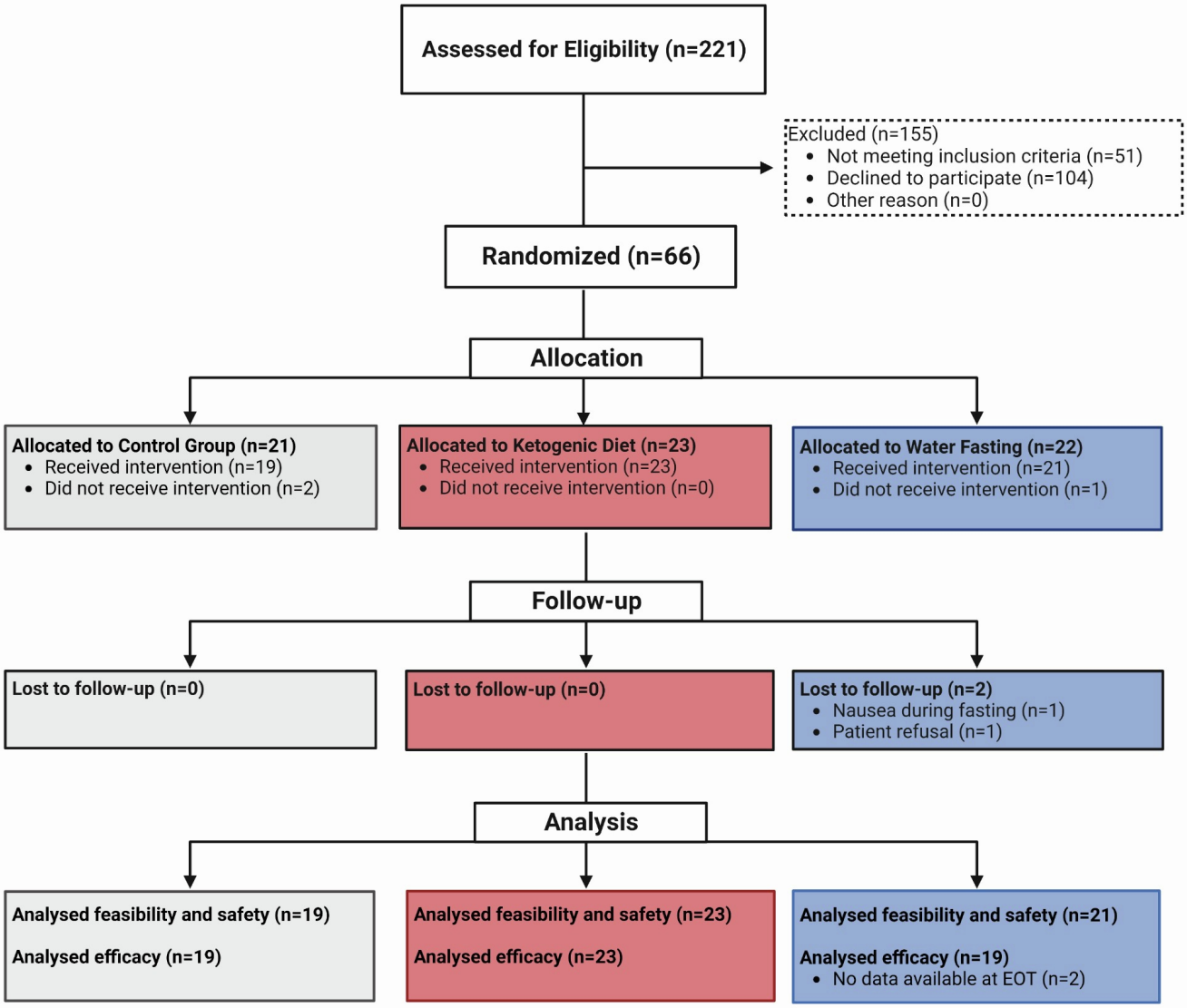
Cell Reports Medicine, Volume *4*

Supplemental information

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

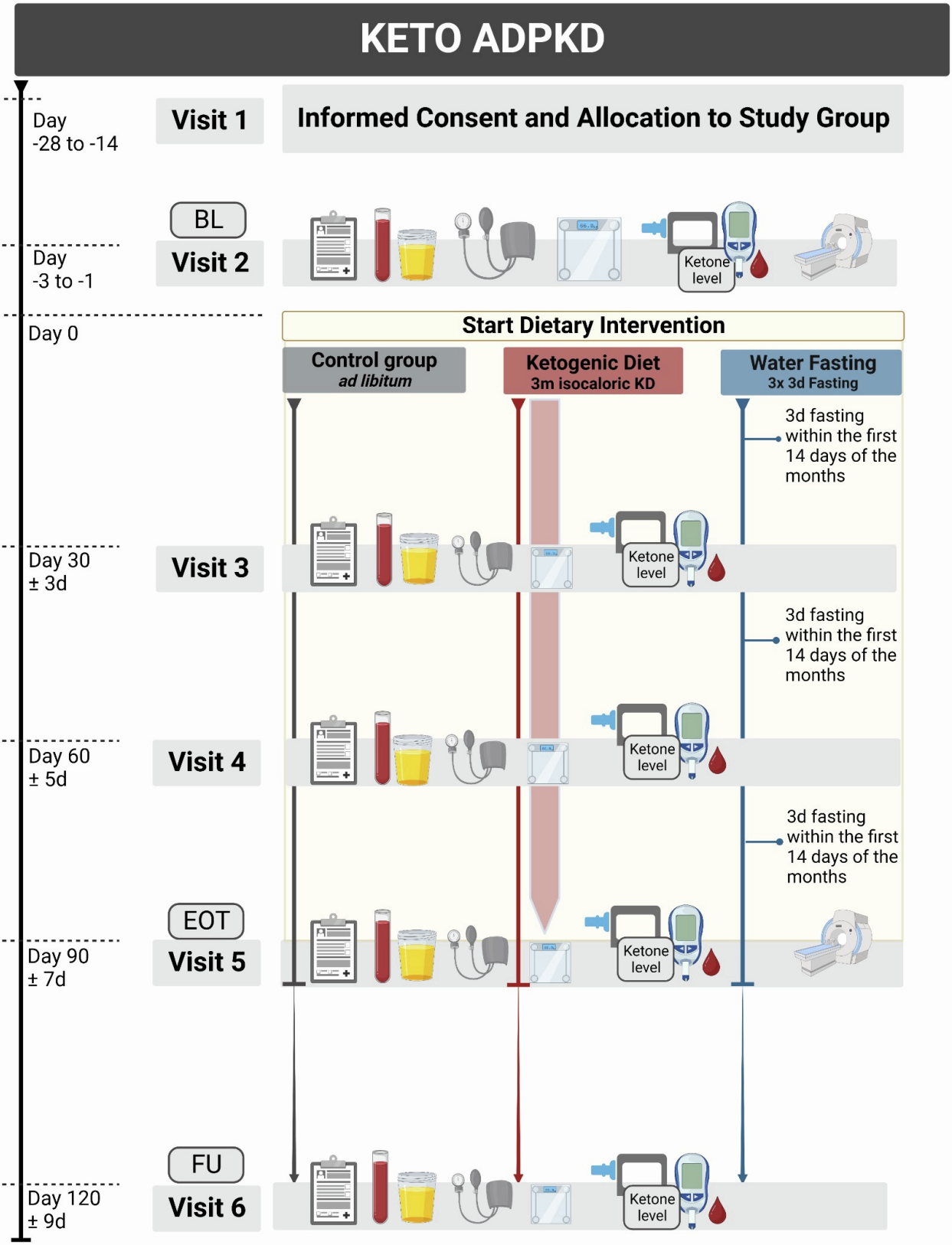
Sadrija Cukoski, Christoph Heinrich Lindemann, Sita Arjune, Polina Todorova, Theresa Brecht, Adrian Kühn, Simon Oehm, Sebastian Strubl, Ingrid Becker, Ulrike Kämmerer, Jacob Alexander Torres, Franziska Meyer, Thomas Schömig, Nils Große Hokamp, Florian Siedek, Ingo Gottschalk, Thomas Benzing, Johannes 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



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

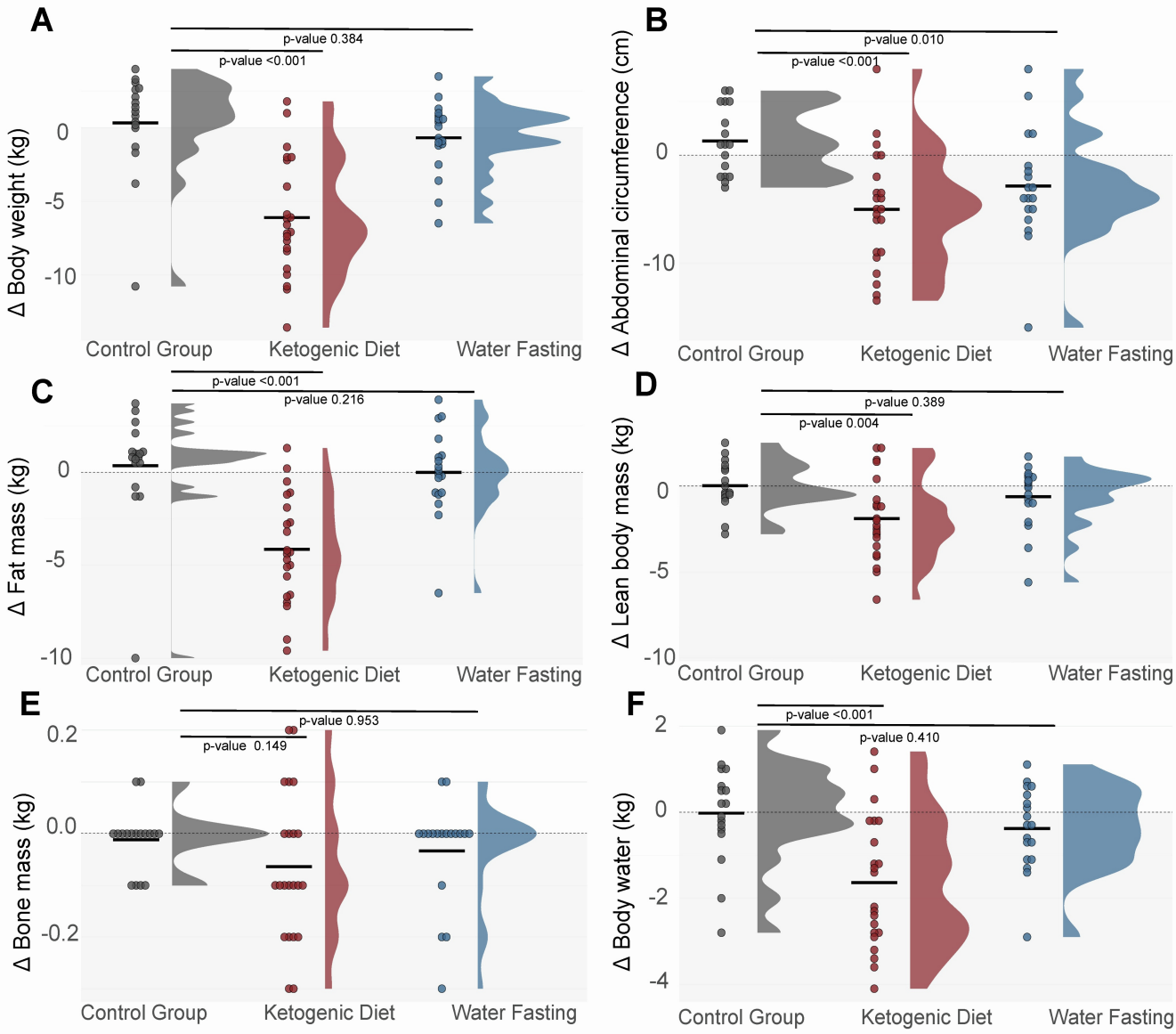
A	Beta-Hydroxybutyrate					
BHB, mean	KD 1.25 mmol/l ±1.25 vs CG 0.32 ±0.80 mmol/l , *p-value 0.001*					
BHB Treshold	reached at 3/3 visits, n(%) of whom also rated diet feasible, n(%)			reached at 2/3 visits, n(%) of whom also rated diet feasible, n(%)		
	KD	**CG**	*p-value*	**KD**	**CG**	*p-value*
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 ±15.60 p.p.m vs. CG 4.18 ±7.22 p.p.m., *p-value < 0.001*		
	KD	**CG**	*p-value*
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		
	KD	**CG**	*p-value*
	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*

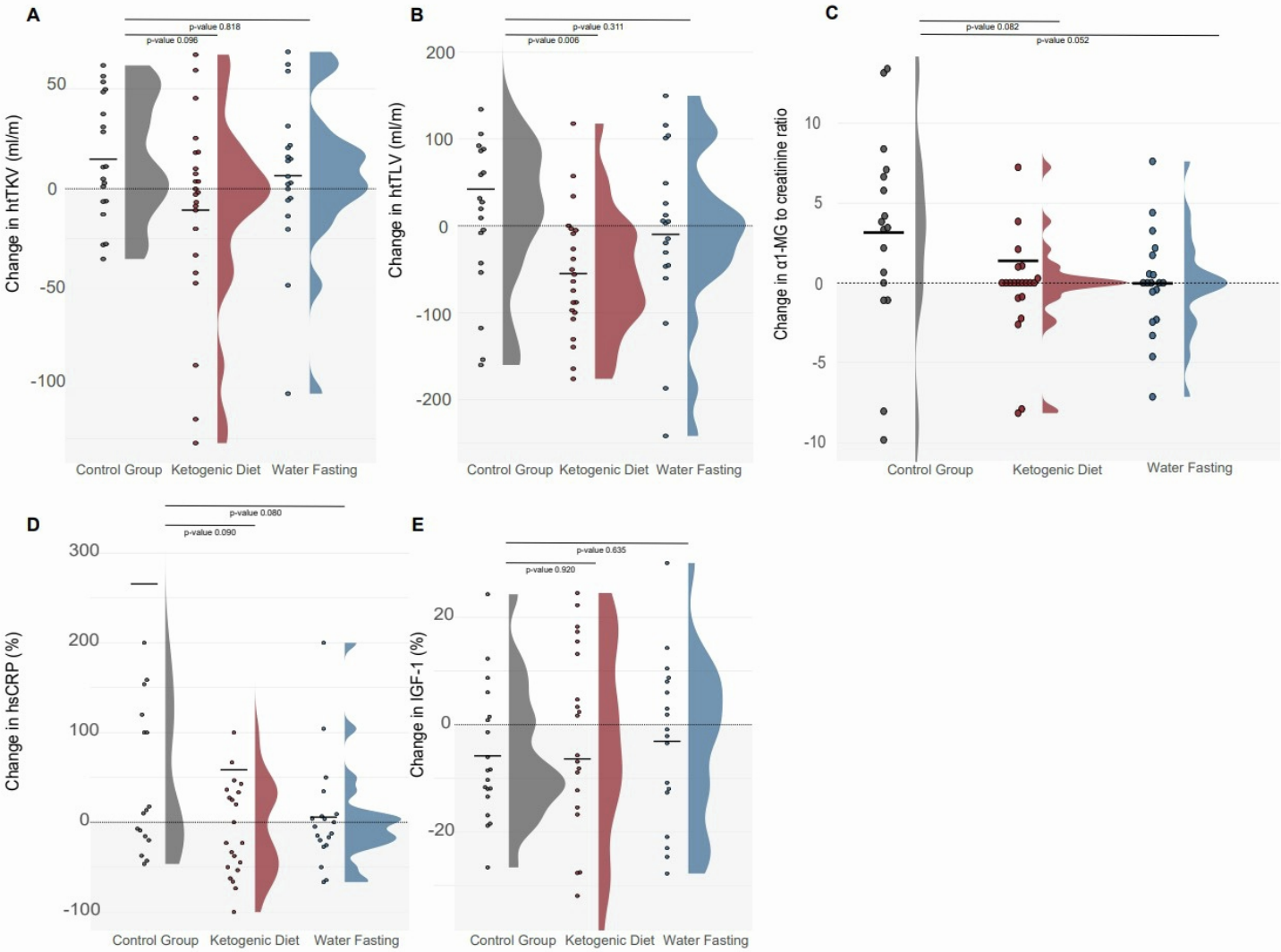
Supplementary Figure 3: In-depth explorative analysis of ketone body changes and anthropometric analyses to assess objective adherence to diet Related to Figure 1. P-values are determined by Chi square testing. 3 patients (2 control, 1 KD) were excluded from body composition analyses due to missing data. (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 ≥ 3% and fat mass reduction of ≥ 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



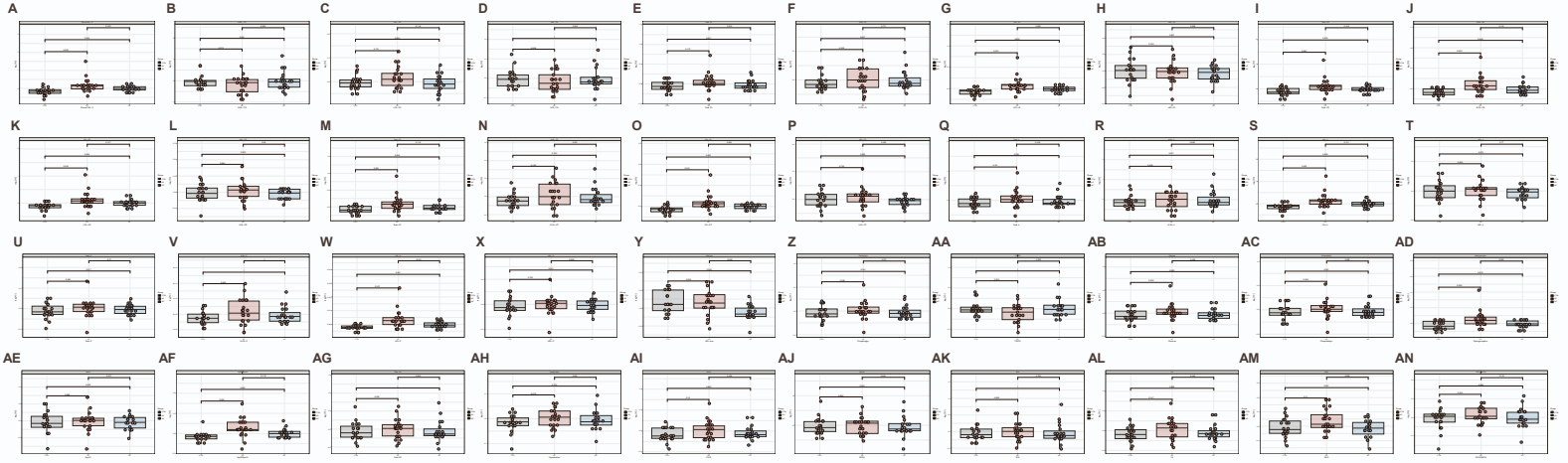
Supplementary Figure 4: Changes of anthropometric parameters from baseline to end of treatment Related to Figure 2. Mean absolute changes of anthropometric parameters are displayed from BL to EOT. The black bar indicates the mean change for the respective group. P-values are provided for the comparison 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 ±30.7 ml/m, KD -10.7 ±48.6 ml/m (p=0.09) WF: 6.5 ±38.9 ml/m (p=0.91). (B) Mean change in htTLV: control +42.1 ±166 ml/m, KD -55.1±72.5 ml/m (p=0.006) and WF -10±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.


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
AA
AB
AC
AD
AE
AF
AG
AH
AI
AJ
AK
AL
AM
AN

Supplementary Figure 6: Full NMR lipid panel

Related to Figure 5. (A-AN) Bar plots showing the log2 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.