Study Protocol and Statistical Analysis Plan
Project Title: Curcumin Therapy to Treat Vascular Dysfunction in Children and Young Adults with ADPKD
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Principal Investigator: Kristen Nowak, Ph.D.
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Specific Aims

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening genetic disease, affecting more than 600,000 Americans [1,2]. Although often considered to be a disease of adults, **complications of ADPKD begin in childhood** [3]. While the hallmark of ADPKD is the development and continued growth of multiple renal cysts that result in ultimate loss of kidney function [4], the leading cause of death among affected patients is cardiovascular in nature [1,2]. As much as 80% of all cardiovascular diseases (CVD) are associated with dysfunction and disorders of arteries [5]. Two of the greatest contributors are vascular endothelial dysfunction, most commonly assessed as impaired **endothelium-dependent dilation (EDD)**, and **stiffening of the large elastic arteries** (aorta and carotid arteries) [6]. Importantly, both measures are independent predictors of future cardiovascular events and mortality [7-10].

Adults with ADPKD demonstrate impaired EDD [11-13] as well as large elastic artery stiffening [14], even in the absence of hypertension. The mechanisms by which arterial dysfunction occurs in ADPKD are incompletely understood, but appear to involve increased oxidative stress and inflammation [15-17]. My preliminary data in children/young adults with ADPKD support that arterial dysfunction develops **very early** in the course of the disease, as evidenced by impaired **brachial artery flow-mediated dilation (FMD_{BA}**; a measure of EDD) and increased **aortic pulse-wave velocity (aPWV**; a measure of large elastic artery stiffness). There are presently very limited treatment options for the prevention of cardiovascular disease in adults with ADPKD, thus, childhood/young adulthood may represent a **critical therapeutic window**.

An increase in **total kidney volume (TKV)** precedes the decline in kidney function, which is typically delayed until the 4th decade of life in patients with ADPKD, and TKV is a prognostic biomarker of future kidney function decline [18]. Thus, a decrease in rate of TKV growth, as measured by **magnetic resonance imaging (MRI)** is an important indicator of kidney disease progression early in the course of ADPKD.

Curcumin is a safe, naturally occurring polyphenol found in the Indian spice turmeric that has a unique ability to activate transcription of key antioxidants, suppress inflammation, and reduce proliferation. Curcumin reduces arterial dysfunction in various rodent models of vascular dysfunction [19,20], and my preliminary data demonstrate that it also reduces age-associated vascular dysfunction in humans. Importantly, curcumin also slows cyst growth in kidney cells *in vitro* [21] and *in vivo* [22]. Thus, curcumin is a promising ***nutraceutical*** to slow progression of renal and cardiovascular disease in children with ADPKD. However, there is presently **no information available** regarding the potential therapeutic benefits of curcumin to treat arterial dysfunction and slow kidney growth in this population.

Accordingly, the **primary aim** of this application is to determine the efficacy of curcumin for improving FMD_{BA} and reducing aPWV in 68 children/young adults with ADPKD. A key **secondary goal** is to obtain insight into the mechanisms by which curcumin therapy may reduce arterial dysfunction. Last, a **third exploratory aim** is to determine if treatment with curcumin slows kidney growth. These aims will be tested in a double-blind, placebo-controlled, randomized clinical trial conducted in children/young adults aged 6-25 years with ADPKD.

Hypothesis 1: Oral curcumin therapy will reduce vascular endothelial dysfunction (increase EDD) and large elastic artery stiffness in children and young adults with ADPKD as compared to placebo.

Specific Aim 1: To determine **non-invasively**: a) FMD_{BA} and b) aPWV before and after 12 months of oral curcumin (25 mg/kg/day) or placebo; and after 6 months in a sub-set of all local patients.

Hypothesis 2: The improvements in arterial function observed with oral curcumin treatment will be associated with reduced oxidative stress and inflammation.

Specific Aim 2: In a subset of patients aged 18-25 years, to measure aPWV and FMD_{BA} during normal vs. reduced (via acute ascorbic acid infusion) oxidative stress, and to measure circulating/urine markers of oxidative stress and inflammation before and after 12 months of oral curcumin or placebo.

Hypothesis 3: Oral curcumin therapy will reduce the rate of kidney growth in children and young adults with ADPKD as compared to placebo.

Specific Aim 3: To quantify TKV measured with MRI before and after 12 months of oral curcumin or placebo.

Impact on the field. This study has the potential to establish **a novel, safe, and easy to deliver therapy** for the treatment of arterial dysfunction, and possibly renal cystic disease, in an understudied population of children and young adults with ADPKD.

Research Strategy

Significance

Autosomal Dominant Polycystic Kidney Disease (ADPKD) and Cardiovascular Disease (CVD). ADPKD is the most common lethal genetic kidney disorder [1,2]. While the hallmark of ADPKD is the development and continued growth of multiple renal cysts that result in ultimate loss of kidney function [4], the leading cause of death among affected patients is cardiovascular in nature [1,2]. The proteins encoded by the PKD-1 and -2 genes, polycystin-1 and -2, are expressed in vascular endothelial cells and smooth muscle cells of all major vessels, resulting in extrarenal manifestations of the disease [23]. There are presently very limited treatment options for the prevention of CVD in patients with ADPKD.

ADPKD in Children and Young Adults. Although often considered to be a disease of adults, ADPKD begins in childhood [3] and can in fact be diagnosed *in utero* [24]. Even in children with ADPKD, evidence of CV abnormalities begin to manifest, such as elevated blood pressure and increased left ventricular mass index [25-27]. There are presently very limited treatment options for the prevention of CVD in adults with ADPKD. Thus, childhood/early adulthood may represent a **critical therapeutic window** to prevent future CV complications.

Vascular Function and CVD. As much as 80% of all CVD is associated with dysfunction and disorders of arteries [5]. Two of the greatest contributors are the development of vascular endothelial dysfunction, most commonly assessed as impaired endothelium-dependent dilation (EDD), and stiffening of the large elastic arteries [6], both of which independently predict of future cardiovascular events and mortality [7-10].

Vascular Function in ADPKD. Adults with ADPKD demonstrate impaired EDD [11-13] as well as large elastic artery stiffening [14]. Endothelial dysfunction occurs early in ADPKD and has been detected in normotensive adult patients with preserved kidney function [11-13]. My **preliminary data** in children and young adults support that vascular dysfunction develops **very early** in the disease, as evidenced by **impaired brachial artery flow-mediated dilation (FMD_{BA}**; a measure of EDD) and **increased aortic pulse-wave velocity (aPWV**; a measure of large elastic artery stiffness). Targeting vascular dysfunction early in the course of ADPKD is more likely to alter the long-term course of the disease compared to later intervention, thus strategies to reduce vascular dysfunction in children and young adults with ADPKD have the potential for huge clinical impact.

Integrative Physiological Mechanisms of ADPKD and Vascular Dysfunction. The mechanisms by which vascular dysfunction develops in ADPKD are incompletely understood; however, reduced nitric oxide (NO) bioavailability is a critical contributor [12,17] and a common mechanism of both impaired EDD and increased arterial stiffness [6,28]. In ADPKD, oxidative stress and inflammation are increased and contribute to the decline in NO bioavailability [15-17]. Oxidative stress is defined as excessive bioavailability of reactive oxygen species (ROS) relative to antioxidant defenses. Physiological stimuli, including inflammatory signaling, promote oxidative enzyme systems to produce ROS, including superoxide anion ($O_2^{\bullet -}$) [29]. Superoxide reduces bioavailability of nitric oxide (NO) by a) reacting directly with NO to form peroxynitrite (ONOO-), another ROS; or b) oxidizing the essential cofactor (tetrahydrobiopterin) for NO synthesis by endothelial nitric oxide synthase (eNOS) [30,31]. Of note, plasma levels of asymmetric dimethylarginine (ADMA), an important inhibitor of eNOS, are increased in adult ADPKD patients with preserved kidney function [17]. Inflammatory signaling can also directly reduce NO bioavailability by down-regulating eNOS activity [32]. In turn, increased ROS stimulate pro-inflammatory gene transcription and protein expression via the redox-sensitive pro-inflammatory transcription factor nuclear factor κ B (NFκB), thus further promoting a pro-inflammatory cascade in a vicious cycle [33,34].

Total Kidney Volume in ADPKD. An increase in **total kidney volume (TKV)** precedes the decline in kidney function, which is typically delayed until the 4th decade of life in patients with ADPKD, and TKV is a prognostic biomarker of future renal insufficiency [18]. TKV is a critical marker of disease progression in children with ADPKD, as renal function remains normal in nearly all affected children despite marked changes in kidney structure [3]. Thus, a decrease in rate of TKV growth, as measured by **magnetic resonance imaging (MRI)** is an important indicator of renal disease progression early in the course of ADPKD.

Therapeutic Potential of Curcumin. Curcumin, from the plant *curcuma longa*, is a safe, naturally occurring polyphenol found in the Indian spice turmeric that has long been used in traditional Indian medicine (see below

for safety information). *In vivo*, supplementation with curcumin improves physiological function in animal models of various diseases [35-37]. Although the exact mechanism of action is unknown, curcumin suppresses both oxidative stress and inflammation *in vitro* and *in vivo* [38-42]. Consequently, curcumin has been proposed as a **safe** and **novel *nutraceutical*** for intervention in several disease states [43].

Curcumin and Vascular Dysfunction. Curcumin administration reduces vascular dysfunction (increases EDD and/or reduces arterial stiffness) in rodent models of hypertension [20], diabetes [36,37], and aging [42]. These improvements are associated with increased vascular NO bioavailability and arterial eNOS expression, reduced vascular oxidative stress (decreased ROS, reduced oxidative damage, and increased antioxidant enzymes), and increased activity of hemoxygenase-1, which can promote anti-inflammatory pathways [20,36,37,42]. In addition, my **preliminary data** support that curcumin also reduces vascular dysfunction associated with aging in healthy humans, consistent with published FMD_{BA} data in post-menopausal women [44]. However, there is presently **no information** available regarding the potential therapeutic benefits of curcumin to treat vascular dysfunction in ADPKD, including children and young adults.

Curcumin and Total Kidney Volume. Curcumin has been of interest in nephrology for over a decade as a potential treatment of acute kidney injury (AKI) or chronic renal impairment in various rodent models of disease. In addition to reducing histological evidence of kidney damage [45-48], its use in these models is associated with reduced evidence of oxidative stress and increased antioxidant activity [46,49,50], as well as reduced activation of the pro-inflammatory transcription factor NFκB and its downstream target tumor necrosis factor-α (TNF-α), concomitant with an increase in the anti-inflammatory transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) [48]. Notably, curcumin also slows cyst growth *in vitro* in a dose-response manner, using both the Madin–Darby canine kidney (MDCK) cell cyst model and an embryonic kidney cyst model [21]. Similarly, in the Pkd-1 deletion mouse model, curcumin improves renal histology and reduces proliferative index, cystic index, and kidney weight (normalized to total body weight) [22]. Thus, curcumin may also decrease the rate of TKV growth in children and young adults with ADPKD.

In summary, cardiovascular complications are the leading cause of death in adults with ADPKD and are characterized by vascular dysfunction, which occurs very early in the course of the disease. Intervening in affected children and young adults may represent a **critical therapeutic window** for the prevention of future cardiovascular events and mortality. Curcumin is a **novel, safe, and easy to deliver** therapy with the potential to treat arterial dysfunction (an independent predictor of future cardiovascular events and mortality), and possibly renal cystic disease, in an understudied population of children and young adults with ADPKD.

Summary of Significance. The biomedical significance of the proposed work includes:

- ADPKD is the most common life-threatening genetic disease;
- There is an urgent need to establish the efficacy of interventions that may minimize CVD risk in patients with ADPKD;
- Presently, there are very limited treatment options for the prevention of CVD in patients with ADPKD;
- Many interventions targeting traditional risk factors have not successfully reduced CVD risk in patients with ADPKD, despite their efficacy in the general population;
- Childhood and young adulthood may be a critical therapeutic window for the treatment of arterial dysfunction and renal cystic disease in ADPKD;
- In a subsample of participants 18-25 yrs, insight will be provided into the integrative physiological mechanisms using **novel translational research techniques** that are non- to minimally-invasive.

Innovation

The innovative features of this application include:

1. Intervening **early in the course of ADPKD** with treatment in children/young adults (6-25 years of age);
2. Assessing two major contributors to arterial dysfunction and CVD risk in ADPKD: vascular endothelial dysfunction and large elastic stiffness;
3. Determining the efficacy of a novel nutraceutical (curcumin) that is an inexpensive, safe, naturally occurring, and easy to deliver substance;
4. In a sub-group of 18-25 year olds, using a translational approach to determine the physiological mechanisms contributing to any improvements in vascular dysfunction with curcumin, including:
    a. functional *in vivo* assessment of the role of oxidative stress in arterial dysfunction using acute antioxidant administration
    b. measurement of urine/circulating markers of oxidative stress and inflammation, utilizing state of

the art mass spectrometry methods

5. Gaining exploratory evidence of the efficacy of curcumin to slow kidney growth, which will provide the basis for a future R01 application employing a longer treatment duration and larger sample size.

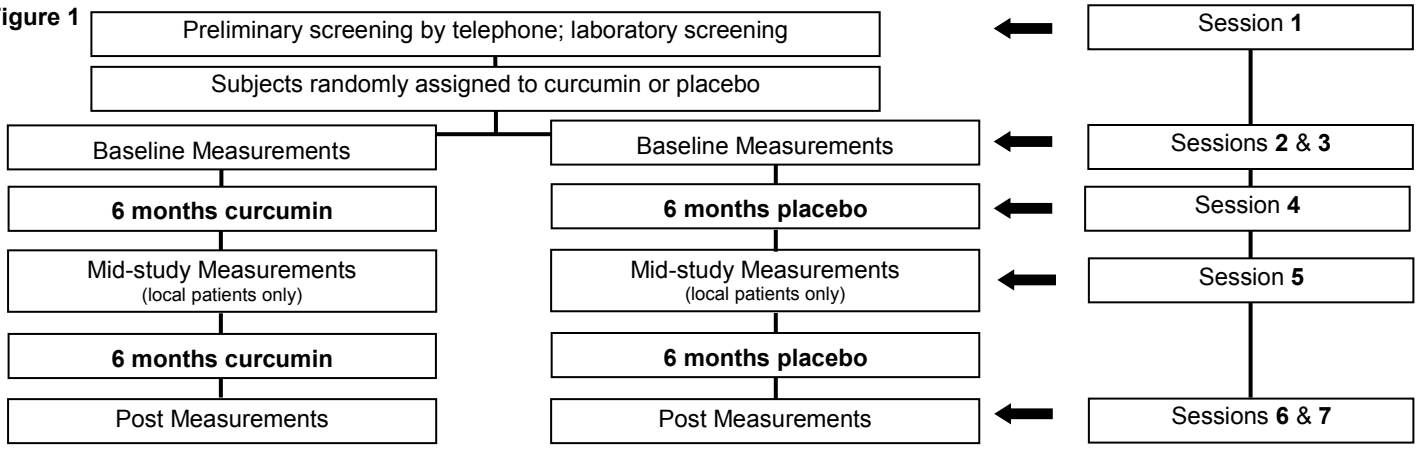
Approach

Subjects. After obtaining their written informed consent (ages 18-25) or assent (ages 6-17; plus parental consent), children and young adults aged 6-25 years with a diagnosis of ADPKD, based on the presence of bilateral renal cysts when there is a positive family history [51], and normal renal function with an estimated glomerular filtration rate (eGFR) >80 mL/min/1.73 m^2 (using CKid Schwartz bedside equation for ages 6-17 [52] and the CKD-EPI equation for ages 18-25 [53]) will serve as subjects. Patients will undergo screening and vascular testing at the University of Colorado Denver Division of Renal Diseases and Hypertension Clinical Vascular Physiology Laboratory, which is part of the Clinical Research Unit. A local contracted laboratory (Quest) and medical records will be used for screening for out of state participants. The University of Colorado Denver ADPKD Center has successfully employed this approach in previous and ongoing ADPKD clinical studies [54,55], including a recently completed trial by the co-mentor (Dr. Cadnapaphornchai) in 110 children and young adults with ADPKD [56]. Laboratory analysis will be performed at the University of Colorado Hospital or by Quest, and mass spectrometry analyses will be performed in the iC42 Clinical Research & Development Center. Renal magnetic resonance imaging (MRI) scans will be performed at the Brain Imaging Center. Major inclusion/exclusion criteria are presented in the table below (Table 1). Those who drop-out/do not complete the study will be encouraged to undergo an end-of study visit which will include identical measurements to the ones planned for session 5.

Table 1 **Inclusion Criteria**	**Exclusion Criteria**
• Aged 6-25 years • ADPKD diagnosis based on bilateral renal cysts + positive family history [51] • Normal renal function with an estimated glomerular filtration rate (eGFR) >80 mL/min/1.73 m^2 (using CKid Schwartz bedside equation for ages 6-17 [52] and the CKD-EPI equation for ages 18-25 [53]) • Ability to provide informed consent or assent	• Currently taking a curcumin supplement • Current smokers or history of smoking in the past 12 months • Marijuana use within 2 weeks prior to FMD_{BA} and aPWV testing • Antioxidant and/or omega-3 fatty acid use within the past 4 weeks prior to FMD_{BA} and aPWV testing and for the duration of the study • Alcohol dependence or abuse • History of hospitalization within the last 3 months • Active infection or antibiotic therapy • Pregnancy, lactation, or unwillingness to use adequate birth control • Body-mass index $\geq$95th percentile in ages 6-17 or >40 kg/m^2 in ages 18-25 (vascular measurements can be inaccurate if severely obese) • Inability to cooperate with/clinical contraindication for MRI including severe claustrophobia, implants, devices, or non-removable body piercings

Experimental Design (Figure 1). A 1-year randomized, placebo-controlled, double-blind design study with curcumin will be conducted. Subjects will undergo telephone and laboratory screening for inclusion/exclusion criteria and will then be randomly assigned to either curcumin or placebo. Members of the investigative team involved in the acquisition and analysis of data will be blinded to the treatment status. Vascular function measurements will be made under supine, overnight fasted (water only) conditions before and after 1-year of the intervention, and at 6-months in local patients to reduce variability over time and better establish timeline of change. Females who are post-pubertal will be scheduled during the first week of their menstrual cycle, when estrogen and progesterone are lowest, minimizing the impact of hormonal variations on vascular function [57].

Figure 1



In state subjects will come to the Clinical Vascular Physiology Laboratory (sessions 1, 2, 4, 5, and 6) or the Brain Imaging Center (sessions 3 & 7) on 7 separate occasions. Out of state participants (approximately half) will travel twice to Colorado. Sessions 2 & 3 and then 6 & 7 will occur on consecutive days during the same trip (**for a total of 2 out of state trips during the study**). Session 1 (and subsequent safety labs) will be arranged at a local contracted laboratory (Quest) for out of state participants.

- **Session 1:** Screening measurements
- **Session 2:** Baseline measurements
    - Vital signs
    - Blood sampling for measurement of circulating curcumin levels, serum creatinine, ALT and AST and urine sampling for markers of oxidative stress (**all subjects**); blood sampling for markers of inflammation (**subset of subjects 18-25 years**)
    - FMD_{BA} (EDD) and aPWV (arterial stiffness) (**all subjects**), with and without acute inhibition of oxidative stress via systemic i.v. infusion of ascorbic acid (**subset of subjects 18-25 years**)
    - Endothelium-independent dilation to sublingual nitroglycerin (**subset of subjects 18-25 years**)
- **Session 3:** Baseline measurements
    - Abdominal MRI for subsequent analysis of TKV (**all subjects**)
    - Begin study treatment (curcumin or placebo)
- **Session 4:** Safety lab check
- **Session 5**: Mid-study measurement of FMD_{BA} and aPWV for **local (in state) patients only;** safety lab check (all patients)
- **Sessions 6** & **7 (month 12):** Identical to Sessions 2 & 3 (note: subjects will not take a dose the a.m. prior to testing session to avoid any acute effects)

Curcumin. Curcumin will be delivered as a dose of 25 mg/kg/day rounded to the nearest 100 mg, delivered as powder measured in 100 mg and 500 mg scoops (Longvida™, Verdure Sciences). The placebo will contain inert substances (soy lecithin, stearic acid, ascorbyl palmitate, carrot extract) of similar proportions and amounts as in the active powder. The powder delivery will improve adherence, particularly among young children who may have difficulty swallowing capsules. The powder will be delivered mixed with a choice of pudding yogurt, or other foods of similar consistency. Absorption of Longvida™ is the same when taken with a small amount of food compared with water or milk.

Curcumin has been investigated extensively for safety and toxicity because of its use in foods. Toxicology studies have been performed on the effects of curcumin in rodents and humans that are summarized below in the overall context of the present study.

Background. Curcumin is the active ingredient in the Indian spice turmeric and gives it a yellowish color. It is found naturally in the root of the plant *Curcuma longa* and has traditionally been used for the prevention and/or treatment of ailments related to the skin, liver, gastrointestinal tract, and the common cold [58]. Epidemiological studies have shown that societies that regularly consume curcumin in their diet have markedly reduced mortality due to coronary heart disease [59]. More recently, curcumin's potential benefits have been assessed in other pathologies such as cancer, arthritis, Alzheimer's disease and hypercholesterolemia. In addition, curcumin has been shown to attenuate oxidative stress and inflammation both *in vitro* and *in vivo* [38,39,41,42,60].

History/Testing. Because of its color, flavor and antioxidant-stabilizing abilities curcumin is commonly used in yellow mustard, pickles and sauces. Curcumin has been used to flavor foods and treat ailments for over 5,000 years and is generally recognized as safe (GRAS) by the FDA (this is also true specifically of Longvida™). Studies conducted in rats and humans using standard toxicology protocols have shown no toxic effects of curcumin *in vivo* at doses as high as **8 grams/day (8,000 milligrams/day)**, ***i.e., several-fold higher than the dose proposed in the present application*** [61-64]***.***

Human Studies. Few side-effects of curcumin have been reported with doses up to 8-12 grams/day (8,000-12,000 milligrams/day) of supplementation in adults [61,62,65] and children [64,66,67]. The few side-effects reported primarily involve gastrointestinal discomfort [61,65,67].

Improving Bioavailability: Choice of Compound. To enhance the bioavailability of curcumin, manufacturers have developed formulations that may prevent glucuronidation, as unprocessed curcumin is insoluble in water and quickly metabolized by glucuronidation. The compound proposed in the current application is a solid lipid curcumin particle (SLCP, Longvida™, Verdure Sciences) that increases plasma levels of curcumin significantly compared to traditional formulations in healthy volunteers [64] and is safe in both this group and osteosarcoma

patients, including children [64]. SLCP supplementation in rats with 720 mg/kg body mass/day, equivalent to 40 g/day (40,000 mg/day) for a 60 kg person, for 90 days had no adverse effects [63].

In summary: 1) curcumin is a naturally occurring and widely used spice in southeastern Asian cultures and poses only minimal risks at very high doses; and 2) all of the safety studies performed in adults and children have used maximal doses of curcumin that are significantly higher than those proposed in the present application (**detailed rationale regarding doses and duration of treatment is below**).

Rationale for Proposed Dose (25 mg/kg/day), Frequency (once a day) and Duration (12 months)

Dose (25 mg/kg/day)

- Dosing is normalized to body mass to account for wide differences across the included age range.
- This normalized dose is based off a 2,000 mg dose delivered to an 80 kg adult, which is similar to published work of others [61,62,64,67] and well below maximal dosing levels. This dose has also been recommended by Verdure Sciences, who is providing the SLCP powder.
- Additionally, 25 mg/kg/day was selected because 2,000 mg/day has been consistently shown to be both safe and well tolerated in adults [61,62,64], and children [64,67], is unlikely to elicit gastrointestinal discomfort [61,67], and improved vascular function in preliminary studies performed in aging adults (see Preliminary Results).
- While older children and young adults could have instead been prescribed to swallow capsules, all participants will ingest the curcumin as powder mixed with pudding or yogurt, to standardize intake across all ages.

Dosing Frequency (once a day)

- The curcumin (or placebo) powder will be administered once a day in the morning for standardization.

Duration of Treatment (12 months)

- Given my previous experience with interventions to improve arterial function in the context of healthy aging, I expect 12 months to be a sufficient duration [68,69]. While I recognized that the duration is likely too short for a significant decrease in rate of TKV growth, any trend from this exploratory aim will be useful to design a future, longer duration R01 grant application.
- Although my preliminary data (see below) support that curcumin has beneficial effects in healthy aging on FMD_{BA} at 3 months, a 12 month treatment period was selected because 1) ADPKD may have a more severe effect on vascular function than healthy aging; 2) reversal of structural properties of the arterial wall associated with arterial stiffness may require a longer duration of treatment; 3) this allows for exploratory insight into changes in TKV, which is supported by cell and animal data [21,22].
- I considered including multiple groups with different outcomes, including a shorter treatment length to evaluate vascular function; however, this design would greatly reduce power and further increase cost of the study. Instead, I opted to add evaluation vascular function in local patients (~50% of the total *n*) at 6 months, in addition to 12 months, in order to account for variations in adherence and changes in development over time (i.e. puberty) that may increase variability.
- Importantly, I recognize that aPWV changes across puberty [70-72], whereas the available data suggest that FMD_{BA} does not [73]. However, this should not limit the interpretation of the results as 1) randomization will ensure similar changes across the active and control groups; 2) puberty will be accounted for in statistical analysis; and 3) vascular measurements at 6 months in local patients will help account for such changes.
- Curcumin has been tested extensively and used in the food/manufacturing industry. It is a *natural* dietary component, and our proposed dose has been repeatedly shown to be well-tolerated and elicit no side effects. Thus, there is no evidence to suggest the possibility of adverse effects associated with the proposed 12 months of increased intake of curcumin.

Study Modifications to Account for COVID-19 Outbreak. On account of COVID-19 and Institutional policies in place regarding work-related travel, the length of treatment of participants in may be extended due to extenuating circumstances. The end-of-study visit may be delayed due to concerns over air travel, in which case participants will be asked to continue taking the curcumin/placebo, as they have been doing for the past year, and we will reschedule their end-of-study visit at the earliest time point that seems appropriate (hopefully within a couple of months). We will obtain in-person data at this time, but will ask participants to go to our contract laboratory for the clinical labs at the planned 12 month time point if possible. We do not see any additional risk posed to participants by extending their treatment period, as curcumin already has a very favorable safety profile.

Summary of risks associated with curcumin. Based on preliminary studies (see below) and an extensive review of literature, the proposed doses of curcumin are both physiologically relevant and predicted to be well-tolerated by the great majority of study participants. The primary risk associated with curcumin supplementation is the possibility of temporary/transitory gastrointestinal distress (flatulence, constipation, diarrhea, nausea) in a very small percentage of individuals [61,65,67].

Measurements.

Screening Measures. Subjects will undergo a medical history and physical exam, including growth parameters and Tanner staging [74-76], aspartate aminotransferase (AST), alanine aminotransferase (ALT), and a pregnancy test for all female participants of possible childbearing potential (Tanner Stage 2 or higher). Serum creatinine will be measured for determination of eGFR using the CKid Schwartz bedside equation for ages 6-17 [52] and the CKD-EPI equation for ages 18-25 [53]. A local contracted laboratory (Quest) and medical records will be used for out of state participants. EMLA cream may be used as needed to numb the skin prior to drawing blood.

Measures of Adherence and Safety:

Adherence. As we expect ~50% of all participants to be from out of state, progress will be reviewed with a monthly phone inquiry of medication supply. Please note, due to the long history and prestige of University of Colorado PKD Center studies, our families have tremendous relationships with our research staff (i.e. investigators and research coordinators). The co-mentor, Dr. Cadnapaphornchai, has successfully employed this approach in previous clinical trials in children/young adults with ADPKD that have included ~50% out of state patients [54,56]. We expect the minimal side-effects associated with curcumin to favor adherence. Participants will have powder mailed every 3 months and will also be asked to mail back the bottle for a volume check.

Safety Checks. While adverse events are not anticipated, a safety questionnaire will be administered over the phone monthly. Serum creatinine, ALT and AST will be measured after 1, 6, and 12 months, and for those out of state, will be performed at a local contracted laboratory (Quest). Home pregnancy tests will be required monthly for all girls of possible childbearing potential ($\geq$Tanner Stage 2). All subjects will be given a digital blood pressure monitor (A&D Medical, UA767) with an appropriately sized cuff and the subject/parent will be instructed in its use. Home blood pressures will be taken monthly and reported during phone safety checks. This approach has been successfully employed previously by the co-mentor, Dr. Cadnapaphornchai [54,56].

Plasma Curcumin Concentrations will be assessed at baseline and 12 months by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the University of Rhode Island [77]. Additionally, curcumin concentrations will be measured by the in red blood cells, the buffy coat, and plasma with the addition of citrate to prevent the degradation of metabolites.

Randomization will be performed by the statistician, using a blocked randomization sequence, with stratification by age group (6-13 or 14-25 yrs).

Outcome Measures. The following measurements will be made at baseline and after 12-months of treatment:

Primary (Functional) Outcomes (Specific Aim 1)

Brachial Artery FMD and Endothelium-Independent Dilation. FMD_{BA} will be determined using high-resolution ultrasonography (Toshiba Xario 200) as described originally by Celermajer et al. [78] and used recently by the applicant [68,79,80]. FMD_{BA} is a widely used, non-invasive method inducing reactive hyperemia to assess endothelium-dependent dilation. It is validated in children [78,81] and is mainly dependent upon NO production [82,83]. Brachial artery endothelium-independent dilation will be determined in the sub-group of participants aged 18-25 yrs by measuring brachial artery dilation for 10 minutes after administration of sublingual nitroglycerin (0.4 mg) [67,84,85]. This assessment is key to the interpretation that any improvements in dilation are indeed dependent upon endothelial production of NO rather than improved smooth muscle relaxation to NO [86]. The use of nitroglycerin is currently safely employed in an NIH-funded, IRB-approved protocol in adults with ADPKD at the University of Colorado Denver. A commercially available software package (Vascular Analysis Tools 5.8.1, Medical Imaging Applications) will be used to acquire and analyze ECG-gated brachial artery diameters. Doppler blood flow velocity will be obtained for estimation of shear rate, which will be entered as a covariate if indicated [87-89].

Aortic Pulse-Wave Velocity. aPWV will be determined as described in detail previously by applicant [69,90] and validated in children [71,91]. Briefly, a transcutaneous custom tonometer (Noninvasive Hemodynamics

Workstation, Cardiovascular Engineering Inc.) will be positioned at the carotid and femoral arteries to non-invasively assess aPWV, as distance/time between the foot of the arterial waveforms [92]. As a secondary index of arterial stiffness, ultrasound imaging of the carotid artery will also be performed for calculation of carotid artery compliance and the β-stiffness index (in conjunction with tonometry), as described previously [96,97]. The images will also be assessed for measurement of carotid artery intimal-medial thickness (IMT), which is used in the calculation of carotid artery compliance and the ß-stiffness index.

Outcomes for Specific Aims 2 (<u>Only in the sub-group aged 18-25 years</u> (except for urinary markers))

Oxidative Stress-Associated Suppression of EDD and Large Elastic Artery Stiffness. The influence of oxidative stress on FMD_{BA} and aPWV (and carotid artery compliance) will be determined by infusing a supraphysiological dose of ascorbic acid (American Regent Labs) known to scavenge superoxide or isovolumic saline, as used previously by the applicant [68,93], and others [84,94-96]. This procedure is currently safely employed in an NIH-funded, IRB-approved protocol in adults with ADPKD at the University of Colorado Denver. The infusion will be performed only if cannulation in a vein on the dorsal aspect of the contra-lateral hand is successful. 0.075 g ascorbic acid/kg fat-free mass dissolved in 150 mL of saline will be infused intravenously at 5 mL/min for 20 min, followed immediately by a "drip-infusion" at 0.5 mL/min over 60 min. Vascular measurements will be made during the "drip infusion" when peak plasma concentrations of ascorbic acid occur [84]. The difference in FMD_{BA} and aPWV (and carotid artery compliance) during ascorbic acid vs. saline infusion indicates the modulation of EDD and arterial stiffness by oxidative stress [84,94]. These measures will be performed in the sub-group (18-25 yrs) only.

Markers of Oxidative Stress. Urinary 8-iso-prostaglandin F2α (8-isoprostane) and 8-hydroxy 2 deoxyguanosine (8-OHdG) will be measured by LC-MS/MS as a markers of lipid peroxidation [97] and oxidative DNA damage [98], respectively, as oxidative damage is increased ADPKD [17]. While Aim 2 is primarily in a sub-group aged 18-25 yrs, these oxidative stress measurements will be performed in all participants (6-25 yrs), as urine can be collected non-invasively.

Inflammatory Markers. Plasma C-reactive protein (immunoturbidimetric method) and plasma interleukin-6 (ELISA; R&D Systems) will be measured by the University of Colorado Hospital as circulating inflammatory markers [79,99], as both are increased in ADPKD [16]. To minimize the amount of blood collected in children <18 yrs, these measures will only be performed in the sub-group aged 18-25 yrs.

Outcome for Specific Aims 3

MRI Measurement of TKV. A 1.5 Tesla Visart System (Toshiba America Medical Systems) or comparable system will be used for all studies. Renal images will be acquired in similar manner and volumetric measurements determined as described for the CRISP study [100]. No contrast agents will be utilized for the study. The radiologist, MRI study technicians and the applicant, who will calculate TKV, will be blinded regarding group assignment. For analysis, DICOM images will be de-identified and evaluated by a single analyst (the applicant) using Analyze software (Analyze 9.0, Mayo Foundation, Rochester, MN). To account for normal growth in children, TKV will be adjusted for height [56]. Feasibility of this aim is supported by the fact that the co-mentor, Dr. Cadnapaphornchai, recently completed an intervention study with MRI measurement of TKV in 110 pediatric and young adult patients with ADPKD [56]. In addition renal parenchyma (TKV – cyst volume) will be measured.

Additional Blood Samples

For subjects signing the Informed Consent Addendum, an additional 5 mL of serum and 9 mL of plasma in adults and an additional 1 mL of serum and 2 mL of plasma in children will be collected for future research. In addition, 20 mL of whole blood will be collected from adult participants only. PBMCs will be isolated from heparinized whole blood using Histopaque-1077 as described previously [106,107]. To provide mechanistic insight into biological pathways that may be altered by curcumin (for example, mammalian target of rapamycin (mTOR)-S6 kinase (S6K) signaling), the PBMCs will be washed and protein will be extracted for Western blot analysis.

Expected Results. In children/young adults with ADPKD, 12 months of curcumin compared to placebo will:

***Hypothesis 1*:**

- ↑ FMD_{BA} (↑ vascular EDD)
- ↓ aPWV (↓ large elastic artery stiffness)

Hypothesis 2 (Only in participants 18-25 years of age):

- Have little or no effect on FMD_{BA} and aPWV in the presence of ascorbic acid, whereas ascorbic acid is expected to significantly improve both of these measures in placebo-treated subjects, indicating a curcumin-mediated reduction in oxidative stress-related suppression of vascular function
- ↓ urinary 8-isoprostane and 8-OHdG, indicating reduced systemic oxidative stress
- ↓ plasma C-reactive protein and interleukin-6, indicating reduced systemic inflammation

Hypothesis 3

- Tend to ↓ the progression of TKV (adjusted for height), which can be examined further in a longer-duration, larger sample size, future R01 grant

Power Calculations and Statistical Analysis. Power calculations are based on clinically meaningful differences in the primary outcomes (FMD_{BA} and aPWV), limited published literature using curcumin in a healthy older population [44], and experience of the applicant regarding the variability of the proposed outcomes [68,69,79]. A sample size of 27 in each group with have 90% power to detect a mean increase in FMD_{BA} of 1.5%, given a standard deviation of 2.0 [68], with $\alpha = 0.025$, adjusted for two primary endpoints. Akazawa et al. [44] demonstrated in healthy post-menopausal women a ΔFMD_{BA} of +1.5% with curcumin compared to a decrease in FMD_{BA} of -0.2% in controls. Similarly, a sample size of 27/group will have 90% power to detect a mean decrease of aPWV of 100 cm/sec given a standard deviation of 125 [69] with $\alpha = 0.025$, adjusted for two primary endpoints. To account for a potential dropout of 20%, 34 subjects/group will be enrolled.

A sub-sample size of 10 in each group (of 18-25 year olds) will have 89% power to detect a 50% reduction in ΔFMD_{BA} with ascorbic acid (given $\Delta FMD = +3.0 \pm 1.0\%$ with ascorbic acid in the placebo group [68]), with a two-sided $\alpha = 0.05$. Aim 3 will be considered exploratory, thus power was not calculated.

Differences between groups at baseline will be assessed by an independent t-test, and a linear mixed-effects model will be fit to compare curcumin vs. placebo (independent variable) with respect to change in outcomes at 6 and 12 months (dependent variables), with covariates and potential confounders (including puberty) incorporated into the model. This approach will account for the use of multiple time points and is flexible to missing (at random) data. Although I do not expect different responses based on sex or race/ethnicity, and the study is not powered to assess these factors, stratified analysis will be performed and any trends will be noted and studied in a larger follow-up study. Dropouts will be handled using an intent-to-treat analysis.

Study Time Line. I will use the initial 8 weeks to begin subject recruitment and screening. The first subjects will begin treatment with curcumin or placebo in month 3 of the proposed overall 5-year funding period. The last patients enrolled will complete the study by approximately month 54. Vascular and MRI measurements will be performed over this period. Mass spectrometry assays will be performed annually to detect any problems early in the course of the study. Statistical analysis and manuscript preparation will be undertaken during the last 6 months (months 54-60). This is an appropriate rate of subject enrollment and experimental testing based on previously completed intervention studies in this population (which also included ~50% out of state patients) by the co-mentor Dr. Cadnapaphornchai [54,56].

Recruitment Plans. Since 1985, the University of Colorado Denver has maintained an ADPKD registry and mailing list. Included in this registry are children and young adult ADPKD patients from the Denver Metro Area and all over the United States. In addition to the University of Colorado Denver registry, I will advertise through the national PKD Foundation. The ADPKD center at the University of Colorado Denver has longstanding expertise and national recognition, and therefore, is able to attract ADPKD patients from all over the United States for clinical studies. Of note, the University of Colorado Denver is currently the only group in the world performing research studies in children with ADPKD. The feasibility of recruitment is supported by the recently completed intervention study performed by the co-mentor Dr. Cadnapaphornchai, which included 110 pediatric and young adult patients with ADPKD (including ~50% out of state) followed for a three-year period [56], and these participants will be recruited for the proposed research study.

Potential Problems, Alternative Strategies, and Future Directions. Although subject recruitment and retention always are challenges, I am confident in my ability to complete the study in the proposed timeline given our location in the densely populated Denver-Boulder metro area and our department's 30 years of experience in studying patients from around the country with ADPKD, including children and young adults. I should have few difficulties with the proposed experimental procedures and protocols as either a) I have expertise in the techniques in other populations; or b) the methodology is already established within our ADPKD research group, and I will be trained to perform these new skills by my mentorship team.

I recognize that alternative approaches exist for examining the effects of curcumin in children and young adults with ADPKD. For example, one alternative would be to undertake a larger study to examine harder

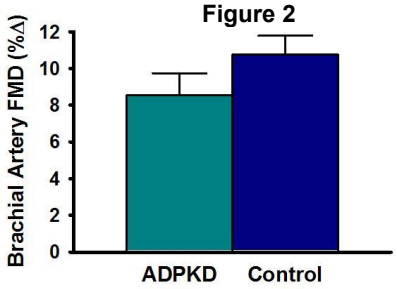
cardiovascular endpoints than the intermediate outcomes proposed in the current application. However, this approach requires very large sample sizes, and the validated endpoints of FMD_{BA} and aPWV will provide initial insight that can guide a future larger-scale clinical trial using hard clinical endpoints. Similarly, I acknowledge that long-term follow-up is required to determine if curcumin has beneficial effects into later adulthood, and this is also a potential future direction following this initial study. I recognize that the proposed study duration and sample size will only provide a trend for any changes in TKV with curcumin, and such exploratory evidence will provide the basis for a future R01 application employing a larger sample size and treatment duration.

Last, I recognize the possibility of negative data; however, such data are still important, as a recent state of the art review on prospective and novel treatments of ADPKD identified curcumin as a potential therapy [101], and either positive or negative results are critical in continuing to advance treatment options. Importantly, the proposed Career Development plan will provide the scientific and professional skills required to conduct future randomized controlled trials in this patient population, and I will continue to pursue other novel interventions through small pilots and in conjunction with my collaborators performing pre-clinical research throughout the award's duration. In the event the study is negative, other potential therapies for a future R01 application include the mitochondria-targeted antioxidant MitoQ and metformin. My training in epidemiology will also allow me to identify other targets for future clinical trials. In addition, I will have a unique dataset of longitudinal changes in vascular function, oxidative stress, and TKV, which may generate novel hypotheses.

Preliminary Results: all data are presented as mean±s.e.

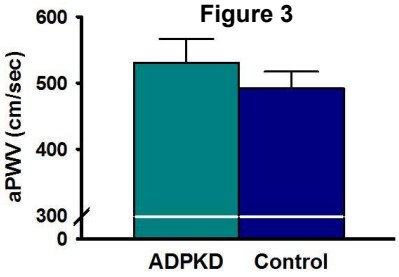
I. Vascular Endothelial Dysfunction in Children and Young Adults with ADPKD

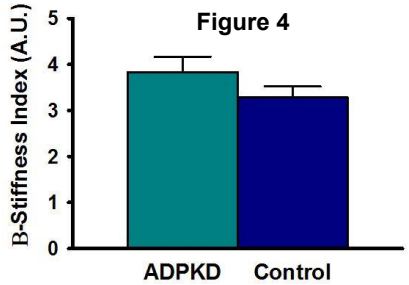
I assessed FMD_{BA} in 9 children/young adults (ages 6-25 yrs) with ADPKD and normal kidney function, and 9 age- and sex-matched healthy controls (16±2 years; 1M/8F). Compared to the healthy control group, FMD_{BA} was impaired (**Figure 2**) in the children and young adults with ADPKD, indicating vascular dysfunction very early in the course of the disease. Baseline brachial artery diameter (3.1±0.09 [ADPKD] vs. 3.1±0.16 cm [control]) did not differ between groups. Blood pressure (108±13/59±7 vs. 109±5/59±2 mmHg) and body-mass index (22.0±2.9 vs. 21.6±1.4 kg/m^2) also did not differ between groups.



II. Large Elastic Artery Stiffness in Children and Young Adults with ADPKD

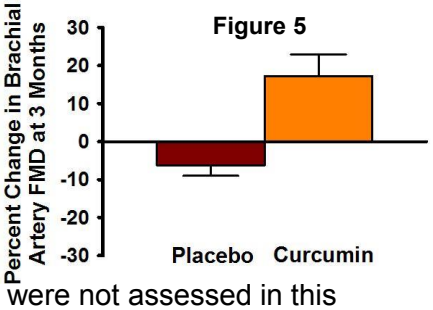
I also assessed aPWV in the same 9 children and young adults with ADPKD and the same 9 age- and sex-matched healthy controls. Compared to the control group, aPWV was greater (**Figure 3)**, indicating increased large-elastic artery stiffness even at a young age. Carotid artery β-stiffness index, a measure of local large-elastic artery stiffness, was also greater in the ADPKD group (**Figure 4**). Resting heart rate did not differ between groups (66±8 [ADPKD] vs. 63±2 bpm [control]).





III. Curcumin to Treat Vascular Dysfunction

My graduate laboratory has assessed the efficacy of curcumin therapy (same company and similar normalized dose as proposed: 2,000 mg/day, Longvida™, Verdure Sciences) to reduce age-associated vascular dysfunction. Compared to placebo, FMD_{BA} was improved (**Figure 5**) in 5 healthy adults (65±3 years, 2M/3F) treated for 3 months with curcumin, as compared to placebo. The curcumin was well tolerated in all participants without any adverse effects. As aging is associated with impaired FMD_{BA} similar to ADPKD, curcumin may also reduce vascular dysfunction in children/young adults with ADPKD. Please note, aPWV and B-stiffness index were not assessed in this preliminary study, as it may require more time to change in response to an intervention.



Human Subjects

1. Risks to the Subjects

a) Human Subjects Involvement and Characteristics.

Subjects. After obtaining their written informed consent (ages 18-25) or assent (ages 6-17; plus parental consent), children and young adults aged 6-25 years with a diagnosis of autosomal dominant polycystic kidney disease (ADPKD), based on the presence of bilateral renal cysts when there is a positive family history [51], and normal renal function with an estimated glomerular filtration rate (eGFR) >80 mL/min/1.73 m^2 (using CKid Schwartz bedside equation for ages 6-17 [52] and the CKD-EPI equation for ages 18-25 [53]) will serve as subjects will serve as subjects. Patients will undergo screening and vascular testing at the University of Colorado Division of Renal Diseases and Hypertension Clinical Vascular Physiology Laboratory. A local contracted laboratory (Quest) and medical records will be used for screening for out of state participants. The University of Colorado Denver ADPKD Center has successfully employed this approach in previous and ongoing ADPKD clinical studies [54,55], including a recently completed trial by the co-mentor (Dr. Cadnapaphornchai) in 110 children and young adults with ADPKD [56]. Laboratory analysis will be performed at the University of Colorado Hospital, and mass spectrometry analyses will be performed in the iC42 Clinical Research & Development Center. Renal magnetic resonance imaging (MRI) scans will be performed at the Brain Imaging Center.

To be eligible to participate in this research, volunteers must meet the following criteria:

Inclusion Criteria:

1) Aged 6-25 years (rationale: 6 was chosen as the lower age limit, as children younger than 6 may have difficulties remaining still for the vascular and MRI measurements; 25 is the upper limit because the focus of the study is in on children and young adults)
2) ADPKD diagnosis of ADPKD based on the presence of bilateral renal cysts when there is a positive family history [51]
3) Normal renal function with an estimated glomerular filtration rate (eGFR) >80 mL/min/1.73 m^2 (using CKid Schwartz bedside equation for ages 6-17 [52] and the CKD-EPI equation for ages 18-25 [53])
4) Ability to provide informed consent or assent

Exclusion Criteria:

1) Currently taking a curcumin supplement
2) Current smoking or history of smoking in the past 12 months (rationale: changes in smoking habits during study involvement could confound the results) [102]
3) Marijuana use within 2 weeks prior to FMD_{BA} and aPWV testing (rationale: use may influence vascular function and cardiovascular changes, including blood pressure and heart rate)
4) Antioxidant and/or omega-3 fatty acid use within the past 4 weeks prior to FMD_{BA} and aPWV testing and for the duration of the study (rationale: use may confound the effect of the acute ascorbic acid infusion on vascular function)
5) Alcohol dependence and abuse
6) History of hospitalizations within the last 3 months
7) Active infection or antibiotic therapy
8) Pregnancy, lactation, or unwillingness to use adequate birth control. All female participants of possible childbearing potential (Tanner Stage 2 or higher) will be required to take a pregnancy test at initiation of the study and then monthly during the study.
9) Body-mass index $\geq$95th percentile using sex, age, and height for children 6-17 years of age, or > 40 kg/m^2 for young adults 18-25 years of age (rationale: vascular measurements can be inaccurate in severely obese patients). The PI and co_I's may use discretion to allow inclusion of children with body-mass index $\geq$95% if vascular measurements seem reasonable to perform.
10) Inability to cooperate with or clinical contraindication for magnetic resonance imaging (MRI) including severe claustrophobia, implants, devices, or non-removable body piercings

Special classes of subjects considered vulnerable populations will not be included in the study. Dr. Chonchol will make final decisions on all patient eligibility.

b) Sources of Research Materials. This is a prospective study of newly recruited human subjects aged 6-25 years with ADPKD. The data collected will be used exclusively for research purposes. All subject identities and records will remain strictly confidential.

c) Potential Risks. We see no psychological, social, or legal risks beyond those of participation in health-related research in general. The potential physical risks of participating in the proposed experiments are reasonably small. The vascular measurements associated with Specific Aim 1 are completely non-invasive. In children 6-17, the only invasive measurement with be collection of a small amount of blood. All of the vascular procedures and the MRI scans have been used previously by the team of investigators in this patient population without complications. Importantly, all of the procedures will be performed in University of Colorado Division of Renal Diseases and Hypertension Clinical Vascular Physiology Laboratory, a facility with on-site full-time supervision including nursing, safety equipment and established emergency procedures. Moreover, the research will be overseen by a study-specific Data Safety Monitoring Board (DSMB) (described in more detail under Data and Safety Monitoring Plan).

The risks associated with the experimental protocols include:

Venous Catheter (**ages 18-25 only**) - Discomfort associated with insertion of the needle; local bleeding and a small hematoma (~10% of cases); risk of infection of a hematoma or significant external blood loss (<1 in 1000), risk of fainting.

Blood draw – Discomfort associated with the insertion of the needle; risk of bruising or feeling lightheaded or faint.

Endothelium-Dependent Dilation (Brachial Artery Flow-Mediated Dilation [FMD_{BA}]) - Inflating the blood pressure cuff during this procedure may cause a mild to moderate intensity "pins and needles or numbing" sensation that resolves when the cuff is deflated.

Magnetic Resonance Imaging - The risk of performing abdominal MRI is minimal. The magnetic field generated within the MRI is not harmful but can cause metal within the body to heat up or electronics to stop working. All subjects will be questioned regarding the presence of metal or electronic devices inside their body. All subjects with either metal implants or implanted electronic devices will be excluded from the study. As the MRI tube is a small round tube, it may make subjects who experience claustrophobia uncomfortable, thus such individuals will be excluded. The most common minor side effect of having an MRI exam is flashing lights in the eyes. This is caused by the magnetic waves and is not harmful. Some people also experience warmth and reddening of the skin, which usually goes away after a few minutes. For all female participants of possible childbearing potential, a negative pregnancy test prior to MRI will be required. Severe claustrophobia is an exclusion criterion for the study. Thus, undue stress due to a confined space during the MRI procedure will not be a potential risk.

Medications/Dietary Supplements - With any medication used in testing, there is small risk of an allergic reaction. Importantly, a physician will be available during the ascorbic acid infusion and nitroglycerin administration (**ages 18-25 only**) for assistance in the unlikely case of an adverse event.

Reactions reported with the use of the specific medications proposed for use include:

Curcumin - Curcumin is the active ingredient in the Indian spice turmeric and is generally recognized as safe (GRAS) by the FDA. Detailed information about curcumin is found in the *Research Strategy.* Few side-effects of curcumin have been reported with either 8-12 grams/day (8,000-12,000 milligrams/day) of short-term supplementation in adults [61,62,65] and children [64,66,67]. The primary risk associated with curcumin supplementation is the possibility of temporary/transitory gastrointestinal distress (flatulence, constipation, diarrhea, nausea) in a very small percentage of individuals and with very high doses [61,65,67]. In the event that symptoms are intolerable, the dose will reduced from 25 mg/kg/day to 15 mg/kg/day. Dr. Chonchol serves as a co-investigator on an ongoing NIH-funded trial using curcumin as a treatment of healthy aging (PI: Seals). As a precaution, monthly negative home pregnancy tests will be required in all female participants of possible childbearing potential.

*Ascorbic Acid (**ages 18-25 only**)* – Similar does of ascorbic acid has been infused intravenously without any unfavorable side effects in healthy subjects, as well as patients with chronic disease, including cardiovascular disease and chronic kidney disease [68,93,103,104]. However, administration of concentrated ascorbic acid may cause irritation to the local area around the infusion. In order to

reduce this risk we will dilute the infusion of ascorbic acid in sterile saline. This procedure is currently safely employed in an NIH-funded, IRB-approved protocol in adults with ADPKD.

*Nitroglycerin (**ages 18-25 only**)* - Nitroglycerin may cause a slight decrease in arterial blood pressure, minor symptoms such as lightheadedness, tingling in the tongue and in the arms and legs, headaches, fainting and/or increased heart rate. The dose of nitroglycerin used is considered safe in the proposed population and is also used in patient populations to relieve angina [105]. This procedure is currently safely employed in an NIH-funded, IRB-approved protocol in adults with ADPKD. Please see below for monitoring information.

*EMLA Cream (**as needed**)* – EMLA cream may cause transient local skin reactions at the application site including paleness, erythema, and edema. Uncommon side effects include a mild burning sensation, itching, or warmth at the application side. In rare cases, allergic reactions or anaphylactic shock may occur.

There are no alternative methods that would provide the same type and accuracy of information as the state-of-the art procedures proposed in this application.

2. Adequacy of Protection Against Risks

a) Recruitment and Informed Consent. I will use the recruitment and adherence strategies and experience previously implemented by myself and my mentorship team. Dr. Chonchol has successfully recruited patients with different kidney diseases, including ADPKD, to clinical trials for many years. Dr. Cadnapaphornchai has been performing research in children and young adults with ADPKD since 2001, including a recently completed 3 year statin study in 110 children and young adults with ADPKD. Patients for the study will be recruited from the ADPKD research center at the University of Colorado Denver Anschutz Medical Campus (UCD), with access to >4800 ADPKD patients around the country, including children and young adults. Since 1985, UCD has maintained an ADPKD registry and mailing list. Included in this registry are children and young adult ADPKD patients from the Denver Metro Area and all over the United States. In addition to the UCD registry, I will advertise through the national PKD Foundation. The ADPKD center at the UCD has longstanding expertise and national recognition, and therefore, is able to attract ADPKD patients from all over the United States for clinical studies. Of note, UCD is currently the only group in the world performing research studies in children with ADPKD. The feasibility of recruitment is supported by the recently completed intervention study performed by the co-mentor Dr. Cadnapaphornchai, which included 110 pediatric and young adult patients with ADPKD followed for a three-year period, including individuals out of state [56], and these participants will be recruited for the proposed research study. The Division of Renal Diseases and Hypertension at the University of Colorado, in general, and Dr. Chonchol and Dr. Cadnapaphornchai specifically, have an excellent track record in recruiting participants and meeting enrollment goals for clinical trials. Dr. Chonchol has served as PI or co-PI in many large multi-center clinical trials and will help monitor and guide the progress of the project.

Written informed consent (participants ages 18-25 years of age and parent(s) of children 6-17 years of age) and assent (children 6-17 years) will be obtained using a standardized forms approved by the University of Colorado Multiple Institutional Review Board (IRB), that provides appropriate information about the study and the potential risks and benefits. The parent(s) and/or child will read the consent/assent form and the investigator will review these forms and will answer any questions that the subject may have prior to obtaining the subject's written consent/assent. The consent form will then be signed by at least one parent and a witness for children under 18, or by the participant if 18-25 years of age, as well as by the investigator as documentation of consent. The assent form will also be signed by children 6-17 years of age and by the investigator and a witness. A copy of the signed consent/assent form(s) will be given to the subject/parent(s). All of the proposed procedures and protocols will be reviewed and approved by the University of Colorado Multiple IRB.

Every attempt will be made to maintain patient's confidentiality, although a potential risk is loss of confidentiality. Since subjects participating in these studies have already been diagnosed with ADPKD, participation is unlikely to affect the ability of to obtain insurance. The University of Colorado PKD research group has 30 years of experience recruiting patients with ADPKD, including patients from out of state. The University of Colorado IRB is aware that our patients travel from out of state and we have taken precautions to ensure confidentiality when patients are traveling or getting blood drawn at a contract laboratory (Quest).

***b) Protection Against Risk*.**
Minimizing General Risks - The potential general risks of the proposed studies will be minimized by:

- Screening for adverse events and allergies to drugs.
- Using only safe, well-established procedures, with only qualified and experienced personnel performing the procedures.
- Ensuring constant personal monitoring of each experimental session by the investigators and clinical staff.
- Providing appropriate clinical supervision and emergency equipment through the Clinical Vascular Physiology Laboratory environment.
- Safety monitoring annually by a data safety monitoring board (DSMB).
- Employing record keeping processes with complete confidentiality. All subject identities and records will remain strictly confidential. Individual subject data will not be associated with subject name.
- Only performing the ascorbic acid infusion and sublingual nitroglycerin administration in a sub-set of young adult participants **18-25 years** of age.
- All blood pressure, blood draws and FMD_{BA} measurements will be performed in the arm not designated for future vascular access for dialysis
- A small venous catheter (for vitamin C intravenous infusion during FMD_{BA} and aortic pulse-wave velocity measurements) will be placed in the arm or dorsal aspect of the hand (**ages 18-25 only**).

Minimizing Specific Risks Related to Drugs/Supplements:

- *Risks to the subjects from curcumin administration* will be minimized by using a dose and a duration of curcumin treatment that have been characterized repeatedly as safe and well tolerated in humans, including children [61,62,64,67]. In addition, scheduled safety checks (phone questionnaire) will occur after one month, three months, six months, and nine months of taking the curcumin (or placebo). Serum creatinine, ALT and AST will be measured after one month, six months, and nine months, and for those out of state, will be performed at a local contracted laboratory (Quest). Home pregnancy tests will be required monthly for all girls of possible childbearing potential (Tanner Stage 2 or higher). All subjects will be given a digital blood pressure monitor (A&D Medical, UA767) with an appropriately sized cuff and the subject/parent will be instructed in its use. Home blood pressures will be taken monthly and reported during phone safety checks. This overall approach has been successfully employed previously by the co-mentor, Dr. Cadnapaphornchai [54,56]. In addition, potential side effects will be reviewed with participants at time of consent and subjects will be instructed to contact the study coordinator if they experience any side effects potentially associated with curcumin therapy.
- *Risks of local irritation from the use of ascorbic acid* (**ages 18-25 only**) will be minimized by diluting the ascorbic acid in saline.
- *Risks associated with sublingual nitroglycerin* (**ages 18-25 only**) will be minimized by screening out subjects who take vasodilating drugs that may interact with sublingual nitroglycerin (such subjects will still be eligible to participate in all other parts of the study), and not administering nitroglycerin to subjects with systolic blood pressure <100-mmHg, mean arterial pressure <70 mmHg, or history of migraines. During nitroglycerin administration, heart rate and blood pressure will be monitored every 2 minutes for signs and symptoms of hypotension. Additionally, an intravenous catheter (also used for ascorbic acid infusion) will be in place for administration of normal saline in the rare event of symptoms due to hypotension.
- *Risks associated with EMLA cream* (**as needed**) – will be minimized by screening for hypersensitivity to prilocaine, lignocaine, or other local anesthetics of the amide type.

Minimizing Specific Risks Related to Confidentiality:
Strict confidentiality of patients' information will be kept in accordance of HIPPA policy. All clinical samples will be stored by code alone. Data will be stored in Research Electronic Data Capture (RedCap), an encrypted, secured, HIPPA compliant website that meets all IRB regulations for secure data management. Paper hard-copies containing any identifiable information, will be locked in the research offices and in a locked cabinet, and will be accessible to members of the research team only on an as-needed basis. The contract laboratory (Quest) will ensure strict confidentiality in accordance of HIPPA for all laboratory data. All information in our database is treated with the same confidentiality as a medical record.

3. Potential Benefits of the Proposed Research to the Subjects and Others
Because the risks of participating in this study are relatively small, the risk-to-benefit ratio also is relatively low. Subjects will receive benefits associated with overall knowledge of their health from any testing performed.

The results of the study have potential benefits for the treatment of vascular dysfunction in children and young adults with ADPKD, and possibly for slowing the progression of renal structural disease, which could potentially reduce overall cardiovascular and renal risk and mortality in this population.

4. Importance of the Knowledge to be Gained

The findings from the proposed research should provide important new information regarding the potential therapeutic effects of curcumin therapy on endothelial dysfunction and arterial stiffness in children and young adults with ADPKD, as there is no available information to date on these questions. In addition, the proposed research should provide important insight into the mechanisms involved in any beneficial effects of curcumin on vascular function, as well as exploratory information regarding the influence of curcumin on total kidney volume. Together, this information will contribute to future interventions to treat vascular dysfunction and reduce cardiovascular risk in this group of patients.

5. Data Safety Monitoring Plan

a) Medical Supervision and Subject Surveillance. The trial will be performed by the University of Colorado Division of Renal Diseases and Hypertension research group located at the University of Colorado Anschutz Medical Campus. The physicians, advanced practice providers, and nursing staff will have the responsibility of performing medical histories and physical examinations as part of the subject screening procedures. The research nursing staff and trained phlebotomists perform the venous catheterizations (ages 18-25 only). Dr. Michel Chonchol, a board certified nephrologist, will provide final decisions regarding subject screening/enrollment and will monitor/oversee clinical status and subject safety as they progress through the intervention.

***b) Data Safety Monitoring Plan*.** A data safety monitoring board (DSMB) including clinicians (a nephrologist and pediatrician) and a statistician (independent of the study investigators but part of the faculty at the University of Colorado, School of Medicine) will be formed to assess potential adverse events. These data will be prepared by the DSMB statistician, ensuring the study statistician remains blinded until the final analysis. The DSMB will meet annually to review the protocol and will follow the guidelines established by the NIH National Center for Research Resources which include: a) monitoring the progress of the protocol (e.g., reviewing subject recruitment, attrition and minority involvement) and the safety of research participants (e.g., reviewing unblinded data for safety); b) assuring compliance with requirements regarding the reporting of adverse events; c) assuring that any action that results in the temporary or permanent suspension of the protocol is reported to all the appropriate monitoring bodies (i.e., IRB, NIH, FDA, etc.); and d) assuring data accuracy and protocol compliance.

***c) Reporting of Side Effects*.** Subjects will be instructed to report side effects to the investigator and their nephrologist, if not part of the investigative team. Any serious adverse events will be reported to the Colorado Multiple IRB, Verdure Sciences, and the FDA, in accordance with the guidelines of each of these groups. Participants who drop out of the study will be handled using an intent-to-treat analysis.

***d) Exit Criteria*.** The primary exit criteria will include completion of the study, , patient request, IRB or DSMB request, or other rationale as determined by the principal investigator or mentors. Other exit criteria are listed below. The number of subjects exiting the study and the reasons for exit will be carefully documented.

Patient Stopping Criteria:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event (AE), laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Pregnancy
- Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness
- Use of an investigational therapy or device other than study medication
- Significant non-compliance with protocol (i.e., procedures, assessments, medication, etc.)
- Serious acute hypersensitivity reactions to investigational drug

Study Stopping Criteria:

The DSMB may recommend study stopping if:

- The data show a significantly increased risk of serious adverse effects in the treatment group.

- It becomes clear that successful completion of the study is not feasible (e.g. there is an excess of patient dropout, missing data, lack of recruitment etc).

Clinical Trials.gov Requirements
The application includes a trial which requires registration at ClinicalTrials.gov. This registration will be performed.

References

1. Fick GM, Johnson AM, Hammond WS, Gabow PA. Causes of death in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol.* 1995;5(12):2048-2056.

2. Ecder T, Schrier RW. Cardiovascular abnormalities in autosomal-dominant polycystic kidney disease. *Nat Rev Nephrol.* 2009;5(4):221-228.

3. Fick-Brosnahan GM, Tran ZV, Johnson AM, Strain JD, Gabow PA. Progression of autosomal-dominant polycystic kidney disease in children. *Kidney Int.* 2001;59(5):1654-1662.

4. Gabow PA, Johnson AM, Kaehny WD Kimberling WJ, Lezotte DC, Duley IT, Jones, RH. Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease. *Kidney Int.* 1992;41(5):1311-1319.

5. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB. Heart disease and stroke statistics--2012 update: a report from the American Heart Association. *Circulation.* 2012;125(1):e2-e220.

6. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. *Circulation.* 2003;107(1):139-146.

7. Covic A, Gusbeth-Tatomir P, Goldsmith DJ. Arterial stiffness in renal patients: an update. *Am J Kidney Dis.* 2005;45(6):965-977.

8. Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Stiffness of capacitive and conduit arteries: prognostic significance for end-stage renal disease patients. *Hypertension.* 2005;45(4):592-596.

9. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation.* 2007;115(18):2390-2397.

10. Shechter M, Issachar A, Marai I, Koren-Morag N, Freinark D, Shahar Y, Shechter A, Feinberg MS. Long-term association of brachial artery flow-mediated vasodilation and cardiovascular events in middle-aged subjects with no apparent heart disease. *In J Cardiol.* 2009;134(1):52-58.

11. Kocaman O, Oflaz H, Yekeler E, Dursun M, Erdogan D, Demirel S, Alisir S, Turgut, F, Mercanoglu F, Ecder, T. Endothelial dysfunction and increased carotid intima-media thickness in patients with autosomal dominant polycystic kidney disease. *Am J Kidney Dis.* 2004;43(5):854-860.

12. Wang D, Iversen J, Wilcox CS, Strandgaard S. Endothelial dysfunction and reduced nitric oxide in resistance arteries in autosomal-dominant polycystic kidney disease. *Kidney Int.* 2003;64(4):1381-1388.

13. Wang D, Iversen J, Strandgaard S. Endothelium-dependent relaxation of small resistance vessels is impaired in patients with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol.* 2000;11(8):1371-1376.

14. Borresen ML, Wang D, Strandgaard S. Pulse wave reflection is amplified in normotensive patients with autosomal-dominant polycystic kidney disease and normal renal function. *Am J Nephrol.* 2007;27(3):240-246.

15. Kocyigit I, Kaya MG, Orscelik O, et al. Early arterial stiffness and inflammatory bio-markers in normotensive polycystic kidney disease patients. *Am J Nephrol.* 2012;36(1):11-18.

16. Menon V, Rudym D, Chandra P, Miskulin D, Perrone R, Sarnak M. Inflammation, oxidative stress, and insulin resistance in polycystic kidney disease. *Clin J Am Soc Nephrol.* Jan 2011;6(1):7-13.

17. Wang D, Strandgaard S, Borresen ML, Luo Z, Connors SG, Yan Q, Wilcox CS. Asymmetric dimethylarginine and lipid peroxidation products in early autosomal dominant polycystic kidney disease. *Am J Kidney Dis.* 2008;51(2):184-191.

18. Chapman AB, Bost JE, Torres VE, Guay-Woodford L, Bae, KT, Landsittel D, Li J, King BF, Martin D, Wetzel LH, Lockhart ME, Harris PC, Moxey-Mims M, Flessner M, Bennett WM, Grantham JJ. Kidney volume and functional outcomes in autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol.* 2012;7(3):479-486.

19. Fang XD, Yang F, Zhu L, Shen YL, Wang LL, Chen YY. Curcumin ameliorates high glucose-induced acute vascular endothelial dysfunction in rat thoracic aorta. *Clin Exp Pharmacol Physiol.* 2009;36(12):1177-1182.

20. Nakmareong S, Kukongviriyapan U, Pakdeechote P, Kukongviriyapan V, Kongyingyoes B, Donpunha W, Prachaney P, Phisalaphong C. Tetrahydrocurcumin alleviates hypertension, aortic stiffening and oxidative stress in rats with nitric oxide deficiency. *Hypertens Res.* 2012;35(4):418-425.

21. Gao J, Zhou H, Lei T, Zhou L, Li W, Li X, Yang B. Curcumin inhibits renal cyst formation and enlargement in vitro by regulating intracellular signaling pathways. *Eur J Pharmacol.* 2011;654(1):92-99.

22. Leonhard WN, van der Wal A, Novalic Z, Kunnen SJ, Gansevoort RT, Breuning MH, de Heer E, Peters DJ. Curcumin inhibits cystogenesis by simultaneous interference of multiple signaling pathways: in vivo evidence from a Pkd1-deletion model. *Am J Physiol Renal Physiol .* 2011;300(5):F1193-1202.

23. Pirson Y. Extrarenal manifestations of autosomal dominant polycystic kidney disease. *Adv Chronic Kidney Dis.* 2010;17(2):173-180.

24. Pretorius DH, Lee ME, Manco-Johnson ML, Weingast GR, Sedman AB, Gabow PA. Diagnosis of autosomal dominant polycystic kidney disease in utero and in the young infant. *J Ultrasound Med.* 1987;6(5):249-255.

25. Ivy DD, Shaffer EM, Johnson AM, Kimberling WJ, Dobin A, Gabow PA. Cardiovascular abnormalities in children with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol.* 1995;5(12):2032-2036.

26. Sedman A, Bell P, Manco-Johnson M, Schrier R, Warady BA, Heard EO, Butler-Simon N, Gabow P. Autosomal dominant polycystic kidney disease in childhood: a longitudinal study. *Kidney Int.* 1987;31(4):1000-1005.

27. Cadnapaphornchai MA, McFann K, Strain JD, Masoumi A, Schrier RW. Increased left ventricular mass in children with autosomal dominant polycystic kidney disease and borderline hypertension. *Kidney Int.* 2008;74(9):1192-1196. PMCID: PMC2666428.

28. Chue CD, Townend JN, Steeds RP, Ferro CJ. Arterial stiffness in chronic kidney disease: causes and consequences. *Heart.* Jun 2010;96(11):817-823.

29. Vila E, Salaices M. Cytokines and vascular reactivity in resistance arteries. *Am J Physiol Heart Circ Physiol.* 2005;288(3):H1016-1021.

30. Cosentino F, Barker JE, Brand MP, Heales SJ, Werner ER, Tippins JR, West N, Channon KM, Volpe M, Luscher TF. Reactive oxygen species mediate endothelium-dependent relaxations in tetrahydrobiopterin-deficient mice. *Arterioscler Thromb Vasc Biol.* 2001;21(4):496-502.

31. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest.* 2003;111(8):1201-1209.

32. Kofler S, Nickel T, Weis M. Role of cytokines in cardiovascular diseases: a focus on endothelial responses to inflammation. *Clin Sci (Lond).* 2005;108(3):205-213.

33. Janssen-Heininger YM, Poynter ME, Baeuerle PA. Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappa B. *Free Radic Biol Med.* 2000;28(9):1317-1327.

34. Chung HY, Sung B, Jung KJ, Zou Y, Yu BP. The molecular inflammatory process in aging. *Antioxid Redox Signal.* 2006;8(3-4):572-581.

35. Smith MR, Gangireddy SR, Narala VR, Hogaboam CM, Standiford TJ, Christensen PJ, Kondapi AK, Reddy RC. Curcumin inhibits fibrosis-related effects in IPF fibroblasts and in mice following bleomycin-induced lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2010;298(5):L616-625.

36. Rungseesantivanon S, Thenchaisri N, Ruangvejvorachai P, Patumraj S. Curcumin supplementation could improve diabetes-induced endothelial dysfunction associated with decreased vascular superoxide production and PKC inhibition. *BMC Complement Altern Med.* 2010;10:57.

37. Majithiya JB, Balaraman R. Time-dependent changes in antioxidant enzymes and vascular reactivity of aorta in streptozotocin-induced diabetic rats treated with curcumin. *J Cardiovasc Pharmacol.* 2005;46(5):697-705.

38. Olszanecki R, Gebska A, Korbut R. The role of haem oxygenase-1 in the decrease of endothelial intercellular adhesion molecule-1 expression by curcumin. *Basic Clin Pharmacol Toxicol*. 2007;101(6):411-415.
39. Parodi FE, Mao D, Ennis TL, Pagano MB, Thompson RW. Oral administration of diferuloylmethane (curcumin) suppresses proinflammatory cytokines and destructive connective tissue remodeling in experimental abdominal aortic aneurysms. *Ann Vasc Surg.* 2006;20(3):360-368.
40. Jain SK, Rains J, Croad J, Larson B, Jones K. Curcumin supplementation lowers TNF-alpha, IL-6, IL-8, and MCP-1 secretion in high glucose-treated cultured monocytes and blood levels of TNF-alpha, IL-6, MCP-1, glucose, and glycosylated hemoglobin in diabetic rats. *Antioxid Redox Signal. 2*009;11(2):241-249.
41. Quiles JL, Mesa MD, Ramirez-Tortosa CL, Aguilera CM, Battino M, Gil A, Ramirez-Tortosa MC. Curcuma longa extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arterioscler Thromb Vasc Biol.* 2002;22(7):1225-1231.
42. Fleenor BS, Sindler AL, Marvi NK, Howell KL, Zigler ML, Yoshizawa M, Seals DR. Curcumin ameliorates arterial dysfunction and oxidative stress with aging. *Exp Gerontol.* 2013;48(2):269-276.
43. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int J Biochem Cell Biol.* 2009;41(1):40-59.
44. Akazawa N, Choi Y, Miyakia A, Tanabe Y, Sugawara J, Ajisaka R, Maeda S. Curcumin ingestion and exercise training improve vascular endothelial function in postmenopausal women. *Nutrition Research.* 2012;32(10)795-9.
45. Shoskes DA. Effect of bioflavonoids quercetin and curcumin on ischemic renal injury: a new class of renoprotective agents. *Transplantation.*1998;66(2):147-152.
46. Sharma S, Kulkarni SK, Chopra K. Curcumin, the active principle of turmeric (Curcuma longa), ameliorates diabetic nephropathy in rats. *Clin Exp Pharmacol Physiol.* 2006;33(10):940-945.
47. Murugan P, Pari L. Influence of tetrahydrocurcumin on hepatic and renal functional markers and protein levels in experimental type 2 diabetic rats. *Basic Clin Pharmacol Toxicol.* 2007;101(4):241-245.
48. Ghosh SS, Massey HD, Krieg R, Fazelbhoy ZA, Ghosh S, Sica DA, Fakhry I, Gehr T. Curcumin ameliorates renal failure in 5/6 nephrectomized rats: role of inflammation. *Am J Physiol Renal Physiol.* 2009;296(5):F1146-1157.
49. Bayrak O, Uz E, Bayrak R, Turgut F, Atmaca AF, Sahin S, Yildirim ME, Kaya A, Cimentepe E, Akcay A. Curcumin protects against ischemia/reperfusion injury in rat kidneys. *World J Urol.* 2008;26(3):285-291.
50. Tirkey N, Kaur G, Vij G, Chopra K. Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. *BMC Pharmacol.* 2005;5:15.
51. Reed B, Nobakht E, Dadgar S, Bekheirnia MR, Masoumi A, Belibi F, Yan XD, Cadnapaphornchai M, Schrier RW. Renal ultrasonographic evaluation in children at risk of autosomal dominant polycystic kidney disease. *Am J Kidney Dis.* 2010;56(1):50-56. PMID: 20452711.
52. Schwartz GJ, Munoz A, Schneider MF,Hak RH, Kaskel F, Warady BA, Furth SL. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol.* 2009;20(3):629-637.
53. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro III AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J, for the Chronic Kidney Disease Epidemiology Collaboration. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-612.
54. Cadnapaphornchai MA, McFann K, Strain JD, Masoumi A, Schrier RW. Prospective change in renal volume and function in children with ADPKD. *Clin J Am Soc Nephrol.* 2009;4(4):820-829. PMCID: PMC2666428.
55. Cadnapaphornchai MA, Masoumi A, Strain JD, McFann K, Schrier RW. Magnetic resonance imaging of kidney and cyst volume in children with ADPKD. *Clin J Am Soc Nephrol* 2011;6(2):369-376. PMICID: PMC3052228.
56. Cadnapaphornchai M, McFann K, George DM, Wang W, Gitomer B, Strain JD, Schrier RW. Effect of pravastatin on total kidney volume, left ventricular mass index, and microalbuminuria in pediatric autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol* 9:889-896, 2014. PMCID: PMC4011448.
57. U.S. Department of Health and Human Services. National Institutes of Health. National Heart, Lung, and Blood Institute. The fourth report on the diagnosis, evaluation, and treatment of high blood

pressure in children and adolescents. Washington, DC. Available at http://www.nhlbi.nih.gov/health/prof/heart/hbp/hbp_ped.pdf. Accessed January 13, 2014.
58. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound assessment of flow-mediated dilation. *Hypertension.* 2010;55(5):1075-1085.
59. Miriyala S, Panchatcharam M, Rengarajulu P. Cardioprotective effects of curcumin. *Adv Exp Biol Med.* 2007;595:359-377.
60. Willcox DC, Willcox BJ, Todoriki H, Suzuki M. The Okinawan diet: health implications of a low-calorie, nutrient-dense, antioxidant-rich dietary pattern low in glycemic load. *J Am Coll Nutr.* 2009;28 Suppl:500S-516S.
61. Jain V, Prasad V, Pal R, Singh S. Standardization and stability studies of neuroprotective lipid soluble fraction obtained from Curcuma longa. *J Pharm Biomed Anal.* 2007;44(5):1079-1086.
62. Baum L, Lam CW, Cheung SK, Kwok T, Lui V, Tsoh J, Lam L, Leung V, Hui E, Ng C, Woo J, Chiu HF, Goggins WB, Zee BC, Cheng KF, Fong CY, Wong A, Mok H, Chow MS, Ho PC, Ip SP, Ho CS, Yu XW, Lai CY, Chan MH, Szeto S, Chan IH, Mok V. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. *J Clin Psychopharmacol.* 2008;28(1):110-113.
63. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, Ko JY, Lin JT, Lin BR, Ming-Shiang W, Yu HS, Jee SH, Chen GS, Chen TM, Chen CA, Lai MK, Pu YS, Pan MH, Wang YJ, Tsai CC, Hsieh CY. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res.* 2001;21(4B):2895-2900.
64. Dadhaniya P, Patel C, Muchhara J, Bhadja N, Mathuria N, Vachhani K, Soni MG. Safety assessment of a solid lipid curcumin particle preparation: acute and subchronic toxicity studies. *Food Chem Toxicol.* 2011;49(8):1834-1842.
65. Gota VS, Maru GB, Soni TG, Gandhi TR, Kochar N, Agarwal MG. Safety and pharmacokinetics of a solid lipid curcumin particle formulation in osteosarcoma patients and healthy volunteers. *J Agric Food Chem.* 2010;58(4):2095-2099.
66. Lao CD, Ruffin MTt, Normolle D, Heath DD, Murray SI, Bailey JM, Boggs ME, Crowell J, Rock CL, Brenner DE. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med.* 2006;6:10.
67. Zuccotti GV, Trabattoni D, Morelli M, Borgonovo S, Schneider L, Clerici M. Immune modulation by lactoferrin and curcumin in children with recurrent respiratory infections. *J Biol Regul Homeost Agents.* 2009;23(2):119-123.
68. Suskind DL, Wahbeh G, Burpee T, Cohen M, Christie D, Weber W. Tolerability of curcumin in pediatric inflammatory bowel disease: a forced-dose titration study. *J Pediatr Gastroenterol Nutr.* 2013;56(3):277-279.
69. Jablonski KL, Fedorova OV, Racine ML, Geolfos CJ, Gates PE, Chonchol M, Fleenor BS, Lakatta EG, Bagrov AY, Seals DR. Dietary sodium restriction and association with urinary marinobufagenin, blood pressure, and aortic stiffness. *Clin J Am Soc Nephrol*. Nov 2013;8:1952-1959. PMCID: PMC3817896
70. Jablonski KL, Racine ML, Geolfos CJ, Gates PE, Chonchol M, McQueen MB, Seals DR. Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. *J Am Coll Cardiol.* Jan 22 2013;61(3):335-343. PMCID: PMC3549053
71. Hidvegi EV, Illyes M, Benczur B, Bocskei RM, Ratgeber L, Lenkey Z, Molnar FT, Cziraki A. Reference values of aortic pulse wave velocity in a large healthy population aged between 3 and 18 years. *J Hypertens.* 2012;30(12):2314-2321.
72. Reusz GS, Cseprekal O, Temmar M, Kis E, Cherif AB, Thaleb A, Fekete A, Szabo AJ, Benetos A, Salvi P. Reference values of pulse wave velocity in healthy children and teenagers. *Hypertension.* 2010;56(2):217-224.
73. Ahimastos AA, Formosa M, Dart AM, Kingwell BA. Gender differences in large artery stiffness pre- and post puberty. *J Clin Endocrinol Metab.*2003;88(11):5375-5380.
74. Kelly AS, Marlatt KL, Steinberger J, Dengel DR. Younger age is associated with lower reactive hyperemic index but not lower flow-mediated dilation among children and adolescents. *Atherosclerosis.* 2014;234(2):410-414.
75. Marshall WA, Tanner JM. Growth and physiological development during adolescence. *Annu Rev Med.* 1968;19:283-300.
76. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44(235):291-303.

77. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child.* 1970;45(239):13-23.

78. Singh SP, Wahajuddin, K. JG. Determination of curcumin in rat plasma by liquid–liquid extraction using LC–MS/MS with electrospray ionization: assay development, Validation and application to a pharmacokinetic study. *J Bioanal Biomed.* 2010;2:79-84.

79. Celermajer DS, Sorensen KE, Gooch VM, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE, Spiegelhalter DJ. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet.* 1992;340:1111-1115.

80. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. *Hypertension.* 2011;57(1):63-69 PMCID: PMC3020150.

81. Jablonski KL, Gates PE, Pierce GL, Seals DR. Low dietary sodium intake is associated with enhanced vascular endothelial function in middle-aged and older adults with elevated systolic blood pressure. *Ther Adv Cardiovasc Dis.* 2009;3(5):347-356. PMCID: PMC3480332.

82. Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, Deanfield JE. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. *J Clin Invest.* 1994;93(1):50-55.

83. Lieberman EH, Gerhard MD, Uehata A, Selwyn AP, Ganz P, Yeung AC, Creager MA. Flow-induced vasodilation of the human brachial artery is impaired in patients <40 years of age with coronary artery disease. *Am J Cardiol.* 1996;78(11):1210-1214.

84. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Luscher TF, Shechter M, Taddei S, Vita JA, Lerman A. The assessment of endothelial function: from research into clinical practice. *Circulation.* 2012;126(6):753-767.

85. Eskurza I, Monahan KD, Robinson JA, Seals DR. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. *J Physiol.* 2004;556(Pt 1):315-324.

86. Eskurza I, Myerburgh LA, Kahn ZD, Seals DR. Tetrahydrobiopterin augments endothelium-dependent dilatation in sedentary but not in habitually exercising older adults. *J Physiol.* 2005;568(Pt 3):1057-1065.

87. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. *Clin Sci (Lond).* Jan 18 2011;120(9):357-375. PMCID: PMC3482987

88. Donald AE, Halcox JP, Charakida M, Storry C, Wallace SM, Cole TJ, Friberg P, Deanfield JE. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. *J Am Coll Cardiol.* 2008;51(20):1959-1964.

89. Widlansky ME. Shear stress and flow-mediated dilation: all shear responses are not created equally. *Am J Physiol Heart Circ Physiol.* 2009;296(1):H31-32.

90. Thijssen DH, Bullens LM, van Bemmel MM, Dawson EA, Hopkins N, Tinken TM, Black MA, Hopman MT, Cable NT, Green DJ. Does arterial shear explain the magnitude of flow-mediated dilation?: a comparison between young and older humans. *Am J Physiol Heart Circ Physiol.* 2009;296(1):H57-64.

91. Jablonski KL, Decker E, Perrenoud L, Kendrick J, Chonchol M, Seals DR, Jalal D. Assessment of vascular function in patients with chronic kidney disease. *J Vis Exp.* 2014;16(88): doi: 10.3791/51478. PMID: 24962357

92. Urbina EM, Williams RV, Alpert BS, Collins RT, Daniels SR, Hayman L, Jacobson M, Mahoney L, Mietus-Snyder M, Rocchini A, Steinberger J, McCrindle B. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: recommendations for standard assessment for clinical research: a scientific statement from the American Heart Association. *Hypertension.* 2009;54(5):919-950.

93. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, Vita JA, Levy D, Benjamin EJ. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation.* 2010;121(4):505-511.

94. Jablonski KL, Seals DR, Eskurza I, Monahan KD, Donato AJ. High-dose ascorbic acid infusion abolishes chronic vasoconstriction and restores resting leg blood flow in healthy older men. *J Appl Physiol.* 2007;103(5):1715-1721. PMID: 17965239

95. Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, Salvetti A. Age-related reduction of NO availability and oxidative stress in humans. *Hypertension.* 2001;38(2):274-279.

96. Moreau KL, Gavin KM, Plum AE, Seals DR. Ascorbic acid selectively improves large elastic artery compliance in postmenopausal women. *Hypertension.* 2005;45(6):1107-1112.
97. Eskurza I, Monahan KD, Robinson JA, Seals DR. Ascorbic acid does not affect large elastic artery compliance or central blood pressure in young and older men. *Am J Physiol Heart Circ Physiol.* 2004;286(4):H1528-1534.
98. Waugh RJ, Murphy RC. Mass spectrometric analysis of four regioisomers of F2-isoprostanes formed by free radical oxidation of arachidonic acid. *J Am Soc Mass Spectrom.* 1996;7(5):490-499.
99. Weimann A, Belling D, Poulsen HE. Measurement of 8-oxo-2'-deoxyguanosine and 8-oxo-2'-deoxyadenosine in DNA and human urine by high performance liquid chromatography-electrospray tandem mass spectrometry. *Free Radic Biol Med.* 2001;30(7):757-764.
100. Donato AJ, Black AD, Jablonski KL, Gano LB, Seals DR. Aging is associated with greater nuclear N F kappa B, reduced I kappa B alpha, and increased expression of proinflammatory cytokines in vascular endothelial cells of healthy humans. *Aging Cell.* 2008;7(6):805-812. PMCID: PMC3032405
101. Grantham JJ, Torres VE, Chapman AB, Guay-Woodford LM, Bae KT, King Jr. BF, Wetzel LH, Baumgarten, DA, Kenney PJ, Harris PC, Klahr S, Bennett WM, Hirschman GN, Meyers CM, Zhang X, Zhu F, Miller JP. Volume progression in polycystic kidney disease. *N Engl J Med.* 2006;354(20):2122-2130.
102. Johnson HM, Gossett LK, Piper ME, Aeschilmann SE, Baker TB, Fiore MC, Stein JH. Effects of smoking and smoking cessation on endothelial function: 1-year outcomes from a randomized clinical trial. *J Am Coll Cardiol.* 2010;55(18):1988-1995.
103. Ghiadoni L, Cupisti A, Huang Y, Mattei P, Cardinal H, Favilla S, Rindi P, Barsotti G, Taddei S, Salvetti A. Endothelial dysfunction and oxidative stress in chronic renal failure. *J Nephrol.* 2004;17(4):512-519.
104. Hornig B, Arakawa N, Kohler C, Drexler H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation.*1998;97(4):363-368.
105. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* 2002;39(2):257-265.
106. de Kreutzenberg SV, Ceolotto G, Cattelan A, et al. Metformin improves putative longevity effectors in peripheral mononuclear cells from subjects with prediabetes. A randomized controlled trial. *Nutr Metab Cardiovasc Dis* 2015;25:686-693
107. Kitada M, Kume S, Takeda-Watanabe A, et al. Calorie restriction in overweight males ameliorates obesity-related metabolic alterations and cellular adaptations through anti-aging effects, possibly including AMPK and SIRT1 activation. *Biochim Biophys Acta* 2013;1830:4820-4827