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Supplemental information

Weight loss and cystic disease progression in autosomal dominant polycystic kidney disease

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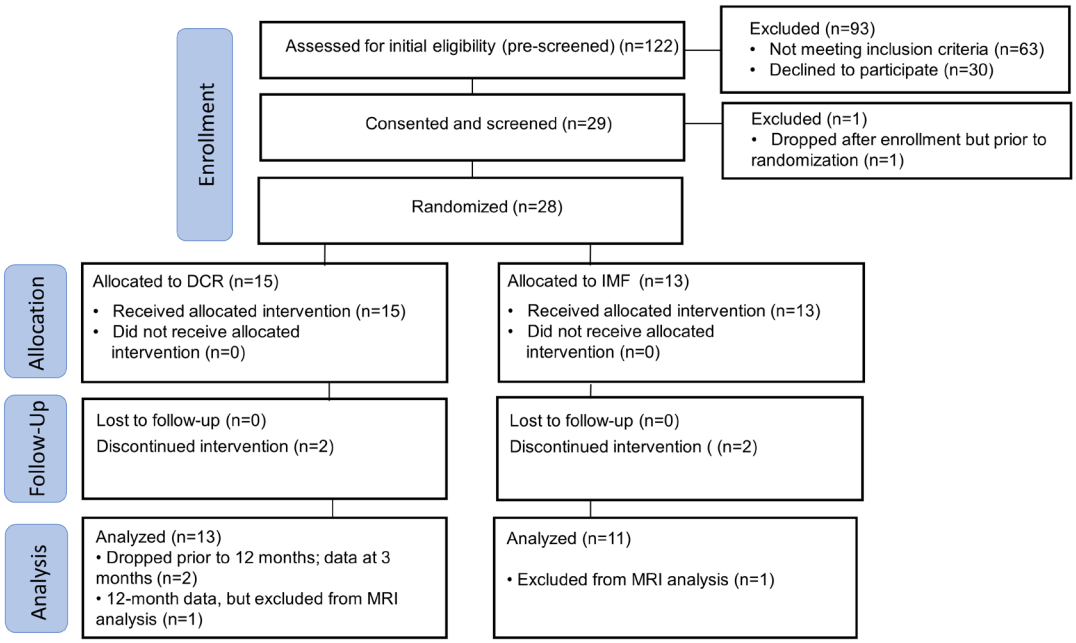

Figure S1. Patient enrollment, randomization, and completion (CONSORT) flow diagram for the human study. Related to STAR Methods. This flow diagram describes enrollment, randomization, and study completion for the pilot clinical trial on daily caloric restriction (DCR) and intermittent fasting (IMF).

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Group	Food Intake													
	Week 1		Week 2		Week 4		Week 8		Week 12		Average		% AL, Actual (Targeted)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
AL	2.11±0.060	2.05±0.34	2.10±0.11	2.05±0.13	2.06±0.020	2.06±0.020	2.49±0.23	1.90±0.16	2.17±0.090	1.84±0.15	**2.18±0.0035**	**1.95±0.024**	100.00 (N/A)	100.00 (N/A)
DCR	1.43±0.0071	1.25±0.067	1.41±0.0071	1.30±0.0071	1.42±0.025	1.27±0.0010	1.49±0.057	1.25±0.011	1.44±0.018	1.29±0.0035	**1.44±0.010**	**1.27±0.016**	65.81 (70)	65.09 (70)
TRF	2.02±0.025	1.83±0.26	2.05±0.16	1.66±0.088	2.07±0.27	1.53±0.22	2.00±0.16	1.92±0.24	2.02±0.15	1.97±0.074	**2.03±0.087**	**1.78±0.059**	92.99 (N/A)	91.27 (N/A)
IMF-CR (Mo,We,Fr)	0.44±0.032	0.37±0.018	0.43±0.0071	0.36±0.025	0.48±0.042	0.37±0.021	0.46±0.025	0.35±0.014	0.44±0.011	0.39±0.0011	**0.45±0.00071**	**0.37±0.0071**	20.54 (20)	18.70 (20)
IMF-AL (Tu,Th,Sa,Su)	3.09±0.33	3.71±0.13	2.86±0.028	3.65±0.14	2.87±0.018	3.28±0.071	3.13±0.26	3.52±0.28	3.74±0.27	3.18±0.49	**3.14±0.19**	**3.47±0.033**	143.58 (N/A)	177.66 (N/A)

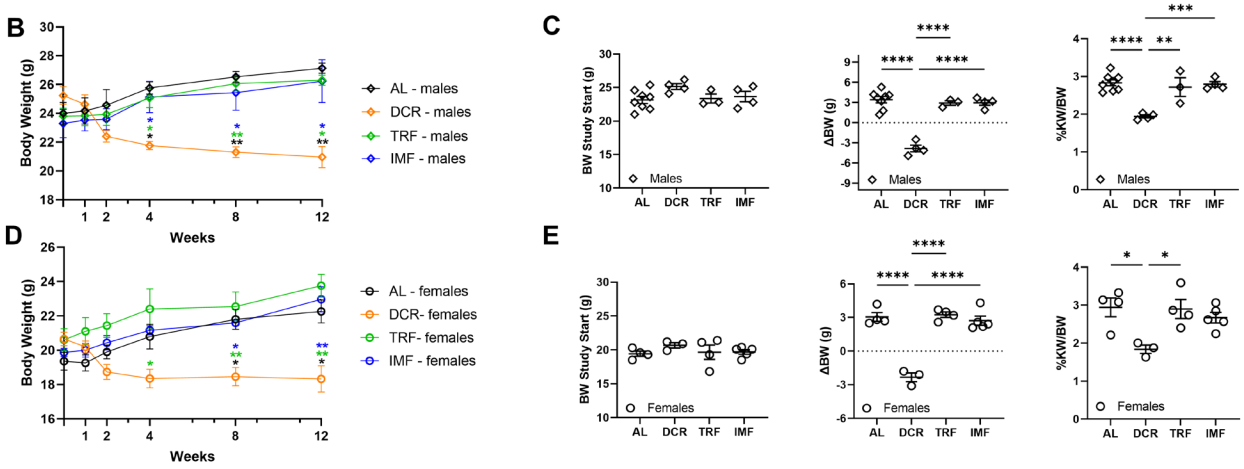


Figure S2. Sex specific details on food intake, body weight change, and %KW/BW in the murine trial. Related to STAR Methods and Figure 2. (A) Food intake of individual mice (Mean±SD shown of 3 males [M], 3 females [F]) throughout the duration of the study. Animals on daily caloric restriction (DCR) averaged ~65% of *ad libitum* (AL) intake (target 70%), animals on time restricted feeding (TRF) reduced their food intake by ~8% compared to AL, and animals on intermittent fasting (IMF) had the targeted 20% of AL food intake on the restricted days (Monday [Mo], Wednesday [We], Friday [Fr]), but overate by ~61% of AL food intake on their *ad libitum* days (Tuesday [Tu], Thursday [Th], Saturday [Sa], Sunday]). (B) [Males]; (D) [Females] Change in body weight throughout the duration of the study (Mean±SD shown of 3M, 3F). Animals on TRF and IMF gained weight comparably to animals on AL feeding. Animals on DCR had the steepest decline in body weight within the first two weeks of study start. Statistical significance is shown compared to AL. (C) [Males]; (E) [Females] Body weight (BW) at start of study, change in body weight from start to end of study, and % kidney weight (KW)/BW at end of study separated by sex. There was no significant difference in the body weight of animals enrolled in the different groups at the start of the study. Independent of sex, only mice on DCR lost significant weight throughout the study and showed a decrease in PKD severity. N: AL (8M, 4F), DCR (4M, 3F), TRF (3M, 4F), IMF (4M, 5F). Mean±SEM shown. Statistics: ANOVA with Tukey's post-hoc. P *<0.05, **<0.01, ***<0.001, ****<0.0001.

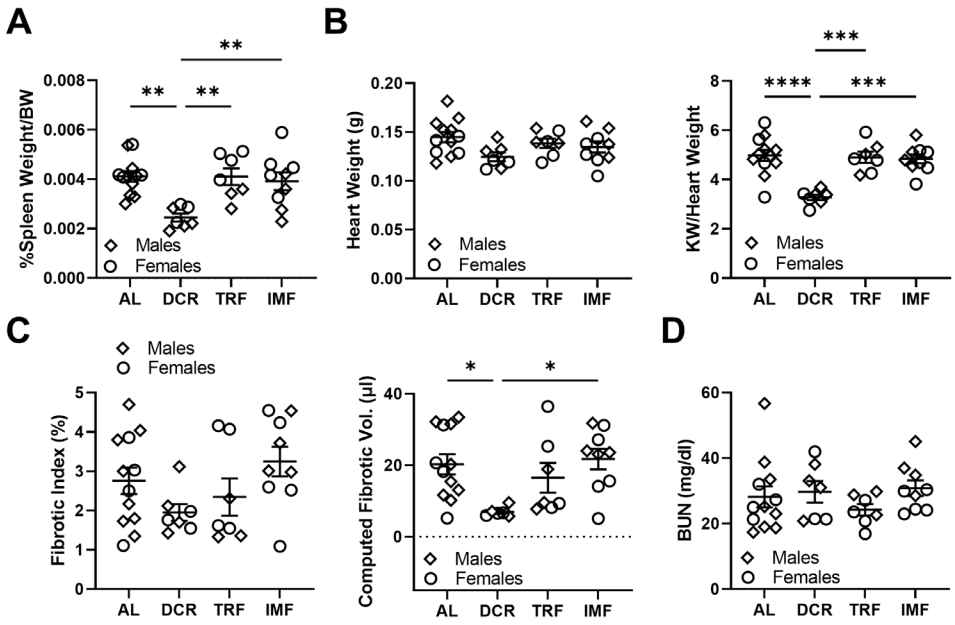


Figure S3. Assessment of PKD-severity and associated pathologies/ physiologies in the murine trial. Related to Figure 2. (A) Spleen weight normalized to body weight (BW). Spleen weight is significantly reduced upon daily caloric intake restriction compared to the other dietary regimens. (B) Heart weight of all animals as measured at end of study. Unlike body weight or femur length, heart weight was not impacted by the dietary intake that the animals were assigned to and hence represents a more suitable normalization factor for kidney weight. Kidney weight/heart weight (KW/HW) as a surrogate to measure PKD severity showed that only animals on daily caloric restriction (DCR) experienced a therapeutic effect based on food intake regimen. (C) Fibrotic Index (fibrotic area/total) and computed fibrotic volume (fibrotic area x kidney weight) as calculated from polarized light images of picrosirius red stained kidney cross sections. Although at 6 months (mo, end of study) the overall fibrotic burden of C57BL/6J *Pkd1*$^{RC/RC}$ animals was minimal, we did observe a significant reduction of fibrotic volume in animals on DCR compared to other dietary regimens. (D) Blood urea nitrogen (BUN) measurements. At study end (6mo) C57BL/6J *Pkd1*$^{RC/RC}$ animals had only slightly elevated levels of BUN compared to age/sex matched wildtype controls and no change was observed among the treatment groups. N: *ad libitum*, AL (8 males [M],4 females [F]), DCR (4M, 3F), time restricted feeding (TRF, 3M, 4F), intermittent fasting (IMF, 4M, 5F). Mean±SEM shown. Statistics: ANOVA with Tukey's post-hoc. P *<0.05, **<0.01, ***<0.001, ****<0.0001.

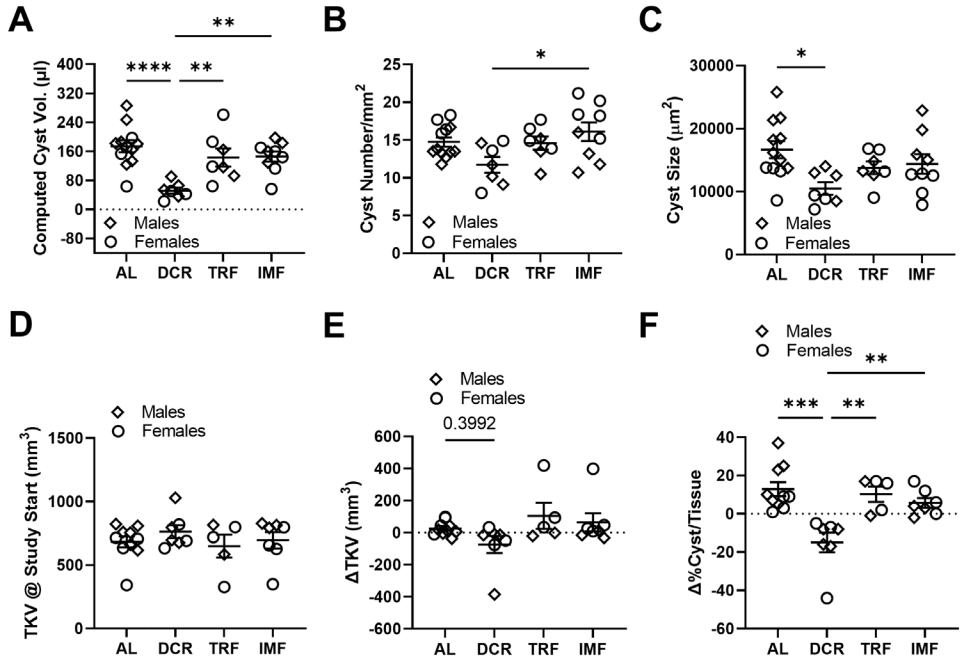


Figure S4. Detailed analysis of kidney cystic burden across study groups within the murine trial. Related to Figure 2. (A) Computed cyst volume calculated based on cystic area of three H&E kidney cross sections per animal and kidney weight at time of sacrifice. (B) Cyst number as measured from three H&E kidney cross sections per animal. (C) Average cyst size as measured from three H&E kidney cross sections per animal. (D) Total Kidney Volume (TKV) at study start as measure by magnetic resonance imaging (MRI). (E) Change in TKV from study start to study end as measured by MRI. (F) Percent change of kidney volume occupied by cysts versus tissue from study start to study end as determined by MRI. For all cystic parameters measured, only animals on daily caloric restriction (DCR) showed a significant decrease in PKD severity. No change was observed for animals on time restricted feeding (TRF) or intermittent fasting (IMF) compared to animals on *ad libitum* (AL) feeding. N: AL (8 males [M], 4 females [F]), DCR (8M,4F), TRF (3M, 4F), IMF (4M, 5F). Mean±SEM shown. Statistics: ANOVA with Tukey's post-hoc. P *<0.05, **<0.01, ***<0.001, ****<0.0001.

Table S1. Questionnaires and Compliance. Related to STAR Methods.

Questionnaires and Compliance	**Daily Caloric Restriction**				**Intermittent Fasting**			
	Baseline	**1 month**	**3 months**	**12 months**	**Baseline**	**1 month**	**3 months**	**12 months**
SF-36 physical health component, score	77±22	N/A	81±21	80±26	83±12	N/A	84±12	91±9
SF-36 mental health component, score	82±10	N/A	79±16	81±12	78±16	N/A	74±13	76±21
POMS vigor activity, score	8.2±3.2	N/A	7.5±3.2	8.2±4.2	7.6±3.0	N/A	7.3±4.5	7.9±5.6
POMS fatigue, score	5.2±5.4	N/A	7.7±7.5	5.2±4.7	6.4±3.9	N/A	6.5±5.4	6.7±4.8
Physical activity, MET hrs/week	7.5±7.7	N/A	6.4±6.5	9.4±78.3	7.9±8.4	N/A	4.9±6.3	5.3±5.1
Visit compliance, % attended	N/A	99±6	90±12	81±15	N/A	89±20	81±21	82±16
Self-reported dietary adherence, score	N/A	9.2±0.7 *	8.2±1.6	7.5±2.5 *	N/A	7.8±1.4	7.4±2.3	4.5±2.8
Self-reported difficulty to adhere, score	N/A	3.8±2.6	4.6±2.4	4.5±2.7 *	N/A	5.4±2.2	6.0±2.5	7.6±2.7
Self-reported likelihood to continue to adhere for next 30 days, score	N/A	9.8±0.4 *	8.7±1.5	7.7±2.6 *	N/A	8.4±1.6	7.6±1.9	5.0±2.8

Self-reported barriers to adherence, n (%)								
Hunger	N/A	4 (31%)	2 (15%) *	2 (18%)	N/A	6 (46%)	9 (75%)	6 (55%)
Holidays/special occasions	N/A	5 (38%)	7 (54%)	1 (9%)	N/A	5 (38%)	7 (58%)	0 (0%)
Vacation/travel	N/A	7 (54%)	6 (46%)	4 (36%)	N/A	2 (15%)	3 (25%)	5 (45%)
Food cravings	N/A	4 (31%)	4 (31%)	2 (18%)	N/A	4 (31%)	4 (33%)	7 (64%)
Stress	N/A	2 (15%)	3 (23%)	4 (36%)	N/A	5 (38%)	5 (42%)	4 (36%)
Too busy	N/A	3 (23%)	3 (23%)	5 (45%)	N/A	4 (30%)	3 (25%)	3 (27%)
Emotional eating	N/A	2 (15%)	3 (23%)	3 (27%)	N/A	0 (0%)	3 (25%)	2 (18%)
Lack of motivation	N/A	0 (0%)	3 (23%)	2 (18%)	N/A	1 (8%)	4 (33%)	5 (45%)
Change in schedule/responsibilities	N/A	2 (15%)	1 (8%)	3 (27%)	N/A	3 (23%)	2 (17%)	0 (0%)
Illness or injury (self or family member)	N/A	0 (0%)	2 (15%)	3 (27%)	N/A	1 (8%)	4 (25%)	1 (9%)
Lack of exercise	N/A	2 (15%)	2 (15%)	2 (18%)	N/A	0 (0%)	0 (0%)	0 (0%)
Lack of support from family/friends	N/A	0 (0%)	1 (8%)	2 (18%)	N/A	0 (0%)	3 (25%)	2 (18%)
Binge eating	N/A	1 (8%)	1 (8%)	1 (9%)	N/A	0 (0%)	0 (0%)	0 (0%)
Lack of weight loss	N/A	1 (8%)	0 (0%)	0 (0%)	N/A	0 (0%)	2 (17%)	2 (18%)

Lack of sleep	N/A	2 (15%)	1 (8%)	1 (9%)	N/A	0 (0%)	0 (0%)	0 (0%)
Side effects from diet	N/A	0 (0%)	0 (0%)	0 (0%)	N/A	1 (8%)	2 (17%)	0 (0%)
Caregiving responsibilities	N/A	0 (0%)	1 (8%)	0 (0%)	N/A	2 (15%)	1 (9%)	1 (9%)
Change in mood	N/A	1 (8%)	1 (8%)	1 (9%)	N/A	2 (15%)	1 (8%)	1 (9%)
Major life event	N/A	1 (8%)	0 (0%)	1 (9%)	N/A	0 (0%)	0 (0%)	0 (0%)

* $p<0.05$ vs. Intermittent fasting (IMF) at same time point by least square mean test between groups at individual time points for continuous variables, and Fisher's exact or chi-squared test vs. the same time point in the across groups for categorical variables. N varies depending on time point, group, questionnaire completion from 10-14. . Visit compliance is percent of education visits attended from baseline through given time point (excludes those that dropped out after previous time point). Self-reported questionnaire is a Likert-scale of 1-10 (months 1, 3, 12) and includes all participants with data available at given timepoint. Other items are baseline, month 1, month 3, and month 12; baseline data included only for participants with at least one follow-up measurement for a given variable. SF-36, 36-Item Short Form Survey; POMS, Profile of Mood States questionnaire. MET, metabolic equivalent of task. Participants could select up to 5 top barriers to adherence.

Table S2. **Adverse Events Related or Possibly Related to the Intervention among Participants who Dropped out of the Study. Related to Table 2.**

Treatment-Emergent Adverse Events	**Intermittent Fasting** (n=2)	**Daily Caloric Restriction** (n=2)
Hunger	1 (50%)	1 (50%)
Gastrointestinal distress (abdominal discomfort, constipation, nausea, or diarrhea)	0 (0%)	1 (50%)
Fatigue	0 (0%)	1 (50%)
Lightheadedness/dizziness	1 (50%)	2 (100%)
Cold Intolerance	0 (0%)	1 (50%)
Change in mood	1 (50%)	0 (0%)
Irritability	0 (0%)	0 (0%)
Insomnia	2 (100%)	0 (0%)
Headache	0 (0%)	0 (0%)
Impaired concentration and/or cognitive difficulties	2 (100%)	0 (0%)
Tremor	0 (0%)	0 (0%)

Data are n (%). These adverse events are among participants who dropped out of the study prior to 3 months. Among the two participants in the daily caloric restriction group who discontinued the intervention between 3 and 12 months, neither reported any adverse events.

Table S3. Changes in Vital Signs, Anthropometrics, Clinical Labs and Circulating Markers. Related to STAR Methods.

Variable	Daily Caloric Restriction			Intermittent Fasting			
	Baseline	**3 months**	**12 months**	**Baseline**	**3 months**	**12 months**	***P*-Value for group-time interaction or comparison of change between groups**
Weight, kg	107±17	95±11****	96±17****	96±10	89±9***	92±9*	0.16
BMI, kg/m^2	33.0±5.8	31.1±5.5****	31.2±6.2***	33.7±4.2	31.8±3.7***	32.02±3.9*	0.28
Waist circumference, cm	115±12	N/A	109±11	108±5	N/A	103±5**	0.99
Hip circumference, cm	119±14	N/A	113±16**	113±11	N/A	111±10	0.89
Waist to hip ratio	0.98±0.14	N/A	0.97±0.09	0.96±0.10	N/A	0.94±0.10	0.24
SBP, mmHg	115±13	115±10	115±14	126±12	123±19	127±14	0.57
DBP, mmHg	74±8	72±6	75±10	84±9	81±13	82±9	0.73
Resting HR, bpm	69±6	66±17	66±10	69±10	65±14	67±11	0.89
Fasting glucose, mg/dL	99±13	88±8	99±8	93±10	96±9	95±6	0.08
Triglycerides, mg/dL	173±83	161±67	111±29**	128±35	117±44	94±36	0.37
Total Cholesterol, mg/dL	187±37	170±34*	164±30**	170±33	169±40	164±36	0.03

LDL Cholesterol, mg/dL	113±31	97±29*	100±24*	102±35	101±43	98±37	0.08
HDL Cholesterol, mg/dL	43±11	45±8	42±9	45±7	45±9	47±8	0.06
HbA1c, %	5.5±0.4	5.3±0.3*	5.3±0.4	5.4±0.3	5.4±0.2	5.3±0.3	0.20
CKD-EPI eGFR, ml/min/1.73m^2	60±23	70±26	60±27	75±17	68±21	76±25	0.42
IGF1, ng/mL	104±27	127±41	103±25	93±32	96±32	101±29	0.21
IGF1-BP, ng/mL	9.4 (1.8, 15.7)	11.1 (7.4, 26.0)*	10.0 (5.8, 19.6*	7.5 (5.2, 11.2)	8.7 (3.1, 18.8)	7.9 (4.4, 10.4)	0.06
IGF1:1GF1-BP, ratio	14.1 (5.3, 68.3)	11.1 (4.7, 16.2)	8.9 (3.9, 20.4)*	13.8 (5.3, 16.2)	9.1 (4.9, 34.1)	11.5 (9.8, 26.0)	0.13
Leptin, pg/mL	38311 (16274, 58448)	30092 (5236, 47858)**	23969 (5529, 55546)*	23904 (15081, 49330)	24004 (5516, 44447)	25725 (6000, 46202)	0.21
Ghrelin, pg/mL	333±119	266±138	358±214	399±119	303±114*	353±124	0.43
Adiponectin, µg/mL	4.7±3.1	4.8±3.1	5.6±2.8	4.8±3.0	4.8±2.6	4.3±2.6	0.55
CRP, mg/L	3.8 (3.3, 4.7)	3.0 (1.8, 10.7)	2.2 (1.4, 11.4)	3.6 (0.9, 5.4)	2.4 (0.6, 4.5)	1.7 (0.5, 5.3)*	0.55
IL-6, pg/mL	7.6±2.1	8.3±2.4	6.8±1.2	6.5±2.3	7.5±2.3	6.7±1.7	0.72
IL-18, pg/mL	598±164	515±134**	503±148**	467±127	452±137	430±151	0.05
TNF-α, pg/mL	9.2±2.0	10.6±3.1	8.2±1.4	8.0±2.1	8.5±1.8	7.7±1.2	0.29
BDNF, pg/mL	2552±621	2377±475	2301±647	2764±647	2741±457	2772±860	0.75
MCP-1, pg/mL	316±72	300±60	265±59	330±112	326±112	349±119	0.58
GLP-1, pM	31.7±14.0	29.6±8.9	29.8±8.5	25.1±7.8	29.2±8.3	25.5±9.2	0.05
Insulin, uIU,mL	23.7±9.5	18.9±7.6*	17.5±9.2***	15.4±4.3	15.6±6.1	14.4±4.8	<0.01
HOMA-IR	5.8±2.7	4.1±2.1*	4.3±2.3*	3.5±0.9	3.7±1.5	3.4±1.2	<0.01

Data are mean±S.D. or median (interquartile range). Participants with at least one follow-up visit for a given variable are included in the analysis; n varies by variable, group, and timepoint (n provided below). P-value in last column is group*time interaction based on a repeated measures analysis with a mixed model or an independent samples t-test comparing change between groups if only 2 time points are available. * p≤0.05; ** p<0.01, *** p<0.001; **** p<0.0001; Tukey-Kramer post-hoc test (adjusted for multiple comparisons) vs. baseline in the same group for repeated measures tests or paired t-test vs. baseline if only 2 time points are available. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per min; LDL, low density lipoprotein; HDL, high density lipoprotein; HbA1c, hemoglobin A1c, CKD-EPI eGFR, estimated glomerular filtration rate by the Chronic Kidney Disease Epidemiology Collaboration equation; IGF1, insulin-like growth factor-1; IGF1-BP, insulin-like growth factor-1 binding protein; CRP, C-reactive protein; IL-6, interleukin-6; IL-18, interleukin-18; TNF-α, tumor-necrosis factor-α; BDNF, bone derived neurotrophic factor; MCP-1, monocyte chemoattractant protein-1; GLP-1, glucagon-like peptide-1; HOMA-IR, Hemostatic Model Assessment of Insulin Resistance.

Table S4. N per group/timepoint., addendum to Table S3. Related to STAR Methods.

Variable	DCR			IMF		
	Baseline	3 months	12 months	Baseline	3 months	12 months
Weight	11	10	11	11	11	11
BMI	11	10	11	11	11	11
Waist circumference	11	N/A	10	11	N/A	10
Hip circumference	11	N/A	10	11	N/A	10
Waist to hip ratio	11	N/A	10	11	N/A	10
SBP	11	8	10	11	6	10
DBP	11	8	10	11	6	10
Resting HR	11	7	10	11	5	10
Fasting glucose	11	10	11	10	9	11
Triglycerides	11	10	10	11	9	10
Total cholesterol	11	10	10	11	9	10
LDL cholesterol	11	10	10	11	9	10
HDL cholesterol	11	10	10	11	9	10
HbA1c	11	10	9	11	9	10
CKD-EPI eGFR	11	10	11	11	9	11
IGF1	10	11	10	11	11	10
IGF1-BP	10	11	10	11	11	10
IGF1:1GF1-BP	10	11	10	11	11	10
Leptin	10	11	10	11	11	10
Ghrelin	10	11	10	11	11	10

Adiponectin	10	11	10	11	11	10
CRP	10	11	10	11	11	10
IL-6	10	11	10	11	11	10
IL-18	10	11	10	11	11	10
TNF-α	10	11	10	11	11	10
BDNF	10	10	10	11	10	8
MCP-1	10	10	10	11	10	8
GLP-1	10	11	10	11	11	10
Insulin	10	11	10	11	11	10
HOMA-IR	10	10	10	11	9	10

Table S5. Comparison of MRI to Historical Data. Related to Figure 1.

	Current Study		Historical Data			
Baseline Characteristics and Kidney Growth	**DCR** (n=10)	**IMF** (n=10)	**CRISP** (n=241)	**HALT-PKD Study A** (n=558)	**TEMPO 3:4** (n=1445)	**DIPAK** (n=309)
Age, years	45±12	47±7	34±9	37±8	39±7	48±7
Sex, % Male	40%	50%	40%	51%	52%	47%
BMI, kg/m^2	35±6	34±5	26±5	27±5	26±5	27±5
CKD-EPI eGFR, ml/min/1.73m^2	60±24	73±17	98±25 (MDRD)	91±17	81±22	51±11 (MDRD)
htTKV, mL/m	921 [605, 1151]	1288 [418, 1994]	504 [407]	692±402	971±499	Placebo: 1029 [723, 1668] Lanreotide: 1138 [790, 1670]
Mayo Classification C, D, or E, n (%)	70%	70%	71%	68%	90%	78%
Annual %Δ htTKV	1.5±3.4	1.7±6.1	5.3±4.0	Std BP: 6.6 (no s.d.) Low BP: 5.6 (no s.d.)	Placebo: 5.5; 95% CI (5.1, 6.0) Tolvaptan: 2.8; 95% CI (2.5, 3.1)	Control: 5.6; 95% CI (4.8, 6.4) Lanreotide: 4.2; 95% CI (3.3, 5.0)

Data are mean±SD or median [IQR]. DCR, daily caloric restriction; IMF, intermittent fasting; CRISP, Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (Chapman et al., 2003, Grantham et al., 2006, Irazabal et al., 2015, Chapman et al., 2012); HALT-PKD, HALT Progression of Polycystic Kidney Disease; TEMPO (Schrier et al., 2014, Irazabal et al., 2017), Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes (Torres et al., 2012, Irazabal et al., 2016, Nowak et al., in press); DIPAK, Developing Interventions to Halt Progression of ADPKD 1 (Meijer et al., 2018), BMI, body mass index; CKD-EPI eGFR, estimated glomerular filtration rate by the Chronic Kidney Disease Epidemiology Collaboration equation; MDRD, Modification of Diet in Renal Disease; htTKV, height-corrected total kidney volume. N=173 class 1 non-Mayo participants used for Mayo classification in CRISP; n=551 participants used for Mayo classification and htTKV in HALT-PKD Study A; n=1391 class 1 participants used for Mayo classification and htTKV in TEMPO 3:4

Supplemental Methods S1. Complete human study protocol; Related to STAR Methods
Protocol #: 17-1327
Project Title: Daily Caloric Restriction and Intermittent Fasting in Overweight and Obese Adults with Autosomal Dominant Polycystic Kidney Disease
Principal Investigator: Kristen Nowak, Ph.D., MPH
Version Date:5/20/2020

Specific Aims

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening genetic disease, affecting 1 in 400 to 1000 individuals[1]. The disease is characterized by development and continued growth of numerous fluid-filled renal cysts that result in ultimate loss of kidney function in the majority of individuals[2]. . 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, thus 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[3]. Interventions that may slow progression of ADPKD are of **considerable clinical importance**.

Similar to the general population, the prevalence of overweight/obesity has been rising in ADPKD patients[4], affecting about two-thirds of individuals[5,6]. While obesity is known to be an independent predictor of incident chronic kidney disease in the general population[7], the role of obesity in ADPKD progression is **surprisingly unknown**. We have novel preliminary data that overweight/obesity is associated with substantially faster kidney growth in adults with ADPKD, independent of baseline kidney size and other risk factors.

Additionally, recent work in multiple rodent models of ADPKD has shown that mild-to-moderate food restriction profoundly slows cyst growth and maintains renal function via mechanisms including activation of the AMP-activated kinase (AMPK) pathway, suppression of mammalian target of rapamycin (mTOR)-S6 kinase (S6K) signaling and insulin-like growth factor-1 (IGF-1) levels, and reversal of ADPKD-associated increased expression of hexokinase 2, the rate limiting step in glycolysis[8,9]. Consistent with these findings, recent evidence indicates that in ADPKD metabolic reprogramming favors enhanced aerobic glycolysis promoting proliferation, which is dependent upon inhibition of the AMPK axis and activation of mTOR signaling[10-12].

Collectively, these data suggest that dietary restriction regimens may slow ADPKD progression. The current standard of care dietary approach for the treatment of obesity is daily caloric restriction (DCR). An alternate approach to traditional DCR is intermittent fasting (IMF), which consists of either fasting or substantially reducing caloric intake 1-3 days per week[13]. Notably, the periods of fasting implemented in the latter approach **may have profound effects upon pathways implicated in ADPKD progression**, including AMPK activation, suppression of mTOR-S6K signaling, and reduced IGF-1 signaling[14,15]. IMF also promotes metabolic reprogramming from carbohydrate to fat metabolism, which could suppress cyst growth, as evidence suggests cysts may rely on glycolysis and be unable to utilize ketones as an energy source[16].

Thus weight loss, caloric restriction, and/or periods of fasting may be beneficial in slowing ADPKD progression. However, there is presently **no information available** regarding the feasibility or efficacy of any dietary restriction approach specifically in patients with ADPKD. Accordingly, the **primary aim** of this application is to determine the feasibility of delivering a 1 year behavioral weight loss intervention (based on either DCR or IMF) in 30 adults with overweight/obesity and ADPKD with normal to moderately declined renal function. Importantly, targeted weekly energy deficit is designed to be similar (~34%) between groups to aid in interpretation of the results. A key **secondary goal** is to evaluate the safety, acceptability, and tolerability of IMF specifically in the ADPKD population as compared to current standard of care (DCR). Last, the **third exploratory aim** is **a)** to obtain mechanistic insight into biological pathways that may be altered; **b)** provide initial insight regarding any changes in TKV with IMF and/or DCR. These aims will be tested in a randomized, two-active arm, pilot clinical trial.

Specific Aim 1: To determine the **feasibility** of delivery of a 1 year group-based behavioral weight loss program in adults with overweight/obesity and ADPKD with normal to moderately declined renal function, with regard to enrollment, retention, and weight loss, to guide a future large-scale randomized, controlled trial.

Specific Aim 2: To evaluate **safety, acceptability, and tolerability** of IMF inf individuals with ADPKD, by comparing evaluation of safety labs, dietary adherence, adverse events, and quality of life measures between groups, to optimize program development in a future large-scale trial.

Specific Aim 3: To measure **makers of biological pathways**, including serum IGF-1/IGF binding protein-1 levels, peripheral blood mononuclear cell expression of p-AMPK and S6K, and serum β-hydroxybutyrate (ketone) levels after 1 year of IMF or DCR, to provide mechanistic insight supporting a large-scale trial.

Impact on the Field. Although weight loss is recommended for individuals with overweight/obesity in the general population[17], clinical studies evaluating the feasibility and efficacy of this recommendation in ADPKD patients, surprisingly, **have *not* been performed**. This work will determine the feasibility of 2 dietary restriction regimens. These results, combined with mechanistic insight obtained, will provide the necessary foundation for a subsequent long-term trial assessing the efficacy of dietary restriction for slowing ADPKD progression.

1. Significance

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common monogenic and potentially fatal disease, affecting 1 in 400 to 1000 individuals[1]. Disease results from a mutation in either of 2 genes, *PKD1* encoding polycystin 1, located on chromosome 16, and *PKD2* encoding polycystin 2, located on chromosome 4[1]. ADPKD is characterized by progressive development and enlargement of renal cysts which eventually destroy the normal parenchyma, leading to end-stage renal disease (ESRD) in the majority of afflicted patients[1,2]. Despite progress in basic research in ADPKD, the prognosis of patients has not substantially changed in the last 20 years[18], emphasizing the need for additional and better treatments.

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[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, and early therapeutic strategies to slow ADPKD progression are indicated.

Overweight and Obesity, Kidney Disease, and ADPKD. Quite similar to the general population, body-mass index (BMI) has been increasing in patients with ADPKD over recent decades[4], and approximately two-thirds of individuals are overweight or obese[5,6]. Obesity is an independent risk factor for incident chronic kidney disease (CKD) and ESRD in the general population[7]. Higher BMI is additionally associated with a faster decline in renal function in individuals with prevalent CKD (non-diabetic)[19], and weight loss can prevent further decline in renal function[20]. However, the role of obesity in progression of ADPKD, surprisingly, has not been evaluated. We have **novel preliminary data** that **overweight and obesity** are associated with **substantially faster kidney growth** (measured by height-corrected total kidney volume [htTKV]) in non-diabetic adults with ADPKD and normal renal function, independent of baseline kidney size and other risk factors. Importantly, an increase in htTKV precedes the decline in kidney function, which is typically delayed until the 4th decade of life in patients with ADPKD, and htTKV is a prognostic biomarker of future renal insufficiency[1].

Dysregulated Metabolic Pathways in ADPKD. The mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine protein kinase, activated by Akt and inhibited by AMP-activated kinase (AMPK)[21]. Overactivation of mTOR and its downstream target S6 kinase (S6K) contribute to ADPKD progression by mediating hyperproliferation of the cystic epithelium[22]. Additionally, AMPK, which is activated in response to low energy (high AMP/ATP ratio)[21], mediates cyst growth both by negatively regulating the cystic fibrosis transmembrane conductance regulator, which promotes cyst fluid secretion, and via mTOR inhibition[23]. Insulin-like growth factor-1 (IGF-1) is also a major regulator of mTOR signaling via the PI3K-Akt pathway and has pleiotropic effects including cell proliferation and differentiation[24]. It has been proposed to be secreted in cystic fluid in ADPKD and possibly contribute to cystogenesis[25].

Dysregulated Metabolic Pathways in Obesity. Notably, obesity is also associated with enhanced mTOR-S6K1 signaling[26], mediated in part by IGF-1 and reduced AMPK-activation[27]. Thus, these shared metabolic pathways could potentially contribute to the greater rate of kidney growth observed in ADPKD patients with overweight/obesity (see **Preliminary Data**). Interestingly, obesity is a known risk factor for many types of cancers, possibly via the mTOR pathway, and ADPKD is characterized by many of the hallmarks of cancer[28].

Metabolic Reprogramming in ADPKD. Also similar to cancer cells, recent evidence supports that cells lacking the PKD1 gene reprogram their metabolism to favor aerobic glycolysis (i.e. the Warburg effect), leading to enhanced cell proliferation and cyst expansion[10,12]. These metabolic abnormalities are dependent upon

AMPK axis inhibition and activation of mTOR signaling[10]. Inhibition of glycolysis with 2-deoxyglucose, a glucose analog that cannot be metabolized, slows kidney growth and decline in renal function via AMPK activation[10,12]. Thus, agents targeting metabolic reprogramming may be effective in slowing ADPKD[10,11].

Dietary Energy Restriction in ADPKD. Recently, mild-to-moderate food restriction (10-40%) was shown to profoundly slow disease progression in multiple rodent models of ADPKD in as little as 2 months, as evidenced by reduced cyst and kidney growth and maintenance of renal function[8,9]. These improvements were mediated in part by suppression of mTOR signaling, AMPK activation, and a reduction in IGF-1[8,9]. Additionally, caloric restriction reversed the ADPKD-associated increase in hexokinase 2 expression, which is the rate limiting step in glycolysis, thus supporting metabolic reprogramming[8]. However, no evidence is currently available regarding the effects of dietary energy restriction in humans with ADPKD.

Potential Benefits of Fasting in ADPKD. Periods of fasting may modify numerous cellular and molecular mechanisms implicated in ADPKD to a greater degree than traditional daily caloric restriction (DCR). Among other pathways, intermittent fasting (IMF) has been shown to stimulate the AMPK pathway, inhibit the mTOR-S6K pathway, and inhibit IGF-1[29]. It can promote metabolic reprogramming via a shift from carbohydrate to fat metabolism, mediated in large part by AMPK[30]. This shift to ketogenesis could suppress growth of cysts, which favor aerobic glycolysis and may not be able to use ketones as an energy source[14]. Related to this rationale, several clinical trials are currently ongoing to evaluate IMF in conjunction with chemotherapy to possibly slow tumor progression in numerous types of cancer, which is supported by studies in animals[31,32].

Current Approaches to Dietary Energy Restriction and Weight Loss: Animal data thus suggests weight loss, caloric restriction, and/or periods of fasting may be beneficial in slowing ADPKD progression; however, this has never been evaluated in humans. The current standard of care dietary approach for treating obesity is DCR[17]. IMF is a novel dietary approach to treating obesity involving 75-100% energy restriction on fast days with habitual intake on fed days. Various IMF regimens have been proposed (e.g. alternate-day fasting, fasting 1-3 days per week)[13] and shown to be safe and tolerable in healthy adults with overweight/obesity, with good adherence and comparable weight loss to DCR[13,33-35]. Prior research has suggested that in contrast to lean adults, obese individuals become habituated to IMF after about 2 weeks, leading to decreased hunger on fasting days and a lack of hyperphagic response on fed days[33]. While both dietary approaches to energy restriction would be expected to produce comparable weight loss (especially if the targeted weekly energy deficit is the same), they may have a profoundly different impact on biological pathways in ADPKD.

Scientific Premise. The high prevalence of overweight and obesity in patients with ADPKD, independent association of overweight and obesity with substantially faster ADPKD progression, and mechanistic animals studies indicating profound slowing of ADPKD with dietary restriction, strongly support the conduction of a pilot and feasibility study of dietary restriction in overweight and obese adults with ADPKD. The lack of previous trials of weight loss in APDKD support a two-arm comparison of traditional weight loss by DCR and a more novel approach of IMF. This pilot and feasibility trial will provide the foundation for a future, large-scale, longer-duration, randomized controlled trial (RCT) of dietary restriction in ADPKD.

2. Innovation

- A clinical trial of weight loss and/or dietary restriction has never been performed in a population of patients with ADPKD.
- Safe, lifestyle-related interventions have the potential for great clinical impact in ADPKD, which slowly progresses across a lifetime.
- IMF is a novel approach to dietary restriction that may affect biological pathways shared by ADPKD pathogenesis.
- Mechanistic insight will be gained by using a translational approach evaluating circulating and peripheral blood mononuclear cell (PBMC) markers of biological pathways in response to DCR and IMF.

3. Preliminary Data

***Recruitment of ADPKD Patients*.** Since 1985, the University of Colorado Denver has maintained an ADPKD registry and mailing list of patients who have participated in longitudinal studies of the natural history of ADPKD progression, as well as clinical trials. Currently, I am recruiting children and young adults with ADPKD for a 1

year clinical trial and recruitment is on track for study completion, with 75% (n=51) already enrolled. Thus, I am confident in my ability to recruit n=15 per group to this pilot and feasibility trial.

Feasibility of a Dietary Restriction Trial. I have prior experience with other dietary interventions, including dietary sodium restriction[36,37]. While I have never conducted a weight loss or dietary restriction intervention, my collaborator, Dr. Victoria Catenacci, has extensive experience in this area, including completion of a pilot trial comparing DCR to IMR in healthy obese adults[34], and will serve as a consultant for this project.

Overweight/Obesity and ADPKD Progression. Using data from the HALT-PKD Study A[5], I evaluated the longitudinal (5-yr) association of overweight/obesity with APDKD progression, measured as ∆htTKV (magnetic resonance imaging). 455 non-diabetic adults with ADPKD and normal renal function were categorized by BMI as normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), or obese (≥30 kg/m^2). In each approach, the initial model was unadjusted, then multivariable adjusted models were performed to include age, sex, race/ethnicity, and randomization group (model 1), model 1 plus SBP, estimated glomerular filtration rate (eGFR), and urinary albumin excretion (model 2), and model 2 + baseline htTKV and liver volume (model 3).

First, a multinomial logistic regression model was used with an outcome of three categories of annual htTKV growth (<5%, 5-7%, and ≥7%), based on the first and last available measurements. The annual percent ∆htTKV was greater with increasing BMI category (normal weight: 5.4±4.2%, overweight: 7.8±4.7%, obese: 9.5±6.0%; p<0.0001). The odds of progressing at ≥7% compared to <5% according to BMI category is shown in **Table 1**. In the HALT-PKD Study A, median annual increase in htTKV was 6.8%, thus categorization as ≥7% annual change represents rapid kidney growth. Next, mixed effects linear regression models were performed considering htTKV measurements at all available time points (baseline, 24 months, 48 months, and 60 months; **Figure 1**). In these models, the main effect of BMI category was considered with all time points incorporated in the model, thus comparing the difference across groups over time.

Model	Normal Weight (BMI 18.5-24.9 kg/m^2) (n=170)	Overweight (BMI 25-29.9 kg/m^2) (n=176)	Obese (BMI ≥30 kg/m^2) (n=109)
Unadjusted	Ref	2.91 [1.80, 4.71]	4.63 [2.58, 8.32]
Model 1	Ref	2.81 [1.67, 4.71]	4.45 [2.40, 8.23]
Model 2	Ref	2.85 [1.68, 4.85]	4.42 [2.35, 8.32]
Model 3	Ref	3.07 [1.79, 5.27]	4.86 [2.54, 9.30]

Table 1. Associations (OR [95% Confidence Interval; CI)] of BMI categories with ≥7% annual percent change in htTKV. In the fully adjusted model, compared to the normal weight group, the obese group had a 4.86 (95% CI): 2.54, 9.30) greater odds of a progressing at a rate of ≥7% compared to <5% htTKV growth. The odds of progressing at a rate ≥7% compared to <5% was also significantly greater in the overweight compared to normal weight.

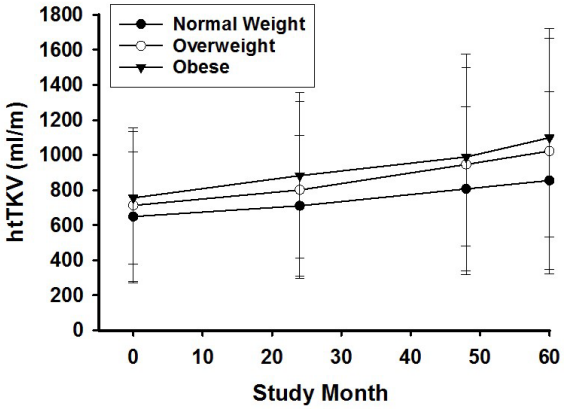


Figure 1. Mean htTKV values according to BMI category and month of measurement**.** In the fully adjusted mixed linear effects regression model, htTKV across all time points was greater in both the overweight (p=0.01) and obese (p=0,005) group compared to the normal weight group, indicating faster progression of ADPKD.

4. Approach

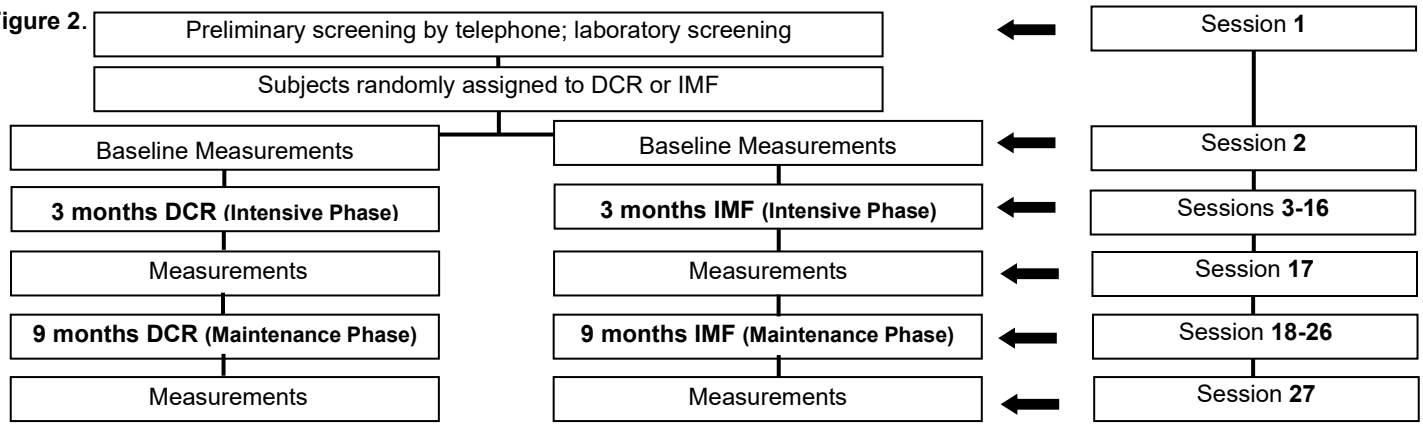
4.1 Subjects. After obtaining their written informed consent, adults (18-65 years) with overweight/obesity (BMI 25-45 kg/m^2) and a diagnosis of ADPKD (modified Pei-Ravine criteria[38]) with preserved to moderately declined renal function (eGFR ≥30 mL/min/1.73 m^2 by the CKD Epidemiology Collaboration [CKD-EPI] equation[39]) will be recruited to participate. Screening and testing will occur at the **University of Colorado PKD Center**. Non-local participants will travel to twice to Colorado (Sessions 2 and 27). Session 1 and subsequent blood draws will be arranged at a local contracted laboratory (Quest) for non-local participants. The behavioral weight loss interventions (focusing on either DCR or IMF as the primary dietary restriction strategy) will be implemented by the Nutrition and Obesity Research Center (**NORC**) Clinical Intervention and Translation **(CIT) Core** of the **Anschutz Health and Wellness Center (AHWC)** and delivered in a web-based manner. Resting energy

expenditure (REE) will be measured at the AHWC and renal magnetic resonance imaging (MRI) scans will be performed at the Brain Imaging Center. Major inclusion/exclusion (I/E) criteria are shown in the table below (**Table 2**), with addition details provided in **Human Subjects**.

Table 2 **Inclusion Criteria**	**Exclusion Criteria**
• Aged 18-65 • ADPKD diagnosis based on the Ravine criteria • BMI 25-45 kg/m^2 • CKD-EPI eGFR ≥30 mL/min/1.73 m^2 • Access to the internet with video chat capabilities • No plans for extended travel without internet access (>2 weeks) during the 3 month intensive period • Not currently participating in another interventional study or weight loss program • Ability to provide informed consent	• Diabetes mellitus • Current nicotine use or history of use in the past 12 months; alcohol/substance abuse • History of hospitalization or major surgery within the last 3 months • Untreated dyslipidemia or uncontrolled hypertension • Pregnancy, lactation, or unwillingness to use adequate birth control • Cardiovascular disease, peripheral vascular disease, cerebrovascular disease, significant pulmonary or gastrointestinal disease, cancer • Medications that may affect weight, appetite, food intake, or energy metabolism • History of clinically diagnosed eating disorder • Weight change of ≥5% in the past 3 months • Untreated hyper- or hypothyroidism • Major psychiatric disorder or current moderate to severe depression • Inability to cooperate with/clinical contraindication for MRI including severe claustrophobia, implants, devices, or non-removable body piercings

4.2 Experimental Design. A 1 year, randomized, two-active-arm, single-blind study with dietary restriction will be conducted (Figure 2). Subjects will undergo telephone and laboratory screening for I/E criteria and will then be randomly assigned to either DCR or IMF. Members of the investigative team involved in the acquisition and analysis of data will be blinded to the treatment status. Due the nature of the intervention, study participants will not be blinded. Both groups will receive a comprehensive, group-based, behavioral, weight loss intervention. Randomized groups will meet in small cohorts, via the web-based platform Zoom (which is HIPAA compliant), separately (Sessions 3-16 and Session 18-26). For non-local participants, diet records will be returned by mail/electronically for session 17 and anthropometric measurements will be collected by utilizing a BodyTrace scale for remote transmission of body weight.

Figure 2.



- **Session 1:** Screening measurements
- **Session 2:** Baseline measurements
    - Resting energy expenditure
    - Anthropometric measurements and vital signs
    - Diet record analysis
    - Questionnaires
    - Blood sampling for measurement of circulating markers and isolation of PBMC (see below)
    - TKV and abdominal adiposity assessment by MRI
    - Meet 1 on 1 with registered dietitian (RD) to orient to program

- **Sessions 3-16:** Online group-based, behavioral intervention sessions (intensive phase; weekly); anthropometric measurements; questionnaires; diet record analysis and (month 1 and 3); home blood pressures (monthly)
- **Session 17:** Blood and urine sampling (completed locally)
- **Sessions 18-26:** Online group-based, behavioral intervention sessions (maintenance phase; monthly to bi-monthly)
- **Session 27:** Identical to session 2, except no resting energy expenditure or 1 on 1 meeting with RD

An additional blood draw(s) may be required if there are safety concerns or unexpected lab errors.

4.3 Dietary Restriction Interventions. Both groups will receive a 1 year group-based, behavioral, weight loss intervention developed and delivered in consultation with the collaborator Dr. Catenacci and administered by the NORC CIT Core. The mentor, Dr. Michel Chonchol (a board-certified nephrologist), will provide medical oversight. Curriculum for the DCR group will be based on the Colorado Weigh behavioral weight loss program, which employs a skills-based approach and cognitive behavioral strategies towards lifestyle modification[40]. Curriculum for the IMF intervention will be developed in consultation with Dr. Catenacci and a RD and will include similar themes to DCR, but with focused behavioral support for IMF. Sessions will be delivered on the HIPAA compliant web-based platform Zoom by the AHWC, last about 60-75 minutes in duration, and will be taught by an RD experienced with group-based behavioral interventions. The initial 3 months will be an intensive phase with weekly sessions focusing on achieving initial weight loss. The final 9 months will be a maintenance with monthly to bi-monthly sessions focusing on strategies to maintain weight loss achieved in the first 3 months. Format will include a mix of large-group discussion, small breakout discussions, visual demonstrations, and written exercises. Topics will include realistic weight loss goal setting, self-monitoring strategies, mindful eating, stress management, cognitive restructuring, and strategies to overcome barriers to healthy eating. Specific topics related to DCR and IMF will also be covered in each respective group. IMF-specific strategies will include: strategies to deal with hunger on fast days, distraction techniques, and choosing a balanced diet/appropriate portions on fed days. Maintenance phase topics will include overcoming weight loss plateaus, screen time and sedentary behavior, negative self-talk, cooking demonstrations, and guest speakers. While the curriculum will primarily be delivered in an online small group setting, there is also an option for 1 on 1 online sessions and/or phone calls as needed. Participant weight will also be tracked weekly at the time of these sessions by a BodyTrace scale.

Both groups will reduce overall energy intake (EI) by ~34% per week, using either DCR or IMF. Recommended macronutrient content (55% carbohydrate, 15% protein, 30% fat) will be the same in both groups. The goal of the DCR group will be an approximately 34% daily energy deficit from baseline individual weight maintenance energy requirements (based on resting energy expenditure [REE] x activity factor of 1.5), which will we measured between 6-10 AM after rest and 24-abstention from exercise using standard indirect calorimetry[41] with the ventilated hood technique. Participants in this group will also be instructed in specific strategies to support DCR including: counting calories, portion size awareness, and daily food logging.

Participants in the IMF group will be instructed to reduce EI to ~20% of estimated energy requirement (delivered as a single meal) three non-consecutive days per week, resulting in a weekly energy deficit of ~34% (similar to the DCR group) (**Table 2**). Sample fast day menus and individualized fast day calorie goals will be provided to assist with achieving EI targets. On fast days, participants will be allowed to consume their calories at the meal of their choice, as a previous study using a similar IMF protocol found that altering times of caloric intake during the modified fast day did not impact weight loss or compliance[42]. On fed days, IMF will eat ad libidum, but encouraged to make healthy food and portion choices. Participants in this group will be instructed in calorie counting, but will be asked to count calories and log food intake only on fast days on a weekly basis (3-day diet records will also be employed as described below). They will also be instructed in specific strategies to support IMF, as described above.

Table 2	Example comparison of kcals per day (and week) with DCR and IMF based on a 2,000 kcal/day energy intake							
Condition	**Sunday**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Weekly Total**
No diet	2,000	2,000	2,000	2,000	2,000	2,000	2,000	14,000
DCR	1320	1320	1320	1320	1320	1320	1320	9,240
IMF	2,000	400	2,000	400	2,000	400	2,000	9,200

1,320 kcals per day for DCR represents a ~34% daily reduction (680 kcals) and a ~34% weekly reduction (4,800 kcals)
400 kcals per day on fasted days for IMF represents a 80% reduction (1,600 kcals); based on previous literature that after ~2 weeks there is no compensation on fed days[32], this also results in a weekly reduction of ~34% (4,800 kcals)

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 with the diet 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). Virtual group meetings will continue to be offered monthly during this time. We will obtain their MRI and the research-specific blood samples 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 diet period, as many plan to continue after the study is completed anyway. Currently enrolled local participants will have the option of returning as scheduled, or also delaying their end-of-study visit if they are more comfortable returning at a later time.

Standardized Adjustments for Participants Experiencing Significant Intervention-Related Adverse Effects. Participants will not be withdrawn by the PI for non-adherence in this intent-to-treat study. Participants will be encouraged to adhere to the dietary prescriptions for the initial 2 weeks as a prior study suggests individuals with obesity become habituated to IMF after ~2 weeks[33]. After the initial 2 weeks, a standardized dietary modification will be offered if a participant: 1) expresses a desire to withdraw due to intolerance of study diet and/or 2) experiences adverse effects related to the study diet (i.e. insomnia, impaired concentration, irritability) that impair ability to function. DCR participants will be allowed to raise their calorie goal to target an energy deficit of 20% from weight maintenance requirements. IMF participants will be allowed to reduce fasting to 2 days per week (i.e. reduce targeted weekly energy deficit to ~20%). Participants will be allowed to continue these strategies for 2 weeks, and will then be asked to re-try the original dietary prescription. If they are still unable to tolerate the original dietary prescription after a second attempt, they will be allowed to continue at the modified levels for the study duration. Percentage of participants in each group requiring intervention modification will be recorded. There will be no adjustments for participants not losing weight.

4.4 Rationale for Intervention Design. We elected to use a two-active-arm study design, as neither weight loss nor periods of fasting have been evaluated previously in humans with ADPKD. Thus, a comparison of IMF to weight loss resulting from a traditional DCR approach is merited. While a control group was considered, given that the primary outcome of this pilot study is feasibility, it was determined that two active groups was a more valuable design than including a control group. A cross-over design was also considered, but concerns about the length of a washout period that would be required between phases favored a parallel group design. The study duration of the intensive phase (3 months) was selected to achieve ~5% weight loss based on the duration of numerous previous pilot and feasibility studies in overweight and obese adults without ADPKD[33,34,43,44], length of animal studies showing changes in biological pathways[8,9], and to inform a large-scale RCT. The 9 month maintenance phase was selected to provide preliminary insight into any changes in TKV at 1 year with each weight loss approach. We elected not to provide all food in order to facilitate translation to a longer duration, large-scale RCT.

4.5. Scientific Rigor and Consideration of Relevant Biologic Variables: Strategies to ensure a robust and unbiased approach include: 1) randomized design, 2) comparison of IMF to the current standard of care dietary approach for weight loss (DCR), 3) guidelines-based behavioral support to both groups, 4) matching of the targeted weekly energy deficit, 5) delivery of the interventions in a real-world setting in which food is not

provided, to avoid bias and ensure translatability of results, 6) development of a detailed curriculum for the weight loss programs to ensure treatment fidelity, and 7) development of a standardized manual of procedures and data collection protocols to enhance transparency and reproducibility. Strategies to ensure consideration of relevant biological variables include: 1) stratification of randomization by sex, 2) reporting results separately for men and women (though not formally powered to compare these groups) and 3) examination of the impact of relevant biologic variables (including age, race/ethnicity, and BMI) on weight loss within both IMF and DCR.

5. Outcome Measures

Feasibility (Aim 1). Careful records will be kept regarding numbers of individuals pre-screened, screened, enrolled, and completed in order to evaluate feasibility. Body weight will be measured on a calibrated digital scale to the nearest 0.1 kg at baseline and month 12 at the University of Colorado PKD Center. Body weight will be collected remotely at the times of group web-based sessions by BodyTrace scales, which allow for remote, secure transmission of data. Percent weight loss will be determined and height will be measured to the nearest 1 mm using a stadiometer, BMI will be calculated, and waist and hip circumference will also be measured and baseline and 1 year.

Safety, Acceptability, and Tolerability (Aim 2). Fasting blood samples will be collected for screening and repeated at 3 and 12 months for analysis including a comprehensive metabolic panel (CMP), complete blood count (CBC), HbA1c, lipid panel, and thyroid stimulating hormone (TSH). Estimated GFR will be calculated using the CKD-EPI equation[39] and a spot urine sample will be collected to measure microalbumin and creatinine excretion at baseline and 3 and 12 months. A pregnancy test will be performed in all premenopausal women at baseline. A standard physical exam and medical history will be administered by a physician or nurse practitioner, and a resting 12-lead electrocardiogram will be performed (baseline and month 12). Additional information about demographics and family history will be collected using the PKD Research Group Contact Form. Subjects will also complete the Beck Depression Inventory (BDI)[54] to screen for depression and the Eating Attitudes Test (EATS-26)[53 45] to screen for eating disorders. Blood pressure (Omron) and other vital signs will also be measured at baseline and month 12, and via home blood pressure monitors monthly.

3-day diet records will be completed and then analyzed by the AHWC nutrition personnel blinded to study group assignment using Nutrition Data System for Research software (University of Minnesota) at baseline, month 1, 3, and 12 to evaluate self-reported EI and macronutrient intake. The IMF will include both a fasted and ad libidum day. We recognize the limitations of self-reported measures of EI[46]; measures will be primarily used to compare intra-individual changes in dietary intake. Additionally, self-reported dietary adherence, effort to adhere, and self-efficacy to adhere will be assessed monthly using a 1-10 Likert scale[47].

Participants will be encouraged to report adverse events to study staff as they occur. Adverse events will be collated and reported to our IRB as per institutional guidelines. We will collect additional parameters to assess tolerability at baseline and months 3 and 12 as follows. Quality of life (QOL) will be assessed with the RAND 36 Item Health Survey (RAND-36)[48] physical and mental health component summary score. Mood state will be assessed with the Profile of Mood States 2 (POMS-2)[49]. Binge eating behavior will be assessed with the Questionnaire on Eating and Weight Patterns-Revised (QEWP-5)[50].

Circulating Markers (Aim 3). Serum IGF-1/IGF binding protein-1 (IGFBP-1) levels (ELISA; DSL/Beckman Coulter) and serum β-hydroxybutyrate (ketone) levels (quantitative enzymatic method)[43] will be measured before and after 3 and 12 months of IMF or DCR using fasting blood samples (**Aim 3)**. Additional secondary outcomes to gain further mechanistic insight will include serum levels of leptin, ghrelin (ELISA, Linco Research Inc.), and brain-derived neurotrophic factor (BDNF; ELISA, Promega)[43] as regulators of appetite. Samples will be collected the morning following a fed day for the IMF group to avoid acute effects of fasting.

PBMC Isolation and Western Blot Analysis (Aim 3). PBMCs will be isolated from heparinized whole blood using Histopaque-1077 (Sigma-Aldrich) as described previously at baseline, 3, and 12 months[51,52]. The PBMCs will be washed and protein will be extracted for Western blot analysis of p-AMPK (Thr 172)[51,52] and S6K (phospho-p70S6K)[51] (Cell Signaling) as indices of AMPK and mTOR activity, respectively (**Aim 3**). Previous studies have shown PBMC protein expression of p-AMPK and S6K significantly change in response to caloric restriction or metformin[51,52]. Samples will be collected the morning following a fed day for the IMF group to avoid acute effects of fasting.

MRI Measurement of TKV (Aim 3). A Siemens 4T system will be used for all studies. Renal images will be acquired at baseline and 12 months in similar manner and volumetric measurements determined as described for the CRISP study[53]. No contrast agents will be utilized. The radiologist, MRI study technicians and the PI, who will calculate TKV using Analyze software (Analyze 11.0, Mayo Foundation, Rochester, MN), will be blinded regarding group assignment. In addition cyst volume and renal parenchyma (TKV – cyst volume) will be measured.

Physical Activity. Participants in both the DCR and IMF may increase physical activity as an additional mechanism to lose weight while participating in the study, and IMF could potentially specifically impact physical activity. Of note, previous studies evaluating changes in free-living physical activity during IMF have shown no change in physical activity as assessed by self-report [54] or activity monitors [33,42]. Self-reported physical activity will be quantified at baseline, 3, and 12 months using the Stanford Physical Activity Questionnaire [55].

Abdominal Adiposity. Using the MRI images obtained for assessment of TKV, abdominal adiposity will be quantified (using de-identified images) in collaboration with Dr. Timothy Kline at the Mayo Clinic, as described previously.[56] Briefly, the automated technique uses the T1-weighted MRI oriented in the axial plane with slice level at the L3 vertebrate to segment and thereby differentiate subcutaneous adipose tissue, muscle, visceral adipose tissue, visceral organ, and bone compartments. From this, regional measurements of both area (2D) and volume (3D) can be used to quantify various body composition parameters.

Additional Blood Samples
For subjects signing the Informed Consent Addendum, an additional 10 mL of serum and 9 mL of plasma will be collected for future research.

6. Statistical Analyses. Randomization (1:1; blocked randomization sequence with stratification by sex) and statistical analysis will be performed by Dr. Zhiying You, biostatistician in the Renal Division. We will aim to recruit 30 participants in smaller cohorts (goal of approximately 6-10 participants). Up to 40 participants will be screened to account for potential screen failures. This sample size was determined to evaluate the feasibility of enrolling, retaining, and performing outcome measurements in a number of participants equal to a single cohort in a future large-scale trial. 3-4 participants per group is also an idea size for a remotely conducted group-based lifestyle weight loss intervention, allowing for optimal group dynamics as well as individualized attention from the RD instructor. Additionally, a total of 12 participants (accounting for 20% drop-out) per group will afford 99% power at an alpha level of 0.05 to detect weight loss of >5% ($-6.2 \pm 0.9\%$)[34] in each group (note, we do not expect significantly different weight loss between groups, thus between group calculations are not appropriate for our hypothesis, and this comparison is to no weight loss).

Aim 1: Feasibility. We will assess overall implementation to guide development of a large-scale RCT. Enrollment: We will assess the ability to enroll 30 participants meeting I/E criteria over the proposed recruitment window (see below). If enrollment is <30, we will determine barriers to enrollment and re-evaluate study design and/or recruitment. We will also assess the number approached compared to enrolled (participation rate) and reasons for declining. Retention: We will assess drop-out rates, reasons for attrition, and compare drop-out between groups. If drop-out exceeds 30% we will consider modifications. Weight loss: We will evaluate weight loss at 3 months (and maintenance at 12 months) as in index that the intervention can be effectively delivered and compare percent weight loss between groups (t-test). These data will be used to estimate an effect size for a large-scale RCT.

Aim 2: Safety, Acceptability, and Tolerability. Safety and tolerability: Rates of study-related adverse events will compared between groups using Fisher's exact test. Any change in eGFR or microalbumin excretion will be compared between groups using two-sample t-tests. Changes in QOL Measures (RAND-36), Mood State (POMS-2), and Binge Eating Behaviors (QEWP-5) will be compared between groups using two-sample t-tests. Acceptability: Changes in self-reported EI and macronutrient intake will be compared between groups using two-sample t-tests. Descriptive statistics will be performed on dietary adherence ratings (1-10 Likert scale).

Aim 3: Mechanistic Markers and Change in TKV. Baseline, 3, and 12 month levels of serum IGF-1/IGFBP-1, PBMC expression of p-AMPK and S6K, and serum β-hydroxybutyrate levels will be compared using paired t-tests within each group, thus evaluating the efficacy of each intervention separately. We expect IGF-1/IGFBP-1 levels to decrease in both groups, but a significant change in p-AMPK, S6K, and β-hydroxybutyrate only in the IMF group. Baseline and 12 month TKV will also be compared using paired t-tests within each

group, again evaluating the efficacy of each intervention separately, and change in TKV over the 12 month duration will be compared to historical data[5].

Post-hoc analysis. To determine the effects of DCR and IMF on abdominal adiposity, we will quantify visceral, subcutaneous, and total adiposity at baseline and mo-12 in each group. For the post-hoc analysis, change in abdominal adiposity (visceral, subcutaneous, total) will assessed within each group using a paired t-test and between groups using an independent samples t-test. For the associative analysis, multivariable linear regression will be used to determine the association of change in abdominal adiposity with change in TKV. We will test for any significant interaction terms between adiposity category and sex and age.

7. Recruitment Plans and Study Time Line. We will use recruitment and adherence strategies and experience previously implemented by the team of investigators. Dr. Chonchol has successfully recruited patients with various etiologies of kidney disease to clinical trials for many years, and I also have over 10 years of experience conducting translational intervention studies. The University of Colorado PKD Center has a rich history of recruiting patients with ADPKD to clinical research, including enrolling n=256 in the recently completed 5-year HALT-PKD studies[5,6]. Participants will be recruited as in small cohorts (goal of approximately 6-10) randomized to either DCR or IMF. Each cohort will undergo baseline measurements and randomization at approximately same time in order to conduct the behavioral intervention in a group setting. The initial 2 months of the award period will be used to obtain regulatory approvals and develop the intervention curriculum. Screening and enrollment will completed over the next ~8 months of the award duration in order to accrue n=30 individuals. The intervention will be delivered during months 2-22, and processing of samples and data analysis will be completed in the final 2 months of the 2 year award period.

8. Potential Problems, Alternative Strategies, and Future Directions. There is a possibility that adherence to the diets may be more difficult in ADPKD patients than other adults with overweight and obesity for unknown reasons; however, the primary aim of this study is to evaluate feasibility. We recognize that use of doubly-labeled water would allow for a more objective measure of total daily energy expenditure and EI and is an important future direction, however, was felt to be beyond the scope of this initial pilot and feasibility study. An alternate approach to weight loss is exercise; however, given that there is less available literature supporting this intervention in ADPKD, we opted for a comparison of dietary restriction strategies (DCR and IMF). We recognize that the proposed study duration and sample size will only provide a trend for any changes in TKV with dietary energy restriction. Importantly, the proposed study will provide the necessary foundation for a subsequent longer duration, larger-scale RCT (R01) assessing the efficacy of dietary restriction for slowing ADPKD progression, including measurement of change in TKV.

9. Human Subjects

9.1 Risks to the Subjects

a) Human Subjects Involvement and Characteristics.

Subjects. After obtaining their written informed consent, 30 adults (18-65 years) with overweight/obesity (BMI 25-45 kg/m^2) and a diagnosis of ADPKD (modified Pei-Ravine criteria[38]) with preserved to moderately declined renal function (eGFR ≥30 mL/min/1.73 m^2 by the CKD-EP equation[39]) will be recruited to participate. Screening and testing will occur at the **University of Colorado PKD Research Center**. A local contracted laboratory (Quest) and medical records will be used for screening for non-local participants. The behavioral weight loss interventions (focusing on either DCR or IMF as the primary dietary restriction strategy) will be implemented by the **AHWC** by the **NORC CIT Core** and delivered via a HIPAA-compliant web-based platform (Zoom). Resting energy expenditure (REE) will be measured at the AHWC and 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 18-65 years
2) ADPKD diagnosis based on the modified Pei-Ravine criteria[38]
3) BMI 25-45 kg/m^2

4) Normal to moderately declined renal function with an estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 by the CKD-EPI equation[39]
5) Access to the internet with video chat capabilities
6) No plans for extended travel without internet access (>2 weeks) during the 3 month intensive intervention period
7) Not currently participating in another interventional study or weight loss program
8) Ability to provide informed consent

Exclusion Criteria:

1) Diabetes mellitus (diagnosis or fasting glucose ≥126 mg/dL or Hemoglobin A1C ≥6.5%)
2) Current nicotine use or history of use in the past 12 months
3) Alcohol or substance abuse (self-report or undergoing treatment)
4) History of hospitalization or major surgery within the last 3 months
5) Untreated dyslipidemia (low density lipoprotein cholesterol > 190 mg/dL or triglycerides >400 mg/dL)
6) Uncontrolled hypertension (systolic blood pressure > 160 or diastolic blood pressure >100 mm Hg)
7) Pregnancy, lactation, or unwillingness to use adequate birth control
9) Cardiovascular disease, peripheral vascular disease, cerebrovascular disease, significant pulmonary or gastrointestinal disease (described below), cancer (within the last 5 years, except skin cancer or other cancers considered cured with excellent prognosis)
10) Abnormal resting electrocardiogram (ECG): serious arrhythmias, including multifocal PVC's, frequent PVC's (defined as 10 or more per min), ventricular tachycardia (defined as runs of 3 or more successive PVC's), or sustained atrial tachyarrhythmia; 2nd or 3rd degree A-V block, QTc interval > 480 msec or other significant conduction defects
11) Significant gastrointestinal disorders including: chronic malabsorptive conditions, peptic ulcer disease, Crohn's disease, ulcerative colitis, chronic diarrhea, or active gallbladder disease
12) Significant pulmonary disorders including: chronic obstructive pulmonary disease, interstitial lung disease, cystic fibrosis, or uncontrolled asthma
13) Regular use of prescription or over-the-counter medications that may affect weight, appetite, food intake, or energy metabolism (e.g. appetite suppressants, lithium, stimulants, anti-psychotics, tricyclic antidepressants; Dr. Catenacci will be consulted as needed; antibiotics started during the intervention period are not an exclusion); regular use of obesity pharmacotherapeutic agents within the last 6 month
14) History of clinically diagnosed eating disorder including anorexia nervosa, bulimia, binge eating disorder. Score >20 on the Eating Attitudes Test (EATS)-26[53] will require further assessment by the Study MD to determine if it is appropriate for the subject to participate in the study.
15) Weight loss >5% in past 3 months for any reason except post-partum weight loss; weight gain >5% in past 3 months requires assessment by PI to determine reason for weight gain and if it is appropriate for the subject to participate in the study.
16) Untreated hyper- or hyperthyroidism (TSH outside of normal range for laboratory or history of uncontrolled thyroid disorder). History of thyroid disorder or current thyroid disease treated with stable medication regimen for at least 6 months in acceptable.
17) Current severe depression or history of severe depression within the previous year, based on DSM-IV-TR criteria for Major Depressive Episode. Score > 18 on the Beck Depression Inventory[54] will require further assessment by the Study MD to determine if it is appropriate for the subject to participate in the study.
18) History of other significant psychiatric illness (e.g. psychosis, schizophrenia, mania, bipolar disorder) which in the opinion of the Study MD would interfere with ability to adhere to dietary interventions.
19) Inability to cooperate with/clinical contraindication for 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. Nowak and Dr. Chonchol will make final decisions on all patient eligibility, in consult with Dr. Catenacci as appropriate.

Any screening results that preclude enrollment will be communicated to potential subjects who will be recommended to consult with their primary care provider.

***b) Sources of Research Materials*.** This is a prospective study of newly recruited human subjects aged 18-65 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 only invasive measurement will be collection of a small amount of blood. Moreover, the research will be overseen by a study-specific DSMB (described in more detail under Data and Safety Monitoring Plan).

The risks associated with the experimental protocols include:

Blood Draw- may result in temporary discomfort, bruising, bleeding, and on rare occasions, infection; however, the potential health risks are minimal.

Magnetic Resonance Imaging (MRI) - 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.

Dietary Weight Loss Interventions – The most common side effects are hunger, fatigue, insomnia, irritability, anxiety, headache, impaired concentration, and cold intolerance. Occasionally subjects can experience constipation, nausea, diarrhea, or abdominal discomfort when changing their usual diet. These conditions usually improved within a few weeks. Occasionally, subjects may also experience weakness, dizziness, lightheadedness, tremor, psychological stress, or cognitive difficulties. The most serious side effect of low calorie diets is gallstone formation, which typically occurs only with extremely low fat diets. Rarely, limiting energy intake may cause dehydration, hypoglycemia, electrolyte abnormalities, confusion, change in mood, arrhythmias, syncope, seizures, worsening of an underlying eating disorder, or impact performance at home, work, or school.

Resting Energy Expenditure – The hood may cause claustrophobia in those susceptible. Participants will have an opportunity to try the hood prior to beginning the measurement.

Group Behavioral Meetings – Due to the nature of the group setting to deliver the intervention, there is a risk of loss of privacy and confidentiality, including external knowledge of diagnosis of ADPKD and participation in a research study.

There is a risk that people outside of the research team will see research information. We will do all that we can to protect information, but it can not be guaranteed.

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.

9.2. Adequacy of Protection Against Risks

a) Recruitment and Informed Consent. I will use the recruitment and adherence strategies and experience previously implemented by the team of investigators. Dr. Chonchol has successfully recruited patients with various etiologies of kidney disease to clinical trials for many years, and the PI also has over 10 years of experience conducting translational intervention studies. The University of Colorado PKD Research group has a rich history of recruiting patients with ADPKD to clinical research, including enrolling n=256 in the recently completed 5-year HALT-PKD studies[5,6]. 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, including local participants from the Denver Metro Area. Since 1985, UCD has maintained an ADPKD registry and mailing list. In addition to the UCD registry, we will advertise through the national PKD Foundation. The ADPKD group at the UCD has longstanding expertise and national recognition. The Division of Renal Diseases and Hypertension at the University of Colorado, in general, and Drs. Nowak, Chonchol, and Catenacci specifically, have excellent track records 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 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 participant 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. Volunteers will meet with a study representative in a private and quiet room and will be permitted ample time to read the document in a quiet room and an unhurried setting. The volunteer's comprehension and autonomy will be assessed by asking subjects to explain the purpose of the study in their own words. The consent form will then be signed by the participant as documentation of consent. A copy of the signed consent form will be given to the subject. All of the proposed procedures and protocols will be reviewed and approved by the University of Colorado Multiple IRB.

***b) Protection Against Risk*.**

Minimizing General Risks - The potential general risks of the proposed studies will be minimized by:

- Ensuring all participants meet the specific inclusion/exclusion criteria.
- Screening for adverse events.
- 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 PKD Research Center environment.
- Safety monitoring annually by a DSMB (described below).
- 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.
- All blood pressure and blood draws will be performed in the arm not designated for future vascular access for dialysis.

Minimizing Specific Risks Related to Dietary Intervention:

- Risks of the dietary interventions will be minimized by ensuring all subjects meet the specific inclusion/exclusion criteria. Subjects will undergo a medical screening including medical history interview and physical exam, screening labs (CBC, CMP, Lipid Profile, TSH, Hemoglobin A1C and in women, a urine pregnancy test) and 12-lead electrocardiogram.
- Subjects in the DCR group will be guided to reduce fat intake to 30% of total calories but will be advised to maintain an intake of at least 10-20 g of fat per day to reduce risk of gallstone formation.
- Subjects in the IMF group will be guided to ensure adequate fluid intake on fast days.
- Subjects in both arms will be monitored closely for adverse events. Subjects will be instructed to report all side-effects and adverse events. Participants will not be discontinued from the study if they fail to comply with the dietary interventions or fail to lose weight, nor will the protocol be modified, as feasibility is the primary outcome. Participants may be discontinued upon request or if deemed medically appropriate by the investigative team.

- Safety will be monitored regularly via frequent contact by study design (weekly during intensive phase; monthly to bi-monthly during maintenance phase)

Minimizing Specific Risks Related to Confidentiality:
Group sessions will only include participants in the research study and other members of the community will not be enrolled in these weight loss sessions. All study participants will be asked to keep the identities of other study participants confidential. In addition to identities, all conversation topics and issues discussed will be asked to remain confidential. Participants will be instructed that they have the right to refuse to answer any question or discuss any issue that makes them feel uncomfortable. Strict confidentiality of subjects' information will be kept in accordance of HIPAA policy. Zoom is a HIPAA compliant web-based platform available through the University of Colorado. All clinical samples will be stored by code alone. Data will be stored as well as collected using Research Electronic Data Capture (RedCap), an encrypted, secured, HIPAA 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. All information in our database is treated with the same confidentiality as a medical record.

9.3. 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. Participants in the study will potentially benefit by decreasing their body weight.

9.4. Importance of the Knowledge to be Gained

The findings from the proposed research should provide important new information regarding the feasibility, safety, acceptability, and tolerability of two behavioral weight loss programs in adults with overweight/obesity and ADPKD with normal to moderately declined renal function, as there is no available information to date on these questions. In addition, the proposed research should provide important insight changes in markers of biological pathways. Together, this information will contribute to will provide the foundation for a subsequent long-term trial assessing the efficacy of dietary restriction for slowing ADPKD progression.

9.5. Data Safety Monitoring Plan

Safety Monitoring

The risks in this study are minimized by the use of extensive inclusion and exclusion criteria, and by close monitoring of the research subjects. In addition, the primary research team is qualified and experienced in all of the study procedures. Nonetheless, since this study places the subjects at more than minimal risk, a data safety monitoring board (DSMB) including clinicians and a statistician (independent of the study investigators but part of the faculty at the UCD 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 after 1 and 3 months, and thereafter every 6-12 months with study investigators 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 of the appropriate monitoring bodies (IRB, NIH, etc.) and d) assuring data accuracy and protocol compliance.

a) Data Safety Monitoring Board

1. The DSMB will be comprised of independent (i.e. not affiliated with the study) faculty members from University of Colorado Anschutz Medical Campus (including a nephrologist, endocrinologist and statistician). We have avoided conflict of interest by recruiting a person who is not affiliated with any member of the research team (i.e. not a collaborator)
2. The DSMB will meet with study investigators at 1 and 3 months during the intensive phase of the study, and every 6-12 months thereafter. During these meetings, the following information will be reviewed:

i. Study progress to date, projected timeline and schedule, discussion of issues or potential problems
ii. Adverse events report (see below)
iii. Recruitment and enrollment statistics including the following information:
    1. Adherence to inclusion/exclusion criteria
    2. Sex, race/ethnicity
    3. Number of subjects who were disqualified prior to randomization and reasons
    4. Number of subjects randomized
    5. Number of subjects who have withdrawn from the study, and the reasons for withdrawal
    6. Adherence to study timeline for enrollment and goals for subject retention

3. Minutes will be taken during the meeting, and after the meeting copies will be distributed. The minutes will be reviewed at the beginning of the next DSMB meeting. The DSMB report will be submitted to the Colorado Multiple Institutional Review Board (COMIRB) at the annual review.

***b) Reporting of Adverse Events*.** Subjects will be instructed to report side effects to the investigator and their nephrologist, if not part of the investigative team. The PI and study MD (Dr. Chonchol) will continually monitor all adverse events during the screening process, during procedures performed as part of this research, and during the weight loss interventions. Detailed information on all adverse events will be recorded by research staff using a standardized adverse event form and will be and evaluated by the PI within 72 hours. Any serious adverse events will be evaluated by the DSMB, PI, and study MD within 24 hours and will be reported to the Colorado Multiple IRB in accordance with the guidelines.

1. Screening Failures: When a screening failure occurs, the individual will be contacted and be informed of the reasons for screening failure and appropriate follow up recommended.
2. Serious Adverse Events: Any serious adverse events that are definitely or probably related to the protocol and any deaths (regardless of relatedness) will be reported to the DSMB and COMIRB within 5 days using the COMIRB Unanticipated Problem Report Form. Serious adverse events are defined as: death, life threatening injuries, inpatient hospitalization, persistent or significant disability/incapacity, or congenital anomaly/birth defect. Any study-related serious adverse events and any deaths (regardless of relatedness) will be reported to the NIH (if applicable) within 2 weeks. For all **serious adverse events determined by COMIRB to be definitely, probably, or possibly related to the study or interventions**, COMIRB will take whatever action(s) it deems appropriate, including but not limited to:
    i. Modification of the protocol
    ii. Modification of the consent form document
    iii. Modification to the timetable for continuing review requirements
    iv. Suspension of new enrollment into the study
    v. Suspension or termination of the study - If the study is suspended or terminated, it will be promptly reported to any agency that has provided funding for the study. All other events not requiring suspension or termination shall be reported during the annual progress report.
3. Unanticipated Adverse Events: Any unanticipated adverse events that are definitely or probably related to the protocol will be reported to the DSMB, COMIRB, and CTRC within 5 days using the COMIRB Unanticipated Problem Report Form and will be included in the annual report to any funding agency
4. Other Adverse Events: All other adverse events will be documented by standard COMIRB procedures and will be reported during the annual DSMB meeting, at the annual COMIRB protocol review, and will be included in the annual report to any funding agency.

***c) Exit Criteria*.** The primary exit criteria will include completion of the study, patient request, IRB or DSMB request, or other medical rationale as determined by the PI or co-investigators. Other exit criteria are listed below. The number of subjects exiting the study and the reasons for exit will be carefully documented. Participants who drop out of the study will be handled using an intent-to-treat analysis.

Individual Stopping Criteria:

An individual subject may be terminated from the study based on the criteria listed below. Any subject that is terminated from the study based on these criteria will be reported to COMIRB during the annual continuing review.

1. Absolute termination criteria

- Request by the volunteer to leave the study
- Pregnancy - Rapid weight gain (>4 kg/month) or secondary amenorrhea in a female volunteer will be cause for a pregnancy test determination
- Alcohol abuse; illicit drug abuse
- Loss of ability to freely provide consent through imprisonment
- Participation in another weight loss program or use of a weight loss medication
- Development of a chronic condition (e.g. hyperthyroidism, hypothyroidism, rheumatoid arthritis, congestive cardiac failure, or neurological disorders such as MS or stroke) **likely** to impact upon ability of the subject to participate
- Development of an acute condition (e.g. myocardial infarction, major depression, accidents outside of the study resulting in physical impairments) **likely** to impact upon ability of the subject to participate

2. Potential termination criteria

- Development of an acute or chronic condition that **may** impair the ability of the subject to participate or impact safety. Subjects will be reviewed on a case-by-case basis and will be reported to the DSMB.
- We do not have a comprehensive list of criteria or causes, but we have prepared plans for:
    - Depression or low mood – subjects will be referred to their primary care provider, and if appropriate, to a psychiatrist. The opinion of the psychiatrist will be used to determine continuance or termination of participation.

Study Stopping Criteria:

There are no efficacy stopping rules in place for this study. It is unlikely that the power to make such a decision would be present during the short timeframe of this study with endpoints related to feasibility and adherence, thus no formal interim analysis is planned. In this relatively minimal risk study it is unlikely that excess adverse events will require stopping the trial. However, as outlined above, we will monitor adverse events injury rates in all participants and alert COMIRB and NIH if a larger than reasonably expected adverse event rate should occur.

The DSMB may recommend study stopping if:

- The data show a significantly increased risk of serious adverse effects in either 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).

Data Monitoring

As described in Section 9.2b Protection Against Risk, study data will be collected and managed using REDCap (Research Electronic Data Capture). The database is hosted at the University of Colorado Anschutz Medical Campus Development and Informatics Service Center (DISC), which will be used as a central location for data processing and management. This server has a high level of security, controlled access, daily back-up, and long-term retention of back-up files. All members of the research group at Colorado Anschutz Medical Campus Development have individual computers that are part of the institution network with institutional oversight of security. Field and range checks will be programmed to minimize data entry errors.

Clinical Trials.gov Requirements

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

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