Reduction of Nocturnal Hypertension in Pediatric Renal Transplant Recipients

PART A. Specific Aims

Nocturnal hypertension and an abnormal decline in nocturnal blood pressure (non-dipping pattern) are commonly found during ambulatory blood pressure monitoring (ABPM) in pediatric renal transplant recipients. These entities are independently associated with progressive renal dysfunction, proteinuria, cardiovascular morbidity and mortality in adults with hypertension and chronic kidney disease. Hence, optimization of anti-hypertensive medications to normalize nocturnal blood pressure and restore the nocturnal dip is a therapeutic target with potential cardiovascular and renal benefit. Tailoring of anti-hypertensive medications to the circadian variation of blood pressure, or chronotherapy, has been utilized as a means of reducing nocturnal blood pressure and converting non-dippers back to the normal dipping pattern. Furthermore, adiponectin, an adipocytokine, has been associated with hypertension. The genetic variation of adiponectin in relationship to dipper status has not been explored.

The proposed study will investigate the effect of chronotherapeutic alteration of anti-hypertensive medication on nocturnal hypertension and end-organ injury in pediatric renal transplant recipients who are non-dippers. Additionally, we aim to examine the association between response to intervention, serum adiponectin levels and adiponectin gene polymorphisms. We hypothesize that (1) evening administration of anti-hypertensive medication will convert subjects from non-dipper to dipper status, improve mean nocturnal blood pressure (BP), and improve nocturnal BP load, (2) evening administration of anti-hypertensive medication will reduce albuminuria, left ventricular mass index (LVMI), rate of decline of glomerular filtration rate (eGFR) and will decrease pulse wave velocity (PWV), and (3) lower adiponectin levels and presence of adiponectin gene polymorphisms will be associated with less of a response to the intervention. The proposed study will explore these hypotheses in a clinical trial. The specific aims of this proposal are as follows:

Specific Aim #1:

To institute a prospective, randomized, open label, blinded end-point trial in pediatric renal transplant recipients who are non-dippers to determine whether adding an evening anti-hypertensive medication improves nocturnal hypertension.

Secondary Aims:

1. To examine whether adding an evening anti-hypertensive medication modifies albuminuria, LVMI, PWV and eGFR slope in pediatric renal transplant recipients who are non-dippers.

2. To determine the association of serum adiponectin levels and single-nucleotide polymorphisms (SNPs) of the adiponectin (ADIPOQ) gene with response to chronotherapeutic alteration of anti-hypertensive medication and outcomes of change in microalbuminuia, LVMI, PWV and eGFR slope in pediatric renal transplant recipients who are non-dippers.

PART B. Significance and Background

Ambulatory Blood Pressure Monitoring

Ambulatory blood pressure monitoring (ABPM) is a non-invasive technique for measuring multiple BP readings over a 24-hour period during regular activities and sleep. This modality has several advantages over conventional casual BP. Namely, ABPM provides measurements outside of the healthcare environment thereby eliminating the white coat effect. ABPM can assess many parameters of BP including averages of systolic and diastolic BP, heart rate, BP variability, and circadian changes during the sleep and awake periods. The modality can also be used to test the efficacy of anti-hypertensive treatment. Perhaps more importantly, ABPM is superior to casual BP in correlating with target organ damage, cardiovascular risk and long-term prognosis in children and adults [1-3].

The use of ABPM in children has been validated [4,5] and recommendations for the standard assessment of ABPM in children and adolescents have been recently developed [4]. Accordingly, the interpretation of ABPM studies is based on a combination of BP parameters. Mean systolic and diastolic BP for the 24 hour, daytime and night-time periods are calculated and referenced against normative data (mean BP greater than the 95% for height and sex indicate hypertension) [6,7]. BP load is another ABPM parameter defined as the percentage of BP measurements above the 95th% for sex and height for the 24 hour, daytime and night-time periods. BP

loads greater than 25% to 30% are considered elevated [4]. Nocturnal dipping refers to the physiological decline in BP during sleep. Nocturnal dipper status is determined by the percent drop in mean systolic BP or diastolic BP between daytime and night-time periods (N/D ratio). Normal dipper status is defined as at least 10% drop (N/D ratio 0.9 or less) in mean BP between daytime and night-time. Non-dipper status is defined as less than 10% drop (N/D ratio >0.9) in mean BP between daytime and night-time [4]. Non-dippers are further classified to reverse-dippers when nocturnal BP is above the daytime mean.

Nocturnal Dipping

ABPM has allowed for the characterization of the 24 hour circadian variation in blood pressure. In most individuals, the normal circadian pattern includes a sharp morning rise in BP, two daytime peaks around 9 a.m. and 7 p.m., a post-parandial dip around 3 p.m. and a decline during sleep [8]. The normal circadian 24 hour BP variation is affected by a variety of external factors and internal factors such as autonomic nervous system tone, catecholamines, the renin-angiotensin-aldosterone axis, hematologic factors, and oxidative stress [8].

In normal dippers, the night-time mean BP is 10 -20% lower than the awake BP. Non-dippers do not demonstrate this physiological decline in blood pressure during sleep, which may represent a pathological condition. A variety of conditions have been associated with non-dipping status, including renal transplantation [9], sleep apnea [10], chronic kidney disease [11-13], essential hypertension [14], diabetes [15] and cerebro-vascular disease [16], to name a few. The mechanisms underlying the lack of blood pressure decline are unclear. Some have postulated that higher nocturnal blood pressures are needed to enhance natriuresis in sodium sensitive individuals [17]. Alterations in the autonomic nervous system [18], renin-angiotensin system [19], and poor sleep quality [20] are also thought to play a role.

The non-dipping phenomenon appears to have clinical significance and is now considered to be a risk factor for cardiovascular disease. In the general adult population, non-dipping and nocturnal BP were found to be strong predictors of cardiovascular morbidity and mortality [21,22]. A prospective study of 1,187 patients with essential hypertension demonstrated that non-dippers had three times as many cardiovascular events than dippers [23]. The non-dipping pattern has also been associated with increased target organ damage such as left ventricular hypertrophy [24], stroke [25], proteinuria [26] and progression of renal insufficiency [27] in adults with hypertension.

Hypertension in Renal Transplantation

Hypertension is a common occurrence after renal transplantation in children. In the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS), which is a registry of greater than 6,500 pediatric renal transplants from 126 centers, 65 – 73% of pediatric transplant recipients were on anti-hypertensive medications 2 years post-transplant, and 59 - 69% were on medications 5 years post-transplant [28]. The use of ABPM is now recognized as the preferred method to diagnose hypertension in the pediatric renal transplant population over casual blood pressure [29]. Giordano et al. assessed 37 renal transplant children and diagnosed hypertension in 62% by ABPM while only 43% were detected by casual blood pressure [30]. Eleven single center studies have evaluated ambulatory blood pressure in pediatric renal transplant recipients to date [30-40]. The prevalence of hypertension in these studies ranged from 36% to 85%, in spite of anti-hypertensive treatment in the majority of children.

The pathogenesis of hypertension in kidney transplant patients is multi-factorial, involving interactions among immunosuppressive therapy, host and allograft risk factors. Corticosteroids increase sodium retention with resultant volume expansion and elevated blood pressure. Calcineurin inhibitors cause vasoconstriction by stimulating vasoactive factors. Host-related factors include pre-transplant hypertension, native kidney disease, high salt diet and obesity. Risk factors related to the kidney allograft include donor age, ischemia time, graft dysfunction, renal artery stenosis and inherited abnormal sodium excretion [41].

Nocturnal Hypertension in Renal Transplantation

Of the published ABPM studies in pediatric transplant recipients, six reported isolated nocturnal hypertension in 22% to 76%. The mean systolic dip ranged from 3% to 8.9% and diastolic dip ranged from 8.7% to 13.9%, indicating that diastolic dipping was more preserved when compared to systolic dipping. When mentioned, an abnormal dipping pattern was found in 23% to 85% of transplant recipients, with systolic non-dipping greater than diastolic non-dipping. Some recipients experienced reversed dipping. McGlothan et al. reported a predominance of nocturnal hypertension in 29 pediatric renal transplant recipients. Isolated nocturnal hypertension was present in 41% with diastolic hypertension being more common. Blood pressure loads were

higher in the night-time compared to the daytime. Systolic nocturnal dip was blunted in 43% and reversed in 17%, and the diastolic nocturnal dip was blunted in 30% of recipients and reversed in 7% [9].

The effect of renal transplantation on circadian variation of blood pressure over time is not known, however some longitudinal studies in adults suggest that it may improve. Covic et al. showed that out of 20 renal transplant recipients who were non-dippers one month after transplant, 40% became dippers at one year [42]. However, 55% remained non-dippers. Lipkin et al. demonstrated an increased prevalence of dippers from 69% after 37 months to 83% after 124 months [43]. In the only longitudinal study of pediatric transplant recipients, Krmar et al. observed that only 11 of 37 recipients maintained consistent dipper status (either dipper or non-dipper) on repeat ABPM with a mean follow-up time of 3.3± 2.2 years (range 1-8) [33]. However, in all these longitudinal studies, antihypertensive therapy was started and titrated over time which may have affected the dipping status. The mechanisms for normalization of the circadian blood pressure profile are not known.

Nocturnal Hypertension and End Organ Effects

Young adults with a history of renal transplantation during childhood and adolescence have a greater than 10 fold risk of cardiovascular death compared to the general population [44]. Hypertension plays a major role in the morbidity and mortality associated with cardiovascular disease, which is the second leading cause of death in pediatric renal transplant patients [37]. Whether pediatric transplant recipients who are non-dippers are at higher risk for cardiovascular outcomes in adult life is unknown. One study in adults with CKD found that daytime and night-time BP were predictive of myocardial infarction, stroke and death [45]. Non-dipping was associated with increased risk, but the relationship did not hold after adjustment for other risk factors. In hemodialysis patients, non-dipping was associated with a hazard ratio of 2.5 for cardiovascular events and 9.6 for cardiovascular death [46]. Toprak et al. reported that adult transplant recipients who were non-dippers had higher LVMI than dippers and nocturnal systolic load was an independent predictor of LVMI [47]. In a study of 28 pediatric renal transplant recipients, LVMI significantly correlated with 24 hour systolic BP, but was not correlated with the absolute value of systolic or diastolic nocturnal dip. There was no mention of LVMI based on dipper status or nocturnal blood pressure [36]. A study of 40 pediatric renal transplant recipients found no association between LVMI and dipping status or blood pressure load [38].

Hypertension in pediatric renal transplant patients has been shown to be associated with allograft dysfunction [48,49]. Due to the lack of prospective studies, no definitive answer can be given to date whether non-dipping or nocturnal blood pressure influences renal function in childhood. In adults with chronic kidney disease (CKD), the non-dipping pattern of ambulatory BP is associated with a faster progression of CKD. In a longitudinal study of 48 hypertensive patients with CKD, Timio et al. demonstrated that non-dippers had a faster rate of decrease in creatinine clearance than dippers [27]. Non-dippers also had greater increase in urinary protein excretion than dippers. A study of 217 veterans with CKD showed that non-dipping and night-time ambulatory BP were stronger predictors of all-cause mortality and the combination of mortality plus progression to ESRD than daytime BP [50]. Davidson et al. likewise showed a greater decrease in GFR in non-dippers (mean change, -15.9%) compared to dippers (mean change, 1.3%) after a median follow-up of 3.2 years independent of blood pressure levels and risk factors for CKD [51]. Similarly, a study of 119 adult renal transplant recipients found that lack of nocturnal dip was associated with poor allograft function and high resistive index on ultrasound [52]. For every 10% nocturnal drop in systolic BP, GFR increased by 4.6 ml/min per 1.73 m^2. Two additional groups found that non-dipper status was associated with lower creatinine clearance [53,54]. In the only pediatric study that evaluated allograft function, Krmar et al. retrospectively showed no difference in the rate of decline of GFR in subjects with nocturnal hypertension compared to those without nocturnal hypertension [33]. Non-dipper status was not evaluated.

Chronotherapy of Hypertension

Given that non-dipper status and nocturnal hypertension are modifiable risk factors for allograft dysfunction, cardiovascular morbidity and mortality, optimization of anti-hypertensive therapy to normalize circadian variation in BP is a potential therapeutic target. Chronotherapy is the practice of administering medications in synchrony with circadian patterns in order to maximize health benefit while minimizing adverse effects [55]. Chronotherapy of hypertension can be accomplished by the specific timing of conventional anti-hypertensive medications based on individual needs. For those with nocturnal hypertension and non-dipping status, evening administration of BP medications may be ideal.

An uncontrolled study in 32 non-dipper patients with CKD showed that shifting one anti-hypertensive medication from morning to evening resulted in restoration of normal dipping status in 87.5% of patients after 8

weeks [56]. Lower nocturnal BP means and office casual BP were also found. Urinary protein excretion was lower after the intervention while GFR and urine sodium excretion remained unchanged. These results were found independent of the class of medication shifted. Portaluppi et al. demonstrated that evening administration of isradipine reduced nocturnal blood pressure, but no information was given regarding the effect on nocturnal dip [57]. In hypertensive adult subjects, evening (vs. morning) administration of valsartan reversed the non-dipping pattern in 73% of patients and reduced urinary albumin excretion by 41% after three months [58]. Currently, there is a prospective study underway of 3,000 adults which will evaluate whether normalizing BP toward a more dipper profile using chronotherapeutic strategies reduces cardiovascular, cerebrovascular and kidney risk [59]. There are no pediatric or renal transplant chronotherapeutic studies published to date.

Adiponectin and Hypertension

Adiponectin is a protein secreted by adipose tissue with insulin sensitizing, vasculo-protective, anti-atherogenic and anti-inflammatory properties. Data suggest that the HMW multimer of adiponectin is the active isoform and that HMW adiponectin or the ratio of HWM adiponectin to total adiponectin (HMWr) is a better biomarker of clinical health and disease than total adiponectin [60]. Reduced levels of adiponectin are thought to play an important role in the development of obesity-related cardiovascular and metabolic diseases, including hypertension. Several studies in adults and children have reported that lower adiponectin correlates with higher BP [61-64]. Furthermore, two studies demonstrated that lower adiponectin levels are found in non-dippers compared to dippers [65,66]. More recently, two prospective clinical studies in adults reported that normotentensive subjects in the lowest percentile group of adiponectin conferred a 2.76 to 3.42 increased risk of developing hypertension 3 to 5 years later when compared to those in the highest percentile group [67,68]. The results of these studies intimate that adiponectin may play a significant role in the pathogenesis of hypertension.

Single nucleotide polymorphisms (SNPs) of the adiponectin gene (ADIPOQ, Gene ID 9370, GeneBank ID NM_004797) have been associated with the metabolic syndrome, cardiovascular disease and diabetes [69-71]. The association between SNPs of adiponectin and hypertension has yet to be fully explored. Iwashima et al. found that the TC genotype of the I164T polymorphism was associated with lower serum adiponectin levels and hypertension in a cohort of Japanese men [62]. Another group reported that the genotype and allele frequency distributions of SNP276 were different in women with pre-eclampsia vs. controls, and that the TT genotype was associated with protection against pre-eclampsia [72]. There are no studies of adiponectin SNPs in renal transplant recipients to date.

FGF-23 and Hypertension

FGF23 is a hormone produced by osteoblasts/osteocytes in bone that acts on the kidney to regulate phosphate and vitamin D metabolism through activation of FGF receptor/α-Klotho co-receptor complexes. High FGF23 predicts graft failure in transplant patients, is independently related with chronic kidney disease (CKD) progression, and associates with endothelial dysfunction in adults. Furthermore, independently of serum phosphate, high FGF23 associates with mortality and left ventricular hypertrophy in dialysis patients and with atherosclerosis in elderly individuals in the general population. FGF23 also predicts a high risk for death and cardiovascular events in predialysis CKD patients and in subjects with coronary artery disease[73]. Elevated circulating FGF23 concentrations have been associated with left ventricular hypertrophy (LVH), and it has been suggested that FGF23 exerts a direct effect on the myocardium. Data on the cardiovascular effects of FGF23 on cardiovascular disease is limited. One study examined found an inverse relationship between FGF23 and renal function in 44 pediatric transplant patients [74]. It is possible that elevated FGF23 may exert its negative impact through distinct mechanisms of action independent from its role as a regulator of phosphorus homeostasis.

Significance of the Research

Nocturnal hypertension and non-dipper status are associated with end-organ damage, cardiovascular morbidity and mortality in adults with hypertension and kidney disease. The long term effects of non-dipping on cardiovascular and allograft outcomes in pediatric renal transplant recipients are not known, however, the likelihood of a similar effect is probable. Thus, it is of interest to find an intervention to normalize the circadian variation in BP toward more of a dipper profile. This study aims to investigate chronotherapeutic alterations of anti-hypertensive medications in pediatric renal transplant recipients with the purpose of improving nocturnal

hypertension and target-organ injury. The effectiveness of a chronotherapeutic approach has not yet been explored in pediatric renal transplant recipients. If this study's hypotheses are proven correct, then this simple strategy may retard progression of renal dysfunction and protect against future cardiovascular morbidity and mortality in pediatric renal transplant recipients.

PART C. Preliminary Studies and Experience of the Investigator

The experience of the candidate in studies pertaining to cardiovascular risk factors and hypertension in renal transplant recipients and her background in epidemiology and biostatistics provide support to the strengths and feasibility of the proposed study. The candidate was the primary investigator in two prospective studies evaluating 1) the relationship between adiponectin and ambulatory blood pressure and 2) cardiorespiratory fitness in pediatric renal transplant recipients. She was also a co-investigator in a study of vascular dysfunction in transplant recipients. In addition, she has written case reports and chapters in the area of hypertension.

I. Ambulatory Blood Pressure in Pediatric Renal Transplant Recipients

We performed a cross-sectional study of thirty-two pediatric renal transplant recipients, aged 14.5 ± 2.8 (range 8 to19) years, at The Children's Hospital of Philadelphia (manuscript attached in Appendix). Subjects were predominantly male (n = 23, 72%), non-black (n = 24, 75%) and non-obese (n = 27, 84%). Median time (inter-quartile range) since diagnosis of end stage renal disease (ESRD) was 4.9 (2.2 to 9.9) years and time since transplant was 1.9 (0.4 to 12.7) years. Median eGFR was 90.0 (70 to 183) ml/min/1.73m^2. All subjects were on prednisone; other immunosuppression included: tacrolimus 26 (81%), mycophenolate mofetil 14 (44%), sirolimus 12 (38%) and azathioprine 4 (13%). Thirteen (41%) subjects were on anti-hypertensive medications: Angiotensin Converting Enzyme Inhibitor (ACEI) 6 (19%), Angiotensin II Receptor Blocker (ARB) 5 (16%), calcium channel blocker 9 (28%) and beta-blocker 4 (13%). Nine subjects held ACEI or ARB medications for one week prior to completing the study due to protocol requirements.

Table 1. 24 hr ambulatory blood pressure monitor results of pediatric renal transplant recipients

Blood Pressure	**N= 32**		
	Mean ± SD	BP Index ± SD	N (%) with BP index >1
24 h systolic BP	115 ± 13 mm Hg	0.94 ± 0.08	8 (25%)
24 h diastolic BP	68 ± 7 mm Hg	0.90 ± 0.08	4 (13%)
Daytime systolic BP	118 ± 12 mm Hg	0.92 ± 0.08	4 (13%)
Daytime diastolic BP	71 ± 7 mm Hg	0.87 ± 0.07	0 (0%)
Night-time systolic BP	108 ± 13 mm Hg	0.97 ± 0.11	13 (41%)
Night-time diastolic BP	62 ± 8 mm Hg	0.94 ± 0.10	12 (38%)

Mean daytime, night-time and 24 hour indexed BP [mean BP divided by the sex and height specific 95th percentiles for systolic and diastolic BP [3,7]] of all subjects were less than one (Table 1). Subjects with hypertension, indicated by BP index greater than one, are shown in Figure 1. Fifteen subjects (47%) had nocturnal hypertension and four subjects (13%) had daytime hypertension. Twelve of the 15 (80%) subjects with nocturnal hypertension on ABPM had normal office casual blood pressures. An abnormal nocturnal dipping pattern was seen in 62% (systolic) and 47% (diastolic) of subjects (Figure 2). The frequency of dippers and non-dippers in years post-transplant is demonstrated in Figure 3. The majority of non-dippers were found in the first two years post-transplant, however, non-dippers were also seen after several years. Ten of the 18 non-dippers were treated with anti-hypertensive medications. Mean BP loads were as follows: daytime systolic 18% and diastolic 12%; night-time systolic 27% and diastolic 29%.

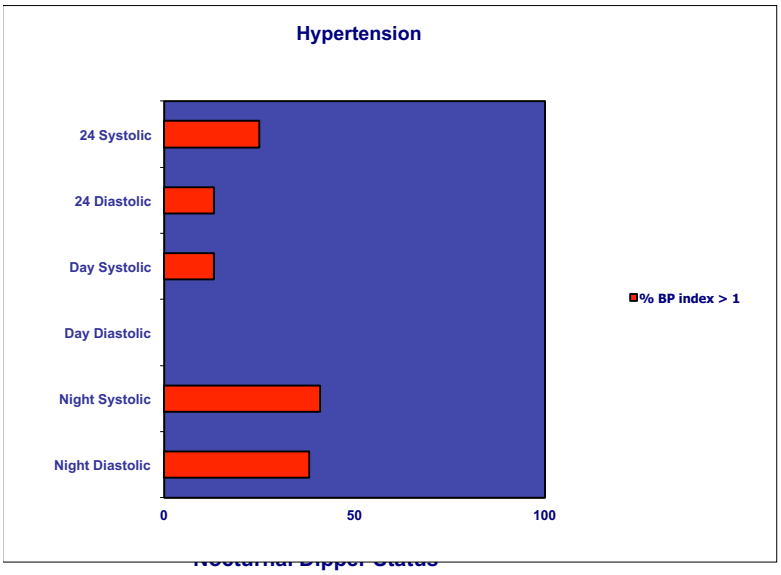


Figure 1. Percent of pediatric renal transplant recipients with blood pressure index greater than one, indicating hypertension.

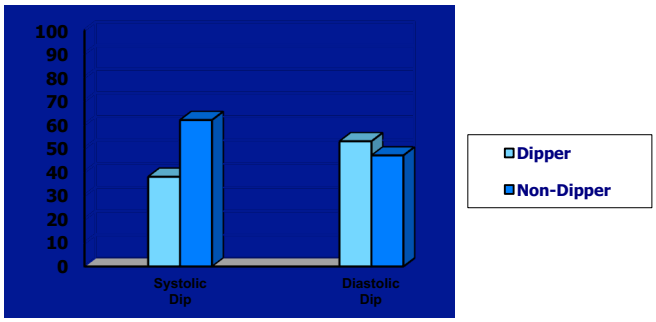


Figure 2. Percent of pediatric renal transplant recipients who are dippers vs. non-dippers.

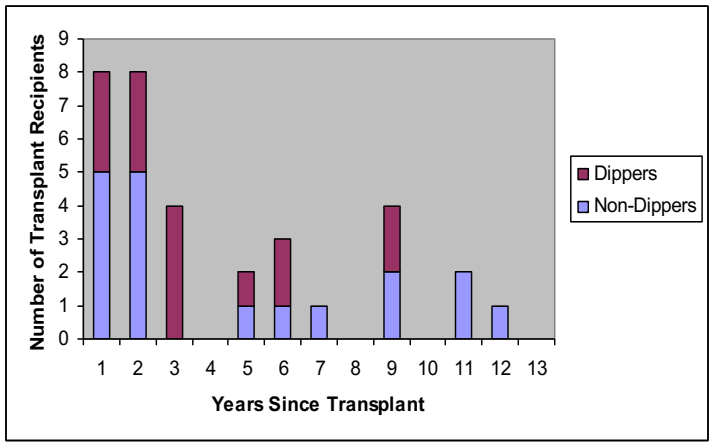


Figure 3. Frequency of dippers and non-dippers years post-transplant.

Our results demonstrated that **half of pediatric renal transplant subjects had nocturnal hypertension and were non-dippers**. Notably, most of these patients had masked hypertension as they had normal casual office blood pressures. Blood pressure loads were also appreciably elevated at night-time compared to the daytime.

II. Adiponectin in Renal Transplant Recipients

Serum total adiponectin and HMW adiponectin to total adiponectin ratio (HMWr) were measured in the same group of pediatric renal transplant recipients. The mean serum total adiponectin level was 9.6 ± 4.9 (median 8.3, range 2.8 to 21.9) µg/ml and HMWr was 0.45 ± 0.11 (median 0.46, range 0.22 to 0.61). Although total adiponectin was lower in male subjects and obese subjects, no significant difference was determined based on sex (male 9.4 ± 5.4 µg/ml *vs.* female 10.0 ± 3.5 µg/ml, p = 0.368), obesity status (obese 7.2 ± 4.2 µg/ml *vs.* non-obese 10.0 ± 4.9 µg/ml, p = 0.223) or immunosuppressive medication. This also was true for HMWr.

To determine the independent anthropometric and clinical predictors of adiponectin in this cohort, variables were entered into stepwise multiple linear regression models. **Tanner stage of pubertal maturation** (stage 1 through 3 as the referent group with an indicator variable for Tanner stages 4 and 5), **history of dialysis** (no dialysis as referent group) and **time since diagnosis of ESRD** were independent predictors of log-transformed total adiponectin (R^2 = 0.46). Mature pubertal stage (β = -0.470, 95% CI: -0.773 to -0.167, p = 0.004) and prior history of dialysis (β = -0.444, 95% CI: -0.756 to -0.133, p = 0.007) independently associated with lower total adiponectin. A 1 month change in time since diagnosis of ESRD was associated with a 0.3% increase in total adiponectin (β = 0.003, 95% CI: 0.0004 to 0.006, p = 0.026). In linear regression models predicting $HMWr^2$ (power transformed, R^2 = 0.53), **pubertal stage, sex, sirolimus use and HDL** were statistically significant determinants. Mature pubertal stage independently associated with lower $HMWr^2$ (β = -0.094, 95% CI: -0.141 to -0.047, p < 0.001). Female sex (β = 0.075, 95% CI: 0.021 to 0.130, p = 0.009) and sirolimus use (β = 0.115, 95% CI: 0.061 to 0.170, p < 0.001) were associated with greater $HMWr^2$. $HMWr^2$ increased by 0.003 (95% CI: 0.001 to 0.004, p = 0.01) for a 1-unit increase in HDL.

We hypothesized that measures of fat mass (total and central) would be associated with adiponectin, however, this was not the case. In addition, adiponectin levels were not significantly lower in obese subjects compared to non-obese subjects, contrary to what was expected. Authors have reported that adiponectin correlates inversely with estimates of fat mass in transplant recipients (BMI) [75,76] while others have not [77,78]. Some postulate that qualitative changes in adipose tissue rather than quantitative changes may mediate the risk of hypertension [79]. The effect of renal transplantation and immunosuppressive medications on the functional aspects of adipose tissue has yet to be determined.

III. Adiponectin and Hypertension in Renal Transplant Recipients

When hypertensive subjects (defined as any BP index > 1) were compared to non-hypertensive subjects, lower total adiponectin (7.3 ± 3.3 µg/ml *vs.* 11.6 ± 5.2 µg/ml, p = 0.01) and HMWr (0.38 ± 0.10 *vs.* 0.50 ± 0.09, p = 0.002) were seen in the hypertensive group (Figure 4). There was a trend for lower adiponectin levels in non-dippers compared to dippers however statistical significance was not met (Figure 5). This may have been a power issue related to the small sample size.

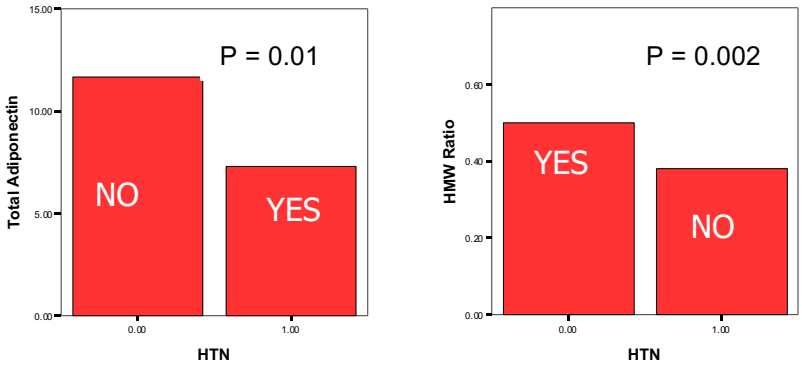


Figure 4. Adiponectin levels in hypertensive subjects vs. normotensive renal transplant subjects.

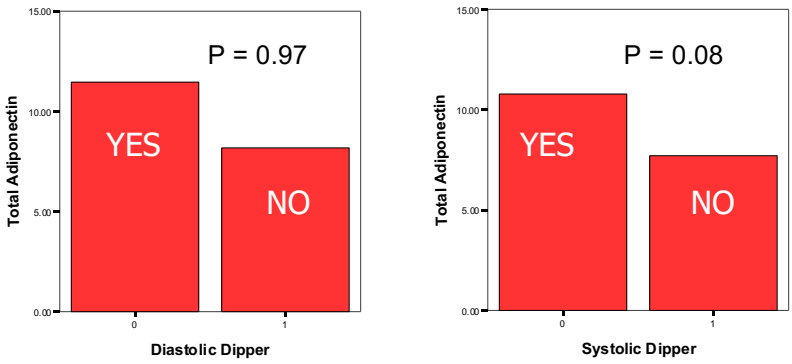


Figure 5. Adiponectin levels in dipper vs. non-dipper.

We found that total adiponectin and HMWr negatively correlated with systolic and diastolic BP in the daytime, night-time and 24 hour periods (Table 2). **Lower total adiponectin was independently associated with greater BP in all ABPM parameters, except for night-time systolic BP** in multiple regression analysis adjusted for fat mass for height z-score, eGFR, age and sex. **HMWr was inversely associated with all ambulatory BP indexes.**

In logistic regression, we demonstrated that **lower total adiponectin and HMWr were significant independent determinants of hypertension in pediatric transplant subjects**. For log total adiponectin, the OR was 0.05 (95% CI: 0.01 to 0.51, p = 0.011), indicating that the odds of having hypertension decreased by 95% when log total adiponectin increased by 1 unit after adjustment for covariates. In the model using $HMWr^2$, the OR was 0.11 (CI: 0.02 to 0.61, p = 0.011), indicating that the odds of having hypertension decreased by 89% when $HMWr^2$ increased by 10 units.

Table 2. Correlation and Regression Coefficients for Adiponectin and ABPM

BP	Total Adiponectin		HMWr	
	Spearman	Regression*	Spearman	Regression*
24 systolic BP index	r = -0.36 p = 0.041	β = -0.067 -0.133 to -0.0001 p = 0.050	r = -0.41 p = 0.021	β = -0.400 -0.769 to -0.031 p = 0.035
24 diastolic BP index	r = -0.43 p = 0.015	β = -0.079 -0.134 to -0.024 p = 0.007	r = -0.42 p = 0.016	β = -0.494 -0.791 to -0.198 p = 0.002
Daytime systolic BP index	r = -0.45 p = 0.011	β = -0.071 -0.129 to -0.013 p = 0.019	r = -0.39 p = 0.026	β = -0.386 -0.725 to -0.071 p = 0.019
Daytime diastolic BP index	r = -0.57 p = 0.001	β = -0.086 -0.135 to -0.036 p = 0.002	r = -0.44 p = 0.011	β = -0.47 -0.759 to -0.198 p = 0.002
Night-time systolic BP index	r = -0.35 p = 0.048	β = -0.073 -0.165 to 0.019 p = 0.114	r = -0.45 p = 0.011	β = -0.556 -1.049 to -0.062 p = 0.029
Night-time diastolic BP index	r = -0.36 p = 0.042	β = -0.093 -.170 to -0.017 p = 0.019	r = -0.42 p = 0.016	β = -0.59 -1.007 to -0.173 p = 0.007

* Total Adiponectin was log transformed. HMWr was power transformed ($HMWr^2$).

IV. Other Cardiovascular Risk Factors in Pediatric Renal Transplant Recipients

Cardiorespiratory Aerobic Fitness

We also studied the non-traditional cardiovascular risk factor of aerobic fitness in 50 pediatric transplant recipients and 70 healthy controls (manuscript attached in Appendix). The impact of body size, fat-free mass (FFM) and fat mass (FM) on cardiorespiratory fitness in pediatric renal transplant recipients has not been established. Study objectives were to assess maximal oxygen consumption (VO_{2max}) in transplants and controls, adjusted for body composition, and to identify risk factors for reduced fitness in transplant recipients. Cycle ergometry and DXA were obtained in subjects, ages 8 to 21 yr. Control recruitment was targeted to

include obese subjects with body mass index Z-scores comparable to transplant recipients. Allometric regression models were utilized. Transplant recipients had significantly lower height Z-scores ($p < 0.001$) and comparable BMI Z-scores. VO_{2max} per body weight (ml/kg/min) and per FFM (ml/kgFFM/min) did not differ between groups. However, **VO_{2max} was 13% lower (95% CI 18, 8; $p < 0.001$) in transplant recipients compared with controls,** adjusted for FM, FFM, sex and race. Greater FFM, lower FM, non-black race, and male sex were independently associated with greater $VO_{2max.}$ Within transplant recipients, hemoglobin levels were positively associated with VO_{2max} ($p = 0.04$) and sirolimus use was associated with lower VO_{2max} ($p < 0.01$). In conclusion, transplant recipients had significant VO_{2max} deficits that were not captured by conventional measures (ml/kg/min). Greater FM was an independent risk factor for low VO_{2max}. Lower fitness in TX may be related to sirolimus effects on skeletal muscle.

Vascular Function

Vascular dysfunction was evaluated in a pilot study of 9 pediatric renal transplant recipients and 18 healthy controls. Pathologic outcome measures included the stiffness of the conduit arteries. Physiologic outcome measures included the vascular response to lack of oxygen (flow-dependant vasodilatation), identification of circulating endothelial cells, and endothelial precursor cells (EPC) that provide estimates of chronicity and extent of vascular injury. Results demonstrated that pediatric renal transplant recipients had **reduced brachial artery reactivity** compared to controls, p= 0.023 (Figure 6). Pediatric renal transplant recipients had **increased pulse wave velocity** compared to controls, p = 0.013 (Figure 7). And, increased circulating hematopoeitic cells with **increased circulating EPC** was demonstrated in the renal transplant group compared to controls (Figure 8). We concluded that pediatric renal transplant recipients have pathological, physiological and biochemical evidence for impaired vascular function. This vascular impairment likely results in accelerated atherosclerosis with premature cardiovascular events in early adulthood.

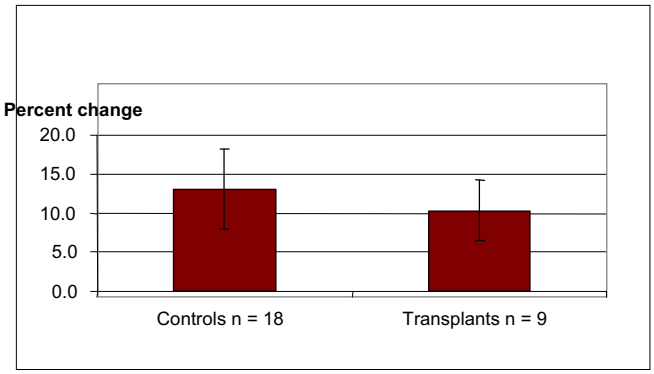


Figure 6. Brachial artery reactivity in pediatric renal transplant recipients compared to controls.

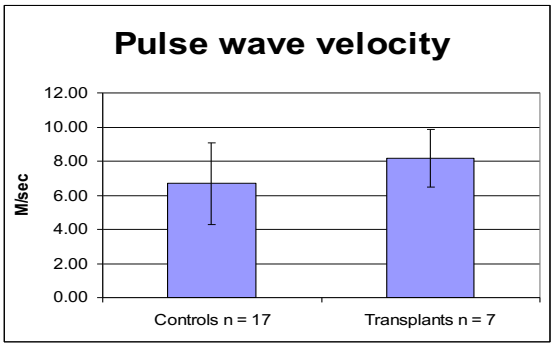


Figure 7. Pulse wave velocity in pediatric renal transplant recipients compared to controls.

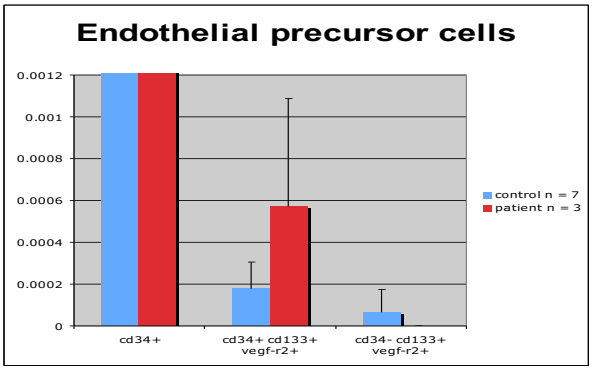


Figure 8. Endothelial precursor cells in pediatric renal transplant recipients compared to controls.

PART D. Experimental Design and Methods

I. Overview of Study Design

A prospective, randomized, open label, blinded end-point trial in pediatric renal transplant recipients who are non-dippers will be conducted to determine the effect of chronotherapeutic alteration of anti-hypertensive medication on nocturnal blood pressure. This study design has been previously validated for use in trials with ABPM measurements [80]. The trial will examine the effects of adding an evening medication. An estimated total of 100 pediatric renal transplant recipients will be enrolled for baseline studies at two pediatric tertiary hospitals over a three year time period. Baseline studies include 24 hour ABPM, echocardiography, PWV, urine albumin to creatinine ratio, and laboratory tests (creatinine, electrolytes, adiponectin, FGF23) and genetic testing for SNPs of the adiponectin gene (ADIPOQ). Subjects identified as non-dippers on baseline 24 hour ABPM will have their blood pressure medication changed and repeat testing will be done at 3 and 6 months. Subjects identified as dippers on ABPM will not continue participation in the study after baseline testing.

The expected sample size of the non-dipper subjects continuing the study is 40. Non-dipper subjects will be randomized to one of two arms: (1) New medication group – a new anti-hypertensive medication will be added in the evening, (2) Control group – anti-hypertensive medications are not altered. 24 hour ABPM, echocardiography, PWV, urine albumin to creatinine ratio, and laboratory tests (creatinine, electrolytes, adiponectin, FGF23) will be tested at baseline, 3 months and 6 months after the intervention. Genetic testing for SNPs of the adiponectin gene (ADIPOQ) will only be performed at baseline. Principles set out in these guidelines will be implemented.

Primary Outcome Measure: The efficacy of chronotherapuetic alteration of anti-hypertensive medication will be tested by comparing the proportion of subjects in each of the treatment arms who change from non-dipper (N/D ratio > 0.9) to dipper status (N/D ratio ≤ 0.9).

Secondary Outcome Measures:

1. Change in night-time systolic/diastolic blood pressure
2. Change in night-time blood pressure load
3. Change in left ventricular mass index
4. Change in albumin:creatinine ratio
5. Change in slope of estimated glomerular filtration rate
6. Change in pulse wave velocity
7. Association of serum adiponectin levels and SNPs of adiponectin with outcomes
8. Association of serum FGF23 levels with outcomes

II. Study Sites

Subjects will be recruited from Cohen Children's Medical Center of North Shore-LIJ Health Systems (CCMC) in New Hyde Park, New York and from The Children's Hospital of Philadelphia (CHOP) in Philadelphia, Pennsylvania.

III. Study Subjects

Patients from both institutions come from a large geographic area with a diverse racial composition. There are currently 200 children greater than 5 years of age who are followed at CHOP and 50 children who are followed at SCH. Pediatric renal transplant recipients who meet eligibility criteria will be invited to have baseline testing done. Eligible subjects who are identified as non-dippers on ABPM will then be invited to continue participation in the study. Recruitment and informed consent will be done in person during nephrology clinics by a member of the study team. No advertisements will be used. Eligibility criteria include:

Inclusion Criteria

1) Subjects age 5-21 years
2) Stable kidney transplant recipients (<30% change in eGFR in past 3 months)
3) eGFR > 30 ml/min/1.73 m^2
4) >6 months since kidney transplant
5) ABPM: Non-dipper status defined as <10% decline (N/D ratio >0.9) in systolic or diastolic blood pressure from daytime to night-time

Exclusion Criteria

1) ABPM: Subjects with daytime mean blood pressure > 95% for height and sex [7] (to avoid confounding effect of necessary addition of daytime anti-hypertensive therapy)
2) Subjects with nephrotic range proteinuria
3) Subjects with major co-morbid conditions such as cardiac disease, pulmonary disease and diabetes mellitus
4) Subjects/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures
5) Subjects who are pregnant will not be eligible for the new medication group

Subjects may voluntarily withdraw from the study at any time.

IV. Randomization and Blinding

Subjects will be randomized to the treatment group or control group utilizing a blocked randomization strategy in order to ensure similar numbers of subjects in each arm. An independent examiner not directly involved in the study will allocate the groups in variable blocks of 2 and 4 in numbered sealed envelopes. The independent examiner will likely be our department secretary. She will have a random assignment of groups generated by a computer in blocks of 2 and 4. She will place the group assignments in sealed numbered envelopes. The research coordinator and study investigators will not know the group assignments prior to enrolling the subjects. As each subject is enrolled, they will be assigned to the group in the envelope in numerical order. Investigators involved in the statistical analysis of the data, laboratory technicians and echocardiographers will be blinded to the subject treatment group.

V. Study Procedures

The outline of study procedures is as follows:

Study Procedures						
	Baseline		**2 week**	**6 week**	**3 Month**	**6 Month**
Review Inclusion/Exclusion Criteria	**X**	**I N T**				
Informed Consent/Assent	**X**					
Randomization	**x**					
Demographics/Medical History	**X**					
Height and Weight	**x**				**x**	**X**
Tanner Stage	**x**					
Pediatric Sleep Questionnaire	**x**					

Serum creatinine/BUN*	x	E	x	x	x	X
Serum electrolytes *	x	R	x	x	x	X
Serum Phosphorus	x	V			x	x
Urine albumin: creatinine ratio	x	E			x	X
CBC with differential	x	N			x	X
Uric Acid	x	T			x	X
Insulin	x	I			x	x
hsCRP	x	O			x	x
Lipid Profile	x	N			x	x
Serum FGF23	x				x	x
Intact PTH	x				x	x
Vitamin D 25 and Vitamin D 1,25	x				x	x
Serum Adiponectin	x				x	X
Adiponectin Genetic Testing	x					
Urine Pregnancy Test**	x		x	x	x	x
24 hr ABPM	X				x	X
Echocardiogram	x				x	X
Pulse Wave Velocity	x				x	X
Treatment Satisfaction Questionnaire						x
Adverse Event/Serious Adverse Event Assessment	x		x	x	x	X

* Subjects started on an ACE inhibitor will have serum creatinine/BUN and electrolytes checked two and six weeks after the intervention. ** Urine Pregnancy tests will be done on subjects started on an ACE inhibitor.

a. Screening Visit

Eligibility and demographic data will be collected on all pediatric renal transplant recipients during the study interval. Subject and disease characteristics will be recorded for all ineligible subjects and those declining participation. Potential subjects will be screened using the protocol inclusion and exclusion criteria. Study subjects who meet the eligibility criteria will be asked to participate in the study in person by a member of the study team. Parental/guardian permission (informed consent) and, if applicable, child assent, will be obtained prior to any study related procedures being performed. 24 hour ABPM and baseline studies will be obtained.

b. Baseline Visit

All subjects who participate in the study will have baseline studies (height, weight, Tanner Stage, Pediatric Sleep Questionnaire, echocardiography, PWV, urine, blood work – 2 tablespoons of blood, genetic testing – 1 teaspoon of blood) completed within two weeks of the blood pressure study. Echocardiography and PWV will be performed at each of the respective institutions. Subjects who meet the 24 hour ABPM criteria (non-dipper status) will then be randomized into one of the treatment groups. Subjects who do not meet blood pressure criteria will not continue in the study past the baseline visit. Female subjects greater than 9 years old (or if post-menarchal) randomized to the new medication group will have urine pregnancy test done.

c. Intervention

Subjects will be randomized into one of two arms:

Control Group - Subjects in the control group will continue to take their medications as usual.

New Medication Group - Enalapril will be added in the evening at 8 pm. If the subject is already on an ACEI or there is a contraindication to starting an ACEI (e.g. worsening renal function, hyperkalemia, history of allergic

reaction to ACEI) the subject will be started on isradipine instead. If the subject is already on an ACEI and calcium channel blocker at baseline, propranolol will then be the new medication added. The choice of medications was made based on the pharmacokinetic profile of the medications (quick-onset and short acting) to lower nocturnal blood pressure with evening administration. Enalapril and isradipine have been shown to effectively change the N/D ratio toward a more dipper profile in clinical trials with adults [57,81]. Subjects started on Enalapril will have urine pregnancy testing done at each visit. Dosing will be as follows:

Medication	Dose
ACEI: Enalapril (half-life 2 hours, peak effect 0.5 - 1.5 hours)	< 40 kg starting dose 2.5 mg titrate to 5 mg > 40 kg starting dose 5 mg titrate to 10mg
Calcium Channel Blocker: Isradipine (half-life 8 hours, peak effect 1 - 1.5 hours)	< 40 kg 2.5 mg > 40 kg starting dose 2.5 mg titrate to 5 mg
Beta Blocker: Propranolol (half-life 3.9 – 6.4 hours, peak effect 1 – 4 hours)	<40 kg starting dose 10 mg titrate to 20 mg >40 kg starting dose 20 mg titrate to 40 mg

d. Safety Visit
Subjects in the new medication group will be evaluated at two and six weeks by the study team to assess for side effects. The dose of the new medication will be titrated up to the target dose if tolerated at the two week visit. Subjects started on ACEI will have serum electrolytes, BUN and creatinine checked (1 teaspoon of blood). Should the subject develop side effects to ACEI (cough, angioedema, hyperkalemia or worsening renal function), the subject will be switched to isradipine.

e. 3 Month and 6 Month Visits
24 hour ABPM, echocardiogram, PWV and laboratory studies will be performed at 3 months and 6 months post-intervention.

f. End of study
At the conclusion of the study, those who were started on a new medication will be given the option to continue the medicine if there was a positive impact on the subject's blood pressure (e.g. lower daytime or night-time blood pressure, restoring of the nocturnal dip). If there was no positive effect of the blood pressure medication, the medication will be weaned off over the time course of one month.

VI. Study Outcomes

Demographic and Clinical Data
The medical record will be abstracted for the following variables: age, age at transplant, race, type of transplant (deceased vs. living donor), current medications, BMI at the time of transplant, history of delayed graft function, history of rejection, prior nephrectomy of the native kidney, and dialysis history. Casual blood pressure from clinic visits will be recorded.

Anthropometric and Pubertal Data
Height and weight will be measured using standardized electronic scales and height boards. Body Mass Index (BMI) will be calculated using the measured height and weight. BMI is defined as weight (kg) / height $(m)^2$, expressed as kg/m^2 and converted to age and gender specific percentiles based on the CDC growth data [82]. Age- and sex-specific Z-scores (standard deviation scores) for height, weight and BMI will be calculated [82]. Obesity is defined as a BMI greater than the 95^{th} percentile for age and sex [83]. The stage of pubertal development will be determined using a validated self-assessment questionnaire [84] and classified according to the method of Tanner [85].

Ambulatory Blood Pressure Monitoring
ABPM will be performed using oscillometric Spacelab 90217 monitors and software (Spacelabs Medical, Redmond, CA) according to recommended guidelines [4]. An appropriate sized blood pressure cuff will be placed on the non-dominant arm with a small monitor attached to a belt. The subject will wear the monitor

continuously for 24 hours, encouraged to carry on with usual activities. BP recordings will be programmed to occur every 20 minutes during the day and every 60 minutes while sleeping. ABPM will be considered satisfactory for analysis if there is a minimum 40 readings during the 24 hours with at least 6 readings at night. Traces will be rejected and ABPM repeated if BP values are missing for a continuous period greater than 2 hours or if greater than 10% of values are artifactual. All ABPM will be interpreted by the primary investigator.

Measured variables include systolic and diastolic mean BP, BP load and BP index for the 24 hour period, daytime period and night-time period. BP load is defined as the percentage of BP values above the sex and height specific 95th percentiles for BP for each subject [3,7]. BP indexes are calculated by dividing the average BP of the subject by the sex and height specific 95th percentiles for systolic and diastolic BP [3,7]. Therefore, a BP index >1 indicates the presence of hypertension. Nocturnal dipper status is determined by the percent drop in mean systolic BP or diastolic BP between daytime and night-time periods (N/D ratio). N/D ratio = [(mean daytime BP – mean night-time BP) / mean awake BP)]. Normal dipper status is defined as at least 10% drop (N/D ratio 0.9 or less) in mean systolic BP or diastolic BP between daytime and night-time. Non-dipper status is defined as less than 10% drop (N/D ratio >0.9) in mean systolic BP or diastolic BP between daytime and night-time.

Laboratory Evaluations

A venous blood sample (2 tablespoons) will be obtained from each subject after an overnight fast for serum urea, creatinine, potassium, sodium, hematocrit, albumin, total cholesterol, triglycerides, glucose, insulin, highly sensitive CRP, phosphorus, PTH, vitamin D 25, vitamin D 1,25 and uric acid. In addition, serum total adiponectin and HMW adiponectin will be measured using a sandwich format enzyme-linked immunosorbent assay kit (Adiponectin Multimeric EIA, Daiichi Pure Chemical, Tokyo, Japan) according to the manufacturer's protocol. HMW adiponectin to total adiponectin ratio (HMWr) will be calculated by dividing HMW adiponectin by total adiponectin. FGF-23 will be measured using a second-generation human FGF23 (C-Terminal) ELISA (Immutopics International, San Clemente, CA). eGFR will be estimated using the Schwartz formula [86]. Homeostasis model assessment of insulin resistance (HOMA-IR) will be calculated according to the following equation: insulin (U/ml) × fasting glucose (mmol/liter) ÷ 22.5 [87]. Subjects will bring first morning urine specimens to study visits, which will be collected at home. First morning spot urine will be collected for albumin and creatinine and a urine albumin to creatinine ratio will be calculated. Urine pregnancy tests will be done on female subjects in trial #2 at baseline, 2 weeks, 6 weeks, 3 months and 6 months. Routine blood and urine will be analyzed by the hospital laboratory at each respective institution. Technicians performing laboratory evaluations will be blinded to the intervention and outcomes. Adiponectin and highly sensitive CRP will be analyzed by the GCRC at SCH.

Genetic Testing

DNA will be extracted from whole blood (1 teaspoon) and the genome will be amplified. Five SNPs of the ADIPOQ known to affect adiponectin levels or are associated with insulin resistance, cardiovascular disease or diabetes will be selected for genotyping [69-71]. These include: rs266729 (5' flanking region), rs1501299 (intron 1), rs1501299 (intron 2), rs2241766 (exon 2) and rs822395 (intron 1).

Echocardiography

Echocardiography will be performed using 2-D echo in M-mode with subjects positioned in the partial left decubitus position. Measurements of the interventricular septal thickness (IVS), posterior wall thickness (PWT), and left ventricular internal dimension (LVID) will be obtained at or just below the mitral valve tips in accordance with the recommendations of the American Society of Echocardiography [88]. Left ventricular (LV) mass will be calculated using the formula by Devereux et al: LV mass (g) = $0.81[1.04(IVS + PWT + LVID)^3 - (LVID)^3] + 0.06$ [89]. Left ventricular mass index will be derived by dividing LV mass in grams by the subject's height in meters raised to the 2.7 power [90]. The technicians and cardiologists will be blinded to the intervention and blood pressure outcomes. Inter- and intra-observer measures of agreement will be monitored using Kappa statistic and intra-class correlation coefficients.

Pulse Wave Velocity

The SphygmoCor Vx pulse wave velocity system (AtCor Medical Pty Ltd, West Ryde (Sydney), Australia) will be used to measure the velocity of the blood pressure waveform between the carotid artery and the radial

artery. The technicians will be blinded to the intervention and blood pressure outcomes. Inter and intra-observer measures of agreement will be monitored using Kappa statistic and intraclass correlation coefficients.

Pediatric Sleep Questionnaire

Obstructive sleep apnea has been associated with abnormal nocturnal dip [73]. Therefore, a 22 item pediatric sleep questionnaire developed by Chervin et al. will be administered to the parent/guardian of the study subject to identify subjects with sleep-related breathing disorders (Appendix) [91]. This questionnaire has been validated and tested for reliability in predicting obstructive sleep apnea. The questionnaire is graded as follows: Yes = 1, No = 0, Don't Know = missing. The total is added and divided by the total number of questions minus those missing answers. A cut off of 33% suggest a diagnosis of a sleep related breathing disorder.

Treatment Satisfaction Questionnaire for Medications

Treatment Satisfaction Questionnaire for Medications (TSQM) developed by Atkinson et al. will be administered to assess patient satisfaction with the change in treatment and the impact of the therapy on quality of life [92]. The tool gathers information about effectiveness of medication, side effects, convenience and global satisfaction.

PART E. Statistical Considerations

a. Statistical Analysis

Statistical analyses will be done using SPSS 15.0 (SPSS Inc., Chicago, IL) statistical package. A two-tailed p-value < 0.05 is the criterion for statistical significance. Intention to treat analysis will be done.

Descriptive statistics will be used to characterize outcome measures in subjects. Means, standard deviations, 95% confidence intervals, medians, and minimum and maximum values will be tabulated and reported for all continuous variables. Frequency counts and percentages will be used for categorical variables. The distributions of each outcome will be examined for normality. Baseline demographic and clinical data between dipper and non-dipper status subjects will be examined. Statistical analyses for both trials will be similar. Differences in means in the treatment group will be compared to the control group using Student's t-test, or the Wilcoxon Rank Sum test if the data are not normally distributed. Differences in proportions will be assessed using the chi-square test.

Specific Aim #1: To analyze the primary outcome, chi-square will be conducted to compare the proportion of subjects who change from non-dipper to dipper status in the two groups at 3 and 6 months. Within-person changes of change in nocturnal dip in each group will also be examined using the paired Student's t- test. Secondary outcome measures of change in systolic/diastolic night-time mean blood pressure and nocturnal blood pressure load between the two groups will be assessed by Student's t-test. Within-person changes in each group will be assessed using paired Student's t-test.

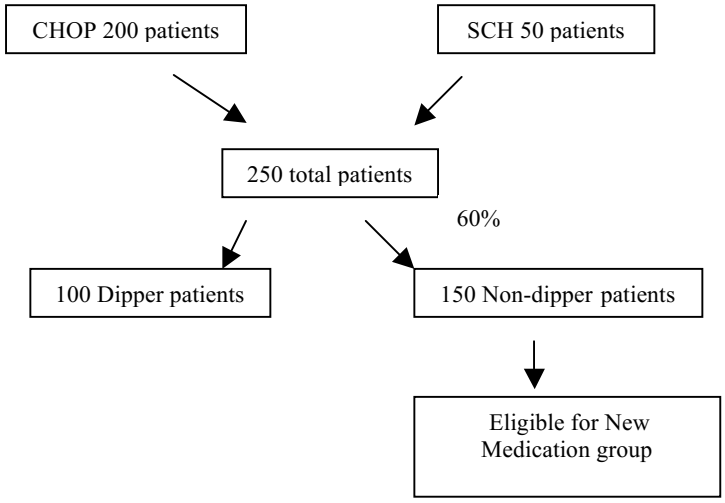
Secondary Aim #1: Student's t-test or Wilcoxon Rank Sum test for non-normally distributed variables will be used to compare values of albuminuria, LVMI and eGFR between groups at each time point. Paired tests will be used to assess within-person changes. Pearson correlation and linear regression will be used to examine the association between change in albuminuria, LVMI, PWV and eGFR and change in nocturnal mean blood pressure, N/D ratio and nocturnal BP load. Regression analysis will be adjusted for sex, Tanner stage, race, Pediatric Sleep Questionnaire Score, BMI z-score and mean blood pressure to evaluate the independent effect of the nocturnal dip. Variables may be log transformed to improve the fit of the models.

Secondary Aim #2: Similar to Aim #1.2, Student's t-test or Wilcoxon Rank Sum test will be used to compare values of total adiponectin and HMWr between groups at each time point. Within-person differences will be done using paired tests. Spearman correlation and linear regression will be used to examine the association between adiponectin and genetic tests with response to outcome (BP, albuminura, LVMI, pulse wave velocity, GFR). Regression analysis will be adjusted for sex, Tanner stage, race, Pediatric Sleep Questionnaire Score and BMI z-score. Variables may be log transformed to improve the fit of the models.

b. Sample Size Estimates

Sample size estimates were calculated for the primary outcome of percent change from non-dipper (N/D ratio > 0.9) to dipper status (N/D ratio ≤ 0.9). The comparison of 14 subjects in each group provides 80% power (α = 0.05) to detect a 50% change from non-dipper status to dipper status in the treatment groups compared to a 5% change in the control group. However, sample size estimates were also calculated for the secondary outcome measure of change in pulse wave velocity. The comparison of 20 subjects in each group provides 80% power (α = 0.05) to detect a 15% change in pulse wave velocity in the treatment group compared to the control group (Reference: Breise et al. 2008, PWV mean 5.43 +/- 0.9)[92]. The investigators believe that a sample size of 20 in each group is attainable and will therefore power the study according to the secondary outcome measure of change in pulse wave velocity.

c. Patient Flow



This patient flow diagram demonstrates that the target sample size is attainable over a three year period. Of note, there will be an estimated 30 new patients followed each additional year eligible for the study.

d. Limitations

The proposed study is limited by the small sample size and lack of a double-blinded placebo controlled design. However, the prospective randomized open label blinded end-point design has been previously used and validated for ABPM trials [79]. The heterogeneity of anti-hypertensive medications is also a limitation, but the design of two studies run in parallel was chosen to address this issue. Also, the children that choose to participate in the study may be more motivated and concerned with their health than those that choose not to participate (selection bias). We will record the subject and disease characteristics for all eligible subjects that decline participation. The difficulty in measuring residual confounding factors such as the complexity of ESRD course, past medical histories and fluctuating values of covariates (e.g. recent rejection episode) is also a limitation. Additionally, six months may be too short a time period to see the effect of changing from non-dipper to dipper status on end organ damage and function. Despite the aforementioned limitations, we believe that this study has many strengths and the results of the proposed study will be generalizable to the pediatric transplant population.

PART F. PROTECTION OF HUMAN SUBJECTS

Risk to Subjects

a. Human Subjects Involvement and Characteristics

The proposed study will involve human subjects, in particular, children and adolescents aged 5-21 years of age. The purpose of the study is to examine treatment of nocturnal hypertension in a pediatric population, therefore, other age groups are excluded from this study. Other inclusion/exclusion criteria are listed elsewhere in the protocol. This vulnerable population will be protected. Assent will be obtained from the participants and participants may withdraw from the study at any time.

b. Sources of Material

The subjects' medical record will be abstracted for clinical data. All other data (blood specimens, echo, pulse wave velocity, ABPM) will be obtained specifically for the proposed research.

c. Potential Risks

The study procedures stipulated in this proposal are non-invasive and pose minimal risk to the subjects. Peripheral blood draws may cause pain, bruising, blood clots under the skin, lightheadedness, fainting, and rarely, fainting. 24-hour ambulatory blood pressure monitoring (ABPM) may cause discomfort from blood pressure cuff inflation, difficulty sleeping, and rarely, small, purplish spots on the upper arm and bruising under the blood pressure cuff. Echocardiogram may cause local skin irritation that generally resolves without taking any action, from the gel used with the device that sends and receives sound waves. Pulse wave velocity measurement may cause local skin irritation from the adhesive on the disc from adhesive on the disc that generally resolves without taking any action.that generally resolves without taking any action. Subjects may feel embarrassed when looking at pictures for measures of growth and development.

The medications started for the intervention have potential side effects and incur moderate risk: Side effects of the new medications include: Enalapril: hypotension, dizziness, cough, hyperkalemia, nausea, vomiting, muscle weakness, rash, angioedema, increased creatinine, hyponatremia, kidney and liver failure. Isradapine: Swelling of the legs or arms, skin flushing, excessive growth of the gums, dizziness, headache, chest pain, disorders of the heart and blood vessels, fast heart rate(edema, tachycardia, dizziness, tiredness, fatigue, nausea, vomiting. Propranolol: Slow heart rate, low blood pressure, sleepiness, fatigue, interference with thinking or concentration, loss of appetite, nausea, vomiting, diarrhea, insomnia, feeling of "pins and needles" or numbness, depression, mental disorder with severe loss of contact with reality, or high blood sugar. Propanolol can interfere with blood clotting. Propanolol can cause skin irritation, rash, bumps, blisters or hives on the palms of the hands and soles of the feet, face, arms or legs. Propranolol can also cause a very serious disease involving the skin called Stevens-Johnson Syndrome. It usually starts with symptoms of a cold (fever, sore throat, chills, headache, tiredness), and sometimes nausea and vomiting, that may last from 1-14 days. This is followed by skin irritations that look like bulls eyes. They develop in clusters and are not itchy. They can occur anywhere, but are usually seen on the hands, and soles of the feet. They may occur inside the mouth and become very sore.

Genetic testing for SNPs of adiponectin also poses a moderate risk. The results of genetic testing will not be disclosed to the subject or treating clinical team. The alternative to participation in the study is to not participate in the study. Renal transplant recipients are not routinely screened for nocturnal hypertension by ABPM and therefore would not have an alternative treatment.

Adequacy of Protection of Risk

a. Recruitment and Informed Consent

Study subjects who meet the eligibility criteria will be approached in person by Nephrology physicians or the study coordinator in the Renal Transplant Clinic. Written informed consent from the participant, participant's parent or legal guardian and assent from the patients will be obtained before any study procedure is initiated. Participants who turn 18 years of age during the course of the study will be approached for informed consent. No study procedure will be continued in these participants unless informed consent is obtained. No advertisements will be used for recruitment of renal transplant patients.

b. Protection Against Risk

We will monitor for clinical adverse events throughout the study. We will report promptly report all unanticipated problems related to research to the IRB and GCRC. Refer to separate DSMP for specifics. Participation in all areas of the study is completely voluntary. Should the subjects become intolerant of any

aspect of the study, their participation (or the specific procedure) will be discontinued. Study investigators will be responsible for communicating to subjects information arising from the study (on harm or benefit), or from other research on the same topic, that could affect subjects' willingness to continue the study. Study investigators will give results of the echocardiography and ABPM to the treating physicians to discuss with the subject. All other aspects of the study will be kept confidential. Data will be stored in a locked cabinet and password protected file.

c. Potential Benefits to Subjects and Others

Ambulatory blood pressure monitoring will allow the medical team to identify patients with undiagnosed hypertension and uncontrolled hypertension. One group might receive more effective treatment and/or have fewer side effects than the other treatment groups. This study has considerable future potential benefit for all children with renal transplants.

d. Scientific Value

The effectiveness of a chronotherapeutic approach has not yet been explored in pediatric renal transplant recipients. If this study's hypotheses are proven correct, then this simple strategy may retard progression of renal dysfunction and protect against future cardiovascular morbidity and mortality in pediatric renal transplant recipients. The potential benefits from the information gathered from this study outweigh the risks.

PART G. INCLUSION OF WOMEN AND MINORITIES

All transplant patients who meet the above inclusion criteria will be invited to participate in the study regardless of gender or race. The study will include females and minorities consistent with the demographics of the transplant program.

PART H. INCLUSION OF CHILDREN

The proposed study will involve children and adolescents aged 5-21 years of age. The purpose of the study is to examine treatment of nocturnal hypertension in a pediatric population, therefore, other age groups are excluded from this study. The investigative team consists of pediatric trained physicians and nurses who have expertise with the pediatric population. Subjects will be recruited from children's hospitals with adequate facilities to accommodate children. Sample size analysis was done to ensure a sufficient number of subjects to contribute to a meaningful analysis relative to the purpose of the study.

PART I. DATA AND SAFETY MONITORING PLAN

Refer to separate DSMP.

IX. Payment to Subjects/Families

Subjects will be compensated $50 per study visit for parking, meals, transportation and the child's time and inconvenience for taking part in this study. Payment will be made at the completion of each visit.

X. Publication Policy

We plan to publish the results (positive or negative) of this study in academic journals and/or national meetings. We will not disclose protected health information about this study. Any proven evidence of falsification of data will be dealt with to take appropriate action against such unacceptable procedures.

PART J. REFERENCES

1. Belsha CW, Wells TG, McNiece KL, Seib PM, Plummer JK, Berry PL. Influence of diurnal blood pressure variations on target organ abnormalities in adolescents with mild essential hypertension. *Am J Hypertens.* 1998;11(4 Pt 1):410-417.
2. McNiece KL, Gupta-Malhotra M, Samuels J, et al. Left ventricular hypertrophy in hypertensive adolescents: analysis of risk by 2004 National High Blood Pressure Education Program Working Group staging criteria. *Hypertension.* 2007;50(2):392-395.
3. Sorof JM, Cardwell G, Franco K, Portman RJ. Ambulatory blood pressure and left ventricular mass index in hypertensive children. *Hypertension.* 2002;39(4):903-908.

4. Urbina E, Alpert B, Flynn J, et al. Ambulatory blood pressure monitoring in children and adolescents: recommendations for standard assessment: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee of the council on cardiovascular disease in the young and the council for high blood pressure research. *Hypertension.* 2008;52(3):433-451.
5. Lurbe E, Redon J. Reproducibility and validity of ambulatory blood pressure monitoring in children. *Am J Hypertens.* 2002;15(2 Pt 2):69S-73S.
6. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics.* 2004;114(2 Suppl 4th Report):555-576.
7. Soergel M, Kirschstein M, Busch C, et al. Oscillometric twenty-four-hour ambulatory blood pressure values in healthy children and adolescents: a multicenter trial including 1141 subjects. *J Pediatr.* 1997;130(2):178-184.
8. Hermida RC, Ayala DE, Portaluppi F. Circadian variation of blood pressure: the basis for the chronotherapy of hypertension. *Adv Drug Deliv Rev.* 2007;59(9-10):904-922.
9. McGlothan KR, Wyatt RJ, Ault BH, et al. Predominance of nocturnal hypertension in pediatric renal allograft recipients. *Pediatr Transplant.* 2006;10(5):558-564.
10. Pankow W, Nabe B, Lies A, et al. Influence of sleep apnea on 24-hour blood pressure. *Chest.* 1997;112(5):1253-1258.
11. Bakris GL, Williams M, Dworkin L, et al. Preserving renal function in adults with hypertension and diabetes: a consensus approach. National Kidney Foundation Hypertension and Diabetes Executive Committees Working Group. *Am J Kidney Dis.* 2000;36(3):646-661.
12. De Nicola L, Minutolo R, Bellizzi V, et al. Achievement of target blood pressure levels in chronic kidney disease: a salty question? *Am J Kidney Dis.* 2004;43(5):782-795.
13. Jafar TH, Stark PC, Schmid CH, et al. Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. *Ann Intern Med.* 2003;139(4):244-252.
14. de la Sierra A, Lluch MM, Coca A, et al. Assessment of salt sensitivity in essential hypertension by 24-h ambulatory blood pressure monitoring. *Am J Hypertens.* 1995;8(10 Pt 1):970-977.
15. Hornung RS, Mahler RF, Raftery EB. Ambulatory blood pressure and heart rate in diabetic patients: an assessment of autonomic function. *Diabet Med.* 1989;6(7):579-585.
16. Shimada K, Kawamoto A, Matsubayashi K, Nishinaga M, Kimura S, Ozawa T. Diurnal blood pressure variations and silent cerebrovascular damage in elderly patients with hypertension. *J Hypertens.* 1992;10(8):875-878.
17. Sachdeva A, Weder AB. Nocturnal sodium excretion, blood pressure dipping, and sodium sensitivity. *Hypertension.* 2006;48(4):527-533.
18. Kario K, Motai K, Mitsuhashi T, et al. Autonomic nervous system dysfunction in elderly hypertensive patients with abnormal diurnal blood pressure variation: relation to silent cerebrovascular disease. *Hypertension.* 1997;30(6):1504-1510.
19. Jensen LW, Pedersen EB. Nocturnal blood pressure and relation to vasoactive hormones and renal function in hypertension and chronic renal failure. *Blood Press.* 1997;6(6):332-342.
20. Thompson AM, Pickering TG. The role of ambulatory blood pressure monitoring in chronic and end-stage renal disease. *Kidney Int.* 2006;70(6):1000-1007.
21. Clement DL, De Buyzere ML, De Bacquer DA, et al. Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *N Engl J Med.* 2003;348(24):2407-2415.
22. White WB. Ambulatory blood-pressure monitoring in clinical practice. *N Engl J Med.* 2003;348(24):2377-2378.
23. Verdecchia P, Porcellati C, Schillaci G, et al. Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. *Hypertension.* 1994;24(6):793-801.
24. Cuspidi C, Meani S, Salerno M, et al. Cardiovascular target organ damage in essential hypertensives with or without reproducible nocturnal fall in blood pressure. *J Hypertens.* 2004;22(2):273-280.

25. Staessen JA, Thijs L, Fagard R, et al. Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. Systolic Hypertension in Europe Trial Investigators. *JAMA.* 1999;282(6):539-546.
26. Bianchi S, Bigazzi R, Baldari G, Sgherri G, Campese VM. Diurnal variations of blood pressure and microalbuminuria in essential hypertension. *Am J Hypertens.* 1994;7(1):23-29.
27. Timio M, Venanzi S, Lolli S, et al. "Non-dipper" hypertensive patients and progressive renal insufficiency: a 3-year longitudinal study. *Clin Nephrol.* 1995;43(6):382-387.
28. (NAPRTCS) NAPTCS. *Annual Report.* 2007.
29. Mitsnefes MM, Portman RJ. Ambulatory blood pressure monitoring in pediatric renal transplantation. *Pediatr Transplant.* 2003;7(2):86-92.
30. Giordano U, Matteucci MC, Calzolari A, Turchetta A, Rizzoni G, Alpert BS. Ambulatory blood pressure monitoring in children with aortic coarctation and kidney transplantation. *J Pediatr.* 2000;136(4):520-523.
31. Calzolari A, Giordano U, Matteucci MC, et al. Hypertension in young patients after renal transplantation: ambulatory blood pressure monitoring versus casual blood pressure. *Am J Hypertens.* 1998;11(4 Pt 1):497-501.
32. Kitzmueller E, Vecsei A, Pichler J, et al. Changes of blood pressure and left ventricular mass in pediatric renal transplantation. *Pediatr Nephrol.* 2004;19(12):1385-1389.
33. Krmar RT, Berg UB. Blood pressure control in hypertensive pediatric renal transplants: role of repeated ABPM following transplantation. *Am J Hypertens.* 2008;21(10):1093-1099.
34. Krull F, Hegerhorst A, Offner G, Hoyer PF, Brodehl J. 24-hour blood pressure monitoring for the control of antihypertensive therapy in pediatric kidney transplant recipients. *Transplant Proc.* 1993;25(4):2575-2576.
35. Lingens N, Dobos E, Witte K, et al. Twenty-four-hour ambulatory blood pressure profiles in pediatric patients after renal transplantation. *Pediatr Nephrol.* 1997;11(1):23-26.
36. Matteucci MC, Giordano U, Calzolari A, Turchetta A, Santilli A, Rizzoni G. Left ventricular hypertrophy, treadmill tests, and 24-hour blood pressure in pediatric transplant patients. *Kidney Int.* 1999;56(4):1566-1570.
37. Mitsnefes MM. Hypertension and end-organ damage in pediatric renal transplantation. *Pediatr Transplant.* 2004;8(4):394-399.
38. Morgan H, Khan I, Hashmi A, Hebert D, McCrindle BW, Balfe JW. Ambulatory blood pressure monitoring after renal transplantation in children. *Pediatr Nephrol.* 2001;16(11):843-847.
39. Serdaroglu E, Mir S, Berdeli A. Hypertension and ace gene insertion/deletion polymorphism in pediatric renal transplant patients. *Pediatr Transplant.* 2005;9(5):612-617.
40. Sorof JM, Goldstein SL, Brewer ED, Steiger HM, Portman RJ. Use of anti-hypertensive medications and post-transplant renal allograft function in children. *Pediatr Transplant.* 2000;4(1):21-27.
41. Buscher R, Vester U, Wingen AM, Hoyer PF. Pathomechanisms and the diagnosis of arterial hypertension in pediatric renal allograft recipients. *Pediatr Nephrol.* 2004;19(11):1202-1211.
42. Covic A, Gusbeth-Tatomir P, Mardare N, Buhaescu I, Goldsmith DJ. Dynamics of the circadian blood pressure profiles after renal transplantation. *Transplantation.* 2005;80(9):1168-1173.
43. Lipkin GW, Tucker B, Giles M, Raine AE. Ambulatory blood pressure and left ventricular mass in cyclosporin- and non-cyclosporin-treated renal transplant recipients. *J Hypertens.* 1993;11(4):439-442.
44. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis.* 1998;32(5 Suppl 3):S112-119.
45. Agarwal R, Andersen MJ. Blood pressure recordings within and outside the clinic and cardiovascular events in chronic kidney disease. *Am J Nephrol.* 2006;26(5):503-510.
46. Liu M, Takahashi H, Morita Y, et al. Non-dipping is a potent predictor of cardiovascular mortality and is associated with autonomic dysfunction in haemodialysis patients. *Nephrol Dial Transplant.* 2003;18(3):563-569.

47. Toprak A, Koc M, Tezcan H, Ozener IC, Oktay A, Akoglu E. Night-time blood pressure load is associated with higher left ventricular mass index in renal transplant recipients. *J Hum Hypertens.* 2003;17(4):239-244.
48. Mitsnefes MM, Omoloja A, McEnery PT. Short-term pediatric renal transplant survival: blood pressure and allograft function. *Pediatr Transplant.* 2001;5(3):160-165.
49. Sorof JM, Sullivan EK, Tejani A, Portman RJ. Antihypertensive medication and renal allograft failure: a North American Pediatric Renal Transplant Cooperative Study report. *J Am Soc Nephrol.* 1999;10(6):1324-1330.
50. Agarwal R, Andersen MJ. Prognostic importance of ambulatory blood pressure recordings in patients with chronic kidney disease. *Kidney Int.* 2006;69(7):1175-1180.
51. Davidson MB, Hix JK, Vidt DG, Brotman DJ. Association of impaired diurnal blood pressure variation with a subsequent decline in glomerular filtration rate. *Arch Intern Med.* 2006;166(8):846-852.
52. Wadei HM, Amer H, Taler SJ, et al. Diurnal blood pressure changes one year after kidney transplantation: relationship to allograft function, histology, and resistive index. *J Am Soc Nephrol.* 2007;18(5):1607-1615.
53. McGregor DO, Olsson C, Lynn KL. Autonomic dysfunction and ambulatory blood pressure in renal transplant recipients. *Transplantation.* 2001;71(9):1277-1281.
54. Kooman JP, Christiaans MH, Boots JM, van Der Sande FM, Leunissen KM, van Hooff JP. A comparison between office and ambulatory blood pressure measurements in renal transplant patients with chronic transplant nephropathy. *Am J Kidney Dis.* 2001;37(6):1170-1176.
55. Hermida RC, Ayala DE, Calvo C, Portaluppi F, Smolensky MH. Chronotherapy of hypertension: administration-time-dependent effects of treatment on the circadian pattern of blood pressure. *Adv Drug Deliv Rev.* 2007;59(9-10):923-939.
56. Minutolo R, Gabbai FB, Borrelli S, et al. Changing the timing of antihypertensive therapy to reduce nocturnal blood pressure in CKD: an 8-week uncontrolled trial. *Am J Kidney Dis.* 2007;50(6):908-917.
57. Portaluppi F, Vergnani L, Manfredini R, degli Uberti EC, Fersini C. Time-dependent effect of isradipine on the nocturnal hypertension in chronic renal failure. *Am J Hypertens.* 1995;8(7):719-726.
58. Hermida RC, Calvo C, Ayala DE, Lopez JE. Decrease in urinary albumin excretion associated with the normalization of nocturnal blood pressure in hypertensive subjects. *Hypertension.* 2005;46(4):960-968.
59. Hermida RC. Ambulatory blood pressure monitoring in the prediction of cardiovascular events and effects of chronotherapy: rationale and design of the MAPEC study. *Chronobiol Int.* 2007;24(4):749-775.
60. Wang Y, Lam KS, Yau MH, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. *Biochem J.* 2008;409(3):623-633.
61. Adamczak M, Wiecek A, Funahashi T, Chudek J, Kokot F, Matsuzawa Y. Decreased plasma adiponectin concentration in patients with essential hypertension. *Am J Hypertens.* 2003;16(1):72-75.
62. Iwashima Y, Katsuya T, Ishikawa K, et al. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension.* 2004;43(6):1318-1323.
63. Kazumi T, Kawaguchi A, Sakai K, Hirano T, Yoshino G. Young men with high-normal blood pressure have lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. *Diabetes Care.* 2002;25(6):971-976.
64. Li HY, Chiu YF, Hwu CM, et al. The negative correlation between plasma adiponectin and blood pressure depends on obesity: a family-based association study in SAPPHIRe. *Am J Hypertens.* 2008;21(4):471-476.
65. Della Mea P, Lupia M, Bandolin V, et al. Adiponectin, insulin resistance, and left ventricular structure in dipper and nondipper essential hypertensive patients. *Am J Hypertens.* 2005;18(1):30-35.
66. Fallo F, Pozza AD, Sonino N, et al. Nonalcoholic fatty liver disease, adiponectin and insulin resistance in dipper and nondipper essential hypertensive patients. *J Hypertens.* 2008;26(11):2191-2197.
67. Chow WS, Cheung BM, Tso AW, et al. Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study. *Hypertension.* 2007;49(6):1455-1461.

68. Imatoh T, Miyazaki M, Momose Y, Tanihara S, Une H. Adiponectin levels associated with the development of hypertension: a prospective study. *Hypertens Res.* 2008;31(2):229-233.
69. Filippi E, Sentinelli F, Romeo S, et al. The adiponectin gene SNP+276G>T associates with early-onset coronary artery disease and with lower levels of adiponectin in younger coronary artery disease patients (age <or=50 years). *J Mol Med.* 2005;83(9):711-719.
70. Hara K, Boutin P, Mori Y, et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes.* 2002;51(2):536-540.
71. Heid IM, Wagner SA, Gohlke H, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. *Diabetes.* 2006;55(2):375-384.
72. Saarela T, Hiltunen M, Helisalmi S, Heinonen S, Laakso M. Adiponectin gene haplotype is associated with preeclampsia. *Genet Test.* 2006;10(1):35-39.
73. Kovesdy CP, Quarles LD. Fibroblast growth factor-23: what we know, what we don't know, and what we need to know. *Nephrol Dial Transplant.*28(9):2228-2236.
74. Wan M, Smith C, Shah V, et al. Fibroblast growth factor 23 and soluble klotho in children with chronic kidney disease. *Nephrol Dial Transplant.*28(1):153-161.
75. Adamczak M, Szotowska M, Chudek J, Karkoszka H, Cierpka L, Wiecek A. Plasma adiponectin concentration in patients after successful kidney transplantation--a single-center, observational study. *Clin Nephrol.* 2007;67(6):381-390.
76. Teplan V, Schuck O, Stollova M, Vitko S. Obesity and adiponectin after kidney transplantation. *Acta Physiol Hung.* 2007;94(1-2):149-157.
77. Chudek J, Adamczak M, Karkoszka H, et al. Plasma adiponectin concentration before and after successful kidney transplantation. *Transplant Proc.* 2003;35(6):2186-2189.
78. Shen YY, Charlesworth JA, Kelly JJ, Peake PW. The effect of renal transplantation on adiponectin and its isoforms and receptors. *Metabolism.* 2007;56(9):1201-1208.
79. Patel JV, Lim HS, Hughes EA, Lip GY. Adiponectin and hypertension: a putative link between adipocyte function and atherosclerotic risk? *J Hum Hypertens.* 2007;21(1):1-4.
80. Smith DH, Neutel JM, Lacourciere Y, Kempthorne-Rawson J. Prospective, randomized, open-label, blinded-endpoint (PROBE) designed trials yield the same results as double-blind, placebo-controlled trials with respect to ABPM measurements. *J Hypertens.* 2003;21(7):1291-1298.
81. Witte K, Weisser K, Neubeck M, et al. Cardiovascular effects, pharmacokinetics, and converting enzyme inhibition of enalapril after morning versus evening administration. *Clin Pharmacol Ther.* 1993;54(2):177-186.
82. Ogden CL, Kuczmarski RJ, Flegal KM, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. *Pediatrics.* 2002;109(1):45-60.
83. Krebs NF, Jacobson MS. Prevention of pediatric overweight and obesity. *Pediatrics.* 2003;112(2):424-430.
84. Morris N, Udry, J. Validation of a self-administered instrument to assess stage of adolescent development *Journal of Youth and Adolescence,.* 1980;9(3):271-280.
85. Tanner JM. *Assessment of skeletal maturity and prediction of adult height (TW2 method).* London ; New York: Academic Press; 1975.
86. Schwartz GJ, Feld LG, Langford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. *J Pediatr.* 1984;104(6):849-854.
87. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care.* 1997;20(7):1087-1092.
88. Lai WW, Geva T, Shirali GS, et al. Guidelines and standards for performance of a pediatric echocardiogram: a report from the Task Force of the Pediatric Council of the American Society of Echocardiography. *J Am Soc Echocardiogr.* 2006;19(12):1413-1430.

89. Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol.* 1986;57(6):450-458.
90. de Simone G, Daniels SR, Devereux RB, et al. Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. *J Am Coll Cardiol.* 1992;20(5):1251-1260.
91. USRDS. Annual Report. 2006.
92. Atkinson MJ, Sinha A, Hass SL, et al. Validation of a general measure of treatment satisfaction, the Treatment Satisfaction Questionnaire for Medication (TSQM), using a national panel study of chronic disease. *Health Qual Life Outcomes.* 2004;2:12.

APPENDICES

Pediatric Sleep Questionnaire

Patient's Name: _____ Date:_____

Name of individual completing questionnaire: _____

Relationship to patient: _____

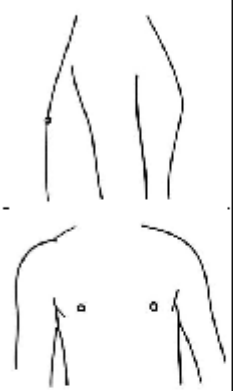
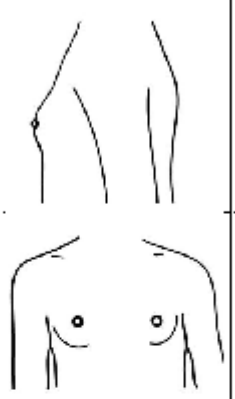
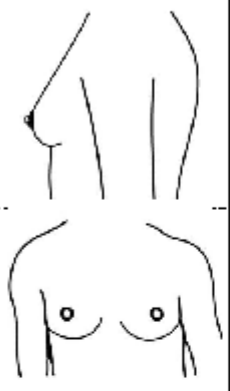
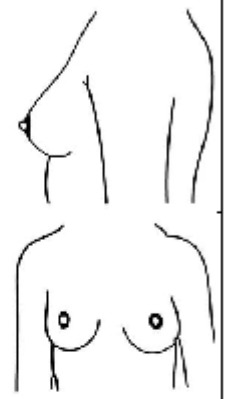
Please answer the following questions by checking the box under the yes, no, or don't know column to the right.

While sleeping, does your child…	YES	NO	Don't know
…snore more than half the time?			
…always snore?			
…snore loudly?			
…have "heavy" or loud breathing?			
…have trouble breathing, or struggle to breathe?			
Have you ever…			
…seen your child stop breathing during the night?			
Does your child…			
…tend to breathe through the mouth during the day?			
…have a dry mouth on waking up in the morning?			
…occasionally wet the bed?			
Does your child…			
…wake up felling *un*refreshed in the morning?			
…have a problem with sleepiness during the day?			
Has a teacher or other supervisor commented that your child appears sleepy during the day?			
Is it hard to wake your child up in the morning?			
Does your child wake up with headaches in the morning?			
Did your child stop growing at a normal rate at any time since birth?			
Is your child overweight?			
This child often…			
…does not seem to listen when spoken to directly			
…has difficulty organizing task and activities			
…is easily distracted by extraneous stimuli			
This child often…			
…fidgets with hands or feet or squirms in seat			
…is 'on the go' of often acts as if 'driven by a motor'			
…interrupts or intrudes on others (e.g. butts into conversations or games)			

From RD Chervin, et. al., Sleep Medicine, 2000, p. 21-32

GIRLS SELECT ONE FROM EACH SET OF DRAWINGS BELOW.

SET ONE: The drawings below show 5 different stages of how the breasts grow. A girl can go through each of the 5 stages as shown. Please look at each drawing and read the sentences that match the drawings. Then, mark an "X" in the box above the drawing that you think is closest to your stage of breast growth.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
The nipple is raised a little. The rest of the breast is still flat.	This is the breast bud stage. In this stage, the nipple is raised more than in stage 1. The breast is a small mound. The areola is larger than stage 1.	The breast and areola are both larger than in stage 2. The areola does not stick out away from the breast.	The areola and the nipple make up a mound that sticks up above the shape of the breast. NOTE: This stage may not happen at all for some girls. Some girls develop from stage 3 to stage 5 with no stage 4.	This is the mature adult stage. The breasts are fully developed. Only the nipple sticks out in this stage. The areola has moved back in the general shape of the breast.

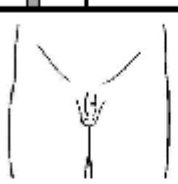
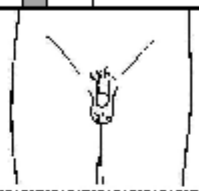
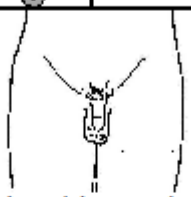
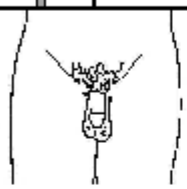
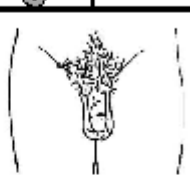
SET TWO: The drawings below show 5 different stages of female pubic hair growth. A girl can go through each of the 5 stages as shown. Please look at each drawing and read the sentences that match the drawings. Then, mark an "X" in the box above the drawing that you think is closest to your stage of pubic hair growth.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
There is no pubic hair at all.	There is a little soft, long, lightly-colored hair. This hair may be straight or a little curly.	There hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a bigger area.	The hair is now as dark, curly, and coarse as that of an adult female. The area that the hair covers is not as big as that of an adult female. The hair has NOT spread out to the legs.	The hair is now like that of an adult female. It covers the same area as that of an adult female. The hair usually forms a triangluar pattern as it spread out to the legs.

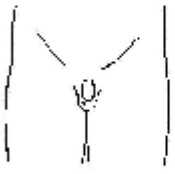
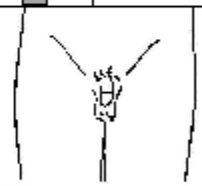
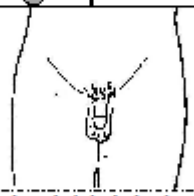
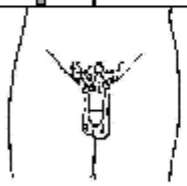
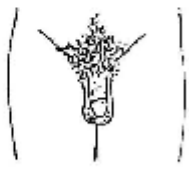
Adapted from: Morris, N M and Udry, J R. (1980). Validation of a Self-Administered Instrument to Assess Stage of Pubertal Development. *Journal of Youth and Adolescence*. 9:271-280.

BOYS SELECT ONE FROM EACH SET OF DRAWINGS BELOW.

SET ONE: The drawings below show 5 different stages of testes, scrotum, and penis growth. A boy can go through each of the 5 stages as shown. Please look at each drawing and read the sentences that match the drawings. Then, mark an "X" in the box above the drawing that you think is closest to your stage of testes, scrotum, and penis growth. Do not look at or select for pubic hair growth with this set of drawings.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
The testes, scrotum, and penis are about the same size and shape as they were when you were a child.	The testes and scrotum are bigger. The skin of the scrotum has changed. The scrotum, the sack holding the testes, has gotten lower. The penis has gotten only a little bigger.	The penis has grown in length. The testes and scrotum have grown and dropped lower than in drawing 2.	The penis has gotten even bigger. It is wider. The glans (the head of the penis) is bigger. The scrotum is darker than before. It is bigger because the testes are bigger.	The penis, scrotum, and testes are the size and shape of an adult man.

SET TWO: The drawings below show 5 different stages of male pubic hair growth. A boy can go through each of the 5 stages as shown. Please look at each drawing and read the sentences that math the drawings. Then, mark an "X" in the box above the drawing that you think is closest to your stage of pubic hair growth. Do not look at or select for penis size with this set of drawings.

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
There is no pubic hair at all.	There is a little soft, long, lightly-colored hair. Most of the hair is at the base of the penis. This hair may be straight or a little curly.	There hair is darker in this stage. It is more curled. It has spread out and thinly covers a bigger area.	The hair is now as dark, curly, and coarse as that of an adult male. The area that the hair covers is not as big as that of an adult male. The hair has NOT spread out to the legs.	The hair has spread out to the legs. The hair is now like that of an adult male. It covers the same area as that of an adult male.

Adapted from: Morris, N M and Udry, J R. (1980). Validation of a Self-Administered Instrument to Assess Stage of Pubertal Development. *Journal of Youth and Adolescence*. 9:271-280.

TSQM *(Version 1.4)*

Treatment Satisfaction Questionnaire for Medication

Instructions: Please take some time to think about your level of satisfaction or dissatisfaction with the medication you are taking in this clinical trial. We are interested in your evaluation of the effectiveness, side effects, and convenience of the medication over the last two to three weeks, or since you last used it. For each question, please place a single check mark next to the response that most closely corresponds to your own experiences.

1. How satisfied or dissatisfied are you with the ability of the medication to prevent or treat your condition?

1 Extremely Dissatisfied
2 Very Dissatisfied
3 Dissatisfied
4 Somewhat Satisfied
5 Satisfied
6 Very Satisfied
7 Extremely Satisfied

2. How satisfied or dissatisfied are you with the way the medication relieves your symptoms?

1 Extremely Dissatisfied
2 Very Dissatisfied
3 Dissatisfied
4 Somewhat Satisfied
5 Satisfied
6 Very Satisfied
7 Extremely Satisfied

3. How satisfied or dissatisfied are you with the amount of time it takes the medication to start working?

1 Extremely Dissatisfied
2 Very Dissatisfied
3 Dissatisfied
4 Somewhat Satisfied
5 Satisfied
6 Very Satisfied
7 Extremely Satisfied

4. As a result of taking this medication do you experience any side effects at all?

1 Yes
0 No (if No, then please skip to Question 9)

5. How bothersome are the side effects of the medication you take to treat your condition?

1 Extremely Bothersome
2 Very Bothersome
3 Somewhat Bothersome
4 A Little Bothersome

5 Not at All Bothersome

6. To what extent do the side effects interfere with your physical health and ability to function (i.e., strength, energy levels, etc.)?

1 A Great Deal
2 Quite a Bit
3 Somewhat
4 Minimally
5 Not at All

7. To what extent do the side effects interfere with your mental function (i.e., ability to think clearly, stay away, etc.)?

1 A Great Deal
2 Quite a Bit
3 Somewhat
4 Minimally
5 Not at All

8. To what degree have medication side effects affected your overall satisfaction with the medication?

1 A Great Deal
2 Quite a Bit
3 Somewhat
4 Minimally
5 Not at All

9. How easy or difficult is it to use the medication in its current form?

1 Extremely Difficult
2 Very Difficult
3 Difficult
4 Somewhat Easy
5 Easy
6 Very Easy
7 Extremely Easy

10. How easy or difficult is it to plan when you will use the medication each time

1 Extremely Difficult
2 Very Difficult
3 Difficult
4 Somewhat Easy
5 Easy
6 Very Easy
7 Extremely Easy

11. How convenient or inconvenient is it to take the medication as instructed?

1 Extremely Inconvenient
2 Very Inconvenient
3 Inconvenient
4 Somewhat Convenient
5 Convenient
6 Very Convenient
7 Extremely Convenient

12. Overall, how confident are you that taking this medication is a good thing for you?

1 Not at All Convenient
2 A Little Convenient
3 Somewhat Convenient
4 Very Convenient
5 Extremely Convenient

13. How certain are you that the good things about your medication outweigh the bad things?

1 Not at All Certain
2 A Little Certain
3 Somewhat Certain
4 Very Certain
5 Extremely Certain

14. Taking all things into account, how satisfied or dissatisfied are you with this medication.

1 Extremely Dissatisfied
2 Very Dissatisfied
3 Dissatisfied
4 Somewhat Satisfied
5 Satisfied
6 Very Satisfied
7 Extremely Satisfied

TSQM Scoring Algorithm

TSQM Scale scores computed by adding the items loading on each factor. The lowest possible score is subtracted from this composite score and divided by the greatest possible score minus the lowest possible score. This provided a transformed score between 0 and 1 that should be multiplied by 100. (see below) [Note that only one item may be missing from each scale before the subscale should be considered invalid for that respondent]

EFFECTIVENESS
([(Item 1 + Item 2 + Item 3) – 3] divided by 18) * 100

If one item is missing

([(Sum(Item 1? + Item 2? + Item 3?)) – 2] divided by 12) * 100

SIDE-EFFECTS
If Question 4 is answered 'No' then score = 100
Else…
([Sum(Item 5 to Item 8) – 4] divided by 16) * 100

If one item is missing

([(Sum(Item5? to Item8?)) – 3] divided by 12) * 100

CONVENIENCE
([Sum(Item 9 to Item 11) – 3] divided by 18) * 100

If one item is missing

([(Sum(Item9? to Item11?)) – 2] divided by 12) * 100

GLOBAL SATISFACTION
([Sum(Item 12 to Item 14) – 3] divided by 14) * 100

If either Item 12 or 13 is missing

([(Sum(Item12? to Item14?)) – 2] divided by 10) * 100

If Item 14 is missing

([(Sum(Item12 and Item13)) – 2] divided by 8) * 100