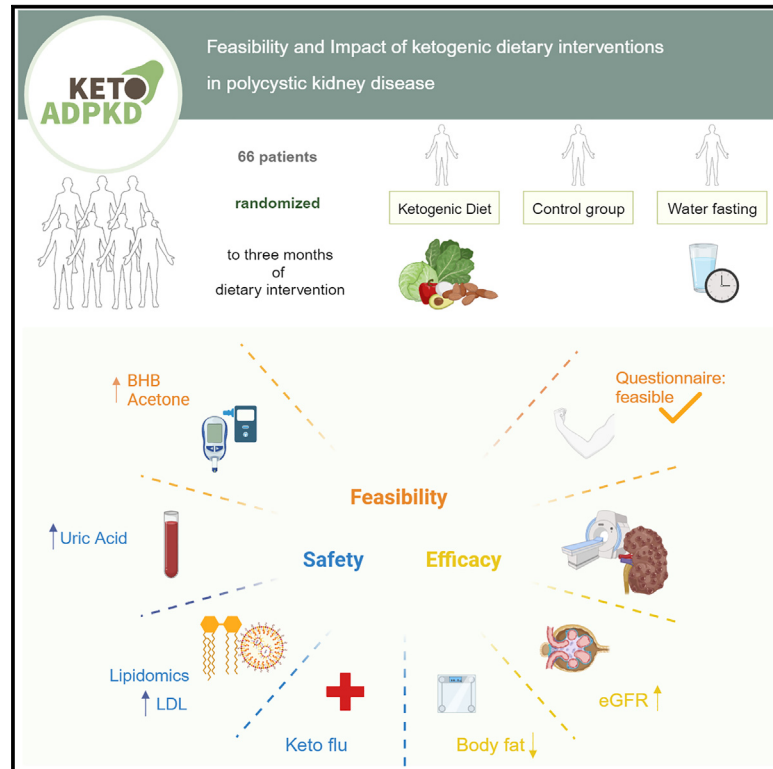


# Feasibility and impact of ketogenic dietary interventions in polycystic kidney disease: KETO-ADPKD—a randomized controlled trial

## Graphical abstract



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## In brief

Cukoski et al. provide the results of a clinical trial examining a ketogenic diet (KETO-ADPKD) as treatment for autosomal-dominant polycystic kidney disease (ADPKD), the most common genetic cause of kidney failure. KETO-ADPKD shows feasibility, provides indications for the effect on kidney function, and highlights lipid changes as a point of interest regarding safety.

## Highlights

- Ketogenic diets are feasible for patients with polycystic kidney disease (ADPKD)
- Ketogenic diets (KD) reduce body weight, primarily changing fat mass
- KD increase glomerular filtration rate (not observed upon repetitive water fasting)
- Future key aim: a long-term trial to confirm sustainable benefits and safety of KD



## Article

# Feasibility and impact of ketogenic dietary interventions in polycystic kidney disease: KETO-ADPKD—a randomized controlled trial

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## SUMMARY

Ketogenic dietary interventions (KDIs) are beneficial in animal models of autosomal-dominant polycystic kidney disease (ADPKD). KETO-ADPKD, an exploratory, randomized, controlled trial, is intended to provide clinical translation of these findings (NCT04680780). Sixty-six patients were randomized to a KDI arm (ketogenic diet [KD] or water fasting [WF]) or the control group. Both interventions induce significant ketogenesis on the basis of blood and breath acetone measurements. Ninety-five percent (KD) and 85% (WF) report the diet as feasible. KD leads to significant reductions in body fat and liver volume. Additionally, KD is associated with reduced kidney volume (not reaching statistical significance). Interestingly, the KD group exhibits improved kidney function at the end of treatment, while the control and WF groups show a progressive decline, as is typical in ADPKD. Safety-relevant events are largely mild, expected (initial flu-like symptoms associated with KD), and transient. Safety assessment is complemented by nuclear magnetic resonance (NMR) lipid profile analyses.

## INTRODUCTION

Autosomal-dominant polycystic kidney disease (ADPKD) is the most common monogenic disease leading to kidney failure and shows a genetic prevalence of ~1:1,000.<sup>1</sup> The course of the disease is characterized by bilateral kidney cysts that increase in number and size with age. About 75% of ADPKD patients need kidney replacement therapy by the age of 70 years.<sup>2</sup> ADPKD is caused primarily by mutations in two genes, *PKD1* and *PKD2* and, on the basis of the function of the encoded proteins, is considered a ciliopathy.<sup>3</sup> The changes in cell biology associated with mutation of these genes are the basis not only to the kidney pheno-

type but to a systemic disorder, most frequently including polycystic liver disease (PLD) and cardiovascular manifestations.<sup>4–6</sup> Prior to 2015, there was no targeted treatment, and the mainstay of therapy was based on supportive measures to protect kidney function and avoid complications.<sup>7</sup> Among others, those measures include optimal control of blood pressure, reduction of salt intake, and sufficient water intake.<sup>8–11</sup> The approval of tolvaptan, a vasopressin-2 receptor antagonist, as the first targeted treatment strategy was a milestone for the field.<sup>12,13</sup> However, therapy-associated adverse events such as hepatotoxicity and massive polyuria limit its universal use.<sup>12</sup> Additionally, tolvaptan only slows the loss of kidney function and does not address



**Table 1. Demographics and baseline characteristics of study participants**

	Overall (n = 63)	Control Group (n = 19)	Ketogenic Diet (n = 23)	Water Fasting (n = 21)
Male	32 (50.79)	10 (52.63)	11 (47.83)	11 (52.38)
Age, years	41.41 ± 9.55	41.26 ± 10.49	41.26 ± 9.94	41.71 ± 8.67
BMI, kg/m <sup>2</sup>	25.65 ± 3.86	25.14 ± 4.28	25.85 ± 3.78	24.98 ± 3.34
htTKV, mL/m	958.04 ± 651.57	838.04 ± 424.85	896.47 ± 511.77	1,134.05 ± 902.75
htTLV, mL/m	1,305.55 ± 825.91	1,394.75 ± 1,047.96	1,106.49 ± 514.93	1,442.87 ± 872.72
Mayo class 1A-B	11 (17.46)	3 (15.79)	5 (21.74)	3 (14.29)
Mayo class 1C-E	52 (82.54)	16 (84.21)	18 (78.26)	18 (85.71)
eGFR, mL/min/1.73 m <sup>2</sup>	84.01 ± 24.00	82.24 ± 22.62	85.77 ± 22.56	83.66 ± 27.52
CKD stage 1	25 (41.27)	6 (31.58)	10 (43.48)	9 (42.86)
CKD stage 2	24 (38.09)	9 (47.37)	8 (34.78)	7 (33.33)
CKD stage 3a	9 (14.29)	2 (10.53)	4 (17.39)	3 (14.29)
CKD stage 3b	5 (7.93)	2 (10.53)	1 (4.35)	2 (9.52)
Urological complications <35 years	26 (41.27)	6 (31.58)	10 (43.47)	10 (47.62)
Arterial hypertension <35 years	53 (84.13)	14 (73.68)	20 (86.95)	19 (90.48)

Categorical values are given as n (%) and continuous data as mean ± SD. There were no statistically significant differences between groups.

BL, baseline; BMI, body mass index; CG, control group; eGFR, estimated glomerular filtration rate; EOT, end of treatment; htTKV, height-adjusted total kidney volume; htTLV, height-adjusted total liver volume; KD, ketogenic diet; WF, water fasting.

extrarenal manifestations in ADPKD. Consequently, novel treatment opportunities are urgently awaited.

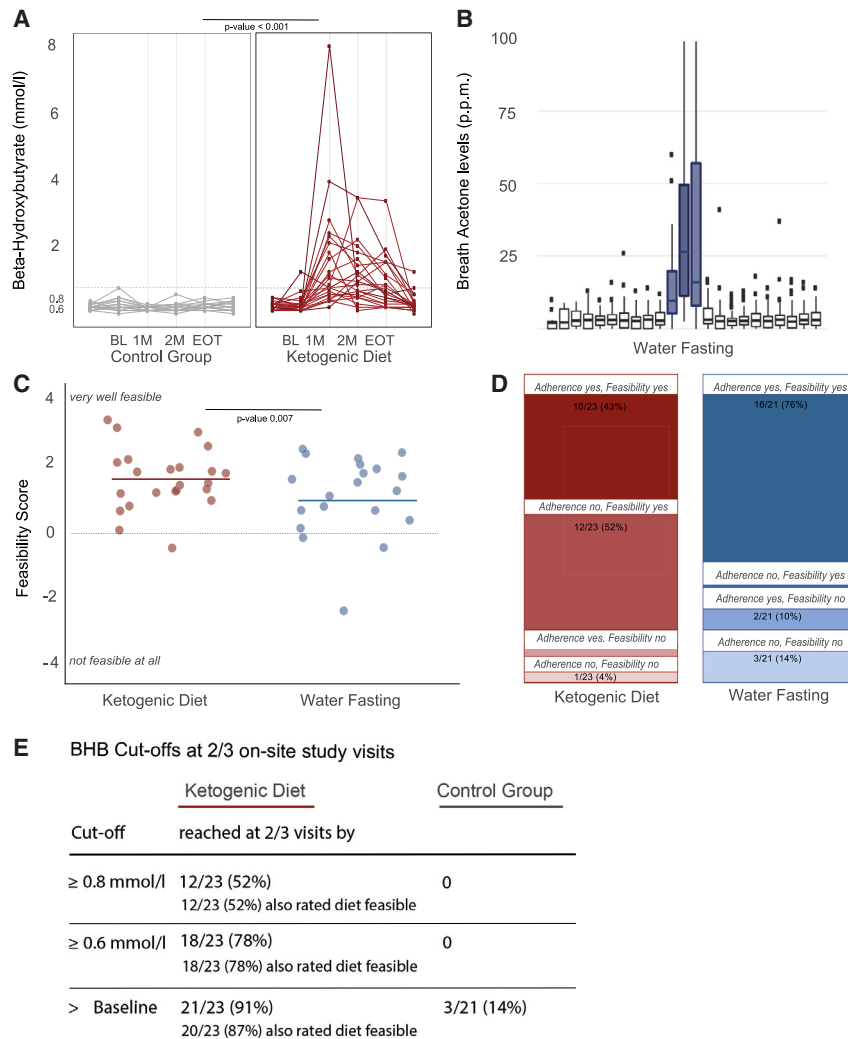
In this regard, cellular metabolic abnormalities observed in ADPKD have gained increasing attention. These changes include a pronounced Warburg effect, glutamine anaplerosis, mitochondrial dysfunction, and defects in tricarboxylic acid (TCA) as well as fatty acid oxidation and synthesis.<sup>14,15</sup> Consequently, cyst-lining epithelial cells have been shown to be glucose dependent and metabolically inflexible.<sup>16–18</sup> Interestingly, a reduction in food intake resulting in ketosis slowed down the increase in kidney weight and the proliferation of cyst-lining cells in two polycystic kidney disease (PKD) mouse models.<sup>19,20</sup> On the basis of this observation, Torres et al.<sup>21</sup> provided evidence in two rodent and a feline PKD model that ketogenic metabolic interventions are highly beneficial to ameliorate cyst growth and loss of kidney function. This raised much attention also among patients, leading to early first steps in human cohorts, including a retrospective case series among ADPKD patients,<sup>22</sup> initial experiences from a supervised ketogenic diet (KD) program for ADPKD patients,<sup>23</sup> and a small pilot trial exposing patients to a short period of ketogenic dietary interventions.<sup>24</sup> Although these studies pointed toward the general feasibility of the diets and positive patient-reported outcomes (PROs), data from randomized trials on longer term interventions extending the knowledge on feasibility and providing actual insight regarding safety and efficacy are missing. To answer these questions, KETO-ADPKD was designed as a clinical trial examining two ketogenic dietary interventions in a randomized and controlled setting.

## RESULTS

### Patient characteristics

A total of 66 patients were randomized into the study and assigned to one of three study arms: a KD group receiving a low-carbohy-

drate (CHO) and high-fat diet, a group undergoing 3-day water fasting (WF) once per month, and a control group receiving routine dietary counseling for ADPKD patients (for details on patient selection, see [STAR Methods](#)). The duration of the intervention phase was 3 months. Twenty-three patients were randomized to the KD group, 22 to the WF group, and 21 to the control group. One participant assigned to the WF group did not meet the inclusion criteria after review of baseline (BL) MRI and was excluded before the BL visit, and two participants assigned to the control group withdrew from the study for personal reasons before starting the intervention. Of 63 patients entering the intervention phase, all patients in the KD group and the control group completed the entire study. Two patients in the WF group were lost to follow-up: one patient dropped out because of feasibility issues regarding WF itself, and the other one specified personal reasons preventing further participation (including travel to the center). These patients were included in the feasibility and safety analyses but not included in all other analyses because of the lack of data availability for the comparison of BL and end of treatment (EOT). Detailed information is provided in the CONSORT (Consolidated Standards of Reporting Trials) diagram in [Figure S1](#); a study flow diagram can be found in [Figure S2](#). In our cohort, 32 participants (50.79%) were men, and the study cohort had a mean age of 41.41 ± 9.55 years. Mean height-adjusted total kidney volume (htTKV) at BL was 958.04 ± 651.57 mL/m, and mean height-adjusted total liver volume (htTLV) was 1305.55 ± 825.91 mL/m. The mean estimated glomerular filtration rate (eGFR) using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation was 84.01 ± 24.00 mL/min/1.73m<sup>2</sup>. A total of 26 patients (41.27%) reported urological complications prior to the age of 35 years, and 53 patients (84.13%) had been diagnosed with arterial hypertension before 35 years of age. At BL, the mean body mass index (BMI) was 25.65 ± 3.86 kg/m<sup>2</sup>. All BL characteristics can be found in [Table 1](#). There were no statistically significant differences in the BL characteristics among the three groups.



**Figure 1. Ketogenic dietary interventions potentially induced ketosis**

Readouts of metabolic efficacy and feasibility are shown ( $n = 63$ ).  $p$  values are provided for the comparison between the control and each intervention group.

(A) Measurements of BHB levels for patients in the KD group and the control group show significantly higher values in the KD group ( $p < 0.001$ ). The threshold of 0.8 mmol/L at 3 of 3 visits was reached by 9 of 23 patients. 18 of 23 KD patients had higher BHB levels at 3 of 3 visits during the diet compared with BL. The horizontal gray dotted lines mark BHB threshold of 0.8 mmol/L. The vertical lines mark visits during the intervention phase.

(B) Breath acetone levels during the course of water fasting periods for patients in the WF group show higher values during the 3 days of fasting compared with days of *ad libitum* diet. Daily mean values of acetone levels are shown.

(C) Average feasibility scores of each diet group are displayed, queried with a dedicated feasibility questionnaire. Twenty-three questions assessed feasibility from  $-4$  (not feasible at all) to  $+4$  (very well feasible). Patients with an average score over all visits during the dietary intervention of  $\geq 0$  were counted for the feasibility endpoint, this included 22 of 23 patients in the KD group and 17 of 21 in the WF group. Mean score was  $1.59 \pm 0.95$  in the KD group and  $0.86 \pm 1.70$  in the WF group and is indicated by the horizontal lines. For the 2 patients who terminated the diet and left the trial we used a score of  $-4$  for all following visits.

(D) Overview of patients that reached both adherence and feasibility (KD, 10 of 23; WF, 16 of 21), only feasibility (KD, 11 of 23; WF, 0 of 21), only adherence (KD, 1 of 23; WF, 2 of 21), and neither adherence nor feasibility (KD, 1 of 23; WF, 3 of 21).

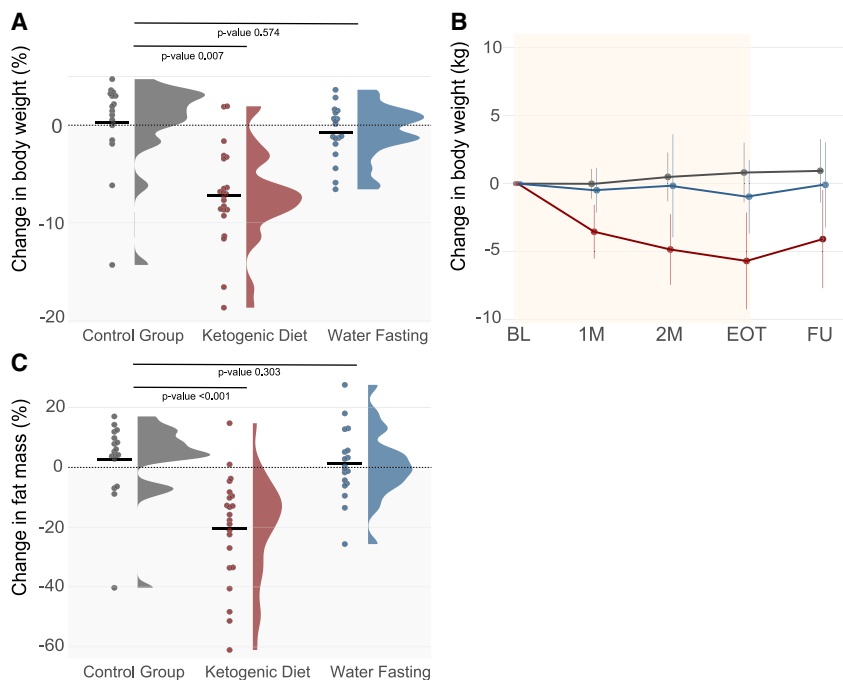
(E) Analysis of exploratory BHB thresholds reached at 2 of 3 visits and their overlap with patient-reported feasibility (see also Figure S3). 1M, 1 month; 2M, 2 months; BL, baseline; BHB, beta-Hydroxybutyrate; CG, control group; EOT, end of treatment; htTKV, height-adjusted total kidney volume; htTLV, height-adjusted total liver volume; KD, ketogenic diet; WF, water fasting; p.p.m., parts per million

### Biochemical efficacy

Beta-hydroxybutyrate (BHB) blood measurements during visits were used primarily to assess ketosis in the KD group. Moreover, both diet groups performed two daily at-home acetone breath analyses. In the WF group, which was not seen at the center during the 3 days of WF, the breath acetone levels served as the primary readout for ketosis.

KD was associated with a clear increase in BHB levels upon initiation of the diet, while no significant changes occurred in the control group (mean BHB level: KD  $1.25 \pm 1.25$  mmol/L vs. control  $0.32 \pm 0.80$  mmol/L,  $p < 0.001$ ; Figure 1A). A predefined threshold of 0.8 mmol/L to assess adherence was met by 9 of 23 patients (39%) in the KD group at all study visits under diet, and two additional patients in this group reached the adherence cut-off in combination with breath acetone measurements, resulting in adherence among 11 of 23 patients (47%) (for details, see STAR Methods).

Considering the high BHB threshold compared with previous studies,<sup>25,26</sup> the fact that the primary endpoint essentially required meeting this threshold at all three on-diet visits, and the finding that all patients in the control group remained well below a BHB level of 0.5 mmol/L (Figure S3), we explored additional targets for objective ketosis. A target value of 0.5 mmol/L was reached by 14 of 23 (61%) at all visits and by 19 of 23 (83%) at two of three visits. BHB levels of  $\geq 0.6$  mmol/L were measured in 10 of 23 (43%) at all study visits and 18 of 23 (78%) at two of three visits. Moreover, 18 of 23 patients (78%) in the KD group showed higher BHB levels at all on-diet visits compared with BL, while only one patient in the control group reached this target ( $p = 0.003$ ; for BHB thresholds met at two of three visits, see Figure S3). Daily acetone measurements also revealed a clear increase in comparison with the control group (mean acetone level: KD  $15.72 \pm 15.57$  ppm vs. control  $4.18 \pm 7.22$  ppm,  $p < 0.001$ ; Figure S3).



**Figure 2. Ketogenic dietary interventions potentially induce weight loss and primarily reduce body fat mass**

Changes in weight and fat mass are shown (n = 63). Black horizontal lines indicate means. p values are provided for the comparison between the control and each intervention group.

(A) Relative changes in body weight from BL to EOT: CG, +0.27%; KD, -7.2%; WF, -0.77%; CG, n = 18; KD, n = 23; WF, n = 19.

(B) Course of body weight for all groups and visits; CG, n = 18; KD, n = 23; WF, n = 19. Mean weight loss CG: 1M,  $-0.01 \pm 1.09$  kg; 2M,  $+0.51 \pm 1.72$ ; 3M,  $+0.32 \pm 1.82$  kg; KD: 1M,  $-3.54 \pm 1.98$  kg; 2M,  $-1.30 \pm 1.31$  kg; 3M,  $-0.84 \pm 1.31$  kg; WF: 1M,  $-0.48 \pm 1.65$  kg; 2M,  $+0.3 \pm 3.01$  kg; 3M,  $-0.8 \pm 2.69$  kg.

(C) Relative changes in body fat from BL to EOT: CG, +2.64%; KD, -20.5%; WF, +1.15%; CG, n = 17; KD, n = 22; WF, n = 18. 4 patients (2 control, 1 KD, 1 WF) were excluded from the body composition analysis because of missing data. 1M, 1 month; 2M, 2 months; BL, baseline; CG, control group; EOT, end of treatment; KD, ketogenic diet group; WF, water fasting group.

In the WF group, acetone levels showed a marked increase during the fasting days compared with days with normal food intake (Figure 1B). Eighteen of 21 WF patients (85%) reached a predefined acetone level of  $\geq 10$  ppm on at least two of three days of each fasting phase.

**Patient-reported feasibility**

Feasibility was assessed using a PRO questionnaire consisting of 23 questions ranging from -4 to +4, with increasing numbers indicating improved feasibility (Table S1). Mean feasibility scores were  $1.59 \pm 0.95$  in the KD group and  $0.86 \pm 1.70$  in the WF group (Figure 1C). Average results above the neutral answer of 0 were considered as an indicator of feasibility in everyday life. An average score  $\geq 0$  over all visits during the intervention period was reached by 22 patients (95%) in the KD group and 18 (85%) in the WF group.

**Combined feasibility endpoint**

The primary combined endpoint was defined as a combination of adherence assessed by metabolic efficacy and patient-reported adherence and feasibility (see STAR Methods for details). A small number of patients reached none (KD, 4%; WF, 14%) of these endpoints. In the KD group 12 of 23 (51%) and in the WF group 2 of 21 (10%) reached either only the adherence or only the feasibility endpoint. The combined endpoint was reached by 16 of 21 patients (76%) in the WF group. In the KD group, a lower number of 10 of 23 patients (43%) reached the combined endpoint, driven mainly by patients not reaching the predefined BHB cutoff of 0.8 mmol/L at all three visits under diet (Figure 1D).

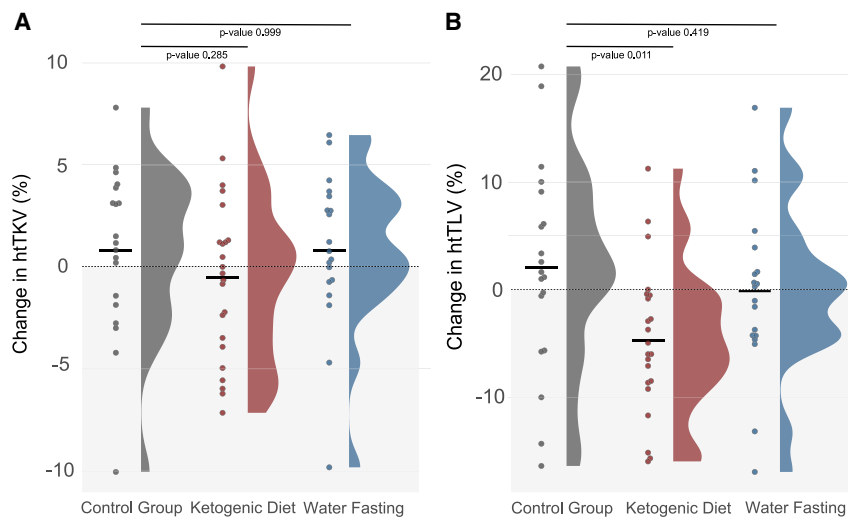
Seventy-eight percent of patients met a combined endpoint using the exploratory BHB cutoff of  $\geq 0.6$  mmol/L on at least

two of three on-diet visits (Figures 1E and S3). This BHB threshold was not reached by a single patient in the control arm. Moreover, 78% of patients showed higher BHB levels at all on-diet visits than at BL and also reported the diet as feasible. Ninety-one percent of patients in the KD group showed BHB levels higher than BL at two of three on-diet visits, and all but one also rated the diet feasible. For details regarding the percentage of patients reaching acetone breath level cutoffs and their relation to patient-reported feasibility, see Figure S3.

**Effects on body weight and body composition**

Mean body weight decreased from BL to EOT in the KD and WF groups while showing a slight increase in the control group (control, +0.27%; KD, -7.2%; WF, -0.77%). This finding was statistically significant for the KD group (p = 0.007) but not the WF group (p = 0.574) in comparison with the control group (Figure 2A). Absolute changes in body weight at all visits including the time point one month after EOT are shown in Figure 2B. The largest fraction of weight loss in the KD group occurred in the first month of diet (Figure 2B) with  $3.54 \pm 1.98$  kg, while  $1.30 \pm 1.31$  kg were lost during the second month and  $0.84 \pm 1.31$  kg in the third month. This weight loss was driven mainly by a reduction of fat mass, which was -20.5% while showing a slightly positive trend in the control (+2.64%) and WF (+1.15%) groups. This finding was statistically significant in the KD group compared with the control group (p < 0.001). There was no significant difference between the WF and control group (p = 0.303) (Figure 2C).

All absolute values resulting from anthropometric analyses are displayed in Figure S4. Mean weight loss from BL to EOT was



**Figure 3. Ketogenic dietary interventions show a signal toward decrease in htTKV and htTLV**

Relative changes of htTKV and htTLV from BL to EOT are displayed (n = 61). One patient in the KD group was excluded from htTLV assessment because of failure of the imaging software to upload the axial images for volumetry. p values are provided for the comparison between control and each intervention group.

(A) Difference in htTKV: CG, +0.79%; KD, -0.55%; WF, 0.8%; CG, n = 19; KD, n = 23; WF, n = 19.

(B) Difference in htTLV: CG, +2.04%; KD, -4.73%; WF, -0.15%; CG, n = 19; KD, n = 22; WF, n = 19. BL, baseline; CG, control group; EOT, end of treatment; htTKV, height-adjusted total kidney volume; htTLV, height-adjusted total liver volume; KD, ketogenic diet group; WF, water fasting group.

-6.11 ± 4.00 kg in the KD group and -0.68 ± 2.49 kg in the WF group. We observed a decrease in abdominal circumference from BL to EOT in the KD and WF groups (control, +1.32 cm; KD, -5.02 cm; WF, -2.86 cm). In comparison with the control group, this reduction was statistically significant for both the KD (p < 0.001) and WF (p = 0.012) groups. On average, patients in the KD group lost 4.15 ± 2.90 kg of body fat. Compared with the control group, this finding was statistically significant (p = 0.005). To a smaller extent, lean body mass was reduced in the diet groups (control, +0.01 kg; KD, -1.90 kg; WF, -0.63 kg). This difference reached statistical significance for the KD group in comparison with the control group (p = 0.004). On average, body water content was lower at EOT than at BL in all groups (control, -0.02 kg; KD, -1.64 kg; WF, -0.38 kg). The marked distinction between the KD group and control group showed statistical significance (p < 0.001). We also considered loss of weight and fat mass as a potential readout of adherence to the dietary intervention. Weight loss of 3% and fat mass reduction of 10% strongly separated the KD group from the control group (Figure S3). These thresholds were reached by 19 of 22 for body mass and 17 of 22 for fat mass among participants in the KD group. More than 75% of these patients (81% losing body weight, 77% losing fat mass) also rated the diet as feasible in the questionnaire. Only 3 of 17 and 1 of 17 patients achieve this weight or fat mass reduction in the control group (p < 0.001 and p = 0.003; Figure S3)

### htTKV

Both the control and WF group showed an increase in mean htTKV between BL and EOT, without a statistically significant difference between groups when reported as relative changes normalized to BL htTKV (control, +0.79%; WF, +0.80%; p = 0.99). In the KD group, we observed a 0.55% decrease in mean htTKV normalized to BL, without reaching statistical significance compared with the control group (p = 0.285) (Figure 3A). Similar findings were obtained when analyzing absolute changes in volume from BL to EOT (control, 14.8 ± 30.7 mL/m;

KD, -10.7 ± 48.6 mL/m; WF, 6.5 ± 38.9 mL/m). Again, comparing the changes in the intervention groups with the change observed in the control group did not provide evidence of a statistically significant difference (KD, p = 0.096; WF, p = 0.818) (Figure S5A).

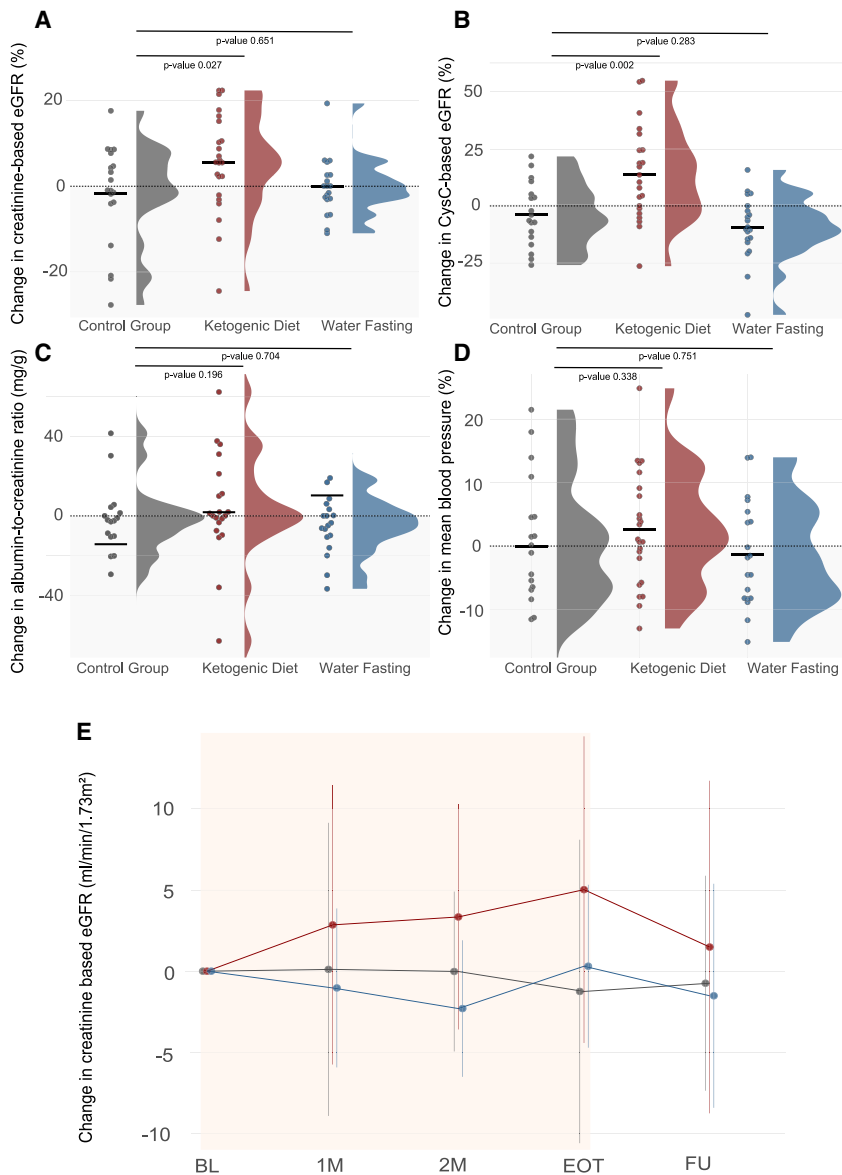
### htTLV

Regarding htTLV, the mean volume increased by 2.04% from BL to EOT in the control group (42.1 ± 166 mL/m). htTLV decreased by 0.15% in the WF group (-10.0 ± 98.2 mL/m) and by 4.73% in the KD group (-55.1 ± 72.5 mL/m) (Figures 3B and S5B). The difference in both the absolute and relative change of htTLV from BL to EOT reached statistical significance between the KD and control groups (relative p = 0.011, absolute p = 0.006). This was not the case comparing the control and the WF group (relative p = 0.419, absolute p = 0.311).

### eGFR, urine albumin, and blood pressure

We analyzed creatinine-based eGFR development from BL to EOT and observed decreases of -1.74% ± 11.7% for the control group and -0.20% ± 6.82% for the WF group but an increase of 5.51% ± 11.4% for the KD group. Compared with the control group, the change in creatinine-based eGFR from BL to EOT was statistically significant in the KD group (p = 0.027) but not in the WF group (p = 0.651) (Figure 4A). Considering the impact of diets and potential changes in muscle mass on creatinine measurements, we also quantified cystatin C (CysC) at BL and EOT. CysC-based eGFR confirmed a significant difference in eGFR development between the KD and control groups (control, -3.62% ± 13.9%; WF, -9.52% ± 14.1%; KD, +13.9% ± 20.6%; Figure 4B).

To examine potential signs of hyperfiltration as a basis to the eGFR increase in the KD group, we analyzed the albumin-to-creatinine ratio. There was no significant difference of the albumin-to-creatinine ratio between the control and the KD/WF groups (KD, p = 0.196; WF, p = 0.704) (Figure 4C). Also, the ratio of alpha-1-microglobulin to creatinine, as a



**Figure 4. Ketogenic dietary interventions have a significant impact on eGFR**

Absolute and relative changes of eGFR throughout the study are displayed (n = 63). Black horizontal lines indicate means. The p values indicate the strength of evidence for a true underlying difference between the control and the two intervention groups; CG, n = 19; KD, n = 23; WF, n = 19.

(A) Mean difference in creatinine-based eGFR from BL to EOT: CG, -1.74%; KD, +5.51%; WF, -0.22%. (B) Mean difference in cystatin C-based eGFR from BL to EOT: CG, -3.62%; KD, +13.9%; WF, -6.21%.

(C) Mean difference of albumin-to-creatinine ratio from BL to EOT: CG, -14.32 mg/g; KD, 1.83 mg/g; WF, 10.30 mg/g. Two patients in the control group who showed a relative difference of 440% and 911%, two patients in the KD group with a relative difference of 457% and 780% and one patient in the WF group, showing 924% difference were removed from the plot but included in calculating the mean and for statistical testing.

(D) Mean difference in blood pressure from BL to EOT, analyzed from average MAP values of 3 measurements per visit: CG, -0.61%; KD, +2.35%; WF, -1.63%. One patient in the control group who showed a relative difference of -23.5% is not displayed in the plot but was included in calculating the mean and for statistical testing.

(E) Line graph showing evolution of absolute changes in creatinine-based eGFR at all visits from BL to FU. Mean change BL to EOT was CG, -1.25 ± 9.35 mL/m; KD, 5.03 ± 9.45 mL/m; WF, +0.31 ± 5.02 mL/m. Mean change between BL and FU was CG, -0.74 ± 1.89 mL/m; KD, +1.49 ± 0.95/10.3 mL/m; WF, -1.52 ± 6.91 mL/m. 1M, 1 month; 2M, 2 months; BL, baseline; CG, control group; FU, follow-up; eGFR, estimated glomerular filtration rate; EOT, end of treatment; htTKV, height-adjusted total kidney volume; htTLV, height-adjusted total liver volume; KD, ketogenic diet group; MAP, mean arterial pressure; WF, water fasting group.

marker of potential differences in tubular damage, showed no significant differences in the change from BL to EOT between groups (Figure S5C). Considering the impact of blood pressure on acute changes in eGFR, we also compared changes in mean arterial blood pressure over time. However, there was no significant difference in the development of blood pressure from BL to EOT across groups (Figure 4D). After switching back to a CHO-rich *ad libitum* diet, we saw a partial loss of the difference in eGFR between the KD and control groups (mean eGFR difference BL-EOT: KD, +5.03 ± 9.45; WF, +0.31 ± 5.02; control, -1.25 ± 9.35 [p = 0.01]; BL to follow-up [FU]: KD, +1.49 ± 10.3; WF, -1.52 ± 6.91; control, -0.74 ± 6.63), and this difference did not reach statistical significance anymore (p = 0.311). Relative changes to BL including all visits until one month after EOT are depicted in Figure 4E).

### Insulin-like growth factor 1 and high-sensitivity C-reactive protein

Mean high-sensitivity C-reactive protein (hsCRP) showed an increase in all three groups from BL to EOT (control, 265.63%; KD, 58.51%; WF, 5.77%). The comparably strong increase in hsCRP in the control and KD group can be explained by underlying infections: One patient in the control group was hospitalized because of a cyst infection at the time of the visit. One patient in the KD group was diagnosed with appendicitis a few days after the visit. Despite the resulting differences in the change in hsCRP between BL and EOT, no statistically significant difference between the control and the intervention groups were found (KD, p = 0.090; WF, p = 0.080) (Figure S5D).

Regarding insulin-like growth factor 1 (IGF-1) levels, all 3 groups showed decreases from BL to EOT, with mean decreases of 5.86% in the control group, 6.41% in the KD group, and 3.13% in the WF group. There was no statistically significant difference between the control group and either of the two intervention groups (KD, p = 0.920; WF, p = 0.635) (Figure S5E).

**Table 2. Safety-relevant laboratory events, events probably related to the intervention and safety-relevant events leading to hospitalization during the intervention period**

	Description	KD, n (%)	WF, n (%)	Control, n (%)
AST/ALT	>ULN to 3.0× ULN (>50–150 U/L)	1 (4)	0	1 (5)
	>3.0× to 5.0× ULN (>150–250 U/L)	0	0	0
Creatinine	up to 1.5× increase baseline	0	0	0
	>1.5–3.0× increase baseline	0	0	0
Uric acid	>ULN without gout specific symptoms (>7 mg/dL)	4 (17)	1 (5)	1 (5)
	>ULN with gout specific symptoms (>7 mg/dL)	0	0	0
Triglycerides	150–300 mg/dL	1 (4)	3 (16)	1 (5)
	>300–500 mg/dL	2 (8)	0	1 (5)
Cholesterol	>ULN (200 mg/dL) to 300 mg/dL	3 (13)	0	0
	>300–400 mg/dL	1 (4)	0	0
Events probably related to the intervention	orthostatic symptoms	4 (17)	4 (21)	0
	keto flu associated	10 (43)*	3 (16)	0
	reflux symptoms	0	1 (5)	0
Safety-relevant event with hospitalization	appendicitis	1 (4)	0	0
	cyst infection	0	0	1 (5)
	nephrolithiasis	1 (4)	0	0

Safety-relevant laboratory events during the intervention period are reported per study arm along with the respective percentage within the study arm. All safety-relevant events were grouped into larger superordinate groups. Superordinate groups (see also Table S3) that contained at least one event which was considered probably associated with the intervention are shown together with the sum of all events contained in this group and the respective percentages per study arm. All safety-relevant events leading to hospitalization after initiation of the intervention period are shown as well as their frequencies and percentages per study arm. Safety-relevant events were defined as new events or a significant worsening of pre-existing conditions during the intervention, thresholds defined according CTCAE criteria. Potential differences between the intervention groups and the control group were investigated using chi squared tests.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; KD, ketogenic diet group; ULN, upper limit normal; WF, water fasting group.

\*Significantly different occurrence of the event in the corresponding intervention group compared with the control group ( $p < 0.05$ ).

### Safety and lab values

A table containing all relevant mean lab values stratified by the different groups for all visits can be found in [supplemental information \(Table S2\)](#).

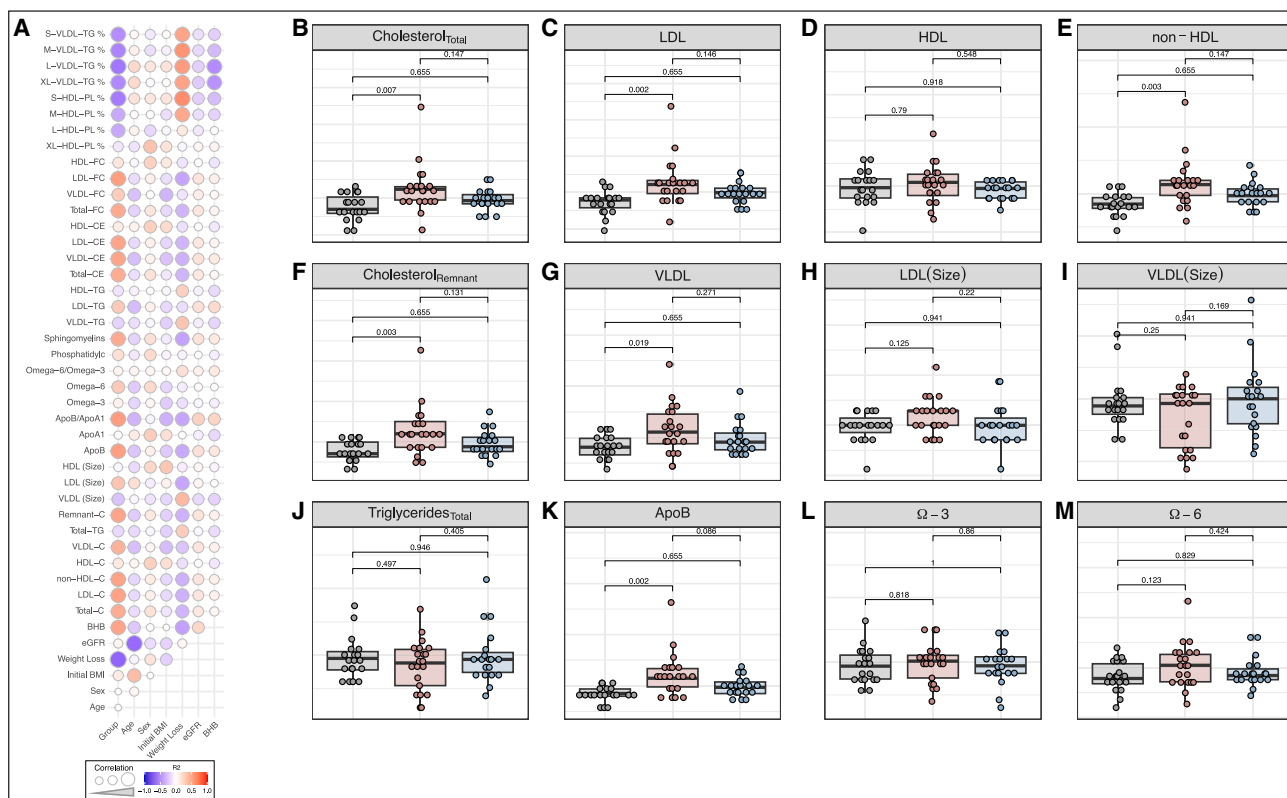
Safety-relevant laboratory events of interest during the diet period as well as all events probably related to the ketogenic dietary interventions and those associated with hospitalization can be found in Table 2. Briefly, in one patient each in the control and KD groups, a slight, asymptomatic increase in transaminases was observed. Four patients in the KD group and one patient each in the control and WF groups showed elevated levels of uric acid. In the KD group, 4 patients developed hypercholesterolemia, while this was the case for none in the other groups. Four patients in each of the ketogenic intervention groups reported orthostasis-related symptoms. Ten patients in the KD group showed symptoms that, regarding type, time of onset, and reversibility over time, are typically reported at the initiation of KD and are usually referred to as “ketogenic flu.” “Ketogenic flu” is a set of symptoms that typically occur 2–7 days after starting a KD. These include primarily headache, foggy brain, fatigue, irritability, nausea, and difficulty sleeping.<sup>27</sup> Three patients in the WF group experienced such symptoms during their 3 days of fasting that are summarized accordingly. All these symptoms in the WF group were self-limiting after the patients resumed their usual diet. Two participants in the KD group and one patient

in the control group experienced safety-relevant events leading to hospitalization after initiating the diet. One of the patients in the KD group developed appendicitis after EOT, and this event was considered not related to the diet. The other patient showed a symptomatic kidney stone during the treatment phase, an event that was scored as possibly treatment related. A second patient in the KD group also developed symptomatic nephrolithiasis. However, this event occurred in the run-in phase before initiation of the diet and was consequently not related to the diet. One patient in the control group developed a cyst infection and was hospitalized to receive intravenous antibiotic treatment. Differences in the number of safety-relevant events as shown in Table 2 were statistically significant only for “ketogenic flu”-associated symptoms in the KD group compared with the control group. All safety-relevant events and their absolute frequencies can be found in [supplemental information \(Table S3\)](#).

### In-depth characterization of the KD-associated lipid profile in ADPKD

To assess the potential clinical relevance of increase in cholesterol levels observed in the KD group, we performed nuclear magnetic resonance (NMR) measurements of 52 lipid parameters using serum samples at BL and EOT. Correlation of the fold change between BL and EOT of the most important markers contained in the panel expectedly showed a strong correlation of





**Figure 5. Ketogenic diet in patients with ADPKD induces significant changes in the serum lipid profile**

NMR analyses of changes in lipid profiles from BL to EOT are shown (n = 63).

(A) Correlation plot showing the relationship between the change of different lipid parameters of serum samples including total cholesterol, LDL, HDL, non-HDL, remnant cholesterol, VLDL, size of LDL particles, size of VLDL particles, total triglycerides, ApoB, omega-3, and omega-6 with clinical parameters including assignment to the KD group vs. control, gender, BMI, weight loss, and eGFR. The correlations were calculated using Pearson's correlation coefficient. The color coding indicates either a positive (red) or negative (blue) correlation, while the size of the circles in the scatterplot indicates the strength of the relationship. (B–M) Bar plots showing the log<sub>2</sub> fold change (BL vs. EOT) of different lipid parameters in all three groups (control, KD, and WF). (B) Total cholesterol, (C) LDL, (D) HDL, (E) non-HDL, (F) remnant cholesterol, (G) VLDL, (H) size of LDL particles, (I) size of VLDL particles, (J) total triglycerides, (K) ApoB, (L) omega 3, and (M) omega 6. Statistical testing was performed using a model correcting for Mayo class, BMI, gender, and age. BL, baseline; CTRL, control group; EOT, end of treatment; KD, ketogenic diet group; WF, water fasting group; ApoB, apolipoprotein B; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance spectroscopy; VLDL, very low-density lipoprotein.

total cholesterol and low-density lipoprotein (LDL) cholesterol with an assignment to KD, while being negatively correlated with weight loss (Figure 5A). Non-HDL (high-density lipoprotein) cholesterol, very low density lipoprotein (VLDL), remnant cholesterol, apolipoprotein B (ApoB), sphingomyelins, and cholesteryl esters in LDL and VLDL as well as total free cholesterol, free cholesterol in LDL, LDL size, and omega-6 fatty acids showed a similar pattern (Figure 5A). In contrast, VLDL size and the ratio of phospholipids to total lipids in small, medium, and large HDL showed opposite correlations with KD group assignment and weight loss (Figure 5A). This was also the case for the ratio of triglyceride to total lipid in all sizes of VLDL particles (Figure 5A). We went on to test significant differences in the fold changes from BL to EOT among all three groups. In general, the WF group was much more similar to the control group than the KD group (Figures 5B–5M and S6). We found a significantly larger rise in the KD group compared with the control group for total cholesterol, LDL, non-HDL cholesterol, remnant cholesterol, VLDL, and ApoB (Figures 5B, 5C, 5E, 5F, 5G, and 5K).

HDL, VLDL size, triglycerides, and omega-3 fatty acids did not show any changes (Figures 5D, 5I, 5J, and 5L). Omega-6 fatty acids and LDL size both appeared to increase in the KD group, but this difference to the control group did not reach statistical significance (Figures 5H and 5M). A row of other lipid parameters, including the phospholipid content in LDL, cholesteryl esters, free cholesterol, sphingomyelins, and the ApoB/ApoA1 ratio, showed an increase from BL to EOT in the KD group significantly different from the control or WF group (Figure S6). No further parameter was reduced by KD.

## DISCUSSION

ADPKD carries a high burden, and currently available therapeutic options are limited and only modestly slow disease progression. Consequently, the unexpectedly large beneficial effect of ketogenic dietary interventions in animal models has received much attention.<sup>21</sup> Human data were limited to a retrospective case series<sup>22</sup> and non-randomized pilot studies in small

cohorts.<sup>23,24,28</sup> KETO-ADPKD now adds a comprehensive analysis of two ketogenic dietary interventions in 63 ADPKD patients on the basis of a randomized controlled design.

Feasibility was a key focus of KETO-ADPKD, as only feasible interventions can have an impact on real-life patient care. Previous studies had indicated good feasibility, but whether this would really be the case in comparison with a control group in a randomized setting had remained unclear.<sup>22–24,28</sup> In line with these studies, nearly all patients in KETO-ADPKD scored the KDIs as feasible and completed the entire study, with only one patient in the WF group terminating her participation prematurely for feasibility reasons. This finding must be considered in the context of both the support provided during the trial with counseling and regular follow-up phone calls. Besides, ADPKD patients show a high degree of motivation regarding therapeutic options, likely partly because of the autosomal-dominant inheritance linked to knowledge on disease courses in other family members. The primary combined feasibility endpoint including both patient-reported feasibility and objective biochemical proof of ketosis was designed to provide an insight into practicability of KDIs containing as much information as possible. This endpoint was reached only by the WF group. This could raise concerns about feasibility of the KD approach. However, the fact that the KD group did not reach its threshold appears to be clearly attributed to a fairly high predefined BHB threshold of  $\geq 0.8$  mmol/L to be reached at 75% of the visits on diet. Essentially, considering that only 3 visits were performed on diet, this led to the unintended need to reach the target at all visits. It is well expected, that patients on a diet over several month will have treatment breaks and “cheat days,” lowering their BHB levels at single visits, and future dietary regimens and study protocols should also account for this. Importantly, the thresholds reached by the KD group were associated with a clear impact on outcome (e.g., body composition, eGFR, and total liver volume [TLV]). Consequently, it is likely that lower BHB target values, as used in previous studies, which are reached on most days of the diet but not necessarily permanently, are sufficient for the beneficial effect.<sup>25,26,29–31</sup> The exploratory analyses regarding different BHB cutoffs in combination with the finding that more than 75% showed BHB levels higher than BL values at all on-diet visits and reached significant body composition thresholds is a clear indicator of feasibility. Beyond ketone body thresholds, patient-reported feasibility itself was high for the KD arm.

In animal models, even short-term KDIs such as 3-day WF already led to detectable changes in cyst burden.<sup>21</sup> However, this rapid effect was not confirmed in the recent human pilot study.<sup>24</sup> In KETO-ADPKD, htTKV slightly decreased in the KD group from BL to EOT while increasing in the other groups, but this difference did not reach statistical significance. The lack of significance is not unexpected considering the generally slow growth of kidneys in ADPKD requiring longer term treatments (e.g., at least one year) to reveal significant differences. However, it is important to note that any reduction in total kidney volume (TKV) is indeed an unexpected finding with important clinical implications, considering that ADPKD usually shows continuous growth of kidneys over time.<sup>12,32</sup> In line with previous findings, htTLV was significantly decreased by KD. However, it is important to note that this is likely a consequence, at least partly, of

glycogen depletion.<sup>24</sup> This phenomenon is also observed under caloric restriction and is used to prepare patients for bariatric surgery.<sup>33</sup> Analyses of cyst fraction may help dissect the liver response further in the future. Nonetheless, considering the lack of therapeutic options for PLD, any new option to limit liver growth in PLD would have a major clinical impact.<sup>34</sup> Repetitive WF periods did not lead to any significant changes in outcome parameters in our study. Consequently, the hypothesis that repetitive short-term ketosis may induce beneficial effects is not supported and potentially abrogated by rebound effects. Such rebound effects are generally known for diets regarding weight loss goals.<sup>35</sup> Consequently, a sustained effect on weight was not expected in the WF arm. Potential acute effects of WF right after the three day period were not the focus of this study and would not have been detected considering that the outcome measurements were not obtained right after fasting.

Kidney failure is the most relevant long-term outcome in ADPKD, and treatment effects on kidney function are the central point of interest regarding novel therapies. ADPKD is characterized by a slow but relentlessly progressive loss of kidney function. Surprisingly, not only was eGFR decrease slowed in the KD group, but kidney function improved over time.<sup>8,12,32</sup> Considering the results of the body composition measurements, it is unlikely that this is a consequence of the impact of muscle mass or meat intake on creatinine measurements. In KETO-ADPKD, body composition mainly showed changes in fat mass and body water, a plausible finding on KD. Using the body composition scales, no single parameter represents muscle mass. Muscle mass is contained in the lean body mass, which slightly decreased in the KD group. However, lean body mass also reflects body water and all other non-fat, non-skeletal parameters, presumably including glycogen. Taken together, muscle mass was probably not affected by KD. The confirmation of the eGFR effect by measurement of CysC strongly supports this point and further provides evidence that this finding is not due to changes in meat intake. Although we cannot exclude that weight loss itself has an impact on kidney function loss beyond the effect of ketosis, weight loss appears unlikely to be the key driver considering both the results of the body composition measurements and the fact that the participants in KETO-ADPKD had a normal weight at BL. An increase of eGFR may also be the consequence of glomerular hyperfiltration, which is considered harmful in chronic kidney disease. Some KD regimens contain high protein amounts, which may lead to hyperfiltration.<sup>36</sup> However, importantly, this was not the case regarding our regimen, which aimed for moderate to low protein intake in line with current recommendations for chronic kidney disease. Besides, neither did albuminuria change as a sign of hyperfiltration<sup>37</sup>; nor, as expected, was blood pressure increased in the KD group, as another confounder regarding eGFR.<sup>38</sup> Acute effects of KD on kidney function in ADPKD could be explained by both a reduction of intrarenal inflammation and cyst-related pressure.<sup>39–42</sup> Nonetheless, hyperfiltration needs to be examined in future studies and only long-term trials will be able to fully confirm a beneficial disease-modifying effect. However, considering the usually relentless progressive loss of kidney function in ADPKD<sup>8,12,32</sup> and the fact that the only available treatment slows eGFR decline by 26%–30% only,<sup>12,43</sup> the findings of our study

would be of very high clinical relevance if a disease-modifying effect can be confirmed in the future.

KD did not have a statistically significant impact on hsCRP in our study, but this may not be the optimal parameter to measure intrarenal inflammation in ADPKD, and small patient numbers as well as confounders need to be considered. In particular, new systemic infections (cyst infection, appendicitis) had a significantly stronger influence on the hsCRP value than a possibly existing mild inflammation in the context of KD. Such mild inflammation would have been superimposed by systemic infections. After returning to a CHO-rich diet for one month the benefit of KD on kidney function was, even though still reaching a potentially meaningful effect size, diminished and did not reach statistical significance anymore indicating the importance for longer term studies to confirm a disease-modifying effect. Increases in eGFR on low-CHO diets had previously been shown in patients with diabetes mellitus,<sup>44</sup> but this work did not address potential influences of muscle mass and diet on creatinine. Regarding the improvement of kidney function in patients on KD, it is important to note that recent work convincingly showed reversibility of the profound changes in renal structure in ADPKD using genetic models.<sup>45</sup> Besides, at least partial reversibility is suggested by the rodent work on ketogenic interventions.<sup>21</sup> It is therefore intriguing to speculate that this may also be the case in humans. However, longer term interventions will be required to answer this question.

Before moving toward longer term trials or real-world recommendations, assessing potential safety signals is a point that deserves special attention. Given the significant weight loss we observed among patients in the KD group within the 3 months of diet, further potentially unfavorable weight loss over longer interventional periods needs to be considered. However, patients undergoing diets typically experience the most significant weight loss in the initial stages, which was also the case in our study. For KD, currently available data point to the fact that weight loss is most pronounced in obese people stabilizing once reaching a normal weight.<sup>46–48</sup> Besides, the marked weight loss at the very beginning is caused by loss of volume (i.e., water and salt) to a large extent, a phenomenon known with KDs.<sup>30</sup> Both points are supported by our body composition measurements. Our extensive safety analyses both at the level of clinical events and laboratory values, identified two areas of interest, blood lipids and kidney stones. One patient showed a symptomatic kidney stone on KD. Whether this is due to the diet cannot be answered at this point, also considering the high prevalence of kidney stones in ADPKD.<sup>49–52</sup> The increase in uric acid observed as well as previous data regarding kidney stones in epilepsy patients on KD indicates that such a connection could be plausible.<sup>52–54</sup> In contrast to many of the KD regimens used our regimen is not protein- or meat-rich, which may partly mitigate this risk. Also, the diet change until symptoms was only 24 days in this patient, a period that is rather short for *de novo* formation of a clinically meaningful kidney stone making passage of a pre-existing stone a possible alternative explanation. Nonetheless, future trials should consider this a safety-related event of special interest and potentially preventive measures on the basis of urine supersaturation profiles, such as citrate supplementation, could be implemented. In ADPKD, there is an interesting

link among crystal formation, citrate, and disease progression, which further underlines the importance of this point.<sup>55–57</sup>

One of the main concerns about KD could be a potentially negative effect on the cardiovascular risk profile, which is of particular relevance in a population of patients with kidney disease. Previous results have been controversial<sup>30</sup> and, among others, reported beneficial effects on cholesterol levels in obese individuals,<sup>58</sup> whereas at least transient increases in the atherogenic lipoproteins were detected in children.<sup>59,60</sup> In the population of KETO-ADPKD, unfavorable ApoB and ApoB-containing lipoproteins including remnant cholesterol increased during KD, supporting the idea of possible negative metabolic effects.<sup>61,62</sup> That being said, it has to be considered that the intervention phase of this study was too short to address the potentially transient nature of these changes. In long-term studies on the effect of KDs in children with epilepsy, a plateau with a downward trend<sup>63</sup> or a normalization of initial increase triglyceride and cholesterol levels<sup>60</sup> was observed, despite initial dyslipidemia. Lipid profiles were also shown to partly improve in long-term studies using low-CHO diets in patients with type 2 diabetes.<sup>44,64</sup>

Of note, most of the available data to date has been obtained in overweight and obese patients, whereas reports on normal weight patients are scarce. One key factor for an increase in LDL cholesterol that has been identified, apart from genetic factors, is the dietary content of saturated fatty acids (SFA). Although the protein content of the ketogenic meal plan in the study was moderate to low, sources of SFA, such as cheese, butter, meat, and coconut oil might contribute to the observed effect of the diet on cholesterol. Besides, currently available data support the notion that lean individuals show the most marked increase, referred to as the lean hyper-responder phenotype.<sup>65</sup> Despite the significant increase in LDL, it is important to note that the difference in the number of safety-relevant events defined according to CTCAE criteria did not reach significance regarding cholesterol. Although there are numerous studies reporting LDL, HDL, and total cholesterol changes upon low-CHO diets, in-depth analyses of the lipid changes in randomized clinical trials are scarce.<sup>30</sup> Small dense LDL particles have been suggested to bear a greater atherogenic potential than other LDL subfractions because of their susceptibility to glycation and oxidation as well as their reduced LDL receptor affinity.<sup>66,67</sup> There is evidence of a decrease of small LDL particles in dietary interventions restricted in CHOs.<sup>68</sup> Our analysis also showed a correlation of increases in LDL size over time with allocation to the KD group. However, the fold change differences from BL to EOT between groups did not reach statistical significance. Additionally, plasma levels of sphingomyelins are considered a risk factor for coronary artery disease<sup>69</sup> and are found to be elevated in the KD group of this trial. If the development of these parameters also depends on the length of the dietary change is still unknown. Serum levels of other markers previously associated with differential cardiovascular risk such as unsaturated and SFAs as well as linolenic acid did not differ between the groups. Taken together, lipid profiles will need to remain a safety aspect of special interest for future long-term trials.<sup>30</sup>

It is important to note that other studies have already contributed important results regarding dietary interventions in ADPKD

before. Specifically, Hopp et al.<sup>70</sup> performed an elegant study comparing intermittent fasting and caloric restriction in obese patients with ADPKD. They found a correlation between slowed kidney growth and weight loss independent from the dietary regimen. Patients in KETO-ADPKD were not obese, and whether the findings also apply to patients with normal weight is not clear. Potentially ketogenesis could explain parts of their results as well, even though both regimens would probably only result in very mild degrees of ketosis. Ketone bodies were not measured in this study. In the future, considering the results in rodents,<sup>21</sup> treatment with exogenous ketone bodies could also be of interest to complement or replace KD, but clinical data are lacking to date. Besides, SGLT2 inhibitors induce ketogenesis, making them attractive therapeutics for ADPKD. Unfortunately, ADPKD was an exclusion criterion in the crucial chronic kidney disease (CKD) trials.<sup>71,72</sup> Metformin, which has already been studied for ADPKD<sup>73,74</sup> and is currently being examined in a multicenter trial (IMPEDE-PKD [NCT04939935]), activates AMP kinase, a mechanism that is also targeted by KD.<sup>21</sup> Consequently, the beneficial effect of ketosis could be exploited from several angles and potentially in a synergistic manner.

In conclusion, KETO-ADPKD provides promising data on the effect of a KD in ADPKD from a randomized controlled trial design. Longer term efficacy studies will be required to obtain a full picture on efficacy and safety. Consequently, this does not result in a general recommendation for a KD as a therapeutic strategy for ADPKD. However, the results already provide a basis for improved counseling of patients with ADPKD choosing to be on a KD for personal motivation. Most important, they are clearly sufficient to motivate the design of larger trials with a treatment duration over several years. Such initiatives can then indeed pave the way toward an entirely novel treatment opportunity for the most common genetic kidney disease.

### Limitations of the study

This study has several limitations. The formal primary combined feasibility endpoint was not reached by the KD group, partly because of an unintended requirement to reach high BHB cutoffs at three out of three on-diets visits by defining a 75% threshold for the endpoint in all study visits. Concerning efficacy outcomes, the intervention period was 3 months only, and the number of participants was limited. Despite the use of both creatinine and CysC to assess GFR, measured GFR would provide additional information to rule out an impact of the diet on the measurements. Future research will be needed to establish whether the increase in eGFR upon KD indicates a beneficial effect on disease severity or may be harmful in the long term because it reflects glomerular hyperfiltration. In this regard, long-term data are required to show real disease-modifying effects. Regarding TKV and TLV, cyst fraction measurements are lacking and should be added in the future. A relevant proportion of patients did not come in a fasting state to at least one of the visits. Consequently, we do not report results on insulin and glucose. Patients in KETO-ADPKD showed quite good kidney function on average. It is thus not possible to extrapolate to later stage disease from the trial findings. Finally, lifestyle factors other than diet (e.g., physical exercise) were not a subject of our study and could further add to beneficial results.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2023.101283>.

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### AUTHOR CONTRIBUTIONS

C.H.L., T.W., J.A.T., R.-U.M., I.B., and F.G. conceptualized the study. U.K. provided advice regarding the dietary interventions. C.L., S.C., P.T., A.K., T.

Brecht, F.M., T.S., N.G.H., I.G., R.-U.M., F.G., and F.S. performed the investigation. S.C., C.H.L., R.-U.M., F.G., P.T., S.A., S.S., S.O., P.A., and N.G.H. were involved in data curation, analysis, validation, and visualization. J.S., C.H.L., S.C., and P.A. performed statistical analysis. R.-U.M., T.W., and T. Benzing acquired funding. S.C., C.H.L., F.G., and R.-U.M. wrote the original manuscript. All authors edited and reviewed the final manuscript.

#### DECLARATION OF INTERESTS

R.-U.M. is a member of the scientific advisory board of Santa Barbara Nutrients and chair of the working group “Genes&Kidney” of the European Renal Association (ERA). T.W. is an inventor on issued and pending patents filed by the University of California, Santa Barbara related to the topic of this article. T.W. is a shareholder of Santa Barbara Nutrients, Inc., and holds a managerial position. T.W. is a scientific advisor and shareholder of Chinook Therapeutics and received research funding from Chinook Therapeutics. The Department II of Internal Medicine (University Hospital Cologne) received research funding from Otsuka Pharmaceuticals not directly related to the study at hand.

#### INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

#### DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

During the preparation of this work the authors did not use generative AI or AI-assisted technologies. We take full responsibility for the content of the publication.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Software and algorithms</b>		
Nightingale Health	Soininen et al. <sup>75</sup> ; Würtz et al. <sup>76</sup>	<a href="https://research.nightingalehealth.com/">https://research.nightingalehealth.com/</a>
R (version 4.0.3)	R Core Team <sup>77</sup>	<a href="https://www.r-project.org/">https://www.r-project.org/</a>
RStudio (version 2022.12.0 + 353)	RStudio Team <sup>78</sup>	<a href="https://posit.co/">https://posit.co/</a>
<b>Other</b>		
Tanita 418 BC MA scale for body composition measurement	Tanita Europe BV	<a href="https://www.tanita.com/en/bc-418/">https://www.tanita.com/en/bc-418/</a>
Portable breath analyzer ACE KETOSCAN mini	ACE instruments	<a href="https://www.ace-technik.com/product/ace-ketoscan-mini-ketosis-test-keto-meter-for-diets-20-mouthpieces-1-calibration-voucher.6872128.html?language_code=en">https://www.ace-technik.com/product/ace-ketoscan-mini-ketosis-test-keto-meter-for-diets-20-mouthpieces-1-calibration-voucher.6872128.html?language_code=en</a>
Portable ketone meter GlucoMen areo 2K	Berlin-Chemie AG	<a href="https://www.glucomenareo.de/jetzt-testen/jetzt-testen-glucomen-aero-2k/">https://www.glucomenareo.de/jetzt-testen/jetzt-testen-glucomen-aero-2k/</a>
Trial registration number: NCT04680780	clinicaltrials.org	<a href="https://clinicaltrials.gov/ct2/show/NCT04680780">https://clinicaltrials.gov/ct2/show/NCT04680780</a>

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Roman-Ulrich Müller ([roman-ulrich.mueller@uk-koeln.de](mailto:roman-ulrich.mueller@uk-koeln.de)).

#### Materials availability

This study did not generate new unique reagents.

#### Data and code availability

- All data reported in this paper will be shared by the [lead contact](#) upon request. Restrictions subject to General Data Protection Regulation (GDPR) may apply.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

### EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

KETO-ADPKD recruited both male and female adult patients from 18 to 60 years of age. More details on baseline characteristics and sample size are provided in [Table 1](#) and the Consort Diagram ([Figure S1](#)). The study was approved by the ethics committee of the medical faculty at University of Cologne and informed consent was obtained from all participants.

### METHODS DETAILS

#### Trial design, inclusion and exclusion criteria

KETO-ADPKD was an exploratory, randomized, controlled, open-label, single-center trial at the University Hospital Cologne. KETO-ADPKD assessed ADPKD patients contained in the database of the AD(H)PKD Registry (NCT02497521) for potential participation based on information contained in the patient records regarding age, kidney function and Mayo Class. 221 patients were contacted by telephone to discuss eligibility and interest to participate. Among these patients, long travel distance to the center was the main patient-reported reason for not participating in KETO-ADPKD. After this pre-screening step, screening was completed based on the newest information on inclusion/exclusion criteria provided by patients. Inclusion criteria were as follows: CKD stage G1-3 AND presence of at least one indicator of rapid disease progression (Mayo class 1C-E OR truncating *PKD1* mutation OR onset of arterial hypertension or urological symptoms <35 years of age OR first- or second-degree family members reaching kidney failure at <60 years of age OR eGFR loss >2.5 mL/min/year OR PROPCKD score >6). Exclusion criteria of the trial were as



follows: Underweight or obese ( $\text{BMI} \leq 18.5 \text{ kg/m}^2$  or  $\geq 35 \text{ kg/m}^2$ ); exposure to a ketogenic diet for more than 2 weeks within the last 6 months; participation in a weight-loss program within the last 6 months; vegan lifestyle; current treatment (or within the last 6 months) with tolvaptan or a somatostatin analog; conditions prohibiting the use of a ketogenic diet; eating disorders; alcohol abuse; type 1 diabetes mellitus, insulin-dependent type 2 diabetes mellitus; contraindications to MRI; pregnancy or breastfeeding; absence of safe contraceptive measures or non-occurrence of menopause. All patients meeting these criteria and willing and able to participate were invited to an on-site visit with a final check of inclusion/exclusion criteria, provision of study information and obtaining informed consent. After enrollment, patients were randomly allocated to one of three treatment arms: ketogenic diet, 3-day water fasting and control group. In case of post-randomization exclusion before the BL visit, patients were replaced and underwent the same randomization process. The study was conducted in accordance with the Declaration of Helsinki and the good clinical practice guidelines by the International Conference on Harmonization. The study protocol will be provided upon request. External monitoring was established to oversee the conduct of the study and review the data periodically to ensure data quality and safety of the participants (carried out by ClinSupport GmbH, 91054 Erlangen, Germany).

### Trial setup

A total of 6 study visits (V1–V6) were planned over the course of this 5-month study, including a 3-month dietary intervention period. The study flow diagram in [Figure S2](#) shows the trial setup in detail. At Visit 1, informed consent was obtained and patients were allocated to their study group. Visit 2 served as baseline (BL) for our analyses. Visit 5 marked the end of treatment (EOT) for the intervention groups. Visit 6 was scheduled to assess a 4-week follow-up period (FU) after return to a carbohydrate (CHO) rich diet.

### Dietary interventions

Between screening and baseline, all patients maintained their usual carbohydrate-rich diet. All patients were advised to stick to general dietary recommendations for ADPKD patients, i.e., low salt intake (<5–7 g/day) and sufficient water intake (>3 L/day). Patients in the control group were allowed to eat *ad libitum*. As per protocol, patients in the KD group followed an isocaloric, classical ketogenic diet for 3 months. They were provided with an individualized meal plan in collaboration with a company providing diet support (Foodpunk, Neubiberg, Germany). Optionally, this included usage of a digital application providing the individual recipes and shopping lists. The content of the diets was independent from this approach. The KD contained a maximum of 30 g carbohydrates per day and a moderate to low target protein intake of 0.8 g/kg body weight, suitable for CKD. Upper limits of intake were defined for: oxalate (100 mg per day), sodium chloride (7 g per day), phosphorus (700 mg per day) and potassium (4000 mg per day). In addition, food preferences, dislikes or intolerances were taken into account, when calculating and customizing the diet plan for each patient. The diet was offered to be vegetarian and through a high percentage of vegetarian recipes, patients were encouraged to limit meat intake. Patients in the 3-day water fasting group performed a fast on 3 consecutive days within the first 14 days of each month. On all other days of the intervention period, they were instructed to eat *ad libitum*. Participants were allowed to consume only non-caloric drinks (e.g., water, tea and coffee without milk) and to eat a broth once a day during the fasting days. Optionally, one of these episodes was permitted to be split into two shorter episodes (1 day and 2 days). During the intervention period all patients were contacted via telephone 15 days after each of their study visits in order to confirm their well-being and to address any potential concerns or possible uncertainties about the intervention. In addition, patients in the WF group were contacted via telephone on a daily basis during the duration of their 3-day fasting periods. All patients of the intervention groups received dietary counseling at BL and at the beginning of each diet month to get an introduction to their specific diet and to clarify any ambiguities. Patients in the interventional diet groups were provided with recipes for permitted snacks as well as protein bars (Adonis Smart Foods) which could be consumed in case of an untamable hunger. As a safety precaution, patients were instructed to ingest a snack consisting of a handful of blueberries or nuts in case of high breath acetone levels of >35 ppm accompanied by symptoms of deep ketosis (e.g., brain fog, “keto flu”) or in case of breath acetone levels of >50 ppm irrespective of any symptoms.

### Trial assessment

Patient history at screening included family history, recording of co-morbidities and intake of any concomitant medication. At each visit, patients were interviewed on their general wellbeing and undesirable symptoms, onset of new diseases were queried. Furthermore, patients were provided with a center-based diet diary for documentation of hunger, overall well-being, potential additional foods deviant from the allocated diet and the daily ketosis measurements. To assess the feasibility and impact of the diets on daily life, a dedicated questionnaire was used at all visits during the intervention to specifically address aspects of the new diet that was based on the questionnaire used in the RESET-PKD trial.<sup>24</sup>

Vital parameters were determined at all visits. Blood pressure and heart rate was measured at three consecutive time points during the visit to obtain mean values for analyses. At each visit from baseline, an electrocardiogram (ECG) was recorded (Schiller, 53567 Buchholz, Germany). Body weight and height were assessed at screening visit, starting from baseline, body composition was measured using the Tanita 418 BC MA scale (Tanita Europe BV, 1101 BE Amsterdam, The Netherlands).

Measurement of ketone body levels in fingerstick-blood and urine as well as breath acetone levels were performed at each in-center visit. Between all visits, patients measured the extent of ketosis in breath twice for three consecutive days of their choice. During the fasting period, patients measured the above-mentioned parameters daily for the entire intervention period. Breath acetone concentrations were measured using a portable breath analyzer (ACE KETOSCAN mini, ACE instruments, 83395 Freilassing, Germany).

Point-of-care BHB blood measurements were achieved using a portable ketone meter (GlucoMen areo 2 K, Berlin-Chemie AG, 12489 Berlin, Germany). All other blood and urine measurements were performed by the central laboratory of the University Hospital Cologne. Regarding laboratory parameters, safety relevant events were defined as new events or a significant worsening of pre-existing conditions during the intervention. Such a worsening was determined by newly exceeding the thresholds defined according to CTCAE criteria.<sup>79</sup>

MRI scans of the abdomen were performed at BL and EOT for all participants in order to measure kidney and liver volumetry. The MRIs were performed by the Department for Radiology of the University Hospital Cologne using a 1.5-T system (Ingenia, Philips Healthcare, Best, The Netherlands). Imaging protocol included both axial and coronal fat-saturated T2w.

### Kidney and liver volumetry

All segmentations were performed using a dedicated research environment (Philips IntelliSpace Discovery, Philips, Best, The Netherlands). Considering the small changes in kidney volume, segmentation of the kidneys was separately performed by two trained and blinded readers on axial T2w under exclusion of the renal hilum; TKV was calculated based on the mean of these segmentations. Segmentation of the liver was conducted on axial T2w imaging as well. Extra-hepatic portions of both, portal vein and bile ducts including the gall bladder were excluded from segmentation allowing for computation of actual TLV.

### Outcomes

Primary endpoint of this trial was a combination of objective adherence and patient-reported feasibility to assess the overall feasibility of the dietary regimens in this trial. Patient-reported feasibility was measured using a previously established questionnaire.<sup>24</sup> To place additional emphasis on long-term feasibility, our analysis encompassed 6 additional questions that aimed at assessing the time frame in which patients perceived themselves capable of adhering to this diet.

The options for answers ranged from  $-4$  to  $+4$  with a higher number indicating better feasibility. The value for each patient was calculated by averaging the single values of the questionnaires from V2 to V5. From these answers, we calculated a mean score for each patient. With scores of 0 indicating a neutral assessment by the participant, we set the feasibility threshold at  $\geq 0$ . Objective adherence was based on ketone body level measurements in blood and breath, as previously established.<sup>24</sup> In the ketogenic diet group objective adherence was achieved if one of the following criteria was met: (1) ketone body levels  $\geq 0.8$  mmol/L in  $\geq 75\%$  of blood measurements at visits per patient OR (2)  $\geq 10$  ppm in  $\geq 75\%$  of breath analyzer measurements and ketone body levels  $\geq 0.8$  mmol/L in  $\geq 50\%$  blood measurements at visits per patient. In the 3-day water fasting group objective adherence was achieved if one of the following criteria was met: (1) full compliance to diet on  $\geq 75\%$  days per patient AND (2) at least 1 ketone body level  $\geq 10$  ppm on at least 2 out of 3 final days of each fasting phase. The primary endpoint was met, when 75% of patients reached both adherence and feasibility.

The following predefined secondary endpoints were investigated (always comparing BL to EOT): between group-difference in htTKV measured by MRI (segmentation); between group-difference in IGF1; between group-difference in hsCRP; between group-difference in blood pressure. Safety-relevant events (determined by lab values, clinical findings and patient-reported symptoms) were evaluated at every visit. Additional secondary endpoints not reported in this manuscript are described in the protocol or the entry on [clinicaltrials.gov](https://clinicaltrials.gov) (NCT04680780). The following exploratory endpoints were analyzed in this study: Between group-difference in htTLV; between group-difference in eGFR; between group-difference in albuminuria; between group-difference in urinary alpha-1-microglobulin; between group-difference in body composition; between group-differences in lipid-associated metabolites (see [NMR metabolomics](#)).

### NMR metabolomics

Serum was obtained by centrifugation right after drawing blood and was immediately stored at  $-80^{\circ}\text{C}$ . Samples were shipped on dry ice for metabolite quantification by Nightingale Health (Helsinki, Finland). The Nightingale biomarker profiling is based on NMR-spectroscopy and quantification of the spectral data using proprietary software. The technology has been described in detail in Soinenen et al.<sup>75</sup> and Würtz et al.<sup>76</sup>

### QUANTIFICATION AND STATISTICAL ANALYSIS

For the primary endpoint feasibility, no sample size calculation as usual was needed, since reaching the defined criteria in  $\geq 75\%$  of patients did not depend on any variability but was a fixed goal. The threshold had been set at 75% assuming that – if a dietary regimen is to be of widespread use in ADPKD-patients in the future after examination in a larger efficacy trial – the majority of individuals should be able to follow this approach in a short-term setting of 3 months. Apart from that, the sample size rationale was 2-fold. For the primary endpoint, that needed a level of 75% adherence to be fulfilled, 21 subjects per treatment arm are sufficient to calculate a 95% exact Clopper-Pearson confidence interval for the true level of adherence per arm that just excludes 50% (i.e.,  $> 52.8$  to 91.8%). For the secondary efficacy endpoint, the experimental diets were assumed to result in ketone body concentrations of 0.8–2.0 mmol/L while in the control arm, we did not expect values to exceed 0.3 mmol/L. Converting the range (0.8–2.0) to a standard deviation of 0.35 and assuming a 50% adherence level we calculate a standardized effect of 1 ( $= (50\% * 1.3 - 0.3) / 0.35$ ) via the rule of three. The two-sample *t*-test requires 17 subjects per group to detect this effect size with power 80% at two-sided type I error 5%. To

compensate for the influence of dropouts and the use of rank-based methods, randomization was continued until 63 subjects had entered the intervention phase. Randomization was performed by centrally prepared sealed randomization envelopes, provided by the Institute of Medical Statistics and Computational Biology of the University of Cologne. Patients were allocated 1:1:1 to the treatment arms, stratified by age (<45 years,  $\geq$  45 years) and gender. In each stratum random permuted blocks of size 3 were used.

Statistical analyses were performed using R (version 4.0.3)<sup>77</sup> and RStudio (version 2022.12.0 + 353).<sup>78</sup> The package ggplot2 was used for graphical visualization.<sup>80</sup>

For a more detailed description of the statistical tests used please see [Table S4](#). In short, the statistical test was selected based on the distribution of the variables tested. In all cases we applied a simple linear model with the group as a factor which results in the comparisons of EOT and FU against BL respectively. p-values for those comparisons were only extracted if the residuals of the model followed a normal distribution (Shapiro-Wilk test), which is the assumption of the lm p value calculation. For those comparisons where the model resulted in a skewed distribution a non-parametric test (pairwise Wilcoxon test) was selected. For categorical variables we used the chi-square test. For numerical variables, mean and standard deviation were also calculated. Statistical testing of secondary and post hoc endpoints was not adjusted for multiple comparisons considering the focused testing strategy described in the publication. Therefore, the results should be interpreted as exploratory. For the analysis of the NMR Metabolomics data we used a linear model as implemented in the limma R package. As our data contained a design using repeated measures (multiple visits by the same patient) we made use of the block design within limma to take this data into consideration. The general model for analysis consisted of a model containing Visit\_Group + Mayo\_Class + BMI + Gender + Age. A contrast matrix was then used to extract the differences between groups and visits as presented in the publication.

#### ADDITIONAL RESOURCES

Registration of the trial on [clinicaltrials.org](https://clinicaltrials.org) (NCT04680780): <https://clinicaltrials.gov/ct2/show/NCT04680780>.

**Cell Reports Medicine, Volume 4**

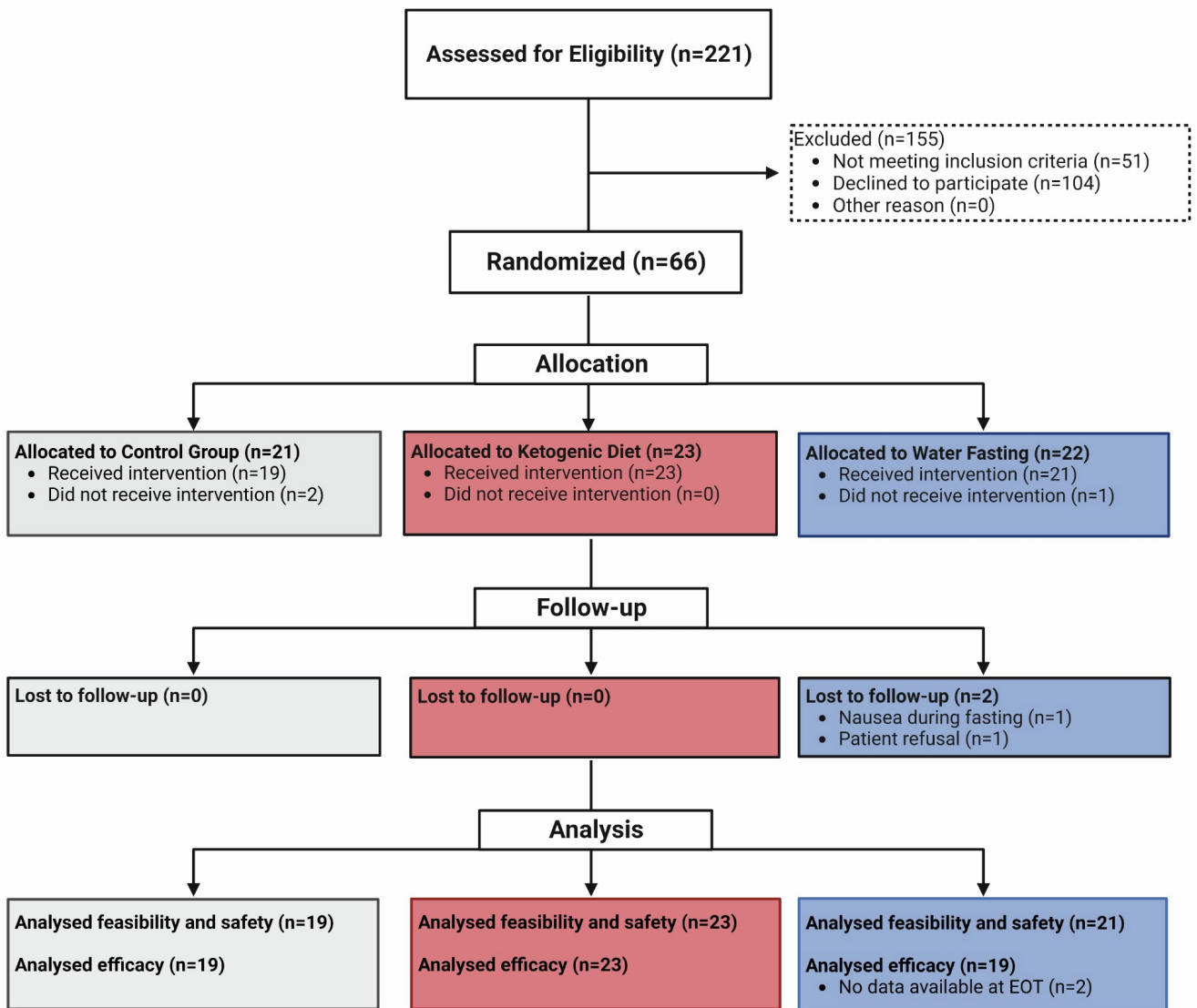
**Supplemental information**

**Feasibility and impact of ketogenic dietary**

**interventions in polycystic kidney disease:**

**KETO-ADPKD—a randomized controlled trial**

**Sadrija Cukoski, Christoph Heinrich Lindemann, Sita Arjune, Polina Todorova, Theresa Brecht, Adrian Kühn, Simon Oehm, Sebastian Strubl, Ingrid Becker, Ulrike Kämmerer, Jacob Alexander Torres, Franziska Meyer, Thomas Schömig, Nils Große Hokamp, Florian Siedek, Ingo Gottschalk, Thomas Benzing, Johannes Schmidt, Philipp Antczak, Thomas Weimbs, Franziska Grundmann, and Roman-Ulrich Müller**

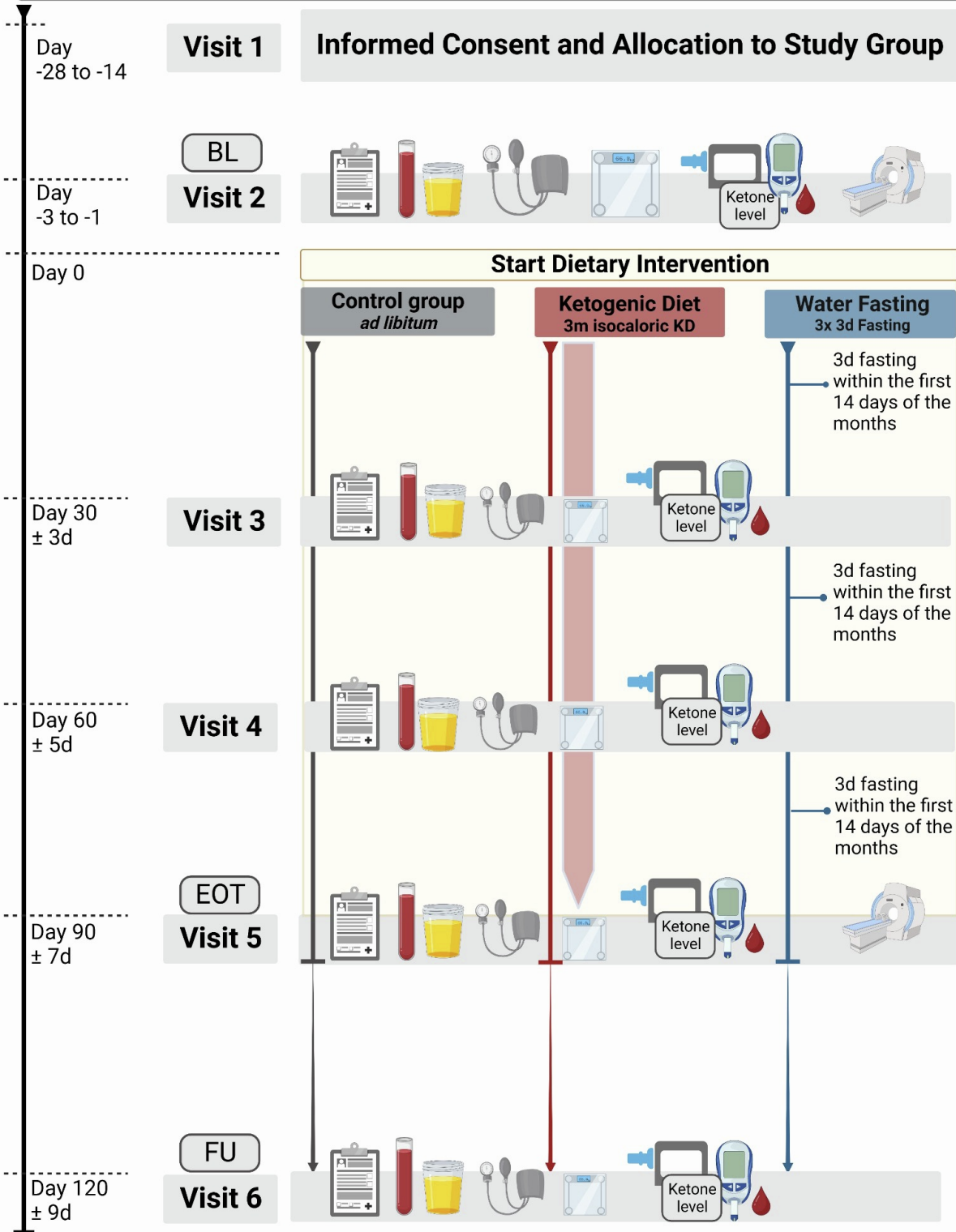


### Supplementary Figure 1: CONSORT Diagram

Related to STAR Methods. CONSORT Diagram illustrating the flow of participants through the study from enrolment to completion. 221 patients were assessed for participation of whom a total of 66 patients were randomized (CG 23, KD 21, WF 21 patients). 63 patients entered the intervention phase, as one participant did not meet the inclusion criteria after review of the baseline MRI and two participants withdrew from the study due to personal reasons before starting the intervention. Of 63 patients, two were lost to follow-up in the WF group. One patient discontinued the KD intervention after 58 days due to hospitalization. The primary endpoint feasibility as well as safety was analyzed for every patient who started the intervention (n=63, CG 19, KD 23, WF 21), all other analyses were carried out for every patient whose data was available at the EOT visit (n=61, CG 19, KD 23, WF 19).

CG, Control group; EOT, End of Treatment, KD, Ketogenic diet; WF, Water fasting

# KETO ADPKD



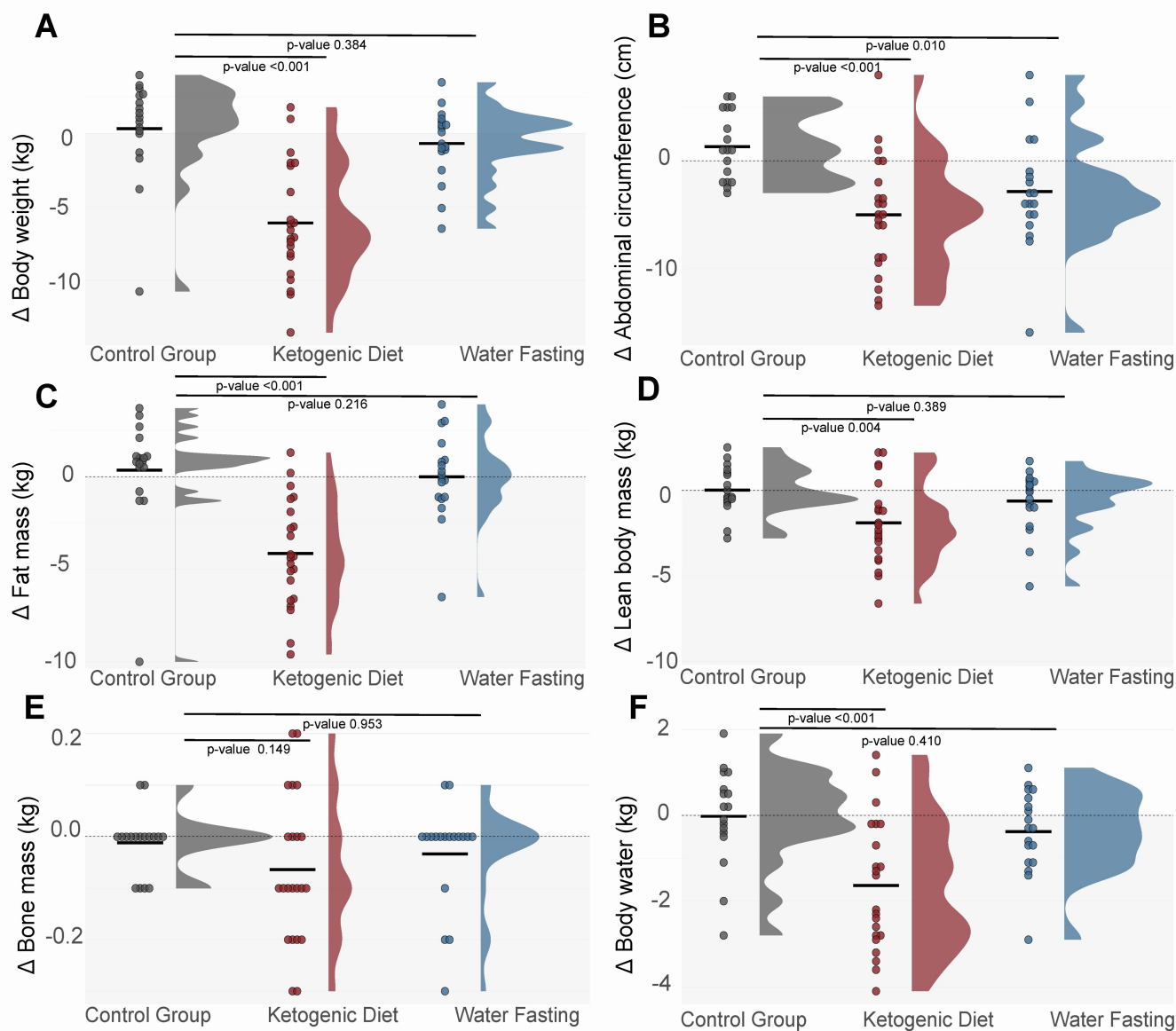
**Supplementary Figure 2: Study Flow diagram** Related to STAR Methods. The study flow diagram illustrates the trial setup and progress of participants throughout the study.

3m, 3 months; BL, Baseline; d, days; EOT, End of Treatment, FU, Follow up

A		Beta-Hydroxybutyrate				
BHB, mean	KD 1.25 mmol/l $\pm$ 1.25 vs CG 0.32 $\pm$ 0.80 mmol/l , <i>p-value</i> 0.001					
BHB Treshold	reached at 3/3 visits, n(%) of whom also rated diet feasible, n(%)			reached at 2/3 visits, n(%) of whom also rated diet feasible, n(%)		
	<b>KD</b>	<b>CG</b>	<i>p-value</i>	<b>KD</b>	<b>CG</b>	<i>p-value</i>
0.8 mmol/l	9/23 (39) 9/23 (39)	0		12/23 (52) 12/23 (52)	0	
0.6 mmol/l	10/23 (43) 10/23 (43)	0		18/23 (78) 18/23 (78)	0	
0.5 mmol/l	14/23 (61) 14/23 (61)	0		19/23 (83) 19/23 (83)	0	
> Baseline	18/23 (78) 18/23 (78)	1/19 (5)	0.003	21/23 (91) 20/23 (87)	3/19 (16)	0.001
B		Breath Acetone				
Acetone, mean	KD 15.71 $\pm$ 15.60 p.p.m vs. CG 4.18 $\pm$ 7.22 p.p.m., <i>p-value</i> < 0.001					
	<b>KD</b>		<b>CG</b>		<i>p-value</i>	
Acetone Treshold	reached by n(%) of whom also rated diet feasible, n (%)					
10 p.p.m	17/23 (74) 16/23 (70)		1/19 (5)		< 0.001	
6 p.p.m.	21/23 (91) 20/23 (87)		3/19 (16)		< 0.001	
> Baseline	22/23 (96) 21/23 (91)		5/19 (26)		< 0.001	
C		Weight and fat loss				
	<b>KD</b>		<b>CG</b>		<i>p-value</i>	
	reached at all measurements, n(%) of whom also rated diet feasible, n (%)					
Weight loss - 3 %	19/22 (86%) 18/22 (81%)		3/17 (17%)		0.001	
Fat mass -10%	17/22 (77%) 17/22 (77%)		1/17 (6%)		0.003	

**Supplementary Figure 3: In-depth explorative analysis of ketone body changes and anthropometric analyses to assess objective adherence to diet** Related to Figure 1. P-values are determined by Chi square testing. 3 patients (2 control, 1 KD) were excluded from body composition analyses due to missing data. (A) BHB levels are displayed for KD and CG. Mean BHB values during dietary intervention significantly differed in the KD vs. CG (*p-value* 0.001). A threshold of 0.5 mmol/l still clearly separated KD from CG. When applying a cut-off of 0.6 mmol/l, 18 patients (78%) showed adherence to the diet at 2/3 visits and rated the intervention feasible. Comparing to baseline, 20 patients (87%) showed higher BHB values under diet and rated the intervention feasible. (B) Acetone levels measured twice daily at home and at on-site visits during the dietary intervention phase for the KD and control group are shown. Mean acetone levels were significantly higher in the KD vs control group. When a cut-off of 0.6 mmol/l was applied, 18 patients (78%) reached this threshold at 2/3 visits and rated the diet feasible. Comparing to baseline, 21 patients (91%) showed higher mean acetone values during the dietary intervention and rated the diet feasible. (C) A weight loss of  $\geq$  3% and fat mass reduction of  $\geq$  10 % clearly separates the KD from the control group. Among patients in the KD group, more than 75 % also rated the diet as feasible (18 (81%) and 17 patients (77%) respectively).

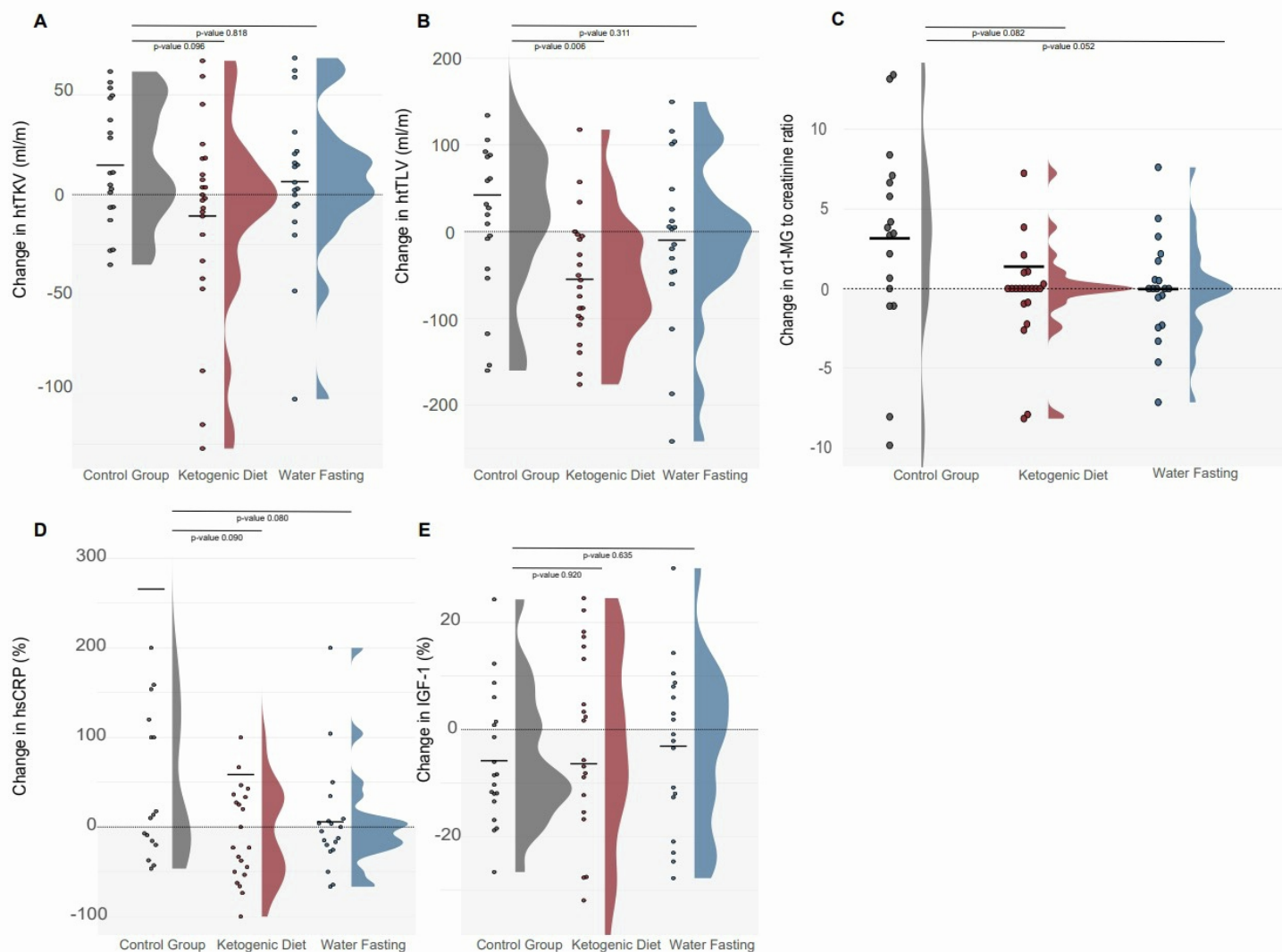
*BHB, Beta-Hydroxybutyrate; CG, Control group; KD, Ketogenic diet, p.p.m, parts per million*



**Supplementary Figure 4: Changes of anthropometric parameters from baseline to end of treatment** Related to Figure 2. Mean absolute changes of anthropometric parameters are displayed from BL to EOT. The black bar indicates the mean change for the respective group. P-values are provided for the comparison between control and each intervention group. 4 patients (2 control, 1 KD, 1 WF) were excluded from the body composition analysis due to missing data. (A) Absolute changes in body weight from BL to EOT: CG +0.34 kg, KD -6.11 kg, WF -0.68 kg; n= CG 19, KD 23, WF 19 (B) Absolute changes in abdominal circumference from BL to EOT: CG +1.32 cm, KD -5.02 cm, WF -2.86 cm; n= CG 19, KD 23, WF 19 (C) Absolute changes in body fat from BL to EOT: CG +0.35 kg, KD -4.15 kg, WF -0.01 kg; n= CG 17, KD 22, WF 18 (D) Absolute changes in lean body mass from BL to EOT: CG +0.01 kg, KD -1.9 kg, WF -0.03 kg; n= CG 17, KD 22, WF 18 (E) Absolute changes in bone mass from BL to EOT: CG -0.01 kg, KD -0.06 kg, WF -0.03 kg; n= CG 17, KD 22, WF 18 (F) Absolute changes in body water from BL to EOT: CG -0.02 kg, KD -1.64 kg, WF -0.38 kg; n= CG 17, KD 22, WF 18

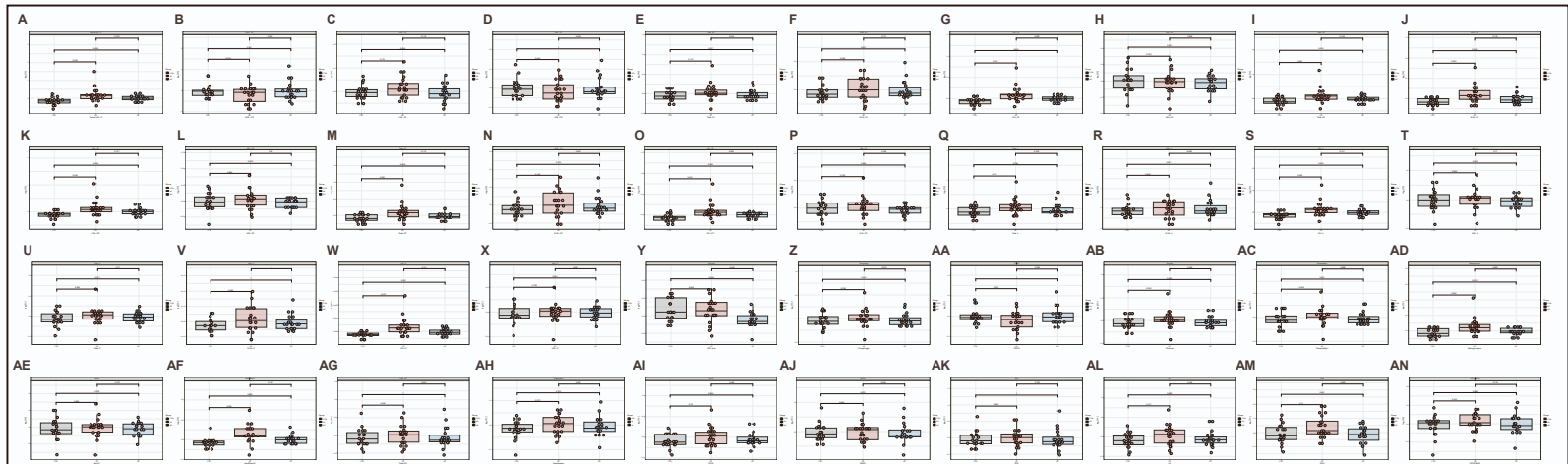
BL, Baseline; CG, Control group; EOT, End of Treatment; KD, Ketogenic diet group; WF, Water fasting group.





**Supplementary Figure 5: Absolute changes of htTKV and htTLV, alpha 1MG, hsCRP and IGF-1 from baseline to end of treatment** Related to Figure 3. Mean absolute changes of htTKV and htTLV are displayed from BL to EOT. The black bar indicates the mean change for the respective group. P-values are provided for the comparison between control and each intervention group. (A) Mean change in htTKV: control  $14.8 \pm 30.7$  ml/m, KD  $-10.7 \pm 48.6$  ml/m ( $p=0.09$ ) WF:  $6.5 \pm 38.9$  ml/m ( $p=0.91$ ). (B) Mean change in htTLV: control  $+42.1 \pm 166$  ml/m, KD  $-55.1 \pm 72.5$  ml/m ( $p=0.006$ ) and WF  $-10 \pm 98.2$  ml/m ( $p=0.317$ ). The two plots show the relative changes of the respective parameter for every individual and mean relative changes for the different groups. The black bar indicates the mean change for the respective group. P-values are provided for the comparison between control and each intervention group. (C) Plot shows mean changes in alpha1MG-to-creatinine ratio from BL to EOT: CG  $+63.68$  mg/g, KD  $-0.77$  mg/g ( $p=0.24$ ), WF  $-8.16$  mg/g ( $p=0.22$ ). (D) Mean change in hsCRP: Control  $265.63\%$ , KD  $58.51\%$  ( $p=0.09$ ), WF  $5.77\%$  ( $p=0.08$ ); n: Control 18, KD 23, WF 19. Two patients of the control group who showed a relative difference of 3233% and 852% respectively are not displayed in the plot but were included in calculation of the mean and statistical testing. The same holds true for two patients of the KD who showed a relative difference of 790% and 725%. (E) Mean change in IGF-1: Control  $-5.86\%$ , KD  $-6.41\%$  ( $p=0.69$ ), WF  $-3.13\%$  ( $p=0.56$ ); n: Control 19, KD 22, WF 18. Two patients of the KD group who showed a relative difference of  $-43.5\%$  and  $-58.9\%$  respectively are not displayed in the plot but were included in calculation of the mean and statistical testing.

EOT, End of Treatment; htTKV, Height-adjusted Total Kidney Volume; htTLV, Height-adjusted Total Liver Volume; hsCRP, High-sensitivity C-reactive protein; IGF-1, Insulin-like growth factor 1; WF, Water fasting group.



## Supplementary Figure 6: Full NMR lipid panel

Related to Figure 5. (A-AN) Bar plots showing the log<sub>2</sub> fold change (BL vs. EOT) of all lipid parameters determined in serum samples by NMR in the three groups (control, KD, and WF) which are not shown in Figure 5. Statistical testing was performed using a model correcting for Mayo Class, BMI, Gender and Age.

*BL, Baseline; CTRL, Control group; KD, Ketogenic diet group; WF, Water fasting group; Clinical LDL-C, clinical LDL cholesterol; VLDL-TG, Triglycerides in VLDL; LDL-TG, Triglycerides in LDL; HDL-TG, Triglycerides in HDL; Total-PL, total phospholipids; VLDL-PL, Phospholipids in VLDL; LDL-PL, Phospholipids in LDL; HDL-PL, Phospholipids in HDL; Total-CE, total esterified cholesterol; VLDL-CE, cholesteryl esters in VLDL; LDL-CE, cholesteryl esters in LDL; HDL-CE, cholesteryl esters in HDL; Total-FC, total free cholesterol; VLDL-FC, free cholesterol in VLDL; LDL-FC, free cholesterol in LDL; HDL-FC, free cholesterol in HDL; Total-L, total lipids in lipoprotein particles; VLDL-L, total lipids in VLDL; LDL-L, total lipids in LDL; HDL-L, total lipids in HDL; Total-P, total concentration of lipoprotein particles; VLDL-P, concentration of VLDL particles; LDL-P, concentration of LDL particles; HDL-P, concentration of HDL particles; HDL\_size, HDL size; Phosphoglyc, Phosphoglycerides; TG/PG, Ratio of triglycerides to phosphoglycerides; Cholines, total cholines; Phosphatidylc, Phosphatidylcholines; Sphingomyelins; ApoA1, Apolipoprotein A1; ApoB/ApoA1, Apolipoprotein B/ Apolipoprotein A1 ratio; Total-FA, total fatty acids; Unsaturation, Degree of unsaturation; PUFA, Polyunsaturated fatty acids; MUFA, Monounsaturated fatty acids; SFA, Saturated fatty acids; LA, Linoleic acid; DHA, Docosahexaenoic acid; PUFA/MUFA, Polyunsaturated fatty acids/Monounsaturated fatty acids ratio.*