
- 10) Image internal carotid artery proximal far wall, (label and store image real time and freeze frame).
- 11) Repeat steps 1-10 on left side.

Intima-media thickness of the distal, mid, and proximal segments of right and left common carotid arteries and carotid bulbs was then measured by a single pediatric cardiologist blinded to the patient's clinical information. A composite of the 8 measurements was used to represent the individual's CIMT. CIMT parameters were compared between the A-A and non-A-A as well as the obese and non-obese transplant groups and the healthy control group. Change in CIMT over time was also assessed longitudinally.

D. Leptin

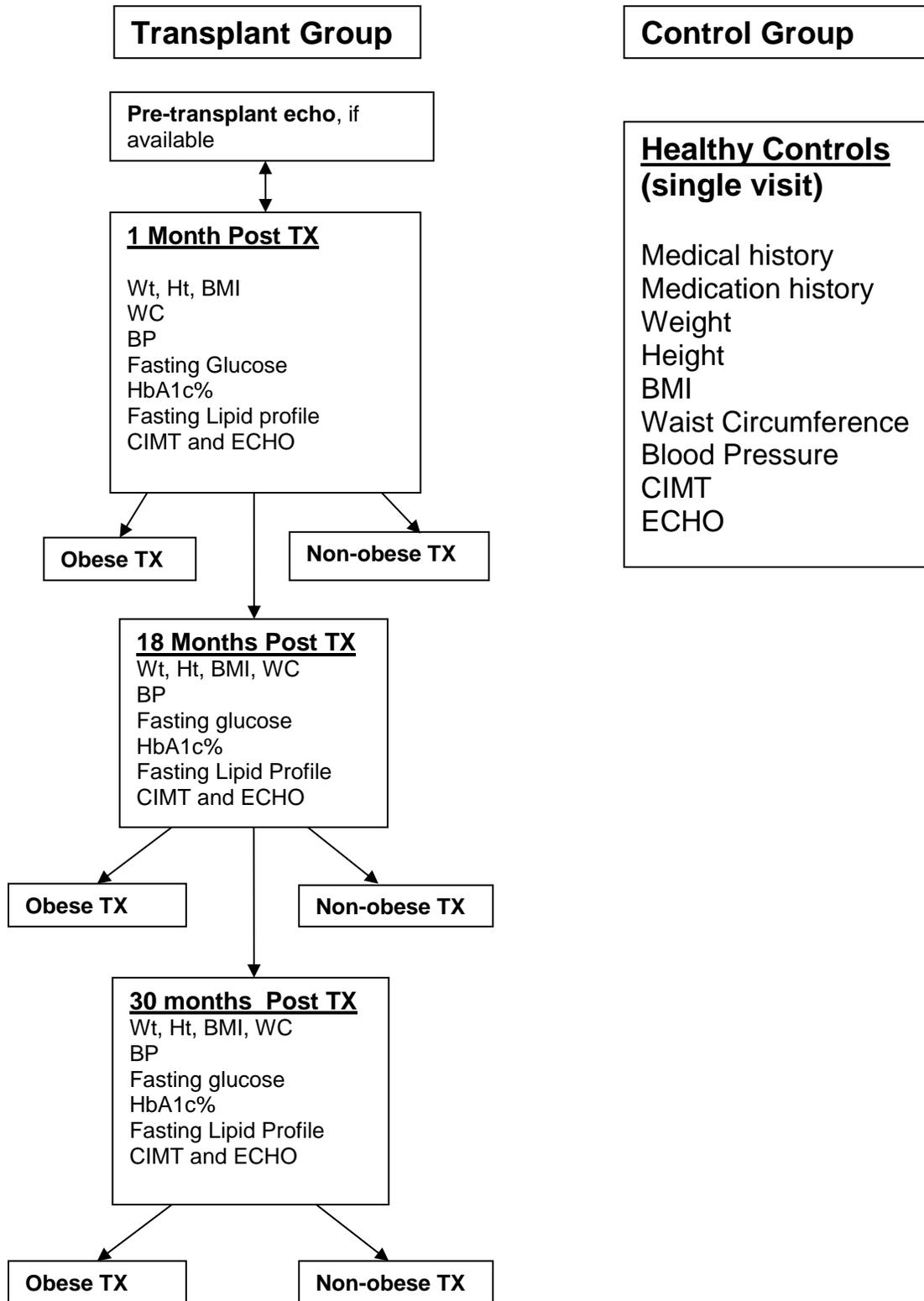
Fasting morning leptin levels were measured in a subset of the transplant group (n=27) between 1-30 months post-transplant. As this test is not part of standard care for transplant recipients, the sample for this analysis was collected from the laboratory by the investigators if blood was left over from the routine sample drawn at the transplant patient's clinic visit. For this reason, blood samples were not available for every subject at every time point. From the blood sample collected, 0.5 mL of plasma was centrifuged, refrigerated, and used for analysis of leptin using enzyme-linked immunosorbent assay (ELISA). Studies have validated ELISA as an accurate method for determining plasma leptin levels (111) .

E. Historical data collection

Historical information was obtained from the participant's electronic medical record at Children's National. This included the following information:

- Medical history and comorbidities
- Duration and modality of dialysis
- Calcium x phosphorus product prior to transplant
- Parathyroid hormone level at time of transplant
- Pre-transplant echocardiogram

Figure 1. Study Design



Statistical analysis

Statistical analysis was performed using STATA 14.0 software.

Sample size and power calculations

Bivariate: Sample size and power calculations for strain are based on our previous study of myocardial strain in a pediatric hemodialysis population, where a significant difference in longitudinal strain rate between the carnitine treated group (n=9) and the control group (n=8) was identified (mean and SD: -1.48 ± 0.33 vs. -1.91 ± 0.36 , $p=0.017$)(112). Sample size calculations for CIMT are based on prior publications in the literature, which demonstrate a 0.03-0.04 mm difference in CIMT as significant (36, 82), and the SD of CIMT in prior studies of healthy children is 0.03-0.04 (113).

Using STATA 14.0 software, it was determined that a minimum sample size of 24 transplant recipients (14 lean transplant and 10 obese transplant) and 24 healthy controls will provide $\geq 80\%$ power to detect a significant difference in CIMT and myocardial strain between groups, assuming a two tailed t-test type 1 error of 5%.

Multivariate: In addition, power analysis for multivariate regression modeling was calculated for strain and CIMT. Based on the published literature, regression models testing associations of obesity with strain and CIMT are estimated to have an expected R^2 value of 0.55 (114, 115) . Assuming that obesity contributes 0.1 to the R^2 , a minimum of 38 observations for strain and CIMT respectively will provide 80% power to detect significant associations for a regression model with 6 predictor variables. Assuming an average of 3 observations per patient, our minimum sample size of 24 transplant patients (a minimum of 72 observations) will be more than adequate to provide 80% power, assuming type 1 error of 5%.

Overall power and sample size: Therefore, considering the above calculations, our goal was to enroll a minimum of 24 transplant recipients and 24 healthy control patients to achieve adequate power for both the bivariate and multivariate analyses. Given the longitudinal nature of the study and the inherent risk of missed visits, drop outs, or losses to follow-up over 30 months duration, we allowed for enrollment of up to 42 transplant participants to ensure that our minimum target sample size of 24 transplant patients would be met each time point.

Univariate analysis

Determine the prevalence of the following morbidities in the transplant group at pre-transplant, 1, 18, and 30 months post-transplant:

- Obesity (*by WC, BMI, and WHr*)
- Dyslipidemia (*high LDL-C, high TG, low HDL-C*)
- Impaired glucose tolerance (*HbA1c%>5.6 or fasting glucose >100*)
- Hypertension (*requiring antihypertensive medication*)
- Uncontrolled hypertension (*BP \geq 95th %ile for age-sex-height*)
- Metabolic Syndrome (*meet \geq 3 of the following 5 criteria: 1) glucose intolerance, 2) hypertension, 3) low HDL-C, 4) high triglycerides, or 5) abdominal obesity (defined by WC and/or WHr methods)*)
- Abnormal CV parameters (*Left ventricular hypertrophy, impaired myocardial strain, increased CIMT*)

Bivariate analysis

1. Comparison of anthropometric measures, blood pressure, and CV outcomes between groups by Student's t-test or Wilcoxon rank-sum
 - Transplant vs. controls (LVH, strain, CIMT)

- Obese vs. lean transplant (LVH, strain, CIMT)
- A-A vs. non-A-A transplant (CIMT)
- Time point comparisons: Pre-transplant*, 1, 18 and 30 months post-transplant

*note that only anthropometrics, biochemical parameters, echo and strain parameters were retrospectively available at the pre-transplant time point.

Measures of WC, WHr, and CIMT were not available pre-transplant.

2. Chi-square test of proportions to assess relationship of pre-transplant hyperparathyroidism with high CIMT at 1 month post-transplant. The purpose of this comparison is to assess effect of abnormal pre-transplant bone and mineral metabolism on CIMT immediately post-transplant.

3. Student's t-test to assess difference in mean leptin levels between lean and obese (by BMI, WHr, and WC) transplant groups.

4. ROC curve analysis to compare ability of BMI, WC, and WHr to detect a composite adverse CV outcome. The adverse CV outcome was defined as the presence of at least 3 of the following 5 criteria: 1) LVH (66), 2) high CIMT ($\geq 95^{\text{th}}$ percentile for race, based on healthy control data), 3) high myocardial strain ($\geq 95^{\text{th}}$ percentile, based on healthy control data), 4) dyslipidemia, and/or 5) hypertension. The measures of diagnostic accuracy (AUC) were used to compare ability of WHr, WC, and BMI to detect obesity-related adverse CV outcome in the transplant population. Sensitivity and specificity are also reported

Multivariate analysis

- GEE multivariate regression was used to determine associations of obesity (*by BMI, WC, and WHr*) and MS with adverse CV outcomes (*LVH, impaired longitudinal and circumferential strain, increased CIMT*) in the transplant recipients. GEE is the most appropriate method of analysis for longitudinal data to

examine associations between predictors and outcomes, accounting for within-subject correlation between the repeated measures over time. The equation for GEE is shown below, where i represents subject, j represents time, β_k is the regression parameter, and V_i is the variance structure:

$$U(\beta) = \sum_{i=1}^N \frac{\partial \mu_{ij}}{\partial \beta_k} V_i^{-1} \{Y_i - \mu_i(\beta)\}$$

- Collinearity Analysis. Prior to constructing the GEE regression models, a correlation matrix was run to evaluate for correlations between the independent variables. Variables that were found to be highly correlated were removed/not included together in the same GEE models. Due to high correlation between BMI-obesity, WC-obesity, WHr-obesity and MS variables, 4 separate GEE regression models were created to examine associations each type of obesity and MS with each adverse CV outcome (*Left ventricular hypertrophy, impaired myocardial strain, increased CIMT*).
- Selection of the “correlation structure” for the GEE regression by QIC.

The equation for QIC is show below:

$$Q(\mu) = \int_y^\mu \frac{y - t}{\phi V(t)} dt$$

Based on the study design, two possible GEE correlation structure options were “exchangeable” or unstructured”. To evaluate these options, the Qasi-likelihood under Independence model Criterion (QIC) values for the exchangeable and unstructured correlation were compared and were found to be similar for the full

model (593.8 vs 594.4), where myocardial longitudinal strain was the dependent variable. Given no significant difference between the two options, the unstructured correlation structure was ultimately selected as the better choice because it does not place any assumptions on the within-subject correlation over time, whereas the exchangeable structure assumes that the within-subject correlation does not vary but remains constant.

- Selection of the best regression model by QIC. GEE regression was performed starting with a full model that included all the independent variables of interest (n=15). The QIC for the full model was generated. Then, a stepwise process was used, whereby a single independent variable was removed from the model, and then the new model run. At each step, the variable with the highest p-value was removed from the model, and QIC value generated for each model, until only independent variables with significant p-values (<0.05) remained. Then, the best and final GEE model was selected based on the model that had the lowest QIC value. Interaction terms were explored after selection of the final model.
- Multivariate Linear and Logistic regression. In addition to the GEE regression described above, linear and logistic regression models were used to evaluate for associations of pre-transplant variables (duration of dialysis prior to transplant, exposure to hemodialysis prior to transplant, history of renal replacement therapy prior to transplant vs. pre-emptive, pre-transplant hyperparathyroidism) with CV outcomes (*longitudinal and circumferential strain, and high CIMT*) at pre-transplant and 1 month post-transplant time points individually. These potential predictors were not included in the GEE longitudinal analysis because they would

be likely to influence CV outcomes only during pre-transplant or at 1 month post-transplant, and would not be expected to continue to influence CV outcomes into the later post-transplant time points.

Chapter 4: Manuscripts

Section 4.1: Manuscript 1

Title: Effects of Obesity and Metabolic Syndrome on Cardiovascular Outcomes in Pediatric Kidney Transplant Recipients: A Longitudinal Study

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Myocardial strain

Speckle tracking echocardiography

Left ventricular hypertrophy

Dyslipidemia

Hypertension

Abstract

Obesity and metabolic syndrome (MS) are common after kidney transplantation, but their contribution to adverse cardiovascular (CV) outcomes in children are not well known. A prospective, controlled, longitudinal cohort study was conducted to investigate effects of obesity and MS on left ventricular hypertrophy (LVH) and myocardial strain in pediatric kidney transplant recipients. Transplant recipients (n=42) had anthropometrics (BMI, waist circumference, waist-to-height ratio), biochemical parameters (fasting glucose, lipid panel, HbA1c%), and echocardiogram with speckle tracking analysis for strain measured at 1, 18, and 30 months post-transplant. Additionally, 35 pre-transplant echocardiograms were analyzed retrospectively. Healthy children (n=24) served as controls. Waist-to-height ratio detected abdominal obesity in 46% of transplant patients, whereas only 8.1% were identified as obese by waist circumference. Ejection fraction and fractional shortening of the transplant group were normal and similar to controls. Prevalence of LVH was 35.2%, 17.1%, and 35.5% at 1, 18, and 30 months. Longitudinal strain of transplant was worse than controls at all time points ($p<0.001$). Hemodialysis independently predicted 21% worse longitudinal strain compared with peritoneal or no dialysis during the pre-transplant period ($p=0.04$). After transplant, obesity, MS, and systolic hypertension predicted increased odds of LVH ($p<0.04$). Worse longitudinal strain was independently associated with obesity, hypertension, combination of MS with elevated LDL-C-cholesterol, and steroid therapy ($p<0.03$), while higher eGFR conferred a protective effect ($p<0.001$). Obesity and MS adversely affect CV outcomes after transplant. Further studies are needed to investigate speckle tracking echocardiography as a tool for early detection of subclinical myocardial dysfunction in this population.

Introduction

Cardiovascular (CV) disease is the second leading cause of morbidity and mortality amongst children after kidney transplant (2). The development of obesity and metabolic syndrome (MS) are common in pediatric kidney transplant recipients, but their contribution to adverse CV outcomes is not well defined in this population. In transplant recipients, typical risk factors for MS, such as excess intake of processed foods and physical inactivity, converge with transplant medication-induced effects of dyslipidemia, glucose intolerance, and hypertension to create a hybridized version of MS unique to this population, the long-term effects of which are not well known (116, 117). Effects of obesity and MS on CV function can be present during childhood, but the signs are likely to be subtle in the early stages (3, 4). As major CV events are rare in children, abnormalities may not become apparent until they are in late stages, when the opportunity for early intervention has been missed. The detection of overt abnormalities such as alteration in measures of systolic left ventricular function by echocardiography, including ejection fraction (EF) or fractional shortening (FS), are rare in the pediatric population (25). Even among children with obesity and MS, EF is typically found to be normal (63). Evaluation of left ventricular mass by traditional echocardiography is also of limited use in detecting CV dysfunction in children with end stage renal disease (ESRD), due to the lack of agreement about how to accurately define left ventricular hypertrophy (LVH) in young children, underestimation of LVH in overweight patients, and lack of reliability in fluid overloaded patients(65, 67). Speckle tracking echocardiography, a newer non-invasive and highly sensitive imaging technique, may offer the opportunity for early detection of subclinical CV disease. Speckle tracking analysis is unaffected by volume and pressure changes, making it well suited for

evaluation of patients with kidney disease (118). In a recent study of adult hemodialysis patients with preserved EF, myocardial strain was a strong independent predictor of mortality, supporting the utility of speckle tracking echocardiography in identifying early subclinical CV risk (102). Little is known about the myocardial strain of pediatric kidney transplant recipients. This is the first longitudinal study to investigate the effects of obesity and metabolic syndrome on myocardial strain in pediatric kidney transplant recipients.

Methods

Study Design and Population

A prospective, controlled, longitudinal cohort study was conducted to investigate the effects of obesity and metabolic syndrome on CV outcomes including assessment of myocardial function by speckled tracking analysis in pediatric kidney transplant recipients. Children who received a kidney transplant at Children's National were eligible to participate in the study. Participants (age 3-20 years) were eligible to enroll between 0 and 18 months post-transplant and were followed until 30 months post-transplant. Those with estimated glomerular filtration rate (eGFR) $<60 \text{ mL/min/1.73m}^2$, multi-organ transplant, and nephrotic-range proteinuria were excluded. Twenty-four healthy children (age 3-20 years) were enrolled as controls. Approval was obtained from the Institutional Review Board (IRB) at Children's National. Informed consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki.

Classification of Obesity and MS

Body Mass Index (BMI) as well as two anthropometric measures of abdominal obesity: waist circumference (WC) and waist- to- height ratio (WHr) were measured at all time points. BMI-obesity was defined as BMI $\geq 95^{\text{th}}$ percentile for age-and-sex (119). Abdominal obesity was

defined as WC $\geq 95^{\text{th}}$ percentile for age-and-sex (106). Since children with kidney disease commonly have short stature, an additional measure of abdominal obesity indexed to height (WHr) was included. WHr-obesity was classified as ≥ 0.539 , based on NHANES III data according to the method of Kahn et al. (19). All transplant patients had blood pressure and biochemical parameters, including fasting glucose, fasting lipid panel, and HbA1c% measured at each time point. MS in transplant patient was defined as those who met ≥ 3 out of the following 5 criteria: 1) abdominal obesity, 2) glucose intolerance, 3) hypertension (controlled or uncontrolled), 4) low HDL-C cholesterol, 5) elevated triglycerides. Glucose intolerance was defined as HbA1c% $>5.6\%$ or fasting glucose >100 mg/dL or on glucose-lowering medication (109). Hypertension was defined by use of antihypertensive therapy; uncontrolled hypertension was defined as having a systolic or diastolic blood pressure z score corresponding to $\geq 95^{\text{th}}$ percentile for age, sex, and height for children 2-18 years old (107) and $\geq 130/85$ mm Hg for those >18 years old. Cut points for low HDL-C and high triglycerides were defined according to 2011 pediatric guidelines (40) for study participants age 3-19, and by Adult Treatment Panel III criteria (108) for >19 years of age.

Echocardiographic Methods

An echocardiogram (echo) was obtained at 1, 18 and 30 months post-transplant. The echos were performed by a single pediatric sonographer using the iE33 xMatrix DS Ultrasound System (Philips North America Corporation, Andover, MA), according to American Society of Echocardiography standards (110). In addition, 35 of the 40 transplant patients had pre-transplant echos that were analyzed retrospectively. Echos were analyzed using Syngo Velocity Vector Imaging (Siemens, Germany) by a pediatric cardiologist blinded to the subject's clinical information. Standard echo parameters included left ventricular mass indexed to height^{2.7}

(LVM/height^{2.7}), FS by standard M-mode method, and EF by Simpson's method. In addition, myocardial strain (%) was assessed in the circumferential (short-axis) and longitudinal (long-axis) directions by speckle tracking analysis. Strain was calculated by measuring the end systolic distance between two speckles of tracked endocardium minus the original distance divided by the original distance. Because the myocardium contracts in the longitudinal and circumferential directions during systole, these values are negative percentages, and more negative values indicate better cardiac contractility. LVH was defined as LVM/height^{2.7} \geq 95th percentile according to the age-specific percentiles of Khoury et al. (66). Echo and strain parameters of the transplant group were compared to controls, and changes over time were assessed longitudinally.

Statistical Analysis

All statistical analyses were conducted using Stata 14.0 (StataCorp LP, College Station, TX). Categorical variables were compared by Chi-square analysis. Student's t-test was used to compare means of normally distributed continuous variables between groups and Wilcoxon rank-sum was used for continuous non-normally distributed variables. Mean \pm SEM and median (IQR) are reported for continuous variables.

Pre-transplant determinants of LVH and strain

In order to understand the differential effects of pre-transplant exposures, we analyzed effects of type and duration of renal replacement modalities on LVH and strain in the pre-transplant period and at 1 month post-transplant. Multivariate linear and logistic regression models were used to evaluate for associations of pre-transplant independent variables (type and duration of dialysis, pre-emptive transplant vs. dialysis) with LVH and strain prior to transplant and at 1 month post-transplant.

Post-transplant determinants of LVH and strain (1-30 months post-transplant)

Generalized estimating equation (GEE) multivariate regression with unstructured correlation was used to conduct longitudinal analysis of determinants of LVH and strain post-transplant. GEE regression accounts for the within-patient correlation inherent to longitudinal serial data, and is robust to missing data (120). Due to collinearity between the three anthropometric measures of obesity and MS, four separate models (1. BMI-obese, 2.WC-obese, 3.WHr-obese, and 4. MS) were created to evaluate associations of each with the CV outcome of interest (LVH, longitudinal strain, and circumferential strain). The additional independent variables included in each full GEE regression model were: age, sex, race, hypertension, glucose intolerance, low HDL-C cholesterol, high triglycerides and, LDL-C cholesterol level, steroid dose, high SBP or DBP z score, and eGFR. Quasi-likelihood under the independence model information criterion (QIC) measure was used to select the best fit model in a stepwise procedure.

Results

Study population

Demographics and characteristics of the transplant and control groups are summarized in Table 1. The study group was comprised of 42 pediatric kidney transplant recipients who were 12.1 ± 0.7 years of age and 50% A-A, with a mean eGFR of 94.4 ± 4.4 mL/min/1.73m² at 1 month post-transplant. All transplant recipients were maintained on standard immunosuppression therapy with Tacrolimus and Mycophenolate Mofetil, and approximately half were receiving steroid therapy in addition. Controls comprised of 24 healthy children of similar age and race distribution.

Of 42 transplant patients, two were excluded from cardiac assessment due to discovery of a bicommissural aortic valve with stenosis in one and refusal of echo in the other case. A total of

100 prospective echos were performed in the remaining 40 patients over the 1 to 30 months post-transplant. A total of 24 echos were performed in the 24 control participants on one occasion.

Prevalence of Obesity and Cardiometabolic Abnormalities

The prevalence of metabolic and CV morbidities in transplant patients at each time point are reported in Table 2. The prevalence of obesity as detected by WHr was significantly higher than by WC alone at 1, 18, and 30 months post-transplant ($p=0.01$, 0.0002 , and 0.05 respectively).

Approximately one-third of patients had post-transplant MS. Hypertension was highly prevalent pre- and post-transplant. Prevalence of hypertriglyceridemia was higher prior to transplant (79.2%), compared with 35.9%, 24.3%, and 29.4% prevalence at 1, 18, and 30 months post-transplant ($p<0.001$). The triglyceride to HDL-C (TG/HDL-C) ratio, an indicator of atherogenic LDL-C particle composition, was significantly higher in transplant recipients with MS (mean TG/HDL-C \pm SEM 4.2 ± 0.8 , 4.9 ± 1.2 , and 4.0 ± 0.9) compared to those without MS (2.1 ± 0.3 , 1.6 ± 0.2 , and 1.9 ± 0.3) at 1, 18, and 30 months post-transplant, $p=0.009$, $p=0.006$, and $p=0.02$, respectively. Details of biochemical indicators of transplant recipients at each time point are summarized in Table 3.

Comparison of CV parameters: transplant and controls

Standard echo parameters

EF and FS of transplant patients were within normal range at all time points. Mean EF was $62.4\pm 0.5\%$, $64.7\pm 0.7\%$, $63.9\pm 0.5\%$ and $64.1\pm 0.6\%$ (normal 55-70%), and FS was $35.5\pm 0.9\%$, $37.0\pm 0.8\%$, $37.4\pm 0.9\%$, $38.0\pm 0.8\%$ (normal 28-40%) at pre-transplant, 1 month, 18 months, and 30 months post-transplant. EF and FS of transplant patients were similar to that of controls, ($64.1\pm 0.5\%$ and $34.8\pm 0.9\%$, respectively). LVM/height^{2.7} (median, IQR) of transplant recipients were higher than controls at all time points (37.8 , IQR $26.1-45.5$) pre-transplant, 38.6 , IQR $31.1-$

43.2 at 1 month, 33.0, IQR 29.7-39.0 at 18 months, and 35.8, IQR 28.2-42.6 at 30 months post-transplant vs. 29.7, IQR 26.1-32.1 g/m^{2.7} in controls, p<0.03).

Myocardial strain

All transplant recipients, both lean and obese, had worse longitudinal strain than controls at all time points, p< 0.001(Figure 1). Circumferential strain of transplant recipients did not differ significantly from controls.

Changes in CV parameters of transplant recipients over time

LVH was present in 37.1%, 35.2%, 17.1%, and 35.5% at pre-transplant, 1, 18, and 30 months post-transplant, respectively (Table 2). Mean longitudinal strain was -17.4±0.67 pre-transplant, improved to -19.7±0.59 and -19.9±0.44 at 1 and 18 months post-transplant (p=0.01 and 0.002 vs. pre-transplant), but did not statistically differ at 30 months post-transplant (-18.7±0.44). Mean circumferential strain was -22.3±0.76 pre-transplant and improved to -25.0±0.70, -25.5±0.58, and -25.7±0.61 (p=0.01, 0.001, and 0.001, respectively), though did not differ from controls.

Determinants of LVH and myocardial strain

Pre-transplant: Adjusted multivariate linear regression indicated that chronic hemodialysis (HD) was the key significant independent predictor of worse longitudinal strain during the pre-transplant period. After adjusting for age, sex, race, hypertension, obesity, and hyperparathyroidism. HD was associated with a 4.0±1.9 impairment in longitudinal strain compared with those who received a pre-emptive transplant or were on PD (p=0.04). No significant associations between type or duration of renal replacement therapy with pre-transplant LVH or circumferential strain were identified, after adjusting for age, sex, race, hypertension, obesity, and hyperparathyroidism.

1 month post-transplant: By 1 month post-transplant, history of receiving HD no longer affected longitudinal strain ($p=0.18$). Obesity became the key independent predictor of worse longitudinal strain at 1 month post-transplant. Obesity was independently associated with a 3.6 ± 1.6 impairment in longitudinal strain at 1 month post-transplant ($p=0.04$). In addition, obesity was an independent predictor of increased odds of LVH at 1 month post-transplant (OR 3.8 ± 1.8 , $p=0.3$). No significant associations were identified between pre-transplant type or duration of renal replacement therapy with circumferential strain at 1 month.

Post-transplant (longitudinal analysis over 1-30 months)

The results of the adjusted longitudinal GEE analysis as classified by the four regression models (BMI-obese, WC-obese, WHr-obese, and MS) are summarized in Table 4.

LVH: BMI-obesity, WHr-obesity, and MS were associated with 3.7 ± 1.9 , 2.8 ± 1.3 , and 3.5 ± 1.7 times higher odds of post-transplant LVH, respectively, whereas WC-obesity was not a significant predictor. In addition, high SBP z-score was independently associated with higher odds of LVH after transplant (OR \pm SEM= 5.2 ± 3.2 , $p=0.007$, 4.1 ± 2.5 , $p=0.02$, and 3.9 ± 2.4 , $p=0.03$, in the BMI, WHr and WC adjusted models, respectively).

Myocardial strain: BMI-obesity, WHr-obesity, and WC-obesity, were independently associated with worse longitudinal strain after transplant (Table 4). In addition, hypertension was independently associated with worse longitudinal strain across all models. Higher eGFR was independently associated with improved longitudinal strain across all models. Neither MS ($p=0.06$) nor LDL-C level ($p=0.2$) were independently associated with longitudinal strain, however, there was a significant interaction between MS and LDL-C level, suggesting that the combination of these variables produces a synergistic effect associated with worse longitudinal

strain, $p=0.01$ (Figure 2). In addition, there a significant interaction between WHr-Obesity and LDL-C cholesterol produced worse longitudinal strain, $p=0.0001$.

No significant associations between obesity, MS, or other determinants with circumferential strain were identified in the multivariate regression.

Discussion

The results of this prospective longitudinal study indicate that obesity and MS are highly prevalent and significantly impact CV outcomes in pediatric kidney transplant recipients. BMI-obesity, WHr-obesity, and MS were significant predictors of increased odds of LVH post-transplant, whereas WC-obesity was not significant. In addition, systolic hypertension was a significant contributor to post-transplant LVH. Speckle tracking echocardiography was able to detect pre and post-transplant impairment in myocardial strain that was not evident by standard measures of left ventricular function, such as EF and FS.

In our cohort, EF and FS of the study group were maintained within normal range and were similar to controls across all time points. However despite the appearance of normal myocardial function by EF and FS, impaired subclinical CV function was revealed by the longitudinal strain of the study participants, which was significantly worse than the controls at all time points.

These findings are in agreement with those of a previous study that reported changes in myocardial strain despite preserved EF in children with end stage renal disease (19 dialysis patients, 17 transplant recipients, and 33 age-matched controls) (104). Similar to the findings of Van Huis, et al.(104), the myocardial dysfunction observed in our transplant cohort was characterized by impaired strain in the longitudinal direction, while circumferential strain did not differ from controls. The reasons for the lack of changes in circumferential strain may be several-fold. In general, longitudinal strain is preferentially used by clinicians because it is more

reproducible than circumferential strain, as the short-axis circumferential motion is technically more difficult to track than the longitudinal base-to-apex contraction of the myocardium (121). Additionally, longitudinal strain is highly sensitive for detecting myocardial disease early in the progression, whereas circumferential strain may remain normal or show exaggerated compensation for preserving left ventricular systolic performance (122). Therefore, longitudinal strain appears to be a more sensitive and accurate indicator of early signs of CV disease in children.

Ours is the first study to investigate the effects of obesity and MS on myocardial strain in pediatric transplant recipients. Consistent with prior reports in the literature, the prevalence of obesity in our cohort (as measured by BMI) approximately doubled after transplantation, from 14.2% pre-transplant to 35.1% at 18 months post-transplant (25). As abdominal obesity measured by WC or WHr is more strongly associated with high metabolic and CV risk than is high BMI in the general pediatric population (19-21), we also examined both of these parameters in our transplant cohort. While the prevalence of abdominal obesity appeared low (8.1%) when assessed by WC-for-age percentiles, it increased to 46% when measured by WHr (Table 2). Given the predominance of short stature amongst children with ESRD, our results suggest that evaluating abdominal circumference without correcting for height may underestimate the true prevalence of visceral obesity in this population. The reported prevalence of post-transplant MS in the pediatric literature is variable (ranging from 14.2%-68%), due in part to differing criteria used for definition of MS (7, 8). In our study, approximately one-third of the transplant recipients had MS, where abdominal obesity was required for the diagnosis.

We examined the effects of pre-transplant variables affecting LVH and strain outcomes. Chronic HD was the key significant independent predictor of worse longitudinal strain during the pre-

transplant period, after adjusting for other pre-transplant factors, including hypertension and obesity. HD was associated with a 21% worsening in longitudinal strain, compared to those who received a pre-emptive transplant or were on peritoneal dialysis prior to transplant. Evidence indicates that children receiving HD are subject to ischemic cardiac injury caused by myocardial stunning (123). This data substantiates the notion that subclinical CV impairment is occurring in children receiving HD, and this should be considered when choosing renal replacement modality. Obesity was not a significant predictor of strain pre-transplant, but emerged as the key player starting as early as 1 month post-transplant.

Obesity was a key independent predictor of worse longitudinal strain over 1-30 months post-transplant (Table 4). Hypertension also conferred to significant risk for worse longitudinal strain. The only variable in the model associated with significant improvement in longitudinal strain was higher eGFR, suggesting that better kidney function confers a protective effect with respect to myocardial function after transplant.

The association of MS with longitudinal strain was not significant ($p=0.06$) in the adjusted regression, however there was a significant interaction between MS and higher LDL-C level resulting in impaired longitudinal strain (Figure 2). A similar interaction was noted between abdominal WHr-obesity and LDL-C level. An association of impaired longitudinal strain with increased serum LDL-C level as well as with myocardial cholesterol concentration has been previously demonstrated in an animal model (124). A possible mechanism for the adverse effect of LDL-C cholesterol on strain may be related to cardiac oxidative stress caused by accumulation of cholesterol in the myocardium. Although LDL-C cholesterol is not a component of the traditional definition of MS, it appears that the combination of MS with higher LDL-C produces an adverse effect on myocardial strain in pediatric kidney transplant recipients. The reason for

this phenomenon may be explained in part by differences in the specific nature of the LDL-C particles found in children with abdominal obesity compared to those without abdominal obesity. Children with abdominal obesity are prone to generate smaller, denser, more atherogenic LDL-C particles, the presence of which can be indicated by an elevated TG/HDL-C ratio of >3.0 (125, 126). A longitudinal cohort study demonstrated that presence of high TG/HDL-C ratio at age 12 was able to predict the occurrence of adverse CV events 3-4 decades later in life (127). In our transplant cohort, children with MS were noted to have an elevated TG/HDL-C ratio (>3.0), while those without MS had normal TG/HDL-C ratio. Further studies are needed to investigate how the combination of MS and elevated LDL-C impacts CV outcomes of pediatric transplant recipients, in whom the effects of MS may not conform to the classical definition.

There were several limitations to our study. Although the longitudinal study design was prospective over time post-transplant, the pre-transplant echocardiograms were obtained from the medical record and retrospectively analyzed. As there were no pre-transplant measures of WC available, we were unable to assess the prevalence of abdominal obesity or MS. Although the distribution of male and female gender was different between the transplant and control group, gender was not significantly associated with any of the CV outcomes across all regression models.

In conclusion, pediatric kidney transplant recipients have a high prevalence of obesity and MS. WHr identifies abdominal obesity in a greater proportion of these patients compared to WC, due to the prevalence of short stature in this population. Additionally these patients have CV abnormalities, which include LVH and impaired myocardial strain. Prior to transplant, exposure to HD results in worsening of longitudinal strain compared with children receiving peritoneal dialysis or those with advanced CKD not receiving dialysis. After transplant, CV dysfunction is

present in children and is exacerbated by obesity, hypertension, and the combination of MS with atherogenic LDL-C cholesterol, highlighting the importance of efforts to manage weight, control blood pressure, and treat dyslipidemia. Higher eGFR appears to confer a protective effect on myocardial strain. Further studies are needed to determine if speckle tracking echocardiography can serve as a useful clinical tool to provide a means of detection of subclinical myocardial dysfunction and opportunity for earlier intervention in pediatric kidney transplant recipients.

Tables and Figures

Table 1. Demographics and clinical characteristics of the transplant and control groups

	Transplant Group n=42 patients	Control Group n=24 patients	p value Transplant vs Controls
Age (years)	12.1± 0.7	11.1 ± 0.5	0.31
Race (%)	50% A-A	58% A-A	0.53
Sex (%)	62% male	25% male	0.004*
<u>Pre-transplant modality</u>		NA	NA
Preemptive	31%		
Hemodialysis	38.1%		
Peritoneal dialysis	30.1%		
<u>Duration dialysis (months)</u>	<u>mean+SEM (95% CI)</u>	NA	NA
Hemodialysis	28.0±5.1(17.7-38.2)		
Peritoneal Dialysis	20.9±3.4(13.9-27.9)		
<u>eGFR (mL/min/1.73m²)</u>	<u>mean+SEM (95% CI)</u>	NA	NA
1 month	94.4±4.4 (85.7-103.0)		
18 months	96.9±4.5 (88-105.9)		
30 months	90.3±3.1 (84.2-96.4)		
<u>Steroid protocol</u>		NA	NA
1 month	52.4%		
18 months	54%		
30 months	55.9%		
<u>Steroid dose of those on steroid therapy (mg/kg/day)</u>	<u>mean+SEM (95% CI)</u>	NA	NA
1 month	0.45±0.05(0.35-0.56)		
18 months	0.17±0.05(0.05-0.29)		
30 months	0.07±0.02(0.04-0.11)		

Table 2. Prevalence of metabolic and CV morbidities in transplant group over time

	Transplant Group (n=42 patients)				p value
	Pre-transplant	1 month	18 months	30 months	
BMI-Obese (BMI \geq 95 th %)	14.2%	22%	35.1%**	27.3%	0.04**
WC-Obese (WC \geq 95 th %)	NA	14.7%	8.1%	15.6%	NS
WHR-Obese (WHR \geq 0.539)	NA	41%	46%	36.3%	NS
Metabolic syndrome	NA	33.3%	29.7%	30.3%	NS
Hypertension	75.7%	59.5%	67.5%	60.6%	NS
High SBP	NA	19.1%	5.4%	12.1%	NS
High DBP	NA	11.9%	2.7%	3.0%	NS
High triglycerides	79.2%	35.9%*	24.3%**	29.4%***	.0009* .0001** .0002***
Low HDL-C	45.8%	43.6%	32.4%	29.4%	NS
High LDL-C	37.5%*	18.0%	13.5%*	21.2%	0.03
Glucose intolerance	NA	15%	29.7%	30.3%	NS
LVH	37.1%	35.2%	17.1%	35.5%	NS
Impaired Longitudinal strain	76.7%	47.1%*	40%**	56.7%	0.002* 0.01**
Impaired Circumferential strain	21.2%	3.0%*	3.1%**	0%***	0.02* 0.02** 0.008***

NS= not significant

*significant difference between pre-transplant and 1 month post-transplant by Chi-square

**significant difference between pre-transplant and 18 months post-transplant by Chi-square

***significant difference between pre-transplant and 30 months post-transplant by Chi-square

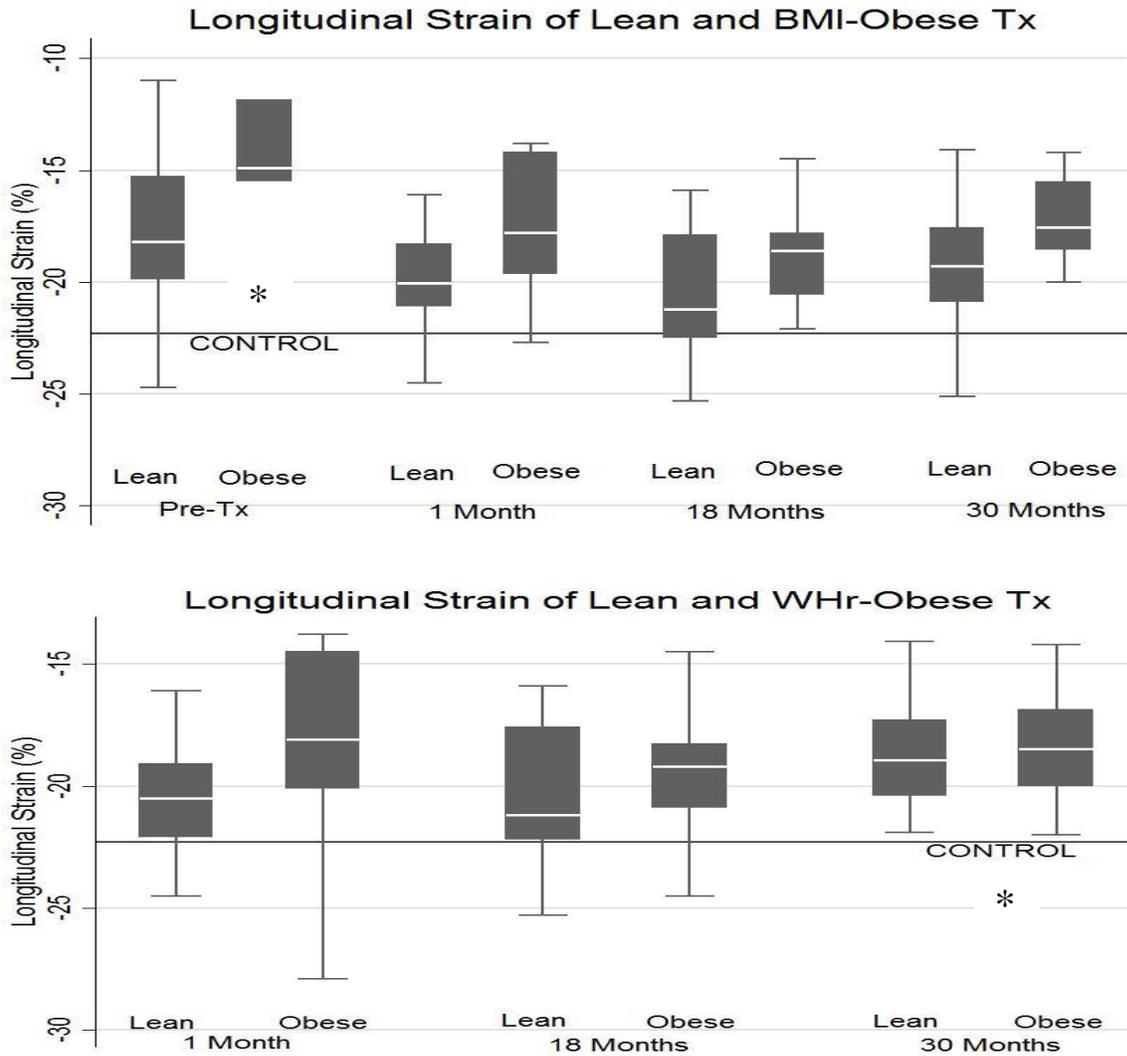
Table 3. Biochemical indicators of transplant recipients at each time point

	Transplant Group (n=42 patients)			
	Pre-Transplant	1 month	18 months	30 months
LDL-C cholesterol (mg/dL) Mean \pm SEM 95% CI Median IQR	101.7 \pm 11.5 (78.8-124.5) 92.5 57.5-141.5	84.3 \pm 4.5 (75.4-93.2) 80 63-104	82 \pm 3.8 (74.5-89.6) 78 67-97	84.2 \pm 4.3 (75.7-92.7) 83 70-98
HDL-C cholesterol (mg/dL) Mean \pm SEM 95% CI Median IQR	44.7 \pm 2.9 (39-50.5) 42.5 33.5-55	44.9 \pm 2.5 (39.8-50) 43 32-51	49.1 \pm 3.1 (42.9-55.3) 46 34-58	47.5 \pm 2.2 (43.2-51.9) 47 39-57
Triglycerides (mg/dL) Mean \pm SEM Median IQR	180 \pm 15.7 ^{##} (148.8-211.1) 155.5 133-216	117.5 \pm 10.6 ^{##} (96.5-138.6) 89 78-143	113.6 \pm 13.5 ^{##} (86.7-140.5) 92 64-124	113 \pm 13.7 ^{##} (85.8-140.3) 83 62-137
Fasting glucose (mg/dL) Mean \pm SEM 95% CI Median IQR	NA NA	91.5 \pm 2.5 86.5-96.5 88.5 82.5-99	92.6 \pm 1.9 88.8-96.4 91 86-96	98.3 \pm 5.9 86.4-110.2 90 87-98
HbA1c% Mean \pm SEM 95% CI Median IQR	NA NA	5.1 \pm 0.05 [#] (5.0-5.3) 5.1 5-5.4	5.3 \pm 0.06 [#] (5.2-5.4) 5.3 5-5.6	5.3 \pm 0.06 [#] (5.2-5.4) 5.3 5.1-5.5
Intact PTH (within 30 days prior to transplant)	452.8 \pm 88.9 (272.5-633.1)	NA	NA	NA
<p>#significant difference between transplant time points, p<0.05 by Student's t-test ##significant difference between transplant time points, p<0.05 by Wilcoxon rank-sum</p>				

Table 4. Significant determinants of LVH and longitudinal strain (1-30 months post-transplant)**

	LVH			Longitudinal Strain (%)	
	OR \pm SEM [95%CI]	p value		β \pm SEM [95%CI]	p value
Model I. BMI-Obese High SBP z	3.7 \pm 1.9 [1.4-9.9] 5.2 \pm 3.2 [1.6-17.2]	0.01* 0.007*	Model I. BMI-Obese Hypertension eGFR	1.5 \pm 0.39 [0.78- 2.3] 1.4 \pm 0.49 [0.43-2.3] -0.04 \pm 0.01[-.06-.02]	0.0001* 0.005* 0.0001*
Model II. WHR-Obese High SBPz	2.8 \pm 1.3 [1.08-7.1] 4.1 \pm 2.5 [1.2-13.8]	0.03* 0.02*	Model II. WHR-Obese Hypertension eGFR	1.1 \pm 0.42 [0.23-1.8] 1.5 \pm 0.46 [0.62-2.4] -0.04 \pm 0.01[-0.06-.02]	0.01* 0.001* 0.0001*
Model III. WC-Obese High SBPz	3.5 \pm 2.7 [0.8-15.5] 3.9 \pm 2.4 [1.2-13.2]	0.09 NS 0.03*	Model III. WC-Obese Hypertension eGFR	1.6 \pm 0.51 [0.63-2.6] 1.4 \pm 0.46 [0.48-2.3] -0.04 \pm 0.01[-0.06-.02]	0.001* 0.003* 0.0001*
Model IV. MS	3.5 \pm 1.7 [1.3-9.3]	0.01*	Model IV. MS Hypertension eGFR	1.90 \pm 0.49 [-0.05-1.8] 1.5 \pm 0.47 [0.55-2.4] -0.04 \pm 0.01[-0.06-.02]	0.06 NS 0.002* 0.0001*
n= 100 observations of 40 patients					
**Longitudinal GEE models were created to examine each of the following independent variables: BMI-obese, WHr-obese, WC-obese, and MS with CV outcomes LVH and longitudinal strain (dependent variables). Additional covariates included in the full model were: age, sex, race, hypertension, glucose intolerance, high triglycerides, low HDL-C, LDL-C level, steroid dose, high SBP z score, high DBP z score, eGFR. QIC measure was used to select the best-fit model in stepwise regression, and the significant associations of the final adjusted model are presented above.					
*significant association p<0.05					

Figure 1. Longitudinal strain of lean and obese transplant recipients at each time point vs. controls



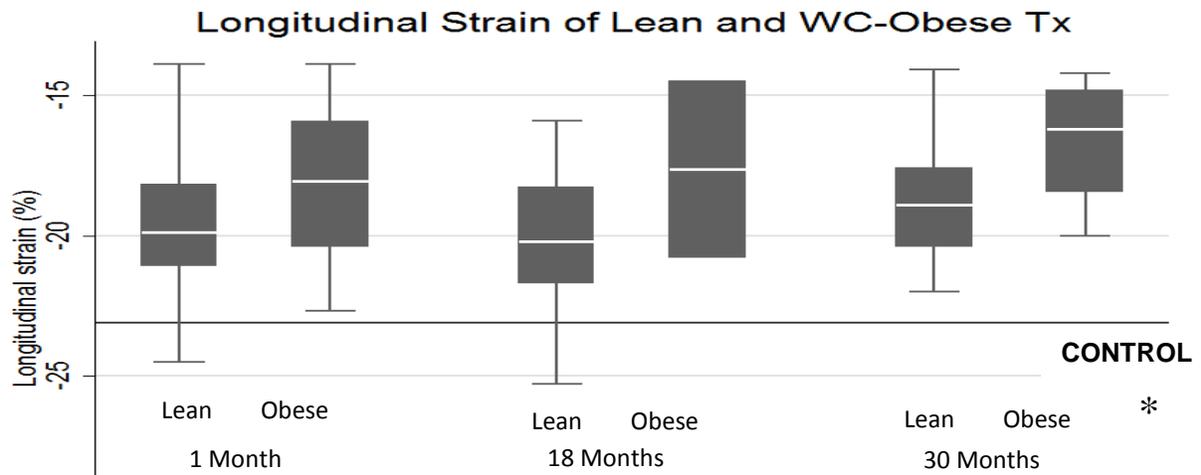


Figure 1 Caption: *Longitudinal strain of all transplants (Tx) both lean and obese, was worse than controls at all time points. WC was not measured pre-transplant, therefore no pre-transplant strain data for WC-obese or WHr-obese is presented.

Figure 2. Interaction between MS and LDL-C on longitudinal strain post-transplant

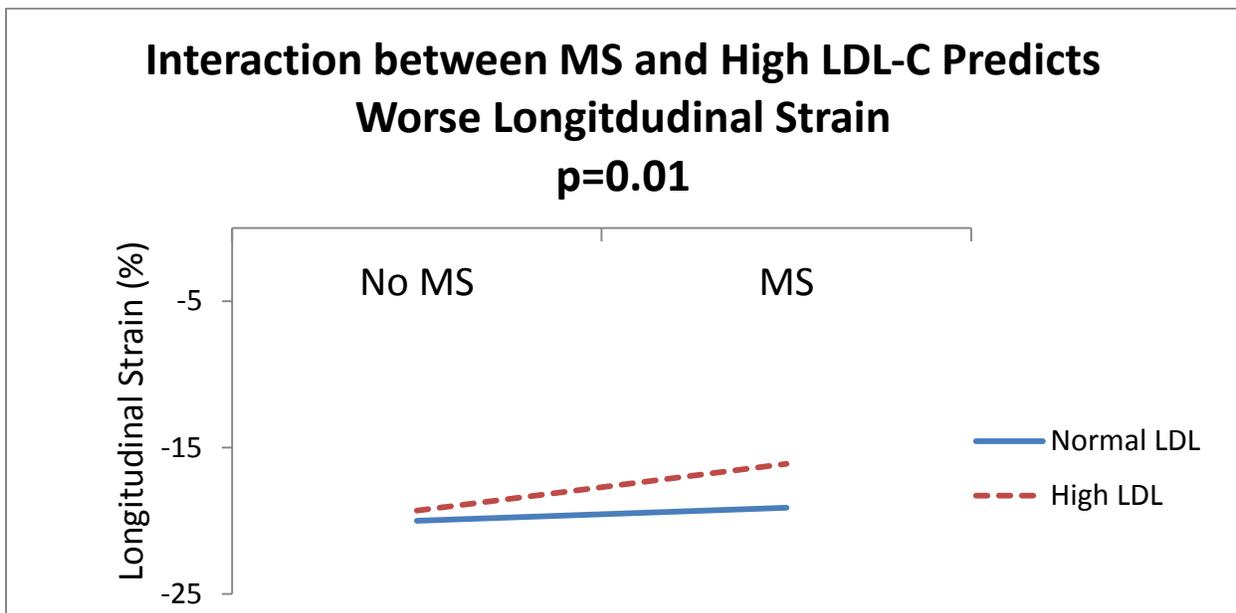


Figure 2 Caption: There was a significant interaction between MS and LDL-C level, indicating the combination of these variables produces a synergistic effect associated with worse longitudinal strain post-transplant, $p=0.01$.

Section 4.2: Manuscript 2

Title: Increased Carotid Intima-Media Thickness in African-American Pediatric Kidney Transplant Recipients

Running Head: Carotid Intima-Media Thickness in African-Americans

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Journal: Pediatric Transplantation

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Abstract

Early signs of subclinical cardiovascular (CV) dysfunction can be detected by ultrasound for carotid intima-media thickness (CIMT). Although African Americans (A-A) are at high risk for CV disease, CIMT of A-A kidney transplant recipients has not been previously investigated. The aim of this prospective, controlled, longitudinal study was to investigate determinants of CIMT in a multi-racial pediatric kidney transplant population, with a focus on A-A. Transplant recipients (n=42) had BMI, waist-to-height ratio, fasting glucose, lipid panel, HbA1c%, and CIMT measured at 1, 18, and 30 months post-transplant. Twenty-four healthy children (14 A-A) served as controls. CIMT of A-A transplant (0.49, 0.49 and 0.48mm) was higher than non-AA transplant (0.43, 0.44, and 0.44mm) at 1, 18, and 30 months and higher than A-A controls (0.47mm). Hyperparathyroidism prior to transplant predicted high CIMT-for-race. A-A race was associated with 10% higher CIMT vs. non-A-A transplant. Metabolic syndrome was associated with 0.03 ± 0.01 mm increased in CIMT among A-A transplant recipients only. In conclusion, A-A kidney transplant recipients have increased CIMT. Metabolic syndrome disproportionately affects CIMT of A-A children post-transplant. Identification of subclinical CV damage, detected by CIMT, may provide an opportunity for early detection of CV risk in this vulnerable population.

Key Words: Metabolic Syndrome, Obesity, Ethnicity, Cardiovascular, Hyperparathyroidism

Introduction

The effects of obesity and metabolic syndrome (MS) on cardiovascular (CV) function are known to begin during childhood, but the clinical signs and symptoms typically do not manifest until adulthood. Autopsy studies have identified fatty streaks and atherosclerotic lesions, attributable to obesity and MS, in the arteries of children and young adults who died of accidental causes (3, 4). As major CV events are rare in children, abnormalities may not become apparent until they are in late stages, thus the opportunity for early intervention is missed. In recent years, non-invasive imaging techniques have become available for the evaluation of carotid intima-media thickness (CIMT), which may provide an early and sensitive marker of subclinical CV dysfunction. Non-invasive ultrasound imaging of the carotid arteries is used to demonstrate the status of the intima-media thickness of the vessel and serves as a reproducible surrogate marker for early atherosclerosis (78). Prospective studies have demonstrated that an increase in CIMT is associated with an increase in the relative risk for stroke and myocardial infarction in the general adult population (79, 80). Increased CIMT has also been found to be associated with MS and its components (obesity, hyperlipidemia, hypertension, glucose intolerance) in the pediatric population (81). In pediatric kidney transplant recipients, traditional risk factors for MS, such as excess caloric intake and physical inactivity, are compounded by transplant medication-induced effects of dyslipidemia, glucose intolerance, and hypertension to create a hybridized version of MS unique to this population. Although obesity, and MS are common post-transplant complications (2), data is lacking with regard to their effects on CIMT in this high risk population. Prior studies investigating CIMT in pediatric kidney transplant recipients have been primarily cross-sectional in nature, have been conducted in predominantly Caucasian

populations, and yielded conflicting results (89, 90, 92). In addition, despite the known higher risks for metabolic morbidities, CV and kidney disease among individuals of African descent (13, 48, 53, 54), no prior studies have focused on the CIMT of pediatric kidney transplant recipients of African ancestry.

The aim of this prospective, controlled, longitudinal study was to investigate the effects of MS on CIMT in a multi-racial pediatric kidney transplant population, with a focus on African American (A-A) pediatric kidney transplant recipients.

Methods

Study Design and Population

A prospective, controlled, longitudinal cohort study was conducted to investigate the effects of MS on CIMT in A-A and non A-A pediatric kidney transplant recipients. Children who received a kidney transplant at Children's National Health System (CHNS) in Washington DC, age 3-20 years, were eligible to participate in the study. Those with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m², multi-organ transplant, and nephrotic-range proteinuria were excluded. Transplant recipients (n=42) enrolled between 0 and 18 months post-transplant and were followed until 30 months post-transplant. Healthy children (n=24, of which 14 were AA), age 3-20 years, served as controls. Approval was obtained from the Institutional Review Board (IRB) at CNHS. Informed consent was obtained from all participants and the study was conducted in accordance with the Helsinki Declaration of 1975.

Classification of CV Risk Factors

Obesity and abdominal obesity were assessed by body mass index (BMI) and waist-to-height ratio (WHr), respectively. BMI-obesity was defined as BMI $\geq 95^{\text{th}}$ percentile (18). WHr-obesity was defined using a cut point of ≥ 0.539 , based on NHANES III data according to the method of

Kahn et al. (19). Transplant patients with MS were defined as those who met ≥ 3 of the following 5 criteria: abdominal obesity, glucose intolerance (109), hypertension (controlled or uncontrolled), low HDL-C cholesterol, or elevated triglycerides. Glucose intolerance was defined as HbA1c% $>5.6\%$ or fasting glucose >100 mg/dL or on glucose-lowering medication (109). Cut points for low HDL-C and high triglycerides were defined according to 2011 Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (40) for study participants age 3-19, and by the Adult Treatment Panel III criteria (108) for those >19 years of age. Hypertension was defined by requirement for antihypertensive therapy. Uncontrolled hypertension was defined as having a systolic blood pressure (SBP) or diastolic blood pressure (DBP) z score corresponding to $\geq 95^{\text{th}}$ percentile for those 2-18 years old (107) and $\geq 130/85$ mm Hg for those >18 years old .

Measurement of CIMT

CIMT was measured at 1, 18 and 30 months post-transplant. B-mode ultrasound imaging of the arterial far wall segments of the right and left common carotid arteries and carotid bulbs was performed according to a standard protocol by a single pediatric Sonographer using aiE33 xMatrix DS Ultrasound System (Philips North America Corporation, Andover, MA). Intima-media thickness of the distal, mid, and proximal segments of right and left common carotid arteries and carotid bulbs was then measured by a single pediatric cardiologist blinded to the patient's clinical information. A composite of the 8 measurements was used to represent the participant's CIMT.

Statistical Methods

Statistical analyses were conducted using Stata 14.0 (StataCorp LP, College Station, TX).

Student's t-test was used to compare means of normally distributed continuous variables between

groups and Wilcoxon rank-sum was used for non-normal continuous variables, with mean \pm SEM and median (IQR) reported. Categorical data were compared by Chi-square analysis.

Multivariate logistic regression was used to evaluate for associations of pre-transplant variables, including duration and modality of dialysis prior to transplant and pre-transplant intact parathyroid hormone (iPTH) with high CIMT at 1 month post-transplant, where high CIMT was a categorical variable defined as CIMT \geq 95th percentile-for-race (to account for the differences between A-A and non A-A), based on our healthy control data.

Generalized estimating equation (GEE) multivariate regression with unstructured correlation was used to evaluate longitudinal association of MS with CIMT. GEE accounts for the within-patient correlation inherent to longitudinal serial data, and is robust to missing data (120). Due to the non-normal distribution of CIMT in the transplant group, continuous CIMT data were log-transformed. Independent variables included in the full GEE regression model were: Age, gender, race, MS, hypertension, glucose intolerance, hypertriglyceridemia, low HDL-C cholesterol, LDL-C cholesterol, steroid therapy, high SBP z score, high DBP z score, and estimated glomerular filtration rate (eGFR). Quasi-likelihood under the independence model information criterion (QIC) measure was used to select the best fit model in a stepwise procedure. Due to the disparate nature of CIMT between AA and non-AA children, the analysis was then stratified by race. CIMT of the A-A transplant group was normally distributed, while CIMT of the non A-A group was log transformed.

Results

Study population

Demographics and characteristics of the transplant and control groups are summarized in Table 1. The study group comprised 42 pediatric kidney transplant recipients who were 12.1 ± 0.7 years

of age and 50% were A-A. All transplant recipients were maintained on standard immunosuppression therapy with Tacrolimus and Mycophenolate Mofetil, and approximately half were receiving steroid therapy in addition. Controls were 24 healthy children of similar age and race distribution. Of 42 transplant patients, three were unable to tolerate or refused the CIMT procedure. A total of 97 CIMT ultrasounds were performed in 39 patients over the 1 to 30 months post-transplant study period. All 24 control participants had CIMT measurement done on one occasion.

Prevalence of metabolic and CV morbidities post-transplant

The prevalence of metabolic and CV morbidities within the transplant study group at each time point are reported in Table 2, and are summarized below.

Obesity: The prevalence of BMI-obesity increased from 14.2% pre-transplant to 35.1% at 18 months post-transplant ($p=0.04$), and trended down to 27.3% at 30 months post-transplant. The prevalence of abdominal obesity by WHr was 41%, 46%, and 36.3% at 1, 18, and 30 months post-transplant respectively.

MS: Approximately one-third of patients had post-transplant MS (33.3%, 29.7%, and 30.3% at 1, 18, and 30 months, respectively). Prevalence of MS did not change significantly over time post-transplant.

Hypertension: Hypertension was highly prevalent pre-transplant as well as post-transplant. Uncontrolled hypertension was less common, with 19.1%, 5.4%, and 12.1% of children affected by high SBP at 1, 18, and 30 months post-transplant, and even fewer had uncontrolled DBP (11.9%, 2.7%, and 3%, respectively). Prevalence of hypertension did not change significantly over time post-transplant.

Dyslipidemia: Dyslipidemia was highly prevalent prior to transplant, with 87.5% of children affected by at least one abnormal lipid parameter (high LDL-C, high triglycerides or low HDL-C). Approximately half of transplant recipients remained dyslipidemic after transplant (59%, 46%, 54.5% at 1, 18 and 30 months, respectively). The prevalence of elevated triglycerides was significantly higher pre-transplant (79.2%) vs. post-transplant (35.9%, 24.3%, and 29.4%, respectively, $p < 0.001$). Elevated LDL-C was also more common pre-transplant vs. 18 months post-transplant (37.5% vs. 13.5%), $p = 0.03$ whereas 1 month and 30 months post-transplant values were comparable to pre-transplant values.

CIMT: The prevalence of high CIMT was 29%, 14.7%, and 16.7% at 1, 18, and 30 months post-transplant, respectively. Within the A-A transplant cohort, the prevalence of high CIMT was 31.3%, 26.6%, and 22.2%, at 1, 18 and 30 months, respectively. There were no significant changes in the prevalence of high CIMT over time post-transplant (Table 2). Similarly, the mean and median values of CIMT did not change significantly over time post-transplant (Table 3).

CIMT of A-A and non A-A transplant and controls

Within the transplant group, the median CIMT of A-A children (0.49, 0.49 and 0.48 mm) was significantly higher than non-AA children (0.43, 0.44, and 0.44 mm) at 1, 18, and 30 months post-transplant, respectively (Table 3). In addition, the mean CIMT of healthy A-A controls was higher compared with healthy non A-A controls (0.47 vs 0.44 mm), $p = 0.02$ (see Figure 1). The CIMT of A-A transplant recipients was greater than that of A-A controls at 1 and 18 month time points (0.49 vs. 0.47 mm, $p = 0.03$), see Table 3. The CIMT of the non A-A transplant recipients did not differ from the non A-A controls at any time point.

Pre-transplant hyperparathyroidism and high CIMT

The mean \pm SEM pre-transplant iPTH of patients with high CIMT at 1 month post-transplant was 736.7 \pm 283.0 pg/mL (95%CI 161.6-1131.9), compared with 337.2 \pm 75.4 pg/mL (95% CI 184.1-490.4) in those with normal CIMT. Chi-square analysis demonstrated that pre-transplant hyperparathyroidism (iPTH level >600 pg/mL measured within 30 days prior to the transplant event) was associated with high CIMT at 1 month post-transplant. Of 27 patients with normal iPTH, only 18.5% had high CIMT at 1 month post-transplant, whereas 57.1% of patients with high iPTH had high CIMT at 1 month post-transplant (p=0.04), see Figure 2.

Pre-transplant determinants of CIMT at 1 month post-transplant

Results of multivariate logistic regression analysis showed that pre-transplant hyperparathyroidism was positively associated high CIMT (defined as \geq 95th percentile-for-race, based of control data) at 1 month post-transplant (3.8 \pm 1.9, p=0.04, pseudo R²=0.45). This indicates that having high iPTH prior to transplant increases the log odds of having high CIMT by 3.8, after adjusting for type and duration of dialysis, dyslipidemia, hypertension, and obesity.

Determinants of CIMT 1-30 months post-transplant

In the multivariate longitudinal GEE regression analysis of the entire transplant cohort, A-A race was the sole significant predictor of increased CIMT. The results indicated that A-A race is associated with a 10.0% \pm 1.0% higher CIMT compared with non A-A transplant recipients, p=0.0001. Due to the significant impact of A-A race on CIMT, the analysis was stratified by race. Results showed that amongst the A-A transplant cohort, the presence of MS was associated with a 0.03 \pm 0.01 mm increase in CIMT (p=0.002). Amongst the non A-A transplant cohort, neither obesity nor MS were significantly associated with CIMT.

Discussion

This study was the first study to investigate CIMT in a cohort of A-A pediatric kidney transplant recipients. Our results reveal that the CIMT of the A-A transplant cohort was significantly higher compared with non A-A transplant recipients. The CIMT of A-A controls were also higher compared with non A-A controls, necessitating the use of race-specific cut-points for CIMT. In addition, the CIMT of the A-A transplant recipients was higher compared to the A-A controls, while the CIMT of the non A-A transplant recipients did not differ from their race-matched controls. We identified pre and post-transplant predictors of race-specific CIMT. Pre-transplant hyperparathyroidism predicted high CIMT-for-race in the early post-transplant period. This finding is in agreement with several other pediatric studies that have identified similar pre-transplant markers of disordered bone and mineral metabolism, such as high calcium-phosphorus product (86, 87) and high cumulative calcitriol dose (90), as predictors of increased CIMT after transplant. This finding highlights the importance of good bone and mineral metabolism management prior to transplant in order to prevent the formation of vascular calcifications that may contribute to increase in CIMT.

MS was found to be a significant independent predictor of increased CIMT in the A-A transplant cohort over 1-30 months post-transplant, but did not affect CIMT of the non A-A children. This disproportionate effect of MS on the A-A children occurred, despite the fact that MS was less common within the A-A compared to the non A-A transplant cohort (26.8% vs. 35.8% prevalence). Thus, our findings indicate that A-A pediatric kidney transplant recipients have increased CIMT, which is negatively impacted by MS, whereas the CIMT of non-AA appears unaffected. CIMT values did not change significantly over time post-transplant amongst either cohort.

Given the disparate nature of CIMT outcomes between our A-A and non A-A transplant cohorts, further studies are needed to elucidate the etiology and the effects of this phenomenon.

Individuals of African descent are generally known to carry higher risk for CV disease and kidney disease than other races (13, 47-49). The reasons for these disparities are not completely understood, and are likely multifactorial in nature. Genetic factors are known to play a role, due to the strong association of the Apolipoprotein L1 gene (APOL1) with risk of kidney disease in individuals of African ancestry. Relationships of APOL1 renal risk variants with increased risk of CV disease among those of African descent are also starting to emerge (50, 51). A-A adults, including young adults, have higher rates of adverse CV events and CV death compared with other ethnic groups (53, 54). Looking at subclinical markers of CV disease in the general population, A-A children and adults have higher left ventricular mass, pulse wave velocity (PWV) and CIMT compared to other ethnicities (55-58). Mittelman, et al. conducted a cross-sectional study examining CIMT in a multiethnic population of 599 healthy children between 6 to 20 years of age (224 Hispanic, 210 European-American, 39 Asian-American, and 126 A-A) (56). They reported that CIMT was greater among the A-A children than any of the other ethnic groups (all $p < 0.05$), while there were no differences in CIMT between the Caucasian, Hispanic and Asian groups; A-A ethnicity remained a significant independent predictor of CIMT in their multivariate analysis ($\beta = 0.01$, $p = 0.02$) (56). Similarly, the results of our study showed that the CIMT of the healthy A-A control group was greater than that of the non A-A control group, which included Caucasian, Hispanic and Asian children (0.47 vs 0.44 mm), $p = 0.02$. CIMT was even further increased in the A-A transplant recipients compared to the A-A controls (0.49 vs. 0.47 mm, $p = 0.03$) at 1 and 18 months post-transplant. In the multivariate analysis of our entire transplant cohort, A-A race was the sole independent predictor of increased CIMT. The etiology

of increased CIMT in the general A-A population is unknown. It remains to be seen whether it may be related to environmental exposures, genetic polymorphisms, or other causes. One study suggests that higher CIMT among individuals of African ancestry may be related to physiological differences in the size of CV structures and lean body mass that vary among different ethnic groups (59), while another study suggested that a blunted nocturnal cortisol rise, caused by psychosocial stress, may account for increased risk for atherosclerosis and CIMT in obese A-A youth (60).

In our study, after stratifying by race, MS was identified as an independent predictor of increased CIMT in the A-A transplant cohort. This highlights the importance of aggressive focus on prevention and early detection of MS for improving CV outcomes. Studies in the general pediatric population suggest that diet and lifestyle changes can be effective in reducing CIMT. Woo et al. conducted a study investigating the effect of diet and exercise on noninvasive markers of atherosclerosis in otherwise healthy obese children, and found a significant improvement in CIMT and percent body fat after one year of intervention (82). While ours was the first study to investigate the effects of MS on CIMT after transplant, a recent multicenter cross-sectional study conducted in Germany examined effects of individual metabolic risk factors (such as obesity defined by BMI and WHr, hypertension, and HDL-C), on CIMT in pediatric kidney transplant recipients (92). In this study, Borchert-Morlins et al. reported no significant associations of metabolic risk factors with CIMT in their Caucasian pediatric transplant cohort, which is in agreement with the lack of significant associations found within our non AA- transplant group. Further studies are needed to investigate why metabolic risk factors may impact the CIMT of A-A pediatric transplant recipients differently.

A limitation to our study was that the distribution of male and female gender was different between the transplant and control groups. However, gender was included in the multivariate regression models, and was not significantly associated with CIMT in any of the analyses. In summary, our findings indicate that A-A pediatric transplant recipients have increased CIMT compared to both A-A controls and non A-A transplant recipients. Pre-transplant hyperparathyroidism contributes to increase in CIMT in the early post-transplant period. MS is common after kidney transplantation, occurring in about a third of non A-A children and about a quarter of A-A children. Early signs of CV damage, as detected by increased CIMT, are present in A-A pediatric kidney transplant recipients. MS is an independent risk factor for increased CIMT among the A-A transplant cohort. Identification of subclinical CV damage, detected by CIMT, may provide an opportunity for early CV risk stratification. Investigation of the effects of racial differences in CIMT and CV risk are critical to creating accurate race-specific risk prediction models for diverse pediatric kidney transplant recipients. At the present time, normative CIMT reference values for children of different racial groups are lacking. Further research, particularly within the high risk A-A pediatric transplant population, is needed to make progress towards delaying or avoiding the occurrence of future major CV events, and increasing the life expectancy of children after kidney transplantation.

Tables

Table 1 Demographics and characteristics of the transplant group and control groups

Number of Patients (n)	Transplant (42)	Control (24)	p value Transplant vs Controls
Age (years)	12.1± 0.7	11.1 ± 0.5	0.31
Race (%)	50% A-A	58% A-A	0.53
Sex (%)	62% male	25% male	0.004*
<u>Pre-transplant modality</u>		NA	NA
Preemptive	31%		
Hemodialysis	38.1%		
Peritoneal dialysis	30.1%		
<u>Duration dialysis (months)</u>	<u>mean+SEM (95% CI)</u>	NA	NA
Hemodialysis	28.0±5.1(17.7-38.2)		
Peritoneal Dialysis	20.9±3.4(13.9-27.9)		
<u>eGFR (mL/min/1.73m²)</u>	<u>mean+SEM (95% CI)</u>	NA	NA
1 month	94.4±4.4 (85.7-103.0)		
18 months	96.9±4.5 (88-105.9)		
30 months	90.3±3.1 (84.2-96.4)		
<u>Steroid protocol</u>		NA	NA
1 month	52.4%		
18 months	54%		
30 months	55.9%		
<u>Steroid dose of those on steroid therapy (mg/kg/day)</u>	<u>mean+SEM (95% CI)</u>	NA	NA
1 month	0.45+0.05(0.35-0.56)		
18 months	0.17+0.05(0.05-0.29)		
30 months	0.07+0.02(0.04-0.11)		

Table 2.

Prevalence of metabolic and cardiovascular morbidities in the transplant group at each time point

	Transplant Group n=42				p value
	Pre-Transplant	1 month	18 months	30 months	
Obesity (BMI \geq 95 th %)	14.2%*	22%	35.1%*	27.3%	0.04**
Abdominal Obesity (WHR \geq 0.539)	NA	41%	46%	36.3%	NS
Metabolic syndrome	NA	33.3%	29.7%	30.3%	NS
Hypertension	75.7%	59.5%	67.5%	60.6%	NS
High SBP	NA	19.1%	5.4%	12.1%	NS
High DBP	NA	11.9%	2.7%	3.0%	NS
Dyslipidemia	87.5%	59%	46%	54.5%	0.03* 0.002** 0.01***
High triglycerides	79.2%	35.9%	24.3%	29.4%	.0009* .0001** .0002***
Low HDL-C	45.8%	43.6%	32.4%	29.4%	NS
High LDL-C	37.5%	18.0%	13.5%	21.2%	0.03**
Glucose intolerance	NA	15%	29.7%	30.3%	NS
High CIMT	NA	29%	14.7%	16.7%	NS

Pre-transplant versus 1 month post-transplant*, Pre-transplant vs 18 months post-transplant **, Pre-transplant vs 30 months post-transplant ***

Table 3.

CIMT of A-A and non-A-A transplant recipients at each time point vs. controls

CIMT (mm)	Controls n=14 A-A n=10 non A-A		Transplant Group n=21 A-A n=21 non A-A					
	n=24		1 month		18 months		30 months	
	A-A	Non A-A	A-A	Non A-A	A-A	Non A-A	A-A	Non A-A
Mean	0.47	0.44	0.49	0.45	0.49	0.44	0.48	0.44
SEM	±.009	±.008	±.007	±0.007	±.007	±0.004	±0.009	±0.006
95% CI	0.45- 0.49	0.42- 0.46	0.47- 0.51	0.43- 0.47	0.47- 0.50	0.43- 0.44	0.46- 0.50	0.43- 0.45
Median	0.46	0.45	0.49	0.43	0.49	0.44	0.48	0.44
IQR	0.45- 0.50	0.42- 0.46	0.47- 0.51	0.43- 0.46	0.46- 0.51	0.42- 0.45	0.46- 0.50	0.42- 0.45
P value A-A vs Non A-A	0.02*		0.001**		0.0001**		0.0007**	
p value trans- plant vs control	NA	NA	0.03*	0.62	0.03*	0.51	0.37	0.79

*significant difference by Student's t-test, p<0.05

** significant difference by Wilcoxon rank-sum, p<0.05

Figures

Figure 1. CIMT of A-A and non A-A transplant at 1, 18, and 30 month time points vs. race-matched controls

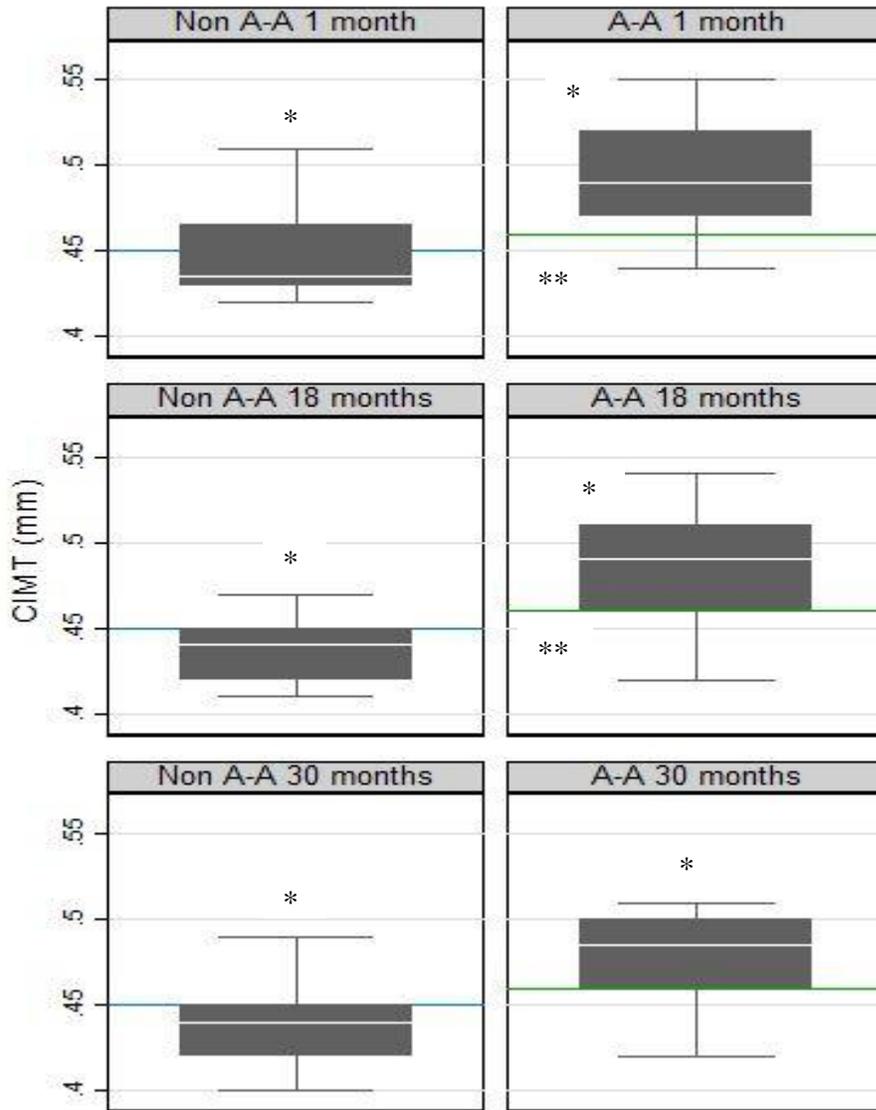
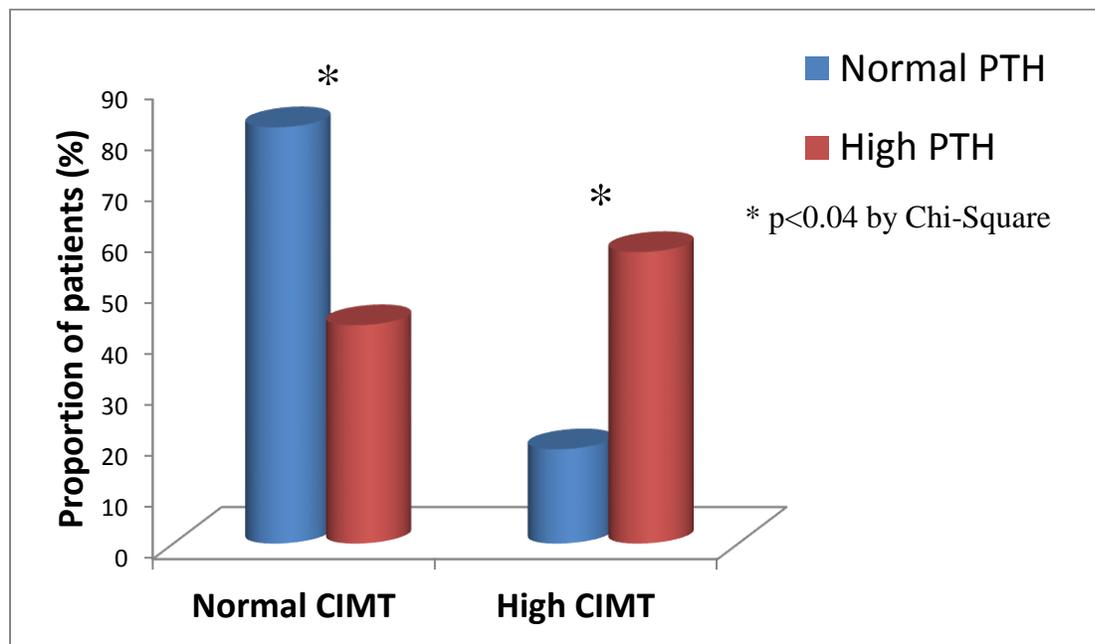


Figure 1. Box plots depict the CIMT of A-A and non A-A transplant recipients, overlaying single line indicating the CIMT of race-matched control patients.

*significant difference between A-A and non A-A transplant recipients

**significant difference between transplant and race-matched controls

Figure 2. Pre-transplant Hyperparathyroidism and High CIMT at 1 month post-transplant



Section 4.3: Manuscript 3

Title: Comparison of BMI, Waist Circumference, and Waist-to-Height ratio for Identification of Subclinical Cardiovascular Risk in Pediatric Kidney Transplant Recipients

Introduction

Cardiovascular (CV) disease is the most common cause of mortality amongst children with end stage renal disease (128). Chronic kidney disease is a life-long chronic illness in which subclinical risks to CV health begin early in the course of the disease and continue to persist after kidney transplantation. Identification of clinical risk factors, such as obesity, that can predict CV dysfunction in children is needed for early and accurate CV risk stratification in this population. However, as major CV outcomes (such as myocardial infarction, stroke, or death) often don't occur until young adulthood or later, evidence linking clinical risk factors to adverse CV outcomes in this vulnerable pediatric population has been lacking. The emergence of newer non-invasive technology, such as carotid intima-media thickness (CIMT) and speckle tracking echocardiography with strain imaging, may provide the opportunity to accurately identify early signs of subclinical CV disease in children (36, 112). Both increased CIMT and impaired myocardial strain have been associated with obesity, hypertension, and dyslipidemia in children (129, 130) and predict major adverse CV events and mortality in adults (80, 101, 102). In general, obese children are known to be at higher risk for metabolic and CV abnormalities, including hypertension and dyslipidemia (14). Abdominal obesity in particular has been more strongly associated with high CV risk than is body mass index (BMI), as obesity-related morbidity is more closely linked to central fat distribution rather than total body fat alone (19, 21). Although BMI is the most common measure of obesity used in clinical practice, evidence shows that both waist circumference (WC) and waist-to-height ratio (WHr) are better predictors of CV risk than BMI in the general pediatric population (19, 20). Obesity is common among

pediatric kidney transplant recipients, as studies show that the prevalence of obesity doubles (from about 15-30%) during the first year after transplantation (25). Factors contributing to post-transplant weight gain include increased appetite and improved taste sensation with the resolution of uremia, liberalization of renal diet restrictions, as well as sedentary lifestyle and poor overall physical fitness, which are pervasive in this population (25). Evidence suggests that BMI does not accurately reflect body composition in children with kidney disease, due to their short stature and altered fat deposition patterns (22, 23). The measure of obesity that best predicts CV risk in pediatric kidney transplant recipients has not been established. The objective of this study was to investigate and compare the ability of BMI, WC, and WHr methods to identify subclinical CV risk in pediatric kidney transplant recipients.

Methods

Study Design and Population

A prospective, controlled cohort study was conducted to investigate the ability of BMI, WC, and WHr to predict subclinical CV risk, as defined by a composite adverse CV outcome, in pediatric kidney transplant recipients. The composite CV outcome was comprised of traditional risk factors (hypertension, dyslipidemia, left ventricular hypertrophy) and non-traditional risk factors (impaired myocardial strain and increased CIMT), all of which have been associated with obesity in children and have been shown to predict future adverse CV outcomes in adults (14, 80, 101, 102, 127, 129, 130). In addition, we examined leptin, a biomarker of obesity associated with CV risk (131), in a subset of lean and obese transplant recipients as classified by BMI, WHr, and WC. Pediatric kidney transplant recipients (1-30 months post-transplant) followed at Children's National Health System (CNHS) in Washington DC, age 3-20 years, were eligible to participate in the study. Those with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m² or

nephrotic-range proteinuria were excluded. Non-obese, healthy children with no history of chronic disease were enrolled as controls. Approval was obtained from the Institutional Review Board (IRB) at CNHS and University of Maryland College Park. Informed consent was obtained from all participants. Transplant recipients had anthropometric measures, blood pressure, fasting lipid panel (triglycerides, LDL-C, and HDL-C cholesterol), CIMT and echocardiogram assessed during study visits at 1, 18, and 30 month post-transplant. A subset of 27 pediatric transplant recipients had leptin levels measured between 1 and 30 months post-transplant. Healthy controls had anthropometric measures, blood pressure, CIMT, and echocardiogram assessed at a single visit.

Classification of Obesity

Obesity was evaluated by BMI and by two different anthropometric measures of abdominal obesity: WC and WHr at 1, 18, and 30 months post-transplant. BMI was calculated and converted to age-and-sex specific percentiles and z-scores, with BMI $\geq 95^{\text{th}}$ percentile defined as obese (119). WC was measured with a Gulick fiberglass tape measure (Country Technology, Gays Mills, WI) at the upper-most lateral border of the right ileum, at the end of an expiration, according to the NHANES procedure (105). WC percentiles and z-scores were determined according to CDC age-and-sex specific tables and obesity defined as WC $\geq 95^{\text{th}}$ percentile (106). WHr was calculated as a ratio of weight in cm divided by height in cm. A WHr cut point of ≥ 0.539 was used to classify obese, based on NHANES data according to the method of Kahn et al. (19).

Echocardiographic Methods

An echocardiogram was obtained at 1, 18 and 30 months post-transplant. The echocardiograms were performed by a single pediatric sonographer using the iE33 xMatrix DS Ultrasound System

(Philips North America Corporation, Andover, MA), according to American Society of Echocardiography standards (110). Echocardiograms were analyzed using Syngo Velocity Vector Imaging (Siemens, Germany) by a pediatric cardiologist blinded to the subject's clinical information. Left ventricular mass indexed to height^{2.7} was evaluated by standard echocardiography. Myocardial strain was assessed in the longitudinal (long-axis) direction by speckle tracking analysis. Strain was calculated by measuring the end systolic distance between two speckles of tracked endocardium minus the original distance divided by the original distance. Because the myocardium contracts during systole, these values are negative percentages, and more negative values indicate better cardiac contractility. Left ventricular hypertrophy (LVH) was defined as left ventricular mass indexed to height^{2.7} $\geq 95^{\text{th}}$ percentile according to the age-specific percentiles of Khoury et al. (66). As there are no established pediatric normative reference values for myocardial strain, the strain data of our healthy control group were used to establish the cut-point for high strain. High strain was thus defined as a value $\geq 95^{\text{th}}$ percentile of our control data.

CIMT Methods

CIMT was assessed at 1, 18 and 30 months post-transplant. B-mode ultrasound imaging of the arterial far wall segments of the right and left common carotid arteries and carotid bulbs was performed according to a standard protocol using the iE33 xMatrix DS Ultrasound System (Philips North America Corporation, Andover, MA). Intima-media thickness of the distal, mid, and proximal segments of right and left common carotid arteries and carotid bulbs was then measured by a pediatric cardiologist, and a composite of the 8 measurements was used to represent the participant's CIMT. As with strain, there are no established normative values for

CIMT in children. Therefore, CIMT data of our healthy control group were used to establish the cut-point for high CIMT. High CIMT was defined as a value $\geq 95^{\text{th}}$ percentile of our control data.

Definition of Composite Adverse CV Outcome

We constructed a composite adverse CV outcome, consisting of five CV risk factors that have been individually linked with obesity in children and are known to predict adverse CV events in adults. The composite adverse CV outcome was defined as the presence of at least three of the following five criteria: 1) left ventricular hypertrophy (defined as left ventricular mass indexed to height^{2.7} $\geq 95^{\text{th}}$ percentile for age and sex (66)), 2) high CIMT (defined as $\geq 95^{\text{th}}$ percentile, based on our healthy control data), 3) high myocardial strain (defined as $\geq 95^{\text{th}}$ percentile, based on our healthy control data), 4) dyslipidemia (defined as the presence of at least one abnormal lipid parameter), and/or 5) hypertension. Cut points for abnormal lipid parameters for HDL-C, LDL-C, triglycerides were defined according to 2011 Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (40) for study participants age 3-19, and by the Adult Treatment Panel III criteria (108) for >19 years of age. Hypertension was defined by requirement for antihypertensive medication.

Statistical Methods

Statistical analyses were conducted using Stata 14.0 (StataCorp LP, College Station, TX). Tests of proportions were performed using Chi-square analysis. Receiver Operator characteristic (ROC) curve analysis was used to compare the ability of BMI, WC, and WHr to detect the composite adverse CV outcome. The measure of diagnostic accuracy defined by the area under the curve (AUC) was used to compare the predictive value of the BMI, WC, and WHr methods. Sensitivity and specificity of each method to detect the composite CV outcome are also reported.

Results

Study population

We conducted an analysis of 116 visits of 42 pediatric kidney transplant recipients over 1 to 30 months post-transplant. Demographics and clinical characteristics of the study participants are shown in Table 1. The study population included 42 pediatric kidney transplant recipients who were 12.1 ± 0.7 years of age. The study population was multi-ethnic, comprised of 50% African American, 28.6% Hispanic, 14.3% European American, 4.8% Middle-Eastern, and 2.3% Asian. The mean BMI z score was 0.73 ± 0.14 , mean WHr was 0.53 ± 0.01 , and mean WC z score was 0.27 ± 0.11 . Transplant recipients were short for age, with a mean height z-score of -1.05 ± 0.21 at 1 month post-transplant. They were maintained on standard immunosuppression therapy with Tacrolimus and Mycophenolate Mofetil, and approximately half were receiving steroid therapy in addition.

Prevalence of adverse CV outcomes among BMI, WHr, and WC-obese transplant recipients

The prevalence and frequency of adverse CV outcomes among the BMI, WHr, and WC-obese transplant recipients, respectively, are shown in Table 2. The prevalence of obesity detected by WHr (43.5%) was significantly higher as compared to BMI (24.1%) and WC (12.0%), $p=0.002$ and $p=0.02$, respectively. The prevalence of dyslipidemia was higher in WHr-obese (76.6%) as compared to the BMI-obese (50%) and WC-obese (44.4%) transplant groups, $p=0.02$ and $p=0.05$, respectively. The proportion of WHr-obese transplant recipients who met the criteria for the composite adverse CV outcome (62.2%) was almost double compared with the proportion of BMI-obese (34.6%) and WC-obese (33.3%) affected by the composite adverse CV outcome.

Distribution of obesity among transplant recipients

Scatter plots were constructed to depict the distribution of BMI, WHr, and WC-obesity in the study population. The distribution reveals the subcategories of discordant observations, allowing

for identification of those participants that were detected as obese by WHr, but not by BMI or WC and vice versa. In a comparison of the WHr and BMI methods, Figure 1 shows that 17.6% (19/108) of observations are categorized as obese by WHr only, whereas no observations are obese by BMI only. Figure 2 compares the WC and BMI methods and shows that less than 2% of observations (2/108) are obese by WC only, whereas 13.9% (15/108) are obese by BMI only. Finally, as shown in Figure 3, a comparison of WHr and WC methods shows that 29.6% (32/108) of observations are categorized as obese by WHr only, whereas no (0/108) observations are obese by WC only.

Leptin

The mean leptin level of obese transplant recipients was significantly greater than that of lean transplant recipients by all three classification methods (BMI, WHr, and WC). Among this cohort of 27 transplant patients who had leptin measured, WHr identified a greater proportion of children as obese by WHR (59.2%) than WC (22.2%) or BMI (18.5%), $p=0.005$ and $p=0.002$, respectively. The difference in leptin between lean and Whr-obese children was 10.8 ± 3.9 vs. 39.5 ± 3.9 pg/mL, $p=0.00001$, respectively, see Table 3.

ROC analysis of composite adverse CV outcome

The results of the comparison of BMI, WHr, and WC-obese methods to predict the adverse CV outcome by ROC analysis are shown in Figure 4. The area under the curve (AUC) for WHr-obese was greatest at 0.77 ± 0.05 (95% CI 0.66-0.87). The AUC for WHr-obese was significantly higher compared to both BMI-obese (0.47 ± 0.06 , 95% CI 0.36-0.59) and WC obese (0.48 ± 0.04 , 95% CI 0.40-0.56), $p=0.0006$. The AUC of WC-obese and BMI-obese did not differ from each other, $p=0.81$.

The sensitivity and specificity of WHr were both good at 75.7% and 73.9%, respectively. The sensitivity of WC was very poor at 11.1%, while the specificity was good at 86.0%. The sensitivity of BMI was also poor at 25.7% while the specificity was comparable to WHr at 71.2%.

Discussion

Our prospective study of three different anthropometric indicators of obesity demonstrated that WHr is a more sensitive method than BMI or WC for identifying a subclinical adverse CV outcome in a population of pediatric kidney transplant recipients. This was the first study to compare the ability of WHr, BMI and WC to predict CV subclinical outcome in children with kidney disease.

The superior ability of WHr to detect adiposity-associated CV risk in our population was evidenced by significantly higher AUC when compared with BMI and WC by ROC analysis (Figure 4). While all three anthropometric methods exhibited good specificity, WHr was a significantly more sensitive indicator of the composite adverse CV outcome. The sensitivity of WHr was 75.7% compared with 25.7% for BMI and only 11.1% for WC. Similarly, when examining the discordant obesity groups, 17.6% of observations were detected as obese by WHr but not by BMI (Figure 1). An even higher percentage, 29.6%, were obese by exclusively by WHr when compared to WC (Figure 3). In addition, WHr-obese pediatric kidney transplant recipients had significantly higher leptin levels compared with lean children. Elevated leptin has been identified as a biomarker for cardiometabolic risk in previous studies of pediatric kidney transplant recipients (132). Taken together, our results suggest that using WHr to assess obesity correctly identifies more pediatric kidney transplant recipients who are at risk for adverse CV

outcomes than BMI or WC. Thus, an important high-risk segment of the population is missed when BMI or WC alone are used to assess obesity in pediatric kidney transplant recipients. Our results are supported by those of a general pediatric population-based analysis that compared the ability of BMI and WHr to identify CV risk among children (n=7657) who participated in the NHANES III study (19). Results of this analysis by Kahn et al. demonstrated that WHr better identified youth with high heart rate, elevated triglycerides, total cholesterol, and LDL-C cholesterol, compared with BMI. The possible reasons behind the stronger association of WHr with CV risk factors are multi-fold. Firstly, as WHr is a measure of abdominal adiposity, WHr-obesity signifies accumulation of excess visceral adipose tissue. Visceral adipose tissue secretes inflammatory cytokines, which induce a variety of unfavorable effects including endothelial dysfunction, glucose intolerance, vasoconstriction, and vascular dysfunction, all of which increased CV risk (17). Even in the setting of normal BMI, excess visceral adipose tissue can accumulate in the abdominal region when dietary lipid intake exceeds the capacity of the peripheral adipocytes to store it, resulting in elevated WHr (133). In our study population, 17.6% of children had abdominal obesity by WHr, despite having a normal BMI. In the NHANES III study, WHr preferentially identified obesity in children who were shorter, had increased central subcutaneous fat distribution characterized by higher subscapular/triceps skinfold ratio, and smaller mid-thigh circumference (19). This body type preferentially identified by WHr in the generally pediatric population closely mirrors the unique body habitus of children with kidney disease. The body composition of pediatric kidney transplant recipients has been characterized by central adiposity, short stature, reduced lean mass, high fat mass, and high trunk:leg fat mass ratio, as revealed by Dual X-ray Absorptiometry (22, 23). Thus, WHr appears well-suited for the assessment of obesity in the pediatric kidney transplant recipients.

Although WC is another measure of central adiposity, WC-for-age percentiles are likely to underestimate abdominal obesity in children with kidney disease, due to the afore-mentioned issues with impaired growth and short stature, which do not allow for accurate comparisons to healthy age-matched peers. To this point, the sensitivity of WC to detect obesity in our transplant study population was extremely low at 11.1%.

Sensitive methods for accurate detection of adiposity-associated CV risk are important in pediatric kidney transplant recipients due to their high risk for obesity, CV morbidity and mortality. As hard CV end-points are uncommon in children, we used a combination of traditional and non-traditional subclinical risk factors to create a composite adverse outcome for the detection of early signs of CV dysfunction in pediatric kidney transplant recipients. We demonstrated that obesity by WHr is a more sensitive method than BMI or WC to detect the subclinical composite adverse CV outcome. Therefore, WHr, a measure of abdominal obesity that is well-suited to assess the unique body composition of pediatric kidney transplant recipients, may be a superior method for detecting CV risk in this high-risk population.

Figures

Figure 1. Distribution of Obesity: Scatter plot of BMI z-score vs WHr

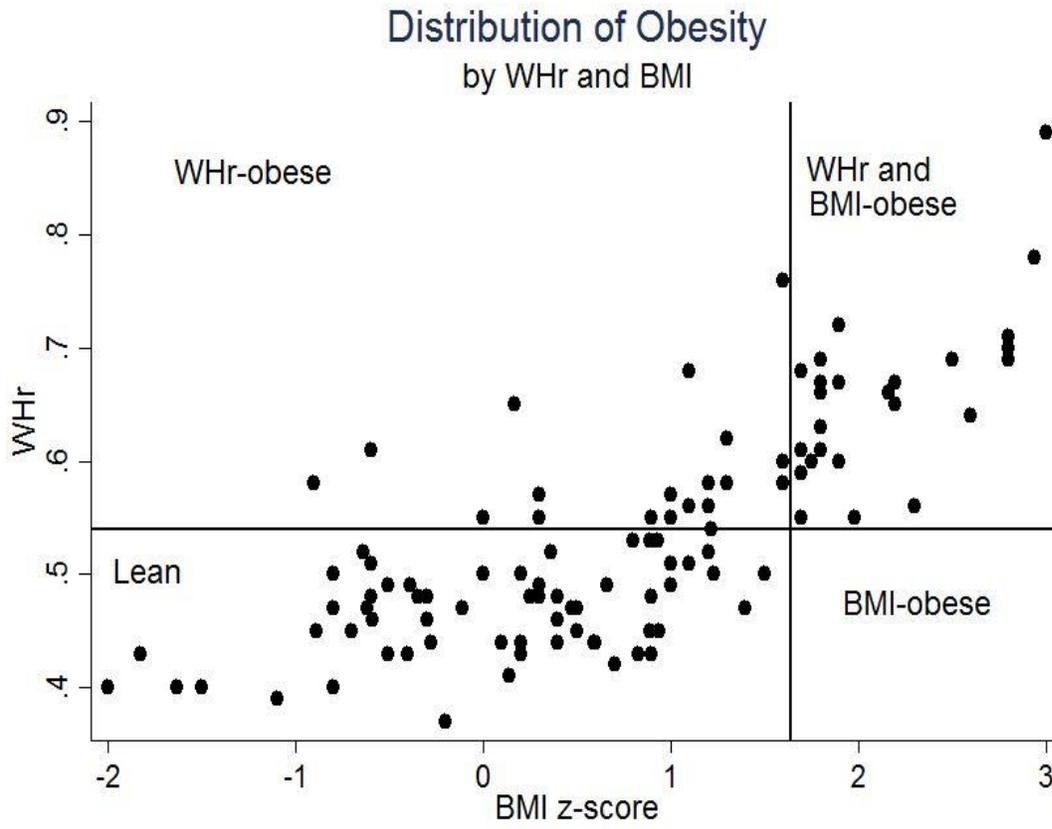


Figure 2. Distribution of Obesity: Scatter plot of WC z-score vs BMI z score

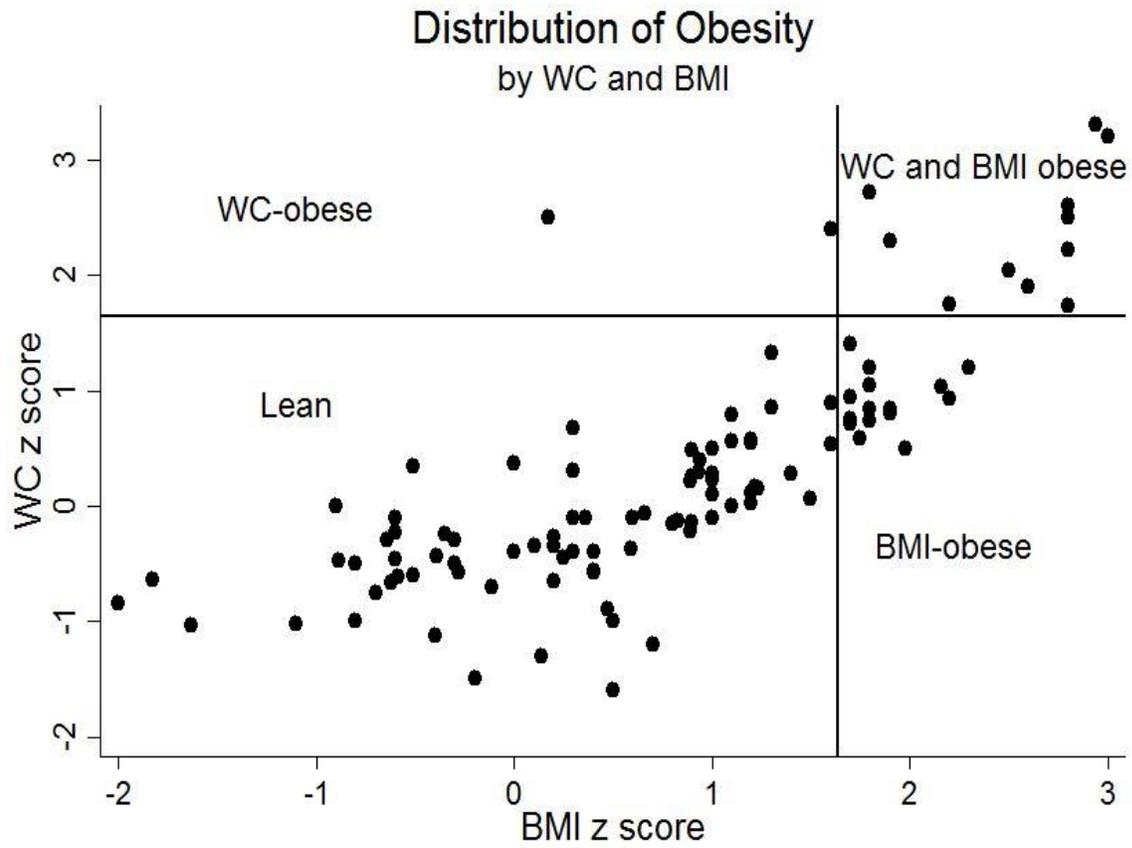


Figure 3. Distribution of Obesity: Scatter plot of WHr vs. WC z-score

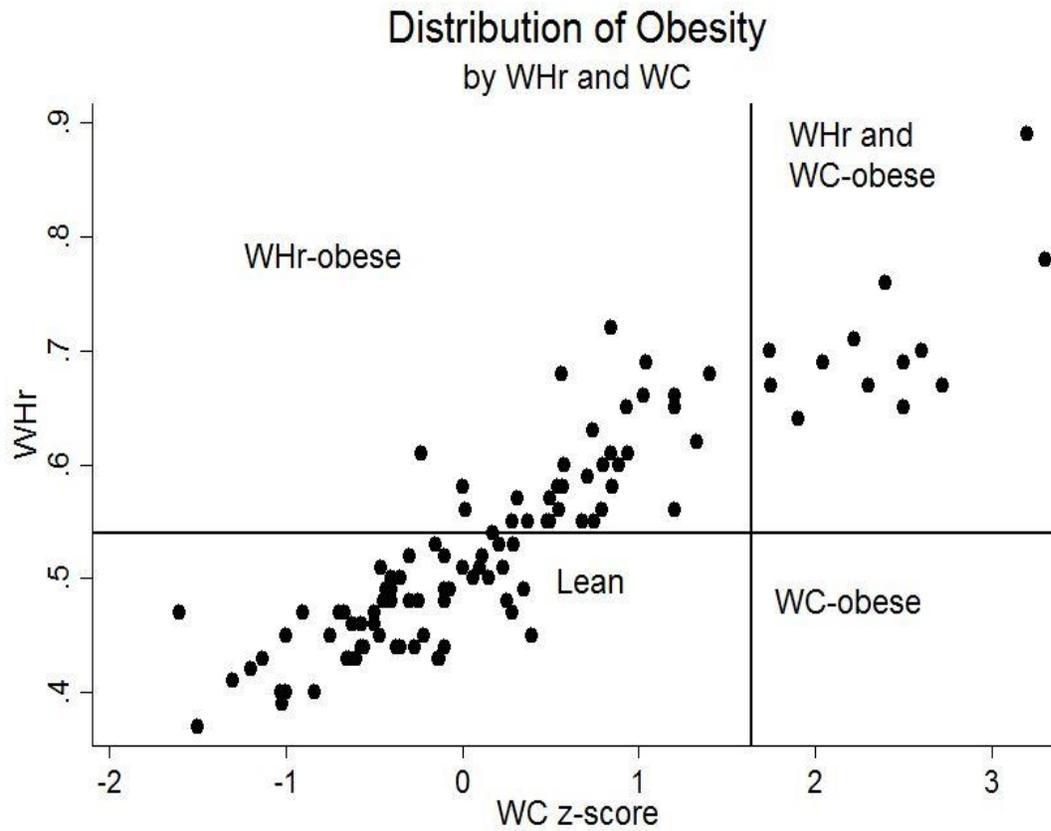
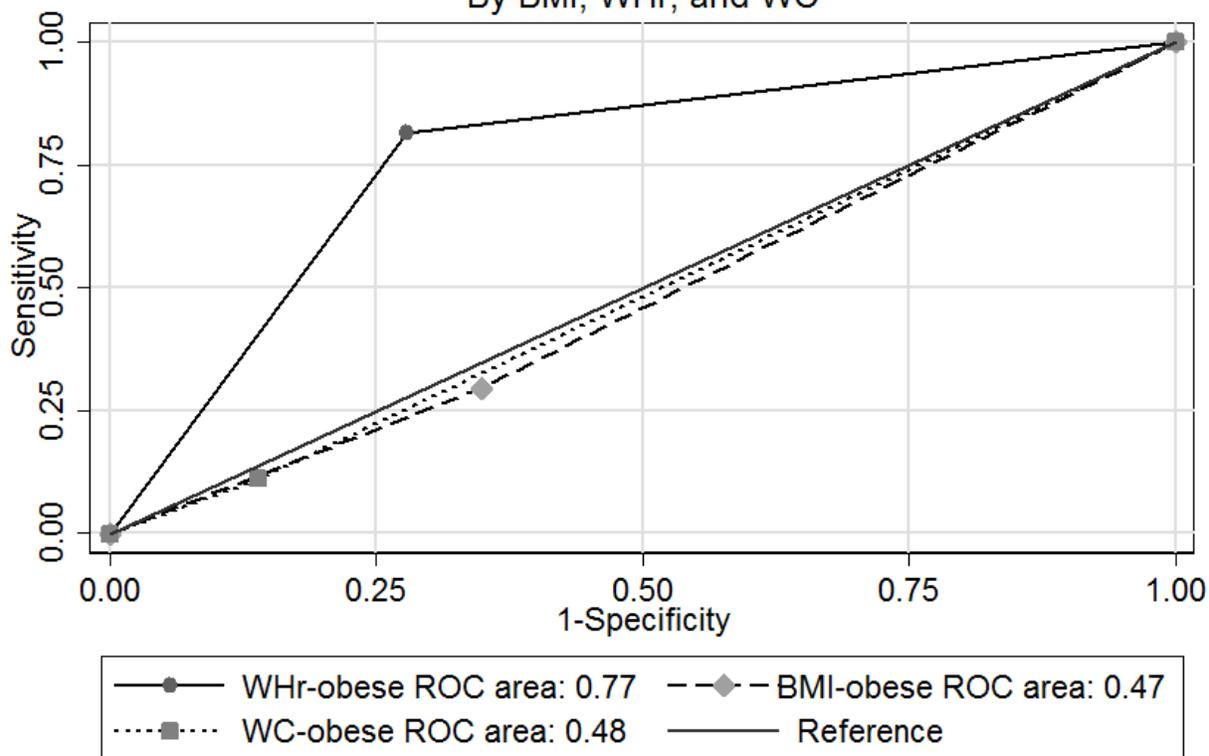


Figure 4 ROC analysis of ability of BMI, WHr, and WC to predict the composite adverse CV outcome

Composite Adverse CV Outcome By BMI, WHr, and WC



Tables
Table 1.

	Transplant Group n=42 patients	Control Group n=24 patients	p value Transplant vs Controls
Age (years)	12.1 ± 0.7	11.1 ± 0.5	0.31
Ethnicity (%)	50.0% African American 28.6% Hispanic 14.3% European American 4.8% Middle-Eastern 2.3% Asian	58.3% African American 4.2% Hispanic 25% European American 4.2% Middle-Eastern 8.3% Asian	0.53 (for % of African American)
Sex (%)	62% male	25% male	0.004*
<u>Pre-transplant modality</u>	<u>% of patients</u>	NA	NA
Preemptive Hemodialysis	31%		
Peritoneal dialysis	38.1%		
	30.1%		
<u>Duration dialysis (months)</u>	<u>mean+SEM (95% CI)</u>	NA	NA

Hemodialysis Peritoneal Dialysis	28.0±5.1(17.7-38.2) 20.9±3.4(13.9-27.9)		
<u>eGFR</u> <u>(mL/min/1.73m²)</u>	<u>mean±SEM (95% CI)</u>	NA	NA
1 month	94.4±4.4 (85.7-103.0)		
18 months	96.9±4.5 (88-105.9)		
30 months	90.3±3.1 (84.2-96.4)		
<u>Steroid protocol</u>	<u>% of patients</u>	NA	NA
1 month	52.4%		
18 months	54%		
30 months	55.9%		

Table 2. Prevalence (%) of CV outcomes within the BMI, WHr, and WC-obese transplant groups

*significant difference in proportions by Chi-square test (p<0.05)

	Prevalence (%) of CV outcomes within the BMI, WHr, and WC-obese transplant groups						
	Obesity	Chronic Hypertension	Dyslipidemia	High Strain	High CIMT	LVH	Composite Adverse CV Outcome
BMI-Obese (≥95 th %)	24.1	57.1	50.0	44.0	12.5	40.0	34.6
WHr-Obese (≥0.539)	43.5	66.0	76.6	62.8	19.5	41.8	62.2
WC-Obese (≥95 th %)	12.0	61.5	44.4	55.5	0.0	33.3	33.3
p value BMI vs WHr	0.002*	0.41	0.02*	0.13	0.47	0.88	0.02*
p value BMI vs WC	0.02*	0.81	0.77	0.55	0.32	0.72	0.93
p value WHr vs WC	0.002*	0.74	0.05*	0.68	0.20	0.64	0.11

Table 3. Mean leptin of lean and obese transplant recipients

	Leptin (pg/mL)	p value
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	Mean \pm SEM 95% CI	
BMI Lean n= 21	18.1 \pm 4.3 (9.2-27.0)	0.03*
BMI Obese n=6	38.1 \pm 6.0 (22.6-53.6)	
WHr Lean n=16	10.8 \pm 3.9 (2.6-19.1)	0.00001*
WHr Obese n=11	39.5 \pm 3.9 (30.8-48.1)	
WC Lean n=22	18.7 \pm 4.1 (10.1-27.3)	0.04*
WC Obese n=5	39.3 \pm 7.2 (19.1-59.4)	

*significant difference between mean leptin of lean vs. obese transplant

Chapter 5: Summary and Future Directions

Summary

In summary, we found that obesity, MS and CV abnormalities are prevalent among pediatric kidney transplant recipients. Comparison of anthropometric methods revealed that WHr is a more sensitive indicator of obesity-associated adverse CV outcomes compared with BMI or WC, due in part to the prevalence of short stature in this population.

Obesity, MS, and hypertension are associated with post-transplant LVH. Exposure to hemodialysis, as opposed to peritoneal or no dialysis, adversely impact longitudinal strain prior to transplant. After transplant, significant predictors of impaired longitudinal strain include obesity and hypertension, whereas higher estimated glomerular filtration rate confers a protective effect. In addition, the interaction of MS with higher LDL-C produces an adverse effect on myocardial strain after transplant. The reason for this phenomenon may be explained in part by the specific nature of the LDL-C particles found in children with abdominal obesity, which are smaller, denser, and more atherogenic. In our study, transplant recipients with MS were noted to have an elevated TG/HDL-C ratio, which suggests the presence of such unfavorable LDL-C particles that may contribute to inflammation and myocardial dysfunction.

CIMT of pediatric kidney transplant recipients is most notably affected by African American race. African American pediatric kidney transplant recipients have increased CIMT, which is negatively impacted by MS, whereas the CIMT of non A-A children appears unaffected after transplant. The etiology and consequences of these differences require further investigation.

In conclusion, obesity, MS, and A-A race adversely affect CV outcomes in pediatric kidney transplant recipients, highlighting the importance of efforts to maintain healthy weight, blood

pressure, blood glucose and lipid profile after transplant. Race-specific pediatric reference values should be utilized, when available. Screening for obesity should include measurements of WHr in order to capture the full complement of adiposity associated CV risk in this population.

Future Directions

Large, multi-center studies are needed to further explore the findings reported in our single-center study. Our results suggest that WHr, a measure of abdominal obesity that is well-suited to assess the unique body composition of pediatric kidney transplant recipients, may be a superior method for detecting CV risk in this high-risk population. Further studies that include measures of percent lean and fat mass in WHr-obese transplant recipients, as well as evaluation of additional biomarkers of adiposity, would help to further clarify the utility of WHr. As with the definition of obesity, the definition of MS in the pediatric kidney transplant population may differ from that of the general pediatric population. Future studies are needed to further investigate how the combination of MS and elevated LDL-C impacts CV outcomes of pediatric transplant recipients, in whom the effects of MS may not conform to the classical definition. Results from our racially diverse pediatric kidney transplant population also highlight the need to assess differences in CV risk factors and outcomes across different racial groups. In particular, further studies are needed to investigate the etiology and consequences of increased CIMT in African American transplant recipients. Imaging techniques such as speckle tracking echocardiography and CIMT may provide a means of detecting subclinical CV dysfunction and provide opportunity for developing age and race-specific risk prediction models in the future.

Chapter 6: Supplemental Tables and Figures

Table S1. Demographics and characteristics of the transplant group and control groups

	Transplant Group n=42 patients	Control Group n=24 patients	p value Tx vs Controls
Age (years)	12.1± 0.7	11.1 ± 0.5	0.31
Race (%)	50% A-A	58% A-A	0.53
Sex (%)	62% male	25% male	0.004*
<u>Pre-transplant modality</u>		NA	NA
Preemptive	31%		
Hemodialysis	38.1%		
Peritoneal dialysis	30.1%		
<u>Duration dialysis (months)</u>	<u>mean±SEM (95% CI)</u>	NA	NA
Hemodialysis	28.0±5.1(17.7-38.2)		
Peritoneal Dialysis	20.9±3.4(13.9-27.9)		
<u>eGFR (mL/min/1.73m²)</u>	<u>mean±SEM (95% CI)</u>	NA	NA
1 month	94.4±4.4 (85.7-103.0)		
18 months	96.9±4.5 (88-105.9)		
30 months	90.3±3.1 (84.2-96.4)		
<u>Steroid protocol</u>		NA	NA
1 month	52.4%		
18 months	54%		
30 months	55.9%		

Table S2. Prevalence of metabolic and cardiovascular morbidities in the transplant group at each time point

	Transplant Group				p value
	Pre-Tx	1 month	18 months	30 months	
Obese (BMI≥95 th %)	14.2%*	22%	35.1%*	27.3%	0.04 (pre vs 18 months)
Obese (WC≥95 th %)	NA	14.7%	8.1%	15.6%	NS
Obese (WHR≥0.539)	NA	41%	46%	36.3%	NS
Metabolic syndrome	NA	33.3%	29.7%	30.3%	NS

Chronic HTN	75.7%	59.5%	67.5%	60.6%	NS
High SBP	NA	19.1%	5.4%	12.1%	NS
High DBP	NA	11.9%	2.7%	3.0%	NS
High triglycerides	79.2%*	35.9%*	24.3%*	29.4%*	.0009 (pre vs 1mo) .0001 (pre vs 18mo) .0002 (pre vs 30mo)
Low HDL-C	45.8%	43.6%	32.4%	29.4%	NS
High LDL-C	37.5%*	18.0%	13.5%*	21.2%	0.03 (pre vs 18mo)
Glucose intolerance	NA	15%	29.7%	30.3%	NS
LVH	37.1%	35.2%	17.1%	35.5%	NS
High Long strain	76.7%*	47.1%*	40%*	56.7%	0.002 (pre vs 1 months) 0.01 (pre vs 18 months)
High Circumferential strain	21.2%*	3.0%*	3.1%*	0%*	0.02 (pre vs 1 months) 0.02 (pre vs 18 months) 0.008(pre vs 30 months)
High CIMT	NA	29%	14.7%	16.7%	NS

*significant difference in proportions by Chi-Square ($p < 0.05$)

Table S3. Skewness and Kurtosis Tests for Normality of Continuous Variables Among Transplant Group

Variable	Pr (Skewness)	Pr (Kurtosis)	Joint Prob > Chi ²
FS	0.127	0.779	0.294
LVMi	0.000*	0.000*	0.000*
EF	0.014*	0.001*	0.001*
Longitudinal Strain	0.562	0.181	0.339
Circumferential Strain	0.795	0.586	0.832
BMI z score	0.916	0.198	0.429
CIMT	0.019*	0.403	0.052

ln CIMT	0.067	0.108	0.058
A-A CIMT	0.106	0.519	0.201
nonA-A CIMT	0.04*	0.78	0.11
lnCIMT nonA-A	0.08	0.46	0.15
WHr	0.001*	0.314	0.007*
WC z score	0.001*	0.215	0.004*
Triglycerides	0.000*	0.001*	0.000*
HDL-C	0.000*	0.013*	0.001*
LDL-C	0.000*	0.002	0.000*
HbA1c%	0.451	0.924	0.745
Fasting glucose	0.000*	0.000*	0.000*
Systolic BPz	0.286	0.807	0.543
Diastolic BPz	0.282	0.671	0.505
*non-normal distribution, p<0.05			

Table S4. Skewness and Kurtosis Tests for Normality of Continuous Variables Among Control Group

Variable	Pr (Skewness)	Pr (Kurtosis)	Joint Prob>Chi ²
FS	0.03*	0.13	0.04*
LVMI	0.04*	0.40	0.08
EF	0.88	0.29	0.54
Longitudinal Strain	0.03*	0.15	0.04*
Circumferential Strain	0.77	0.28	0.51
CIMT	0.22	0.78	0.42
BMI z score	0.45	0.05	0.10
WHr	0.43	0.46	0.52
WC z score	0.87	0.89	0.98
Systolic BPz	0.88	0.55	0.83
Diastolic BPz	0.73	0.07	0.16
*non-normal distribution, p<0.05			

Table S5. Comparison of anthropometric and blood pressure parameters of transplant and healthy controls groups

		Transplant Group n=42			Control Group
		1 month	18 months	30 months	Single point n=24
BMI z-score	Mean±SEM (95% CI)	0.47±0.17 (0.13-0.81)	0.88±0.18* (0.5-1.21)	0.81±0.20 (0.39-1.22)	0.30±0.17* (-0.04-0.66)

	Median (IQR)	0.5 (-.5-1.23)	1.0 (.3-1.7)	.89 (0-1.6)	0.4 (-.4-1.1)
WHr	Mean±SEM (95% CI)	0.53±0.01 (0.51-0.56) (n=38)^	0.54±0.01 (0.50-0.57)	0.54±0.02 (0.50-0.58)	0.45±0.01 (0.43-0.47)
	Median (IQR)	0.51** (0.48-0.61)	0.51** (0.47-0.6)	0.52** (0.45-0.58)	0.46** (0.43-0.49)
WC z- score	Mean±SEM (95% CI)	0.28±0.17 (-0.07-0.63) (n=38)^	0.26±0.16 (-0.08-0.59)	0.35±0.19 (-0.03-0.73)	-0.23±0.13 (-0.51-0.05)
	Median (IQR)	0.03 (-0.35-0.68)	0.1 (-0.5-0.8)	0.11 (-0.4-0.93)	-0.2 (-0.68-0.18)
SBP z- score	Mean±SEM (95% CI)	0.71±0.12* (0.46-0.95)	0.66±0.12* (0.42-0.89)	0.63±0.15* (0.33-0.92)	-0.15±0.19* (-0.56-0.24)
	Median (IQR)	0.71 (0.18-1.4)	0.7 (0.21-1.1)	0.52 (0.0-1.2)	-0.06 (-0.5- -0.37)
DBP z- score	Mean±SEM (95% CI)	0.40±0.14* (0.11-0.68)	0.37±0.11* (0.15-0.60)	0.36±0.12* (0.10-0.60)	-0.28±0.18* (-0.67-0.10)
	Median (IQR)	0.33 (-0.27-1.1)	0.63 (-0.27-0.86)	0.52 (-0.3-0.81)	-0.24 (-1.1-0.41)
<p>*significant difference between means by Student's t-test, transplant vs. control, p<0.05 #significant difference between means by Student's t-test, transplant time points, p<0.05 **significant difference between medians by Wilcoxon rank-sum, transplant vs. control, p<0.05 ##significant difference between medians by Wilcoxon rank-sum, transplant time points, p<0.05 ^unless otherwise noted</p>					

Table S6. Significance (p values) for transplant and control group comparisons of anthropometric and blood pressure variables

	P values				
	1 vs 18 months	1 vs 30 months	Control vs 1 months	Control vs 18 months	Control vs 30 months
BMI z-score	0.11	0.21	0.52	0.03*	0.08
WHr	0.80	0.91	0.0008**	0.002**	0.002**
WC z-	0.95	0.96	0.06	0.06	0.06

score					
SBP z-score	0.76	0.67	0.0001*	0.0002*	0.001*
DBP z-score	0.90	0.82	0.004*	0.001*	0.003*
<p>*significant difference between transplant and control, p<0.05 by Student's t-test #significant difference between transplant time points, p<0.05 by Student's t-test **significant difference between transplant and control by Wilcoxon rank-sum, non-parametric, p<0.05 ## significant difference between transplant time points by Wilcoxon rank-sum, non-parametric, p<0.05</p>					

Table S7. Comparison of anthropometric and blood pressure parameters of the obese and lean transplant groups

	Transplant Group						Control n=24
	1 month		18 months		30 months		
	Obese	Lean	Obese	Lean	Obese	Lean	
BMI							
BMI z-score	1.9±.11 (1.7-2.2) (n=9)	.07±.15 (-.23-.4) (n=33)	1.9±.16 (1.6-2.3) (n=13)	.22±.17 (-.13-.57) (n=24)	2.2±.18 (1.8-2.7) (n=9)	.26±.17 (-.09-.61) (n=24)	.30±.17 (-.04-.66)
P value Tx vs control	.00001 [#]	.13	.00001 [#]	.71	.00001 [#]	0.84	
P value BMI-obese vs. lean	.00001*		.00001*		.00001*		
Waist Circumference							
	Obese	Lean	Obese	Lean	Obese	Lean	Control
WC z-score	2.3±.15 (2.0-2.6) (n=5)	-.02±.12 (-.28-.23) (n=33)	2.7±.32 (2.1-3.3) (n=3)	.03±.12 (-.2-.27) (n=34)	2.3±.27 (1.8-2.9) (n=5)	0±.13 (-.27-.27) (n=28)	-.23±.13 (-.51-.05)
P value Tx vs. control	.0007 ^{##}	0.27	0.006 ^{##}	0.31	0.0007 ^{##}	0.30	
P value WC-obese vs. lean	.0004**		.004**		.0005**		
Waist to Height Ratio							
WHr	.62±0.01 (.59-.65)	.47±.01 (.45-.48)	.62±.02 (.59-.66)	.46±.01 (.44-.48)	.64±.02 (.59-.7)	.46±.01 (.44-	.45±.01 (.43-.47)

	(n=16)	(n=22)	(n=17)	(n=20)	(n=14)	.48 (n=19)	
P value Tx vs control	0.00001 ^{##}		0.005 ^{##}		0.006 ^{##}		
P value WHr-obese vs. lean	0.00001 ^{**}		0.00001 ^{**}		0.00001 ^{**}		
Systolic Blood Pressure z score of BMI-obese and lean							
SBP z score Mean±SEM 95% CI	.49±0.2 (.03-0.95)	.77±.15 (.46-1.1)	.94±.19 (.52-1.3)	.48±.14 (.18-.79)	.47±.24 (-.1-1.0)	.69±.18 (.3-1.1)	-.15±.19 (-.56-.24)
Median IQR	.41 .1-1.6	.92 .19-1.5	.94 .45-1.2	.59 0-1.1	.45 .05-.67	.65 -.05-1.2	-.06 -.5-.37
P value Tx vs control	.05	.0004 [#]	.0007 [#]	.01 [#]	.07	.003 [#]	
P value BMI-obese vs lean Tx	0.37		0.07		0.53		
Systolic Blood Pressure z score of WC-obese and lean							
SBP z score Mean±SEM 95% CI	.68±.34 (-.26-1.6)	.65±.14 (.35-.95)	1.3±.59 (-1.2-3.8)	.6±.11 (.37-.84)	.94±.38 (-.11-2.0)	.57±.16 (.24-.9)	-.15±.19 (-.56-.24)
Median IQR	.96 .41-1.05	.53 .12-1.4	.93 .45-2.4	.69 1.8-1.1	.52 .45-1.7	.54 -.1-1.2	-.06 -.5-.37
P value Tx vs control	.06	.001 [#]	.02 [#]	.0008 [#]	.0001 [#]	.006 [#]	
P value WC- obese vs lean Tx	0.95		0.12		0.39		
Systolic Blood Pressure z score of WHr-obese and lean							
SBP z score Mean±SEM 95% CI	.58±.2 (.14-1.0)	.69±.17 (.33-1.1)	.85±.15 (.52-1.2)	.49±.16 (.15-.84)	.53±.19 (.12-.94)	.70±.22 (.23-1.1)	-.15±.19 (-.56-.24)
Median IQR	.38 .14-1.0	.71 .12-1.4	.93 .39-1.1	.6 -.07-1.1	.48 -.1-.88	.58 0-1.2	-.06 -.5-.37
P value Tx vs control	0.01 [#]	.002 [#]	.0004 [#]	0.01 [#]	0.02 [#]	.006 [#]	
P value WHr- obese vs lean Tx	0.67		0.13		.59		
Diastolic Blood Pressure z score of BMI-obese and lean							
DBP z score Mean+SEM	.39±0.35 (-.43-1.2)	.40±0.16 (.08-.72)	.34±.2 (-.1-.77)	.32±.13 (.05-.59)	.25±.21 (-.24-.73)	.40±.15 (.08-.72)	-.28±.18 (-.67-.10)

Median IQR	.32 -.21-.67	.34 -.27-1.1	.63 -.86-.85	.34 .27-.88	.26 .1-.61	.52 -.32-.9	-.24 -1.06- .41
P value Tx vs control	.07	.007 [#]	.03 [#]	.008 [#]	.10	.006 [#]	
P value BMI-obese vs lean Tx	0.96		0.95		0.59		
Diastolic Blood Pressure z score of WC-obese and lean							
DPB z score Mean \pm SEM 95%CI	1.08 \pm 0.4 (-.01- 2.2)	.31 \pm .12 (-.02- .65)	.76 \pm .34 (-.71- 2.2)	.34 \pm .12 (.1-.58)	.64 \pm .18 (.13- 1.1)	.31 \pm .14 (.01-.6)	-.29 \pm .18 (-.66- .08)
Median IQR	.67 .65-1.1	.3 -.39-.98	.68 .22-1.4	.48 -.3-.86	.71 .26-.81	.49 -.87- .34	-.24 -1.06- .41
P value Tx vs control	.003 [#]	.02 [#]	.05	.004 [#]	.03 [#]	.01 [#]	
P value WC- obese vs lean Tx	0.09		0.31		0.35		
Diastolic Blood Pressure z score of WHr-obese and lean							
DBP z score Mean \pm SEM 95%CI	.37 \pm .27 (-.2-.95)	.41 \pm .19 (.02-.8)	.44 \pm .18 (.05- .84)	.32 \pm .12 (.02- .61)	.45 \pm .17 (.05- .84)	.28 \pm .18 (.02- .62)	-.29 \pm .18 (-.66- .08)
Median IQR	.31 -.44-.66	.46 -.25-1.1	.67 -.3-.85	.21 -.83-.89	.52 .22-.72	.46 -.4-.9	-.24 -1.06- .41
P value Tx vs control	.04 [#]	.01 [#]	.009 [#]	.01 [#]	.008 [#]	.03 [#]	
P value WHr- obese vs lean Tx	0.92		0.58		0.51		

*significant difference between lean and obese transplant groups by Student's t-test, p<0.05

#significant difference between transplant and control groups by Student's t-test, p<0.05

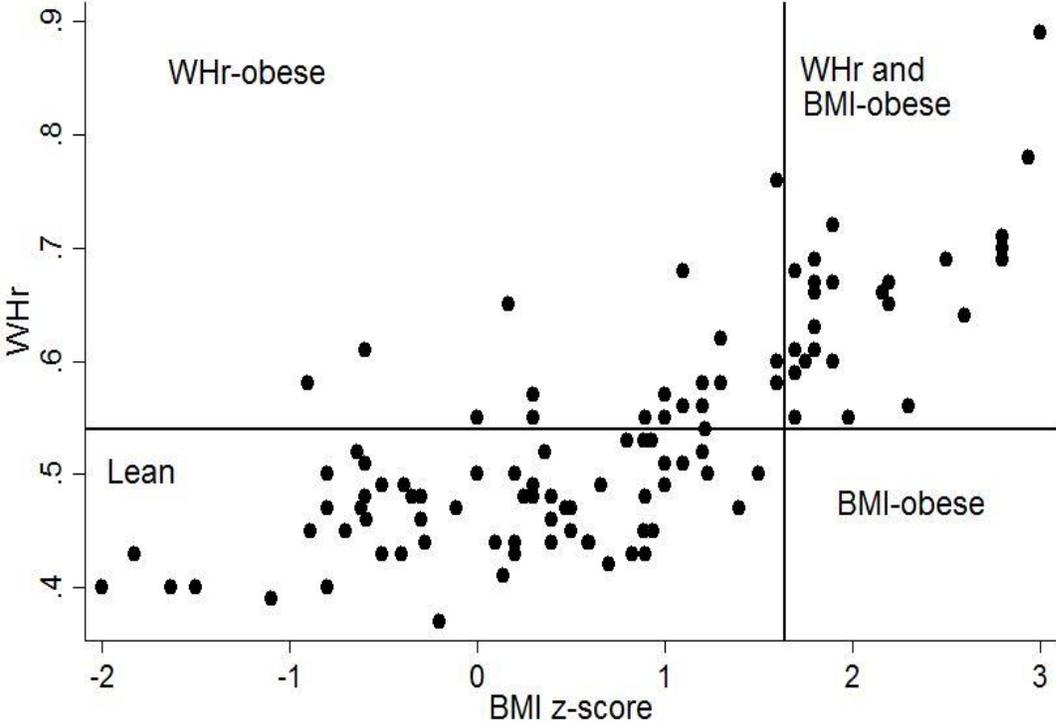
**significant difference between lean and obese transplant groups by Wilcoxon rank-sum

significant difference between transplant and control groups by Wilcoxon rank-sum

Figure S1. Distribution of Obesity Amongst Transplant Population

Scatter plots BMI vs WHr, BMI vs WC, and WC vs WHr

Distribution of Obesity by WHr and BMI



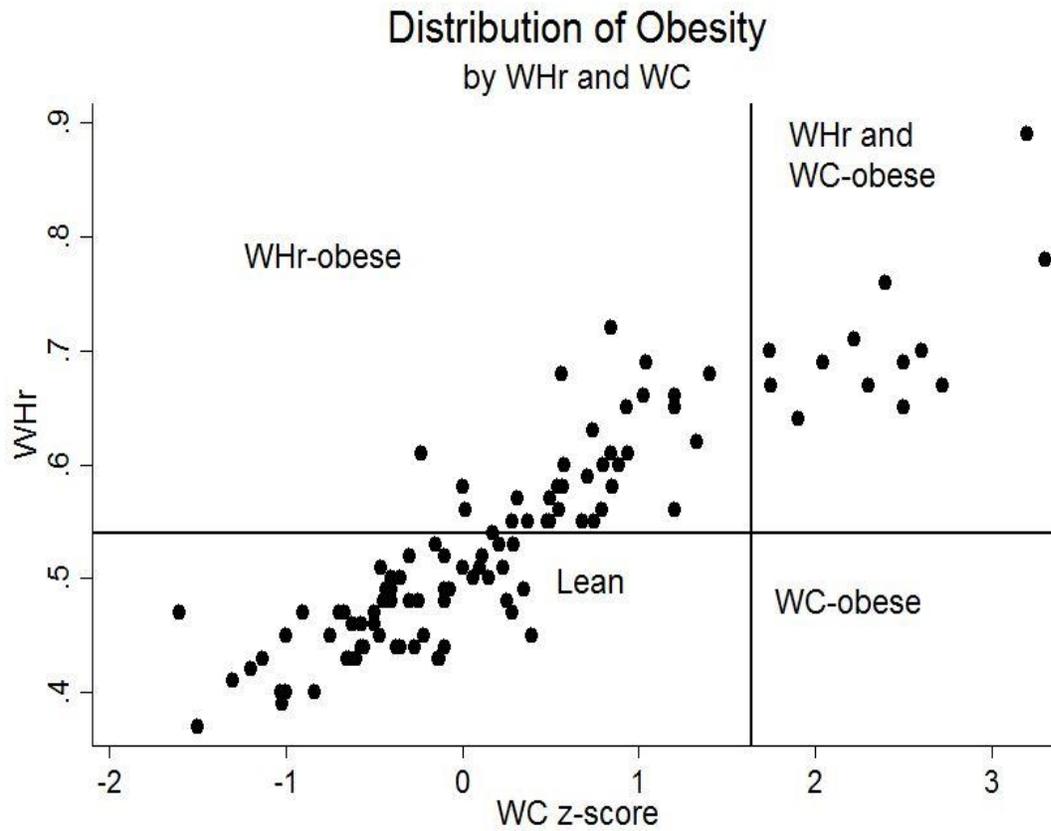


Figure S2. ROC analysis to compare the ability of BMI, WHr, and WC obesity classification methods to identify composite adverse CV outcome after transplant

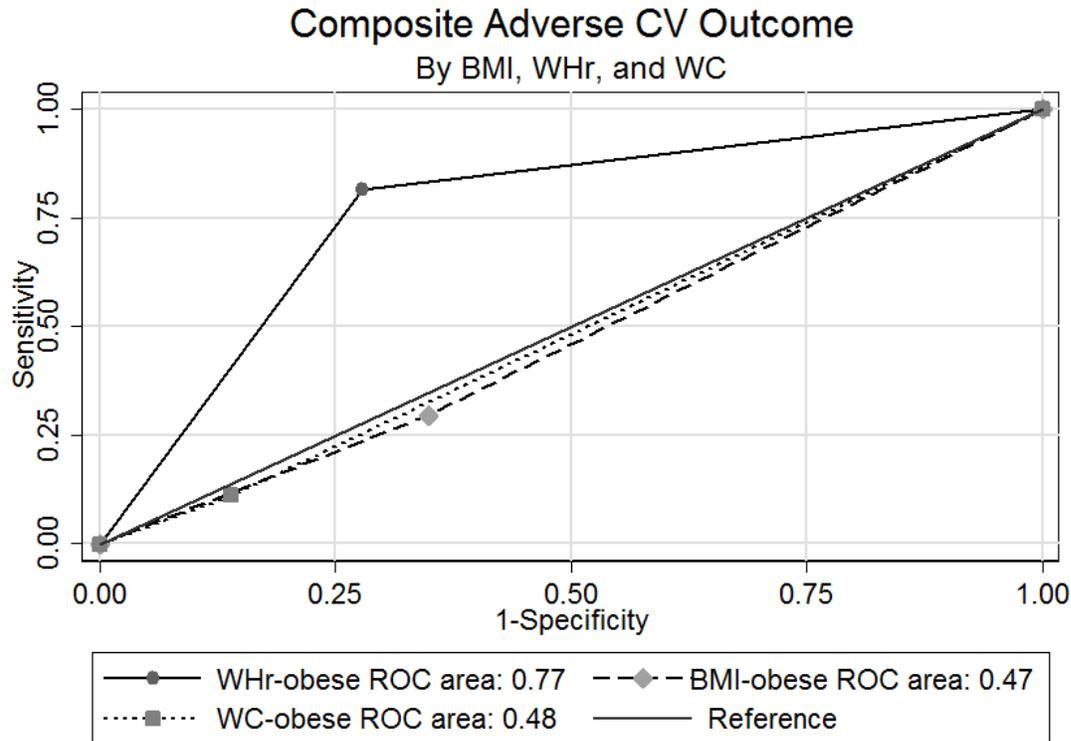


Table S8. Measures of standard echo, myocardial strain, and CIMT in Transplant and Controls

		Control Group	Transplant Group n=42			
		n=24	Pre-Tx	1 month	18 months	30 months
Fractional shortening (%)	Mean	34.8	35.5#	37.0	37.4	38.0#
	SEM	± 0.93	± 0.86	± 0.84	± 0.88	± 0.84
	95% CI	(32.9-36.8)	(33.8-37.2)	(35.4-38.7)	(35.6-39.1)	(36.2-39.6)
	Median	33.8**	36.1	36.3	37.1**	38.0**
	IQR	(31.6-36.9)	(31.6-37.6)	(33.5-41.1)	(33.3-40.3)	(35.6-41.5)
Ejection fraction (%)**	Mean	64.1	62.4	64.7	63.9	64.1
	SEM	± 0.48	± 1.08	± 0.68	± 0.52	± 0.63
	95% CI	(63.1-65.1)	(60.2-64.6)	(63.3-66.0)	(62.8-64.9)	(62.8-65.3)
	Median	64.0	62.7	63.8	63.1	63.8
	IQR	(61.8-66.2)	(58.4-	(62-67)	(61.9-	(61.7-

			64.9)		65.8)	66.6)
LVMI/Ht ^{2.7} (g/m ^{2.7})	Mean SEM 95%CI	29.8 ±1.1 (27.5-32.2)	40.3 ±2.8 (34.7-45.9)	39.2 ±2.4 (34.4-44.0)	34.3 ±1.4 (31.5-37)	35.5 ±1.5 (32.4-38.6)
	Median IQR	29.7** (26.1-32.1)	37.8** (26.1-45.5)	38.6** (31.1-43.2)	33.0** (29.7-39.0)	35.8** (28.2-42.6)
Long strain (%)	Mean SEM 95%CI	-23.1 ±0.63 (-24.3- -21.8)	-17.4 [#] ±0.67 (-18.8 - -16.1)	-19.7 [#] ± 0.59 (-20.9- -18.5)	-19.9 [#] ± 0.44 (-20.8- -19.0)	-18.7 ±0.44 (-19.6- -17.8)
	Median IQR	-22.3** (-25.3- -21.3)	-18.1** (-19.1- -15.2)	-19.8** (-21.1- -18)	-20.2** (-21.7- -17.9)	-18.6** (-20- -17.1)
Circumferential strain (%)	Mean SEM 95%CI	-23.9 [#] ±0.65 (-25.5- -22.5)	-22.3 [#] ± 0.76 (-23.9- -20.7)	-25.0 [#] ± 0.70 (-26.5- -23.6)	-25.5 [#] ± 0.58 (-26.7- -24.3)	-25.7 [#] ±0.61 (-27- -24.5)
	Median IQR	-24.6 (-25.5 - -21.4)	-21.0 (-25.8 - -19.4)	-25.0 (-27.4- -22)	-25.7 (-27.9- -23.3)	-25.4 (-28- -23.4)
CIMT (mm)	Mean SEM 95%CI	0.46 ±0.007 (.45-.48)	NA	0.47 ±0.006	0.46 ±0.006	0.46 ±0.006
	Median IQR	0.46 (.44-.48)	NA	0.47 (.44-.50)	0.45 (.44-.49)	0.46 (.44-.49)

	P values Control vs. Transplant			
	Control vs Pre-tx	Control vs 1 months	Control vs 18 months	Control vs 30 months
FS	0.47	0.09	0.04**	0.01**
EF	0.06	0.84	0.79	0.83
LVMI	0.01**	0.002**	0.01**	0.02**
Long Strain	0.00001**	0.0001**	0.0004**	0.00001**
Circumferential	0.14	0.28	0.07	0.05

Strain				
CIMT	NA	0.42	0.89	0.95
	*significant difference between transplant and control, p<0.05 by Student's t-test **significant difference between transplant and control, p<0.05, by Wilcoxon rank-sum, non-parametric			

	P values Transplant Time Points				
	1 vs 18 months	1 vs 30 months	Pre-tx vs 1 months	Pre-tx vs 18 months	Pre-tx vs 30 months
FS	0.80	0.45	0.21	0.14	0.04#
EF	0.57	0.84	0.01 ^{##}	0.10	0.07
LVMi	0.11	0.40	0.97	0.16	0.89
Long Strain	0.69	0.21	0.01 [#]	0.002 [#]	0.11
Circumferential Strain	0.58	0.46	0.01 [#]	0.001 [#]	0.001 [#]
CIMT	0.45	0.38	NA	NA	NA
	#significant difference between transplant time points, p<0.05 by Student's t-test ##significant difference between transplant time points, p<0.05 by Wilcoxon rank-sum, non-parametric				

Figure S3. Change in FS over time in transplant group

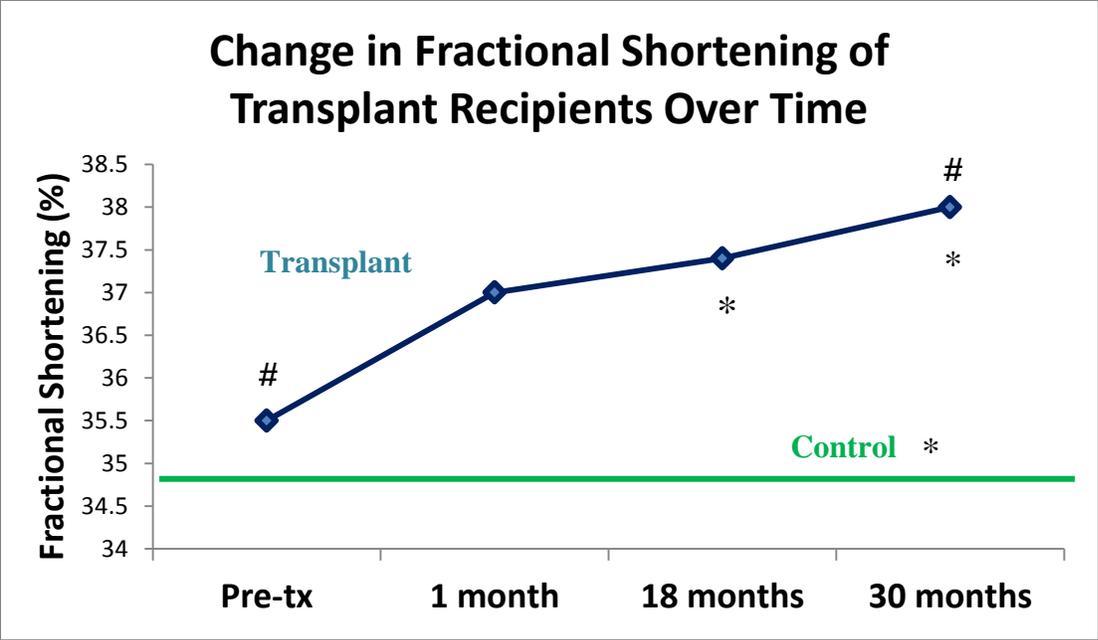


Figure S4. Change in EF over time in transplant group

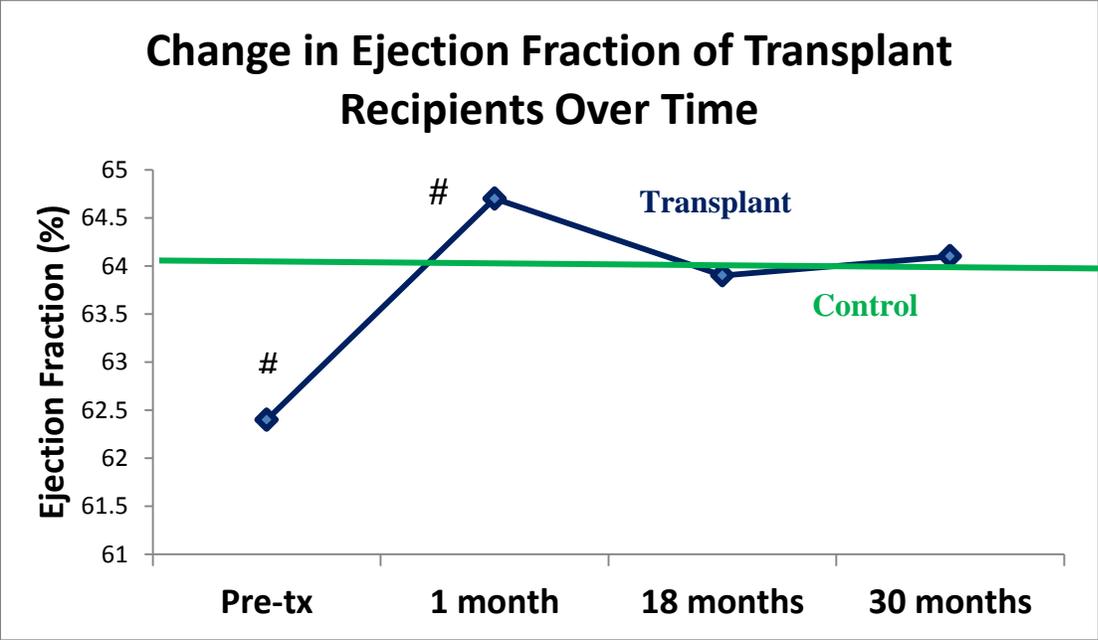


Table S9. LVMI of BMI-obese and lean transplant recipients at each time point vs controls

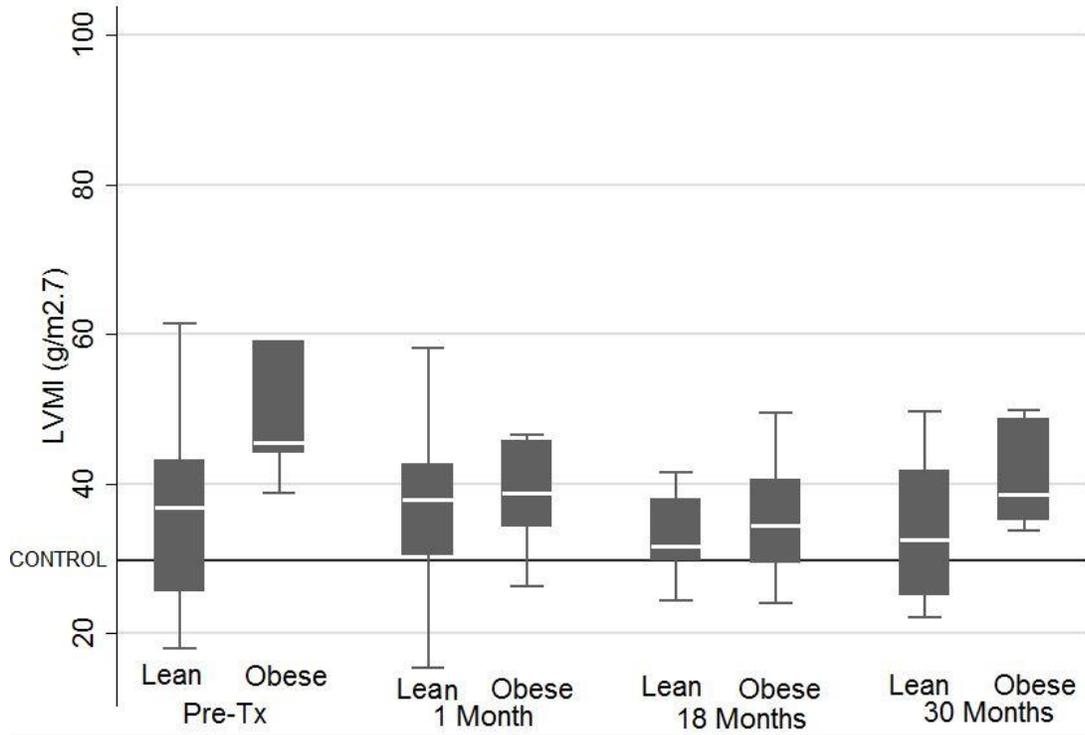
BMI	Control Group	Transplant Group n=42							
LVMI g/m ^{2.7}	n=24	Pre-Tx		1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean
Mean	29.8	56.7	37.6	39.0	39.3	35.0	33.0	41	33.6
SEM	±1.1	±10.	±2.6	±2.7	±3.0	±2.2	±1.6	±1.5	±1.7
95%CI	(27.5-32.2)	4 (27.9 - 85.4)	(32.3 - 42.8)	(32.4 - 45.6)	(33.1 - 45.5)	(30.2 - 39.9)	(29.5 - 36.3)	(35.1 - 46.9)	(30.0 - 37.3)
Median	29.7	45.5	36.9	38.7	37.8	34.3	31.6	38.5	32.5
IQR	(26.1-32.1)	(44.1 -59)	(25.6 -43)	(30.4 - 42.6)	(34.2 - 45.6)	(29.3 - 40.5)	(29.7 - 37.9)	(35- 48.6)	(25- 41.7)
P value Obese vs lean	NA	0.03**		0.51		0.57		0.05	
P value Tx vs control	NA	.0009 **	0.04 **	.005 **	.008 **	0.02 **	0.04 **	0.0006 **	0.20

WHr	Control Group	Transplant Group n=42					
LVMI g/m ^{2.7}	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	29.8	43.9	35.8	35.5	33.3	41.0±2.5	33.6±1.8
SEM	±1.1	±3.8	±3.1	±2.2	±1.8	(35.1- 46.9)	(30.0- 37.3)
95%CI	(27.5-32.2)	(35.7- 52.1)	(29.5- 42.1)	(30.7- 40.2)	(29.6- 37.1)		
Median	29.7	40.3	33.5	32.7	33.2	35.8	36.5
IQR	(26.1-32.1)	(37.5- 46.5)	(25.8- 41.6)	(28.4- 41.6)	(29.7- 38)	(31.3- 41.0)	(25- 42.6)
P value Obese vs lean	NA	0.04**		0.77		0.57	
P value Tx vs control	NA	.0002 **	0.07	0.02**	0.04**	0.009**	0.15

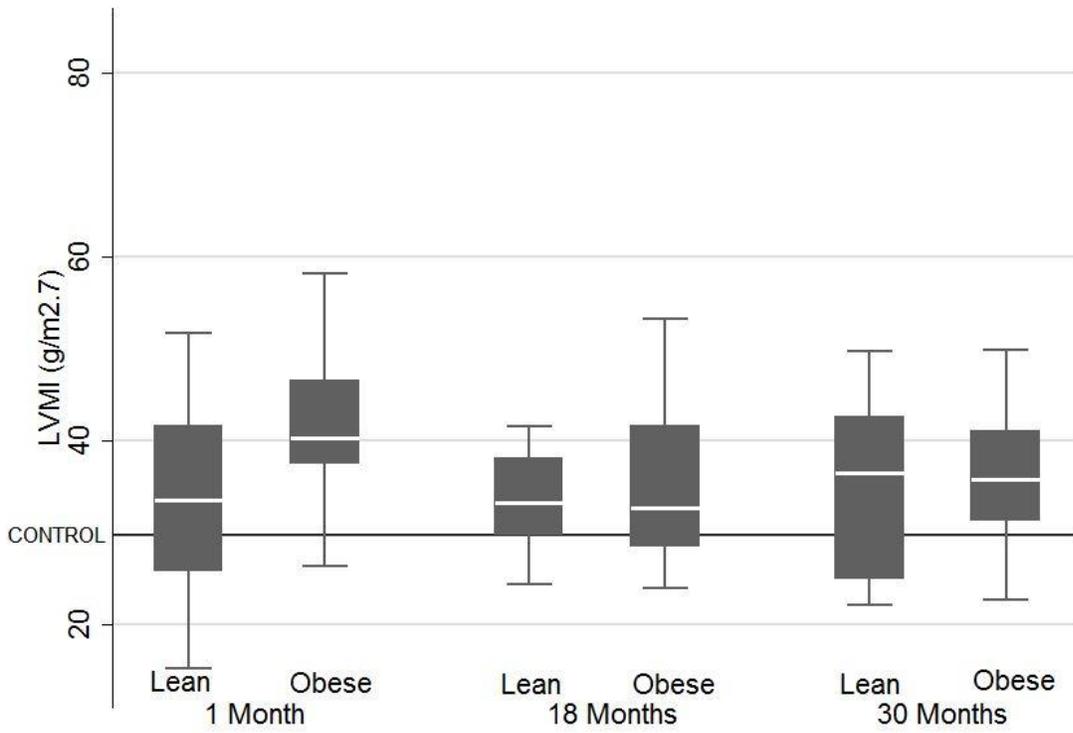
WC	Control Group	Transplant Group					
LVMI g/m ^{2.7}	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	29.8	39.4	39.2	40.2	33.9	41.0	34.7
SEM	±1.1	±0.8	±2.7	±4.2	±1.4	±5.1	±1.6
95%CI	(27.5-32.2)	(36.0-42.8)	(33.7-44.7)	(-13.1-93.5)	(31.0-36.8)	(24.6-57.4)	(31.4-38.0)
Median	29.7	37.8	38.7	40.2	32.1	42.0	35.8
IQR	(26.1-32.1)	(38.6-41)	(30.4-43.5)	(36-44.4)	(28.0-39.5)	(32.2-49.8)	(27.4-41.7)
P value Obese vs lean	NA	0.71		0.18		0.23	
P value Tx vs control	NA	0.02**	.004**	0.04**	0.02**	0.01**	0.05

Figure S5. LVMI of BMI-obese and lean transplant recipients at each time point vs controls

LVMI of Lean and BMI-Obese Tx



LVMI of Lean and WHr-Obese Tx



LVMl of Lean and WC-Obese Tx

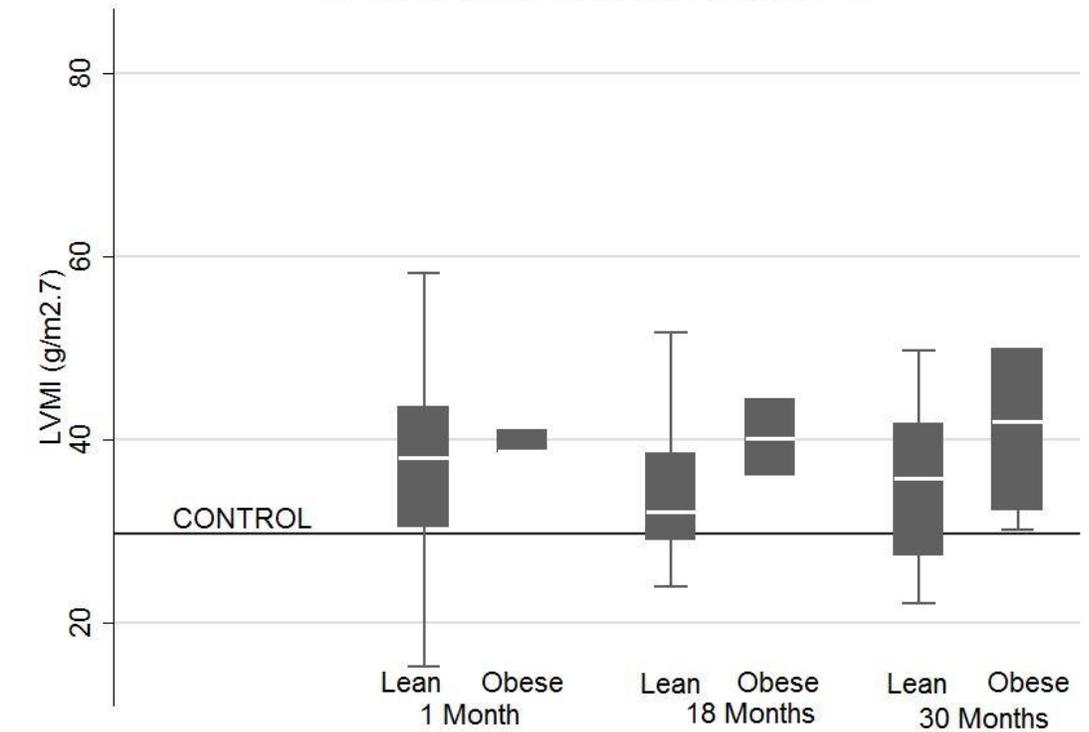


Table S10. Longitudinal strain of obese and lean transplant recipients at each time point vs controls

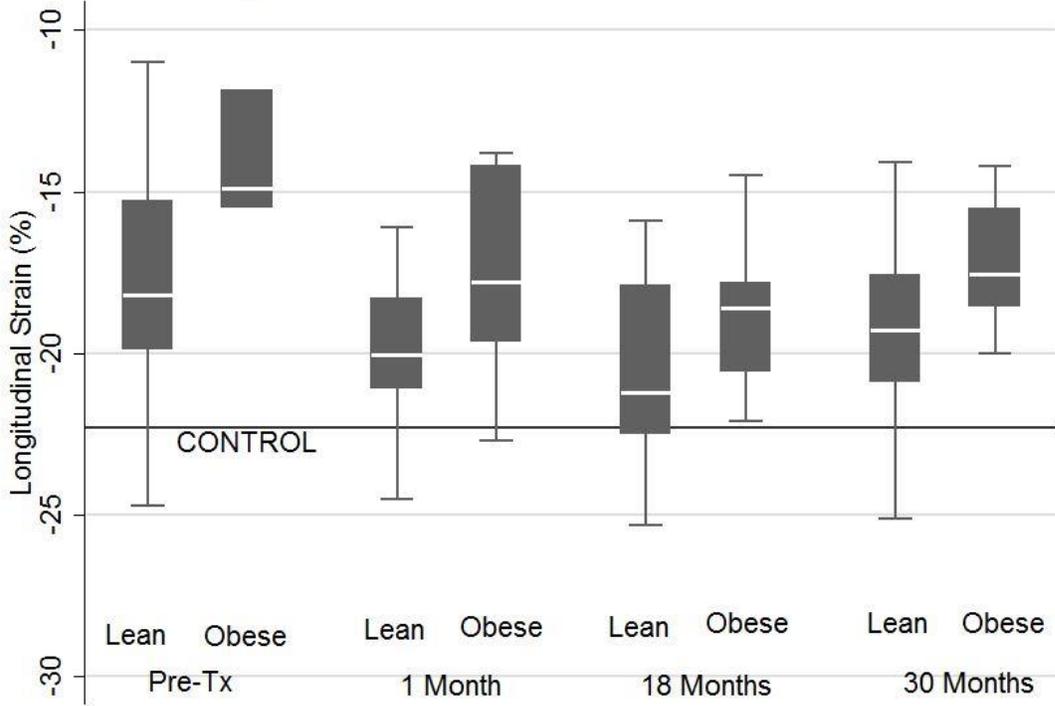
BMI	Control Group	Transplant Group							
		Pre-Tx		1 month		18 months		30 months	
Long Strain (%)	n=24	Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean
Mean	-23.1	-14.1	-17.8	-17.5	-20.3	-18.8	-20.6	-17.2	-19.1
SEM	±0.63	±1.1	±0.7	±1.1	±0.6	±0.5	±0.6	±0.7	±0.5
95% CI	(-24.3- -21.8)	-18.9 - -9.3)	-19.2- -16.4	-20.2- -14.8	-21.6- -19.0	-20.1- -17.6	-21.8- -19.4	-18.8- -15.5	-20.3- -18.2
Median	-22.3	-14.9	-18.2	-17.8	-20.1	-18.7	-21.2	-17.5	-19.3
IQR	(-25.3- -21.3)	-15.5- -11.9	-19.9- -15.3	-19.6- -14.2	-21.1- -18.3	-20.3- -18.3	-22.5- -17.9	-18.5- -15.5	-20.9- -17.6
P value Obese vs lean	NA	0.09		0.04*		0.05*		0.04*	
P value Tx vs control	NA	.00001*	.00001*	.00001*	.003*	.00001*	.006*	.00001*	.00001*

WHr	Control Group	Transplant Group					
Long Strain (%)	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	-23.1	-18.3	-20.7	-19.4	-20.4	-18.4	-18.9
SEM	±0.63	±0.96	±0.66	±0.62	±0.62	±0.66	±0.60
95% CI	(-24.3- -21.8)	-20.4- -16.3	-22.1- -19.3	-20.7- -18.0	-21.7- -19.1	-19.9- -17.0	-20.2- -17.7
Median	-22.3	-18.1	-20.5	-19.2	-21.2	-18.1	-19.0
IQR	(-25.3- -21.3)	-20.1- -14.5	-22.1- -19.1	-20.9- -18.1	-22.2- -17.6	-20.0- -16.9	-20.4- -17.3
P value Obese vs lean	NA	0.04*		0.26		0.58	
P value Tx vs control	NA	.0001*	0.01*	0.004*	.0001*	.00001*	.00001*

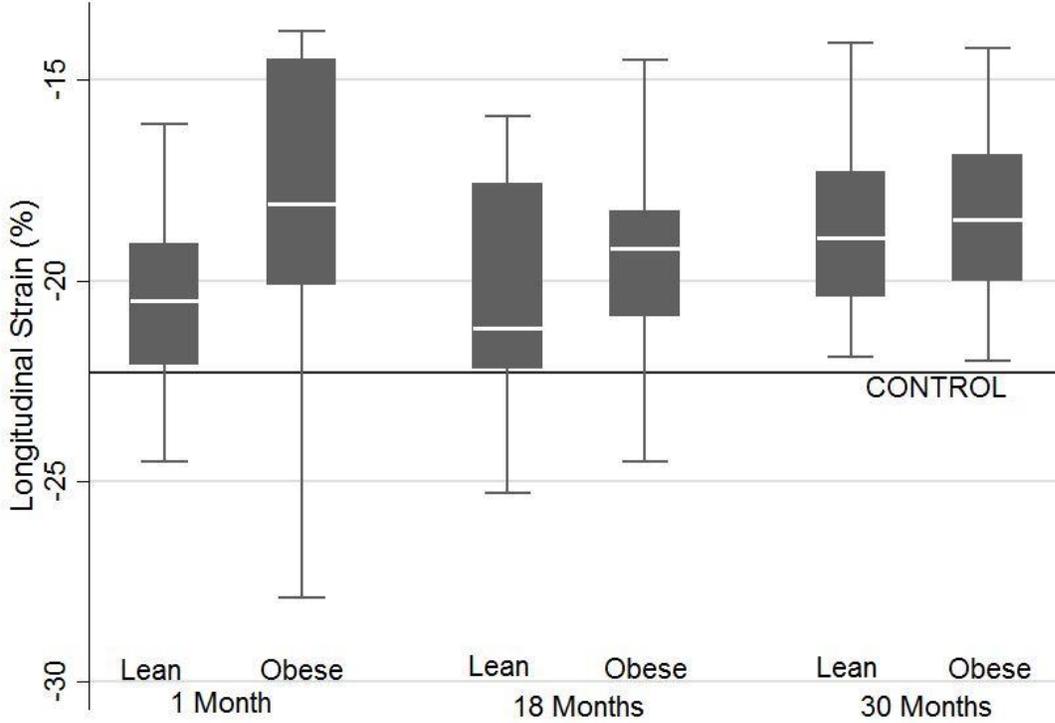
WC	Control Group	Transplant Group					
Long Strain (%)	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	-23.1	-18.2	-19.9	-17.7	-20.1	-16.6	-19.0
SEM	±0.63	±1.8	±0.6	±3.1	±0.4	±1.2	±0.4
95% CI	(-24.3- -21.8)	-23.9- -12.5	-21.1- -18.6	-57.7- 22.4	-21.0- -19.1	-20.6- -12.7	-20.0- -18.1
Median	-22.3	-18.0	-19.9	-17.7	-20.2	-16.2	-18.9
IQR	(-25.3- -21.3)	-20.4- -16.0	-21.1- -18.2	-20.8- -14.5	-21.7- -18.3	-18.5- -14.9	-20.4- -17.6
P value Obese vs lean	NA	0.37		0.21		0.07	
P value Tx vs control	NA	.005*	.0007*	0.02*	.0002*	.0004*	.00001*

Figure S6. Longitudinal strain of obese and lean transplant recipients at each time point vs controls

Longitudinal Strain of Lean and BMI-Obese Tx



Longitudinal Strain of Lean and WHr-Obese Tx



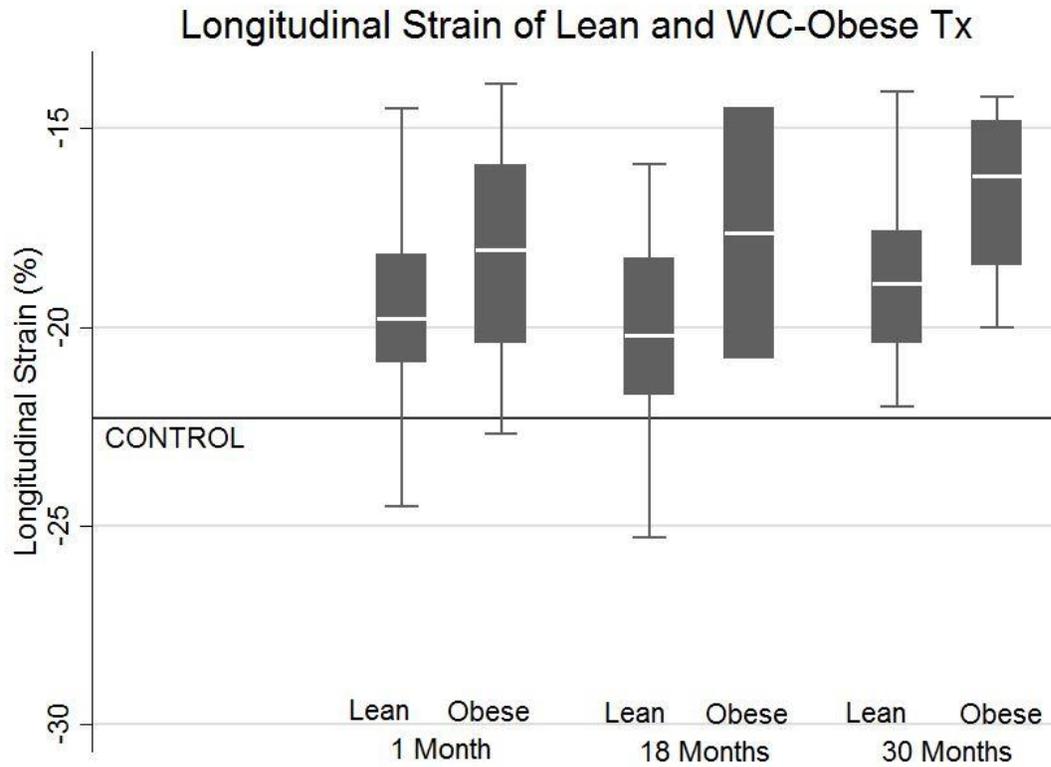


Table S11. Circumferential strain of obese and lean transplant recipients at each time point vs controls

BMI	Control Group	Transplant Group							
		Pre-Tx		1 month		18 months		30 months	
Circumferential Strain	n=24	Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean
Mean	-23.9	-20.0	-22.8	-24.5	-25.1	-25.1	-25.7	-24.8	-26.6
SEM	± 0.65	± 2.2	± 0.8	± 1.5	± 0.8	± 0.6	± 0.8	± 1.2	± 0.7
95% CI	(-25.5- -22.5)	-26.0- -13.9	-24.4- -21.1	-28.1- -20.9	-26.8- -23.5	-27.5- -24.0	-26.6- -23.7	-27.7- -22.0	-27.5- -24.5
Median	-24.6	-20.4	-22.2	-25.0	-25.0	-25.8	-25.6	-25.0	-25.4
IQR	(-25.5 – -21.4)	-21.7- -19.0	-26.0- -19.5	-27.0- -21.4	-27.6- -22.2	-26.5- -24.9	-29.1- -22.9	-27.9- -21.4	-28.0- -23.7
P value Obese vs lean	NA	0.19		0.69		0.66		0.41	

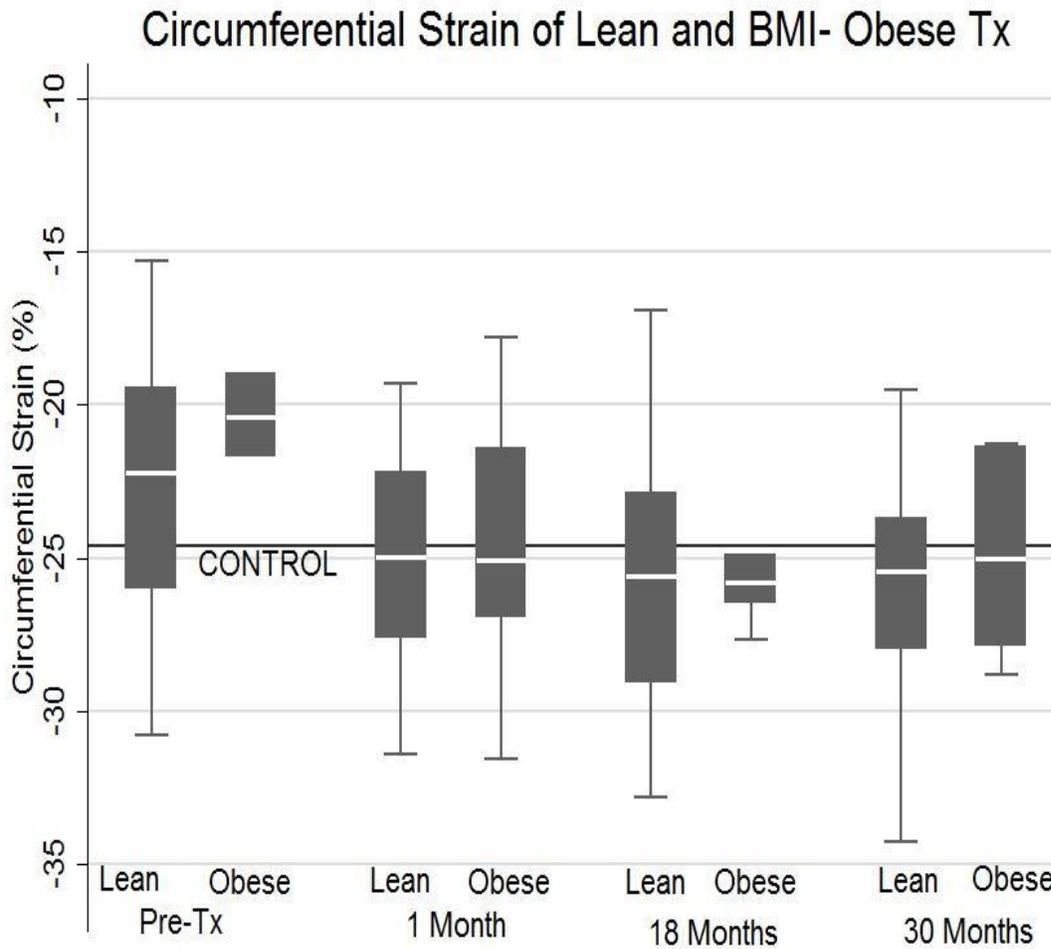
P value Tx vs control	NA	0.03*	0.29	0.67	0.23	0.25	0.11	0.50	0.04*
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WHr	Control Group	Transplant Group					
Circumferentia l Strain	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	-23.9	-24.3	-25.6	-25.7	-25.4	-25.5	-25.9
SEM	±0.65	±0.90	±1.0	±0.65	±0.90	±0.76	±0.91
95% CI	(-25.5- -22.5)	-26.2- -22.4	-27.8- -23.4	-27.1- -24.3	-27.3- -23.6	-27.2- -23.8	-27.8- -24.0
Median	-24.6	-24.3	-25.1	-26.0	-25.4	-25.9	-25.4
IQR	(-25.5 - -21.4)	-26.5- -22	-29.1- -21.3	-27.2- -25.0	-29.1- -22.7	-28.0- -23.5	-28.7- -23.4
P value Obese vs lean	NA	0.37		0.84		0.74	
P value Tx vs control	NA	0.71	0.15	0.07	0.17	0.13	0.07

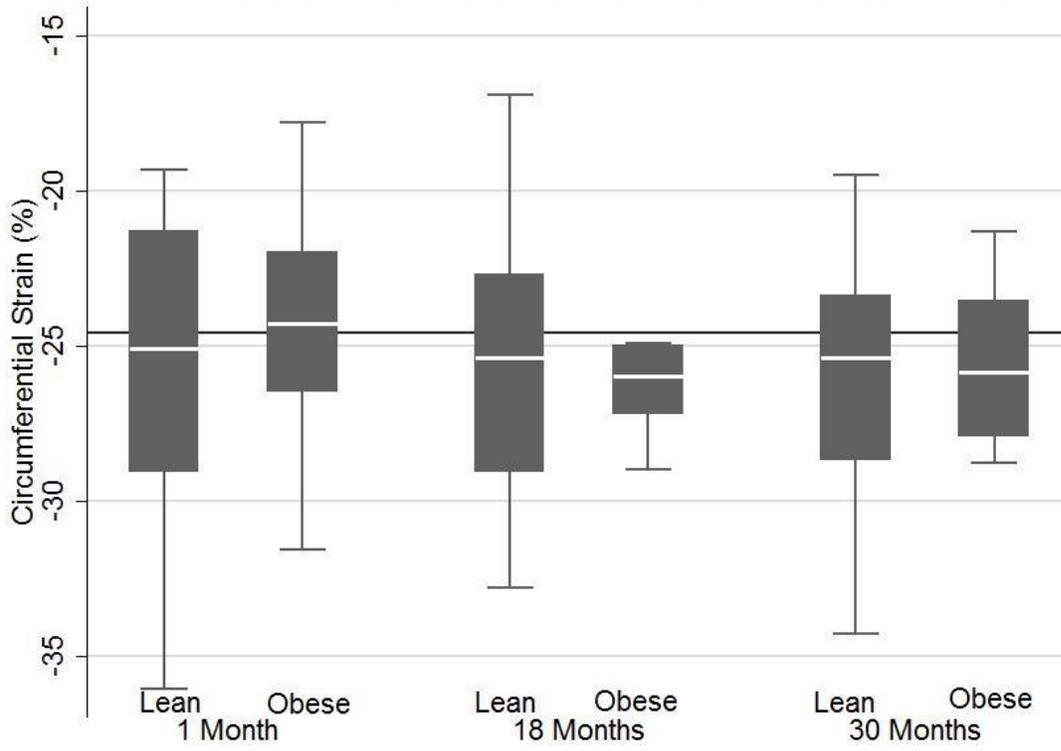
WC	Control Group	Transplant Group					
Circumferentia l Strain	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	-23.9	-24.5	-25.1	-27.7	-25.5	-24.7	-25.9
SEM	±0.65	±2.5	±0.7		±0.6	±1.6	±0.7
95% CI	(-25.5- -22.5)	-32.5- -16.4	-26.6- -23.6		-26.7- -24.3	-29.7- -19.6	-27.2- -24.5
Median	-24.6	-23.1	-25.0	-27.7	-25.6	-24.7	-25.4
IQR	(-25.5 - -21.4)	-28.0- -21.0	-27.4- -22.2		-28- -22.9	-27.3- -22.0	-28.0- -23.7
P value Obese vs lean	NA	0.77		NA		0.50	

P value	NA	0.75	0.25	NA	0.10	0.64	0.04*
Tx vs control							

Figure S7. Circumferential strain of BMI- obese and lean transplant recipients at each time point vs controls



Circumferential Strain of Lean and WHr-Obese Tx



Circumferential Strain of Lean and WC-Obese Tx

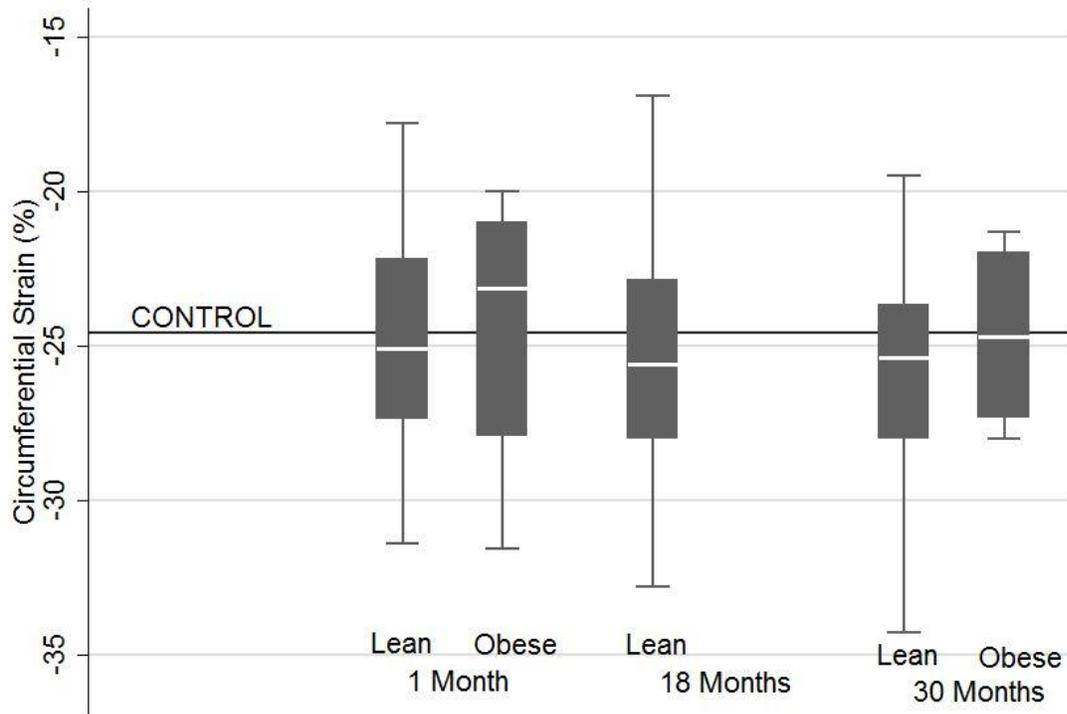


Table S12. CIMT of obese and lean transplant recipients at each time point vs controls

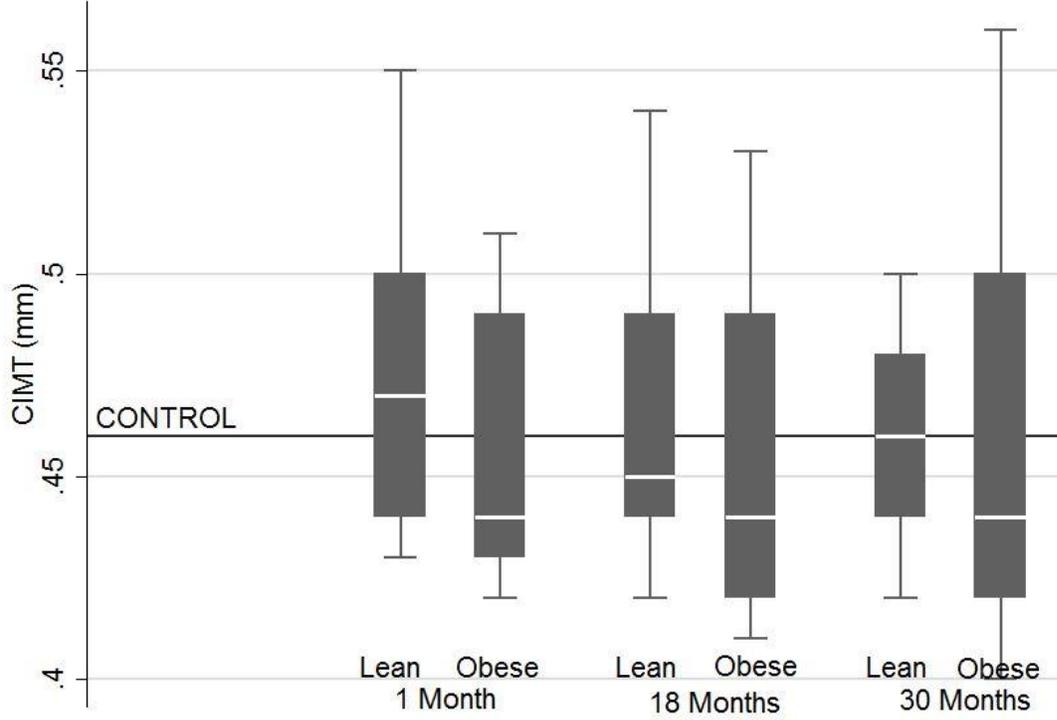
BMI	Control Group	Transplant Group					
CIMT	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	0.46	0.45	0.47	0.46	0.47	0.46	0.46
SEM	± 0.007	± 0.01	± 0.007	± 0.01	± 0.008	± 0.02	± 0.006
95%CI	(.45-.48)	0.43-0.48	0.46-0.49	0.43-0.48	0.45-0.48	0.42-0.50	0.45-0.48
Median	0.46	0.44	0.47	0.44	0.45	0.44	0.46
IQR	(.44-.48)	0.43-0.49	0.44-0.50	0.42-0.49	0.44-0.49	0.42-0.50	0.44-0.48
P value Obese vs lean	NA	0.09		0.27		0.73	
P value Tx vs control	NA	0.40	0.14	0.51	0.78	0.75	0.91

WHr	Control Group	Transplant Group					
CIMT	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	0.46	0.47	0.47	0.46	0.47	0.47	0.46
SEM	±0.007	±0.01	±0.008	±0.01	±0.008	±0.01	±0.005
95% CI	(.45-.48)	0.44-0.49	0.45-0.49	0.44-0.48	0.45-0.48	0.44-0.50	0.45-0.47
Median	0.46	0.46	0.47	0.44	0.45	0.45	0.46
IQR	(.44-.48)	0.43-0.50	0.44-0.50	0.42-0.49	0.44-0.49	0.43-0.50	0.44-0.48
P value Obese vs lean	NA	0.69		0.35		0.90	
P value Tx vs control	NA	0.80	0.30	0.61	0.82	0.90	0.94

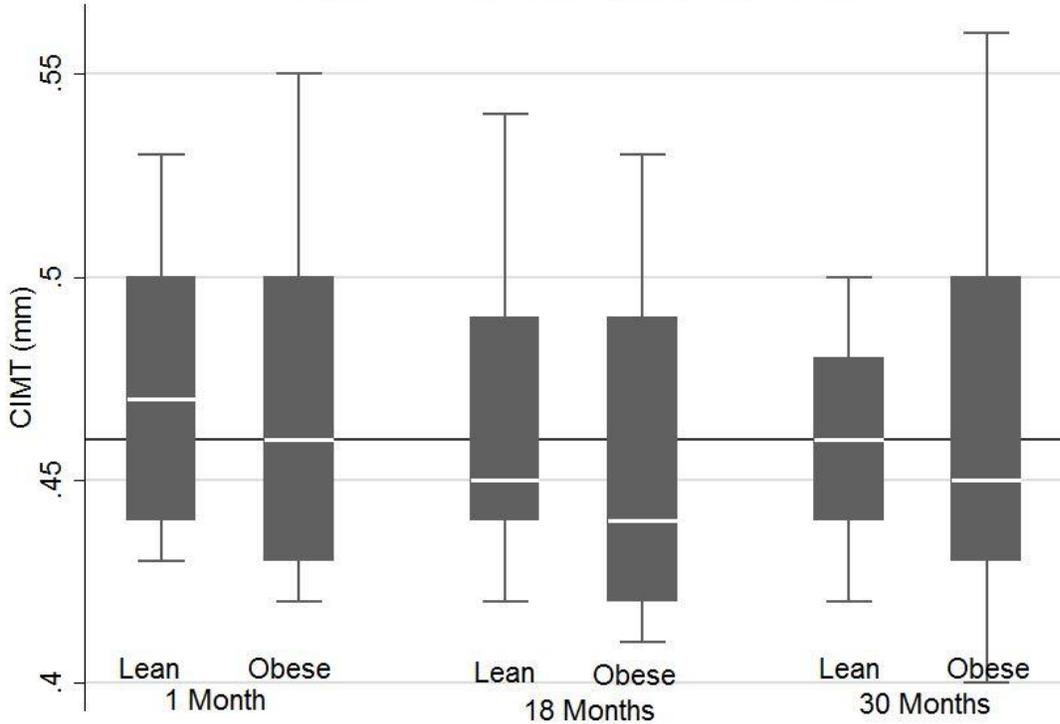
WC	Control Group	Transplant Group					
CIMT	n=24	1 month		18 months		30 months	
		Obese	Lean	Obese	Lean	Obese	Lean
Mean	0.46	0.48	0.47	0.48	0.46	0.44	0.47
SEM	±0.007	±0.02	±0.007	±0.04	±0.006	±0.02	±0.007
95% CI	(.45-.48)	0.45-0.48	0.42-0.53	-0.1-1.05	0.45-0.48	0.39-0.49	0.45-0.48
Median	0.46	0.49	0.46	0.49	0.45	0.43	0.46
IQR	(.44-.48)	0.46-0.50	0.43-0.50	0.44-0.53	0.43-0.49	0.42-0.44	0.44-0.49
P value Obese vs lean	NA	0.57		0.55		0.10	
P value Tx vs control	NA	0.27	0.62	0.66	0.83	0.13	0.65

Figure 8. CIMT of obese and lean transplant recipients at each time point vs controls

CIMT of Lean and BMI-Obese Tx



CIMT of Lean and WHr-Obese Tx



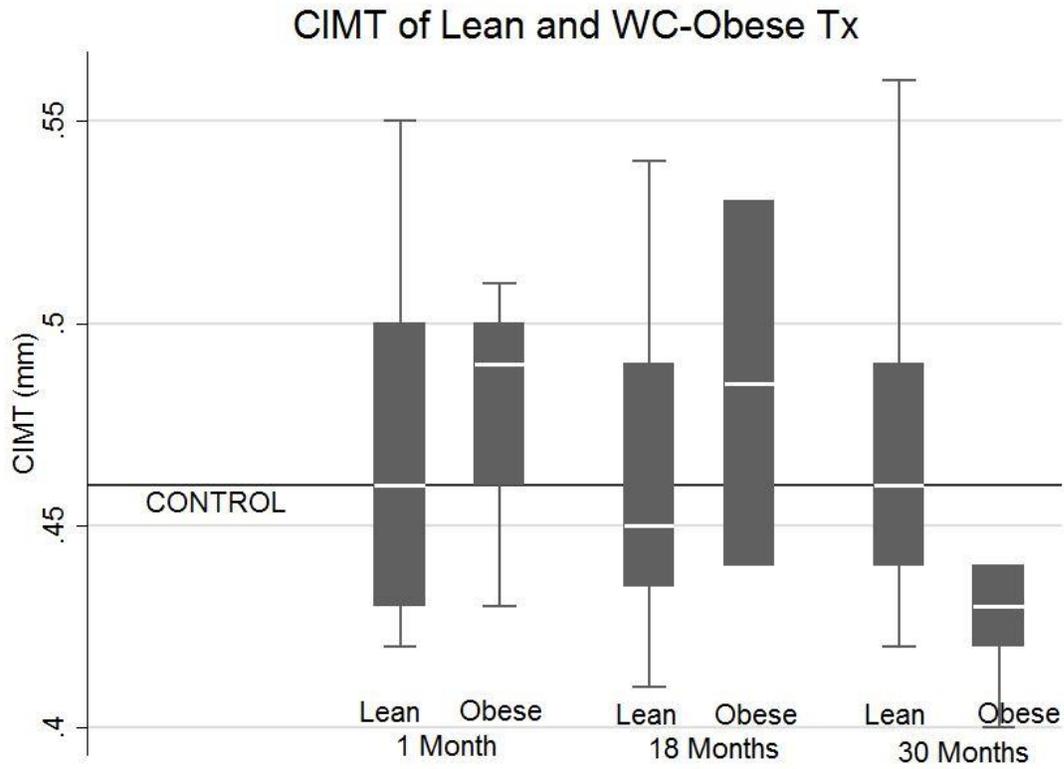


Table S13. CIMT of A-A and non-AA transplant recipients at each time point vs controls

CIMT	Control Group n=14 A-A n=10 nonAA		Transplant Group n=21 A-A n=21 non A-A					
	n=24		1 month		18 months		30 months	
	A-A	Non A-A	A-A	Non A-A	A-A	Non A-A	A-A	Non A-A
Mean	0.47	0.44	0.49	0.45	0.49	0.44	0.48	0.44
SEM	±.009	±.008	±.007	±0.007	±.007	±0.004	±0.009	±0.006
95% CI	0.45-0.49	0.42-0.46	0.47-0.51	0.43-0.47	0.47-0.50	0.43-0.44	0.46-0.50	0.43-0.45
Median	0.46	0.45	0.49	0.43	0.49	0.44	0.48	0.44
IQR	0.45-0.50	0.42-0.46	0.47-0.51	0.43-0.46	0.46-0.51	0.42-0.45	0.46-0.50	0.42-0.45
Pvalue A-A vs Non	0.02*		0.001**		0.0001**		0.0007**	

A-A								
P value Tx vs control	NA	NA	0.03*	0.62	0.03*	0.51	0.37	0.79

*significant difference by Student's t-test

** significant difference by Wilcoxon rank-sum, $p < 0.05$

Figure S9. CIMT of A-A and non-AA transplant recipients at each time point vs controls

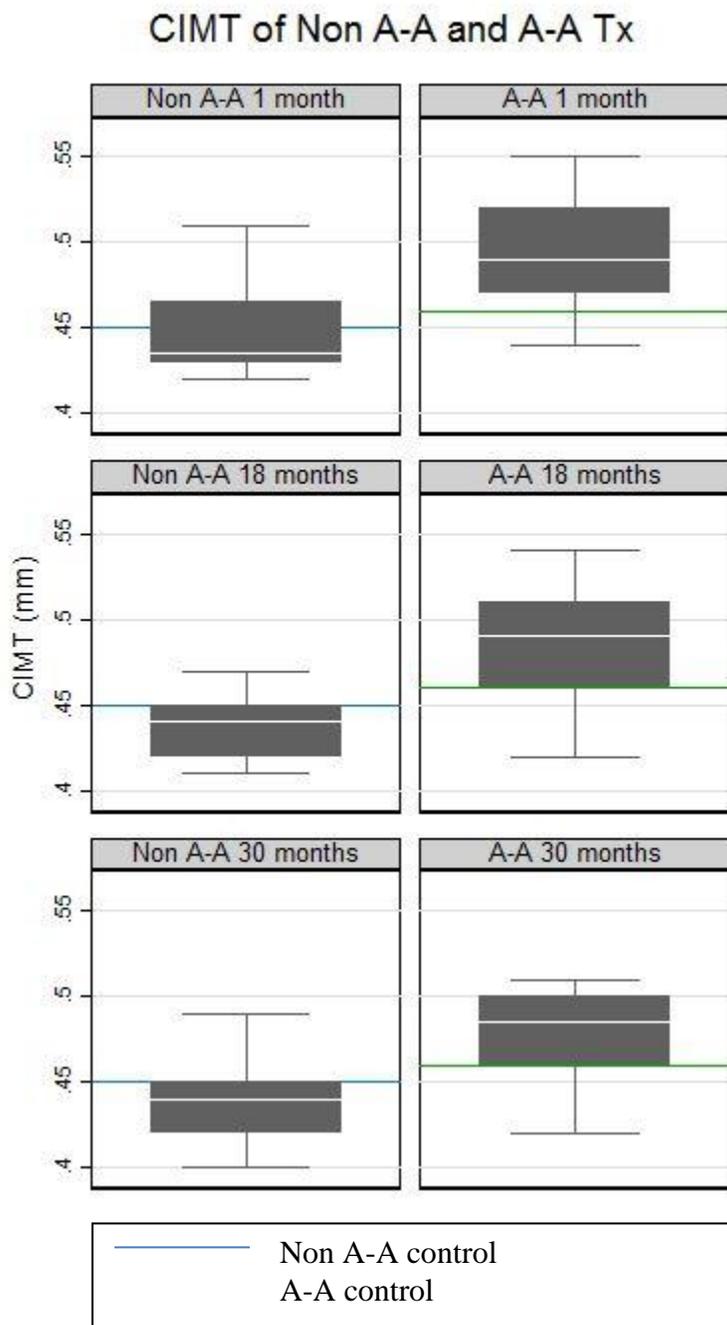


Figure S10. CIMT of lean and obese Tx stratified by race, obesity defined by WHr

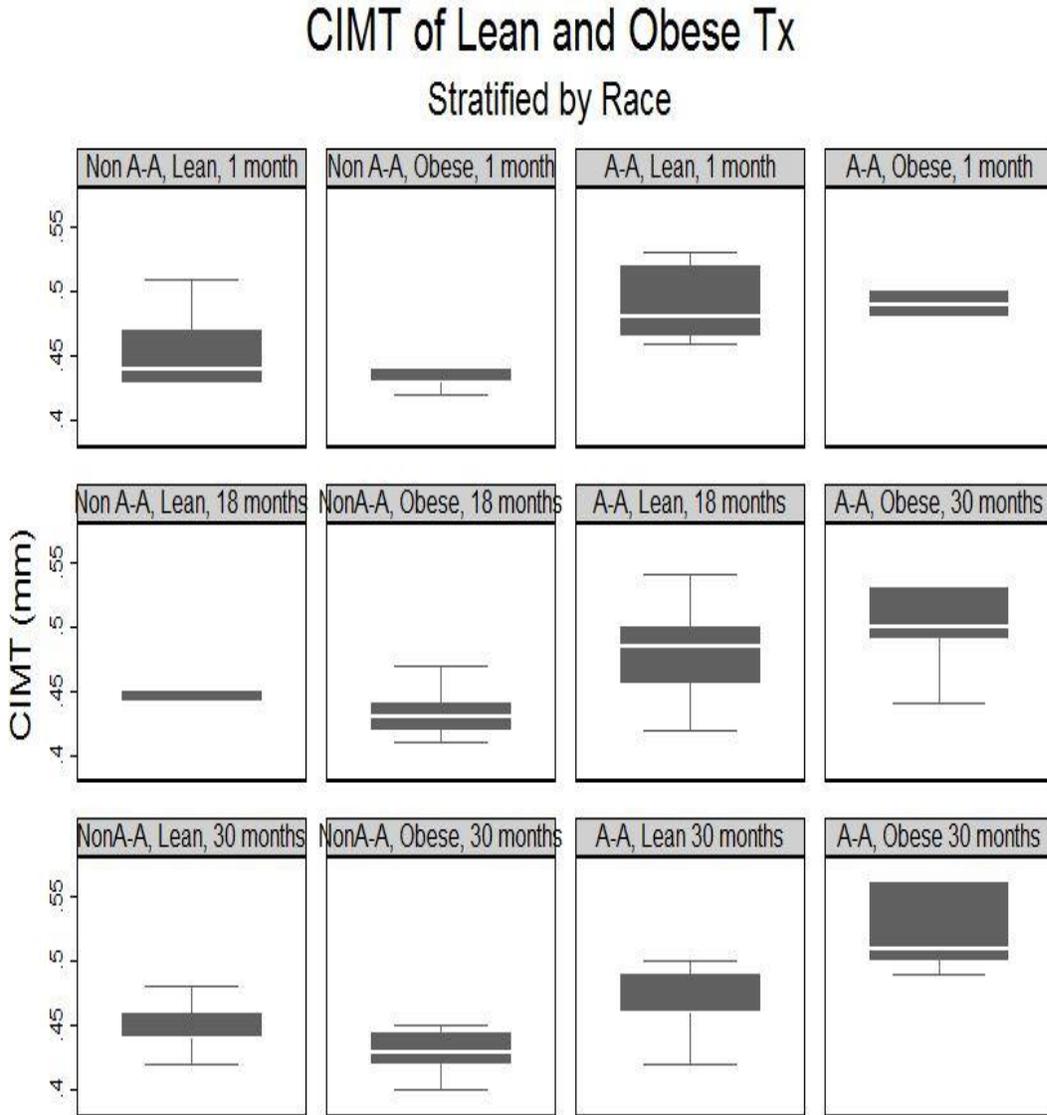


Figure S11. Pre-transplant Hyperparathyroidism and High CIMT at 1 month post-tx

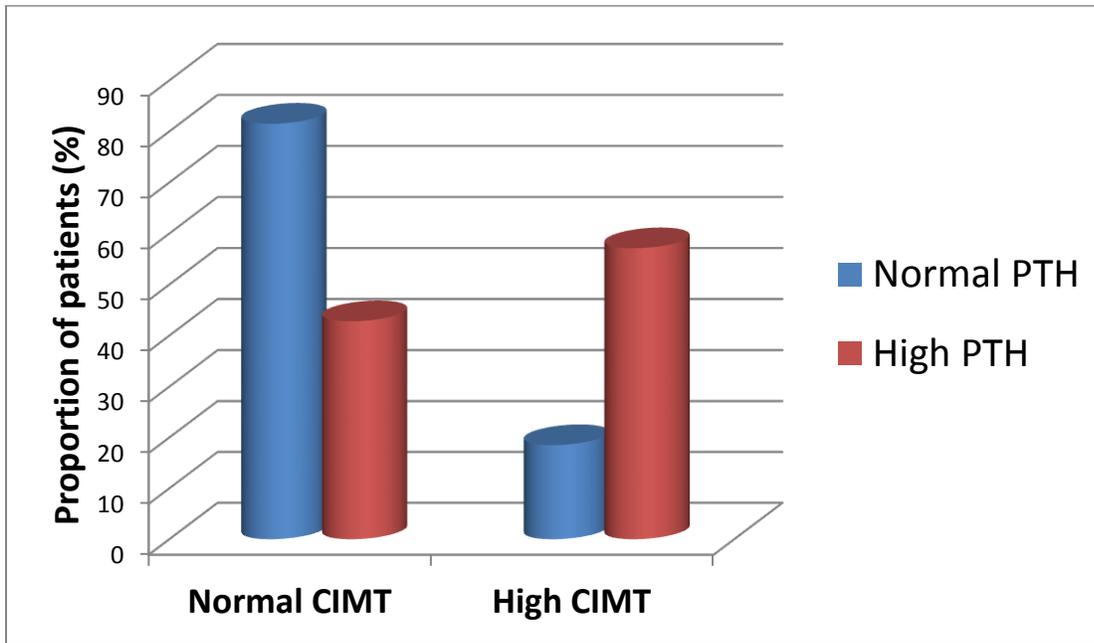


Table S14. Chi-square table of High PTH and High CIMT

	Normal PTH	High PTH	Total
Normal CIMT	22	3	25
High CIMT	5	4	9
Total	27	7	34

Table S15. Biochemical indicators of transplant recipients at each time point

	Transplant Group n=42			
	Pre-Tx	1 month	18 months	30 months

LDL-C cholesterol (mg/dL) Mean+SEM 95%CI	101.7±11.5 (78.8- 124.5)	84.3±4.5 (75.4-93.2)	82±3.8 (74.5-89.6)	84.2±4.3 (75.7-92.7)
Median IQR	92.5 57.5-141.5	80 63-104	78 67-97	83 70-98
HDL-C cholesterol (mg/dL) Mean+SEM 95%CI	44.7±2.9 (39-50.5)	44.9±2.5 (39.8-50)	49.1±3.1 (42.9-55.3)	47.5±2.2 (43.2-51.9)
Median IQR	42.5 33.5-55	43 32-51	46 34-58	47 39-57
Triglycerides (mg/dL) Mean + SEM	180±15.7 ^{##} (148.8- 211.1)	117.5±10.6 ^{##} (96.5-138.6)	113.6±13.5 ^{##} (86.7-140.5)	113±13.7 ^{##} (85.8- 140.3)
Median IQR	155.5 133-216	89 78-143	92 64-124	83 62-137
Fasting glucose (mg/dL) Mean+SEM 95%CI	NA	91.5±2.5 86.5-96.5	92.6±1.9 88.8-96.4	98.3±5.9 86.4-110.2
Median IQR	NA	88.5 82.5-99	91 86-96	90 87-98
HbA1c% Mean+SEM 95%CI	NA	5.1±0.05 [#] (5.0-5.3)	5.3±0.06 [#] (5.2-5.4) *(n=35)	5.3±0.06 [#] (5.2-5.4)
Median IQR	NA	5.1 5-5.4	5.3 5-5.6	5.3 5.1-5.5
Intact PTH (at Tx)	452.8±88.9 (272.5- 633.1)	NA	NA	NA
Calcium x Phosphorus product (pre-Tx)	58.2±3.0 (52-64.4)	NA	NA	NA

*unless otherwise noted

	P values Transplant Time Points				
	1 vs 18 months	1 vs 30 months	Pre-tx vs 1 months	Pre-tx vs 18 months	Pre-tx vs 30 months
LDL-C	0.79	0.84	0.37	0.28	0.39
HDL-C	0.29	0.22	0.93	0.45	0.35
Triglyceri des	0.27	0.19	0.0004 ^{##}	0.0001 ^{##}	0.0004 ^{##}

Glucose	0.46	0.79	NA	NA	NA
HbA1c%	0.03 [#]	0.04 [#]	NA	NA	NA
	#significant difference between transplant time points, p<0.05 by Student's t-test ##significant difference between transplant time points, p<0.05 by Wilcoxon rank-sum, non-parametric				

Figure S12. Change in mean triglyceride level of transplant recipients over time

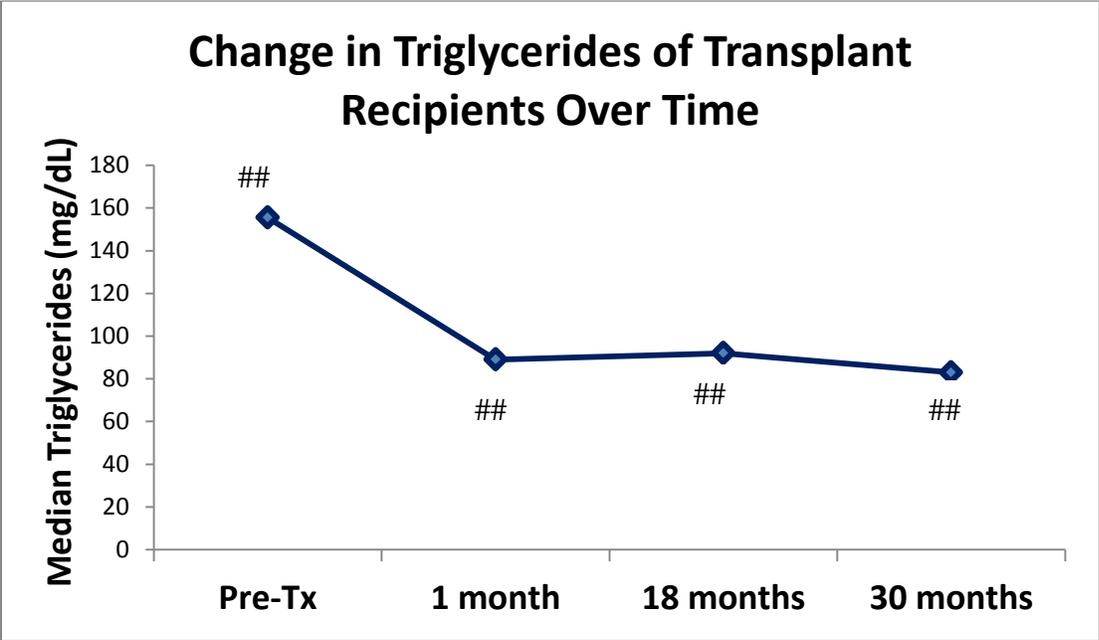


Figure S13. Change in mean HbA1c% over time post-transplant

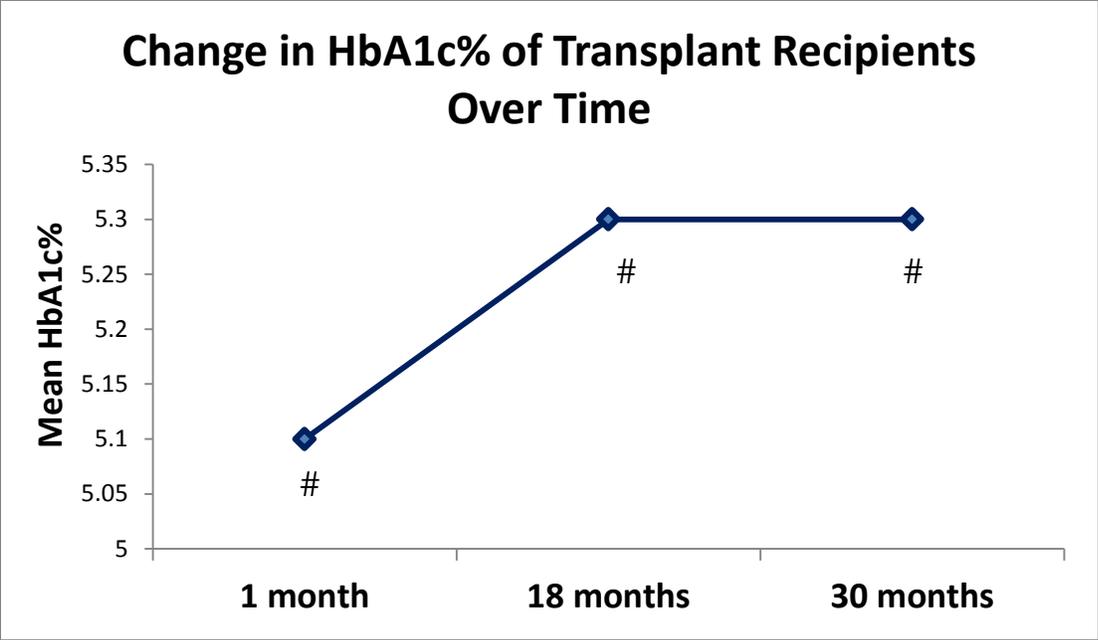
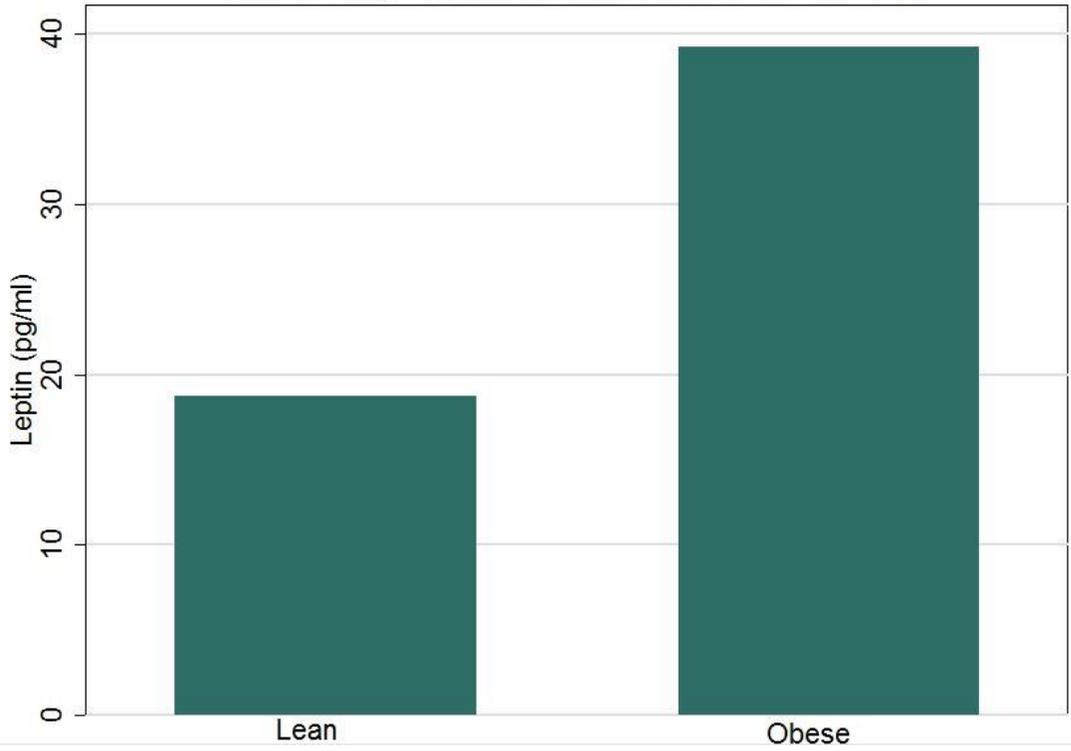
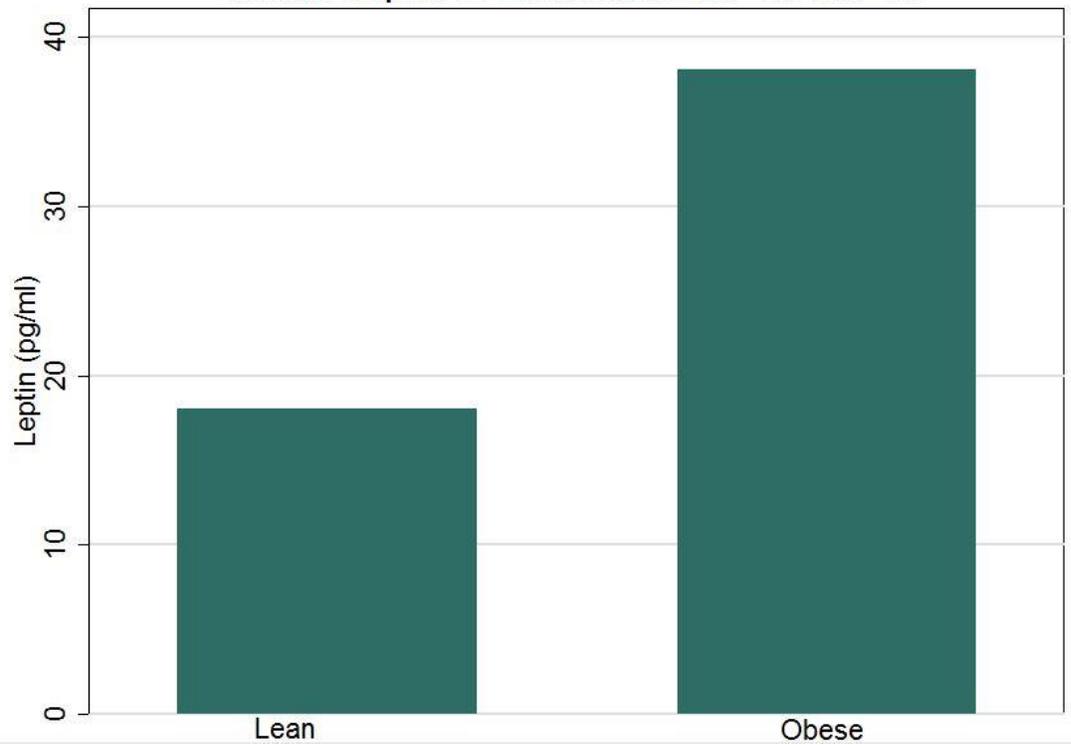


Figure S14. Mean leptin of lean and obese transplant recipients

Mean Leptin of Lean and WC-Obese Tx



Mean Leptin of Lean and BMI-Obese Tx



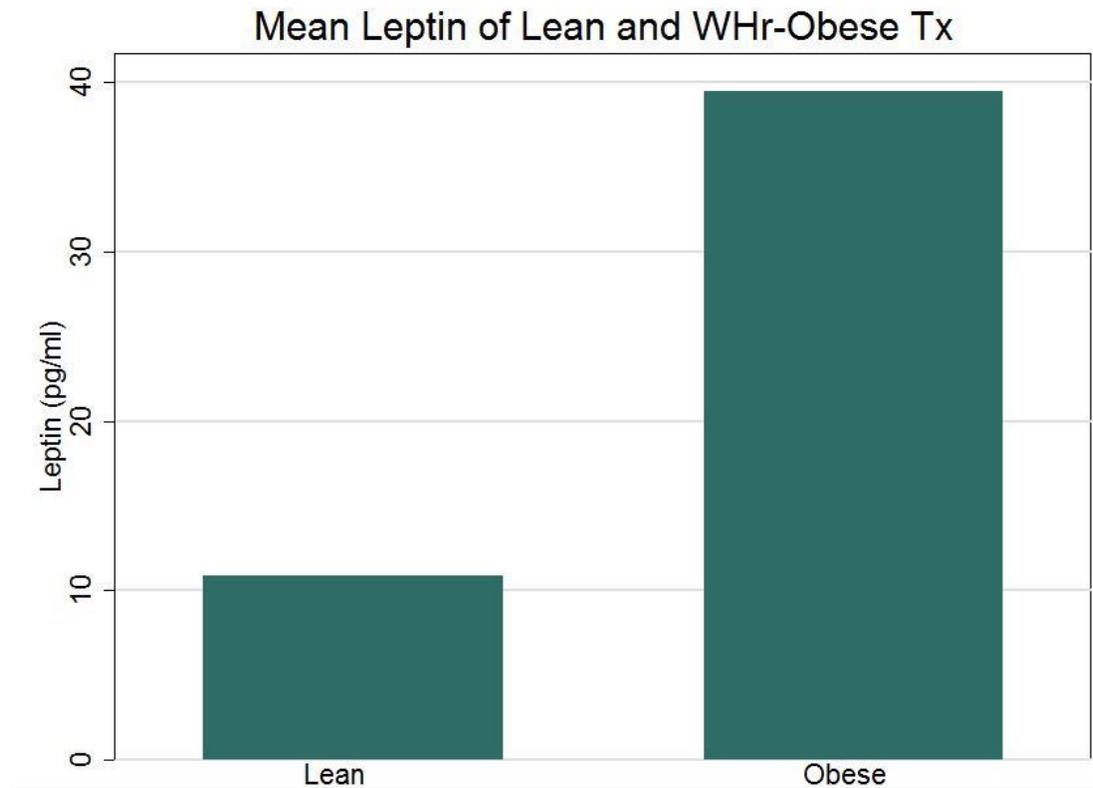


Table S16. Leptin levels of lean and obese transplant recipients (n=27)

	Leptin (pg/mL) Mean \pm SEM 95% CI	p value
BMI Lean n= 21	18.1 \pm 4.3 (9.2-27.0)	0.03*
BMI Obese n=6	38.1 \pm 6.0 (22.6-53.6)	
WHR Lean n=16	10.8 \pm 3.9 (2.6-19.1)	0.00001*
WHR Obese n=11	39.5 \pm 3.9 (30.8-48.1)	
WC Lean n=22	18.7 \pm 4.1 (10.1-27.3)	0.04*
WC Obese n=5	39.3 \pm 7.2 (19.1-59.4)	

*significant difference between mean leptin of lean vs. obese transplant

Multivariate Analysis

Table S17. Output of multivariate GEE stepwise regression

Model Name LVH n=40 patients 100 obs	Output of final/best model after stepwise GEE regression			
BMI-obese	Odds Ratio	<u>SEM</u>	p	[95% CI]
	BMI-Obese	3.7 \pm 1.9	0.01	1.4-9.9
	High SBPz	5.2 \pm 3.2	0.007	1.6-17.2
	High DBPz	4.7 \pm 4.4	0.10	0.74-30.0
	Age	4.4 \pm 3.8	0.09	0.81-23.9
	<u>Interactions: NS</u>			
WHR-obese	OR	<u>SEM</u>	p	[95% CI]
	WHR-obese	2.8 \pm 1.3	0.03	1.08-7.1
	High SBPz	4.1 \pm 2.5	0.02	1.2-13.8
	Age	3.1 \pm 1.6	0.03	1.1-8.5
	<u>Interactions: NS</u>			
WC-obese	OR	<u>SEM</u>	p	[95% CI]
	WC-obese	3.5 \pm 2.7	0.09	0.80-15.5
	High SBPz	3.9 \pm 2.4	0.03	1.2-13.2
	High DBPz	5.0 \pm 4.7	0.09	0.79-31.6
	Age	5.2 \pm 3.3	0.01	1.5-17.7
	<u>Interactions: NS</u>			
MS	OR	<u>SEM</u>	p	[95% CI]
	MS	3.5 \pm 1.7	0.01	1.3-9.3
	High SBPz	3.7 \pm 2.5	0.05	0.98-13.8
	Age	3.3 \pm 2.2	0.08	0.88-12.8
	<u>Interactions: NS</u>			
Model	Output of final/best model after stepwise GEE			

Name L. Strain n=40 patients 100 obs	regression			
BMI-obese	Coef.	±SEM	p	[95% CI]
BMI-Obese	1.5	±.39	0.0001	.78- 2.3
Hypertension	1.4	±.49	0.005	.43- 2.3
Glucose Intol	.33	±.43	0.45	-.52- 1.2
LDL-C	.01	±.01	0.29	-.01-.03
High SBPz	-.72	±.72	0.32	-2.1-.68
Steroid dose	.63	±1.0	0.55	-1.4- 2.7
eGFR	-.04	±.01	0.0001	-.06-.02
cons	-17.7	±1.3	0.0001	-20.4-15.1
	<u>Interactions: NS</u>			
WHR-obese	Coef.	±SEM	p	[95% CI]
WHR-obese	1.06	±.42	0.01	.23-1.8
Hypertension	1.5	±.46	0.001	.62-2.4
Glucose Intol	0.37	±.45	0.41	-.51-1.2
LDL-C	0.01	±.01	0.33	-.01-.03
Steroid dose	0.59	±1.0	0.55	-1.4-2.6
High SBPz	-.91	±.75	0.22	-2.4-.56
Race	-.07	±.46	0.87	-.97-.83
Sex	0.18	±.45	0.68	-.7-1.1
eGFR	-.04	±.01	0.0001	-.06 -.02
cons	-17.6	±1.4	0.0001	-20.4 -14.8
	<u>Interactions:</u>			
	WHR-obese#LDL-C	.07	±.02	0.0001 .04-.10
WC-obese	Coef.	±SEM	p	[95% CI]
WC-obese	1.6	±.51	0.001	.63-2.6
Hypertension	1.4	±.46	0.003	.48-2.3
Glucose Intol	.56	±.42	0.18	-.25-1.4
Low HDL-C	.14	±.45	0.76	-.75- 1.0
LDL-C	.01	±.01	0.26	-.01-.03
Steroid dose	.85	±1.02	0.40	-1.1-2.8
High SBPz	-1.1	±.66	0.09	-2.4-.17
eGFR	-.04	±.01	0.0001	-.06-.02
Race	-.12	±.45	0.78	-1.0-.76

	Sex cons	.10±.44 -17.5±1.5	0.81 0.0001	-0.75-0.96 -20.6- -14.5
	<u>Interactions:NS</u>			
MS		Coef. ± SEM	p	[95% CI]
	MS	0.90±.49	0.06	-.05-1.8
	Hypertension	1.5±.47	0.002	.55- 2.4
	Glucose Intol	0.38±.50	0.45	-.60-1.3
	LDL-C	0.01±.01	0.30	-.01-.03
	Steroid dose	0.54±1.04	0.59	-1.5-2.6
	High SBPz	-.95±.77	0.22	-2.6-.55
	Sex	0.12±.48	0.53	-.82-1.0
	Race	-0.15±.47	0.75	-1.1-.77
	eGFR	-0.04±.01	0.0001	-.06-.02
	cons	-17.5±1.4	0.0001	-20.2 -14.7
	<u>Interactions:</u>			
	MS#LDL-C	0.05±0.02	0.01	0.01-0.09

Model Name In CIMT	Output of final/best model after stepwise GEE regression			
BMI-obese		Coef. ± SEM	p	[95% CI]
	BMI-obese	-0.02±0.01	0.16	-0.04-0.01
	Steroid therapy	-0.02±0.02	0.09	-0.06-0.004
	Race	0.09±0.01	0.0001	0.06-0.12
WHr-obese		Coef. ± SEM	p	[95% CI]
	WHr-obese	-0.01±0.01	0.68	-0.03-0.02
	Steroid therapy	-0.02±0.01	0.11	-0.05-0.005
	Race	0.09±0.01	0.0001	0.06-0.12
WC-obese		Coef. ± SEM	p	[95% CI]
	WC-obese	0.01±0.02	0.63	-0.03-0.05
	Steroid therapy	-0.03±0.01	0.09	-0.06-0.004
	Race	0.10±0.01	0.0001	0.06-0.13

MS	Coef. <u>±</u> SEM	p	[95% CI]
MS	0.01+0.01	0.16	-0.01-0.04
Steroid therapy	-0.02+0.01	0.10	-0.05-0.005
Race	0.10+0.01	0.0001	0.06-0.13

Table S18. Race stratification longitudinal analysis of CIMT

Model Name	Output of final/best model after stepwise GEE regression			
A-A CIMT	Coef. <u>±</u> SEM	p	[95% CI]	
BMI z score	BMI z	0.01+0.006	0.06	-0.001-0.02
	Dyslipidemia	0.01+0.01	0.25	-0.01-0.03
	Steroid therapy	0.02+0.02	0.11	-0.01-0.06
	Sex	-0.01+0.02	0.46	-0.04-0.02
	Cons	0.43+0.02	0.0001	0.38- 0.48
WHr	WHr	0.09+0.09	0.31	-0.1-0.28
	SBP z score	-0.01+0.004	0.23	-0.01-0.003
	Age	0.004+0.01	0.75	-0.02-0.02
	eGFR	0.001+0.0002	0.01	0.0001-0.0009
	Cons	0.36+0.05	0.0001	0.26-0.45
WC z score	WC z	-0.004+0.005	0.48	-0.01-0.01
	Chronic HTN	0.005+0.009	0.60	-0.01-0.02
	SBP z score	-0.004+0.003	0.17	-0.01-0.02
	Steroid therapy	0.001+0.02	0.96	-0.03-0.03
	Age	-0.002+0.01	0.87	-0.02-0.02
	eGFR	0.0006+0.0001	0.0001	0.0002-0.0009
	Cons	0.39+0.02	0.0001	0.35-0.44
MS	MS	0.03+0.01	0.002	0.01-0.05
	Steroid therapy	-0.007+0.01	0.80	-0.02-0.02
	Sex	0.003+0.01	0.79	-0.02-0.02
	eGFR	0.000004+0.0002	0.98	0.0003-0.0003
	Cons	0.40+0.03	0.0001	0.35-0.45

Model Name In CIMT non-AA	Output of final/best model after stepwise GEE regression			
BMI z score	Coef.	± SEM	p	[95% CI]
	BMI z	-0.02+0.02	0.18	-0.05-0.01
	Steroid therapy	-0.05+0.03	0.09	-0.11—0.01
WHr	Coef.	± SEM	p	[95% CI]
	WHr	0.10+0.12	0.40	-0.14-0.34
	Steroid therapy	-0.04+0.02	0.06	-0.1—0.001
WC z score	Coef.	± SEM	p	[95% CI]
	WC z	-0.001+0.01	0.97	-0.03-0.03
	Steroid therapy	-0.04+0.03	0.16	-0.09-0.01
MS	Coef.	± SEM	p	[95% CI]
	MS	-0.01+0.02	0.67	-0.05-0.03
	Steroid therapy	-0.05-0.03	0.06	-0.1-0.001

Table S19. Pre-transplant variables with LVH and strain

Model Name Pre-transplant LVH	Output of final/best model after stepwise multivariate logistic regression			
	Coef.	± SEM	p	[95% CI]
Pre-transplant:	No significant associations identified.			
Model Name 1 Month Post-tx LVH	Output of final/best model after stepwise multivariate logistic regression Pseudo R2= 0.45			
	Coef.	± SEM	p	[95% CI]
HD History	-1.3	±1.3	0.33	[-3.9-1.3]
Hypertension	-1.4	±1.1	0.20	[-3.7-0.7]
Race	2.7	±1.4	0.06	[-.09-5.4]
BMI-Obese	3.8	±1.8	0.03	[0.32-7.3]
High iPTH	1.8	±1.6	0.25	[-1.3-5.0]
Duration dialysis	.14	±.09	0.12	[-.03-.32]

Model Name DV:Pretransplant Longitudinal Strain	Output of final/best model after stepwise multivariate linear regression R2= 0.37			
	Coef.+ SEM	p	[95% CI]	
Dialysis History	1.2±2.1	0.57	(-3.2-5.6)	
HD History	4.0±1.9	0.04*	(0.1-7.9)	
Hypertension	0.36±1.7	0.84	(-3.3-4.0)	
Race	-1.7±1.7	0.31	(-5.2-1.8)	
Age	-1.4±1.8	0.47	(-5.3-2.6)	
BMI-Obese	4.5±2.4	0.08	(-0.6-9.7)	
High iPTH	-0.89±1.8	0.63	(-4.7-3.0)	
cons	-18.9±2.0	0.0001	(-23.3- -14.7)	
Model Name 1 Month Post-tx Longitudinal Strain	Output of final/best model after stepwise multivariate linear regression R2 0.32			
	Coef.+ SEM	p	[95% CI]	
Dialysis History	2.4±1.7	0.18	(-1.2-6.0)	
HD History	-1.1±1.7	0.52	(-4.6-2.4)	
Chronic HTN	0.4±1.2	0.73	(-2.0-2.8)	
Race	0.1±1.3	0.91	(-2.6-2.9)	
Age	0.1±1.3	0.47	(-1.7-3.7)	
BMI-Obese	3.6±1.6	0.04*	(0.19-6.9)	
High iPTH	-0.26±1.5	0.86	(-3.4-2.9)	
cons	-22.7±1.4	.0001	(-25.5- -19.8)	

Model Name Pretransplant Circumferential Strain	Output of final/best model after stepwise multivariate linear regression R2 = 0.45			
	Coef.+ SEM	p	[95% CI]	
History of HD	-4.6±2.6	0.11	(-10.5-1.3)	
Race	-0.5±2.9	0.86	(-7.3-6.3)	
Sex	1.1±2.5	0.67	(-4.7-6.9)	
BMI-Obese	4.4±4.0	0.30	(-4.9-13.8)	
High iPTH	0.9±2.7	0.76	(-5.4-7.2)	
Dyslipidemia	1.3±3.3	0.71	(-6.4-9.1)	
cons	-21.0±4.3	0.0001	(-31.0- -11.0)	

Model Name 1 Month Post-tx High CIMT	Output of final/best model after stepwise multivariate logistic regression Pseudo R2 0.45		
	Coef+SEM	p	[95% CI]
High iPTH	3.8±1.9	0.04	0.16-7.6
Hypertension	2.7±1.5	0.08	-.33-5.8
Hx dialysis	-3.1±1.7	0.07	-6.4-.23
Dyslipidemia	-2.5±1.3	0.06	-5.1-0.1
Age	0.62-1.3	0.64	-2.0-3.2

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