GREASE II. A phase II randomized, 12-month, parallel-group, superiority study to evaluate the efficacy of a Modified Atkins Diet in Autosomal Dominant Polycystic Kidney Disease patients

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ABSTRACT

Introduction: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a condition that causes progressive renal function decline. Preclinical data suggest the presence of a profound metabolic derangement in ADPKD. Cystic cells shift their energy metabolism from oxidative phosphorylation to aerobic glycolysis, show inhibition of fatty acid oxidation and become glutamine and arginine dependent. Recent preclinical experiences have suggested beneficial effect in terms of reduction of cystic size, interstitial fibrosis and disease progression, targeting these deregulated metabolic pathways by ketosis induction. The dietetic approach to ADPKD, because of low cost and absence of toxicity, represents an interesting therapeutic strategy.
Methods and analysis: The protocol describes a phase II clinical trial that will evaluate the effect on Total Kidney Volume, safety and tolerability of a ketogenic diet in a selected ADPKD population. The trial will have, as secondary objective, the evaluation of the ability of the ketogenic diet to slow down the renal function decline. This will be a 12-month randomized, parallel group, two arm, superiority trial with 1:1 allocation to evaluate the efficacy of a Modified Atkins Diet protocol compared to a balanced normocaloric diet on 90 ADPKD patients.
Dissemination: The study results will be released to the patients and the medical community.

1. Introduction

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a Mendelian disease caused by mutations involving PKD1, PKD2 and few other rarer genes and characterized by the formation and enlargement of multiple renal cysts that cause progressive renal function decline [1]. ADPKD is the fourth leading cause of end stage renal disease (ESRD) in Europe [2] and the United States [3]. No treatments are available to completely halt the progression of the disease [4]. To date, the only therapeutic option available is Tolvaptan, a selective oral vasopressin V2-receptor antagonist, able to slow the loss of annual renal function by about a third [5]. Its adoption in the ADPKD population is, however,

restricted by some limiting factors: the drug is approved in subjects with an aggressive form of the disease [6]; because of the significant aquaresis, many patients are unable to reconcile therapy with their lifestyle and decline to start the treatment [7]. Among the patients who start the treatment a certain quota (about 15 %) [5] end the therapy mainly because of the same side effect of extreme aquaresis or in a limited number of subjects (about 4%) because of liver toxicity [8]. In conclusion, ADPKD still represents an illness with a significant unmet medical need. We believe that achieving a significant slowing of the disease in the total ADPKD population requires an approach using several combined strategies. In this regard, we propose a dietetic approach which represents a strategy of extreme interest because of its

relatively low cost [9] and absence of toxicity.

The literature reports suggestive preclinical data that metabolic deregulation exists in ADPKD. Among these alterations, a deregulation in glucose metabolism has been identified. In particular, the data suggest that cystic cells shift their energy metabolism from oxidative phosphorylation to aerobic glycolysis [10], an alteration of the energy metabolism also described in neoplastic cells (Warburg effect) [11,12]. This alteration is closely linked to the alteration of many cellular metabolic sensors. Upregulation of the mammalian target of rapamycin complex 1 (mTORC1) [13], inhibition of the AMP-activated kinase (AMPK) [10,14,15] and activation of the Sirtuins [16] were all documented in ADPKD. In addition to the alterations attributable to glucose metabolism, other metabolic pathways appear altered in ADPKD. Various evidence suggests a dysregulation of lipid metabolism mediated by the Hepatocyte nuclear factor 4α (Hnf4α) and by the peroxisome proliferator-activated receptor α (PPARα) [17]. Furthermore, alterations in the amino acid metabolism and in particular of glutamine (through the upregulation of glutaminase 1) [18,19] and arginine [20] have also been documented. All of these metabolic control targets have been the subject of drug manipulation attempts in preclinical or RCT studies [4], but in addition to possible pharmacological interventions, most recent experiences have suggested the possible role of dietary manipulations targeting the same metabolic sensors. Warner et al. have applied a caloric restriction of 40 % compared to an ad libitum feeding in a mouse model of ADPKD, obtaining an extraordinary reduction of the cystic growth [21]. Kipp et al. have shown in their preclinical mouse model that a substantial benefit can be maintained even with a smaller reduction of food intake (23 % reduction of food intake) [22]. Recently the same group confirmed in animal models that the advantage in reducing cystic growth should not be attributed to the reduction of caloric intake, but rather to the induction of ketosis [23]. In fact, through various dietary manipulation techniques able to generate ketosis (time-restricting feeding, ketogenic diet, acute fasting and oral administration of β-hydroxybutirrate), applied to different PKD animal models (rats, mice and Persian cats) they succeeded in demonstrating the beneficial effect of ketosis induction in terms of reduction of cystic size, interstitial fibrosis and disease progression [23]. This result was likely mediated by mTOR signaling inhibition [23]. Our group recently concluded a pilot study that assessed the effect of the ketogenic diet in a small group of patients with ADPKD. The three tested subjects showed a good compliance to the modified Atkins diet (MAD); they reached a considerable level of ketosis and showed a significant decrease of glycemia [24]. This preliminary study confirms the feasibility of a clinical trial testing the effect of a ketogenic diet in an ADPKD population. In light of these preclinical data, we therefore want to conduct a clinical study to evaluate the efficacy of this approach. The greater clinical experience in the use of ketogenic diets in literature and in our hospital is related to its application in pediatric populations with forms of drug resistant epilepsy [25]. Long-term adoption of the ketogenic diet in adult patients is less documented [26], while, with only the exception of our pilot study [24], there is no experience in ADPKD patients.

1.1. Objectives

The objective of this project is a clinical trial that will evaluate the effect on TKV, safety and tolerability of a ketogenic diet in a selected ADPKD population. The trial will have, as a secondary objective, the slowing of renal function decline. This will be a 12-month randomized, parallel-group, two-armed superiority trial with 1:1 allocation to evaluate the efficacy of a MAD protocol compared to a balanced normocaloric diet on ADPKD patients.

2. Methods

The SPIRIT reporting guidelines [27] were used in compiling this protocol.

2.1. Participants, interventions and outcomes

The study will be conducted as a monocentric trial in the Clinic of the Renal Genetic Disease of the Academic Hospital AOU Policlinico di Modena, Italy.

The study will be submitted to the local Ethical Committee (Modena, Italy). Patients must provide written, informed consent before any study procedures occur.

2.1.1. Inclusion criteria

Patients eligible for the trial must comply with all of the following at randomization:

- ADPKD diagnosis according to clinical criteria [28].
- Subjects of both sexes.
- Aged 18–60 years.
- Renal function larger than 24 mL/min/1.73m^2 according to the CKD-EPI formula.
- Mayo score 1C-1D-1E [29] (TKV calculated by MRI).

2.1.2. Exclusion criteria

- Types I and II Diabetes.
- Low Body Mass Index (< 20 Kg/m^2).
- Gastrointestinal malabsorption problems.
- Eating behavior disorders (anorexia, bulimia).
- Personal history of renal stones.
- Dyslipidemia.
- Familial hypercholesterolemia.

2.1.3. Interventions

Eligible patients will be randomized in equal proportions between MAD protocol and balanced normocaloric diet (BND).

The MAD protocol will consist of a mainly plant-based diet with the following bromatological breakdown of total energy intake:

- 20 g of carbohydrates (about 4–6 % of total caloric intake).
- Proteins 25–30 %.
- Lipids 60–70 % (mainly polyunsaturated fats, medium chain triglycerides will be present).

A sugar-free multivitamin supplement will be provided to correct deficiencies of micronutrients. K or Mg Citrate supplement will be provided to decrease the risk of renal stone formation.

The Balanced Normocaloric Diet (BND) will consist of a diet based on the following bromatological breakdown of total energy intake:

- Carbohydrates 55 − 60% (of which oligosaccharides < 15 %).
- Proteins 10–15 %.
- Lipids 25–30 % (of which monounsaturated < 7%, polyunsaturated > 10 %).
- Daily total fibers 30 g.

K or Mg Citrate supplement will be provided.

In both arms, the calorie intake will be adjusted during the study to obtain stable body weights (the calorie intake will be regulated based on the basal metabolic rate calculated with the Schofield formula and with multivectorial bio-impedancemetry and will be suitable for individual energy expenditure. The calorie intake will be adjusted during the trial to minimize weight change).

2.1.3.1. *Interventions—modifications or interruption*. In the case of patients randomized to the MAD protocol who are no longer able to follow the diet prescription due to a subjective dietary intolerance or because of personal preference, a switch to the BND protocol will be applied. The researcher can decide to switch from MAD to BND in any

case that suggests a detrimental effect of the diet on the subject (i.e., prolonged and severe dyslipidemia of more than 6 months, stone formation in patients without previous history of renal stones and others). Regardless of any decision to modify or discontinue their assigned intervention, study participants will be retained in the trial whenever possible to enable follow-up data collection and prevent missing data.

2.1.3.2. Interventions—concomitant care. Patients in active treatment with Tolvaptan are eligible for this trial. A stratification strategy for Tolvaptan and renal function is implemented in the protocol (see section on 'Statistical Analyses'). Patients are not allowed to start Tolvaptan treatment after randomization. The therapies for hypertension management are permitted without limitation. For dyslipidemia treatment, the introduction of statin or fenofibrate is not allowed during the study. However, patients already using these drugs (statin or fenofibrate) before enrollment will be allowed to continue the therapy.

2.1.3.3. Intervention adherence. Patients will meet dietitians monthly to verify compliance with nutritional therapy and to identify strategies to overcome encountered difficulties. Patients will be provided with different aids for the correct execution of the diet at home, such as personalized recipes and instructions for calculating variations to recipes, without modifying the bromatological composition of the assigned diet. A specific phone app (KETAPP, Copyright by University of Modena and Reggio Emilia) will help patients follow their diet. KETAPP is a tool designed to facilitate adherence to the treatment of all patients on a ketogenic diet. The mobile app is available in both IOS and Android environments. The app is designed to help patients organize ketogenic meals by having them select the desired items from a menu, which is calibrated on daily caloric intake. It is possible to organize the meals for the entire week, to help patients plan their weekly shopping.

2.1.4. Adherence assessments
To enhance validity of data, multiple methods will be used to assess diet adherence, including evaluation of data collected by KETAPP and evaluation of glycemia and ketonemia levels, obtained by a twice-daily finger puncture and a portable blood analyzer.

2.1.5. Outcomes
The following outcomes will be recorded:

2.1.5.1. Primary endpoint of efficacy (TKV variation). The primary endpoint of this study is the evaluation of the effect of the MAD protocol on the modification of the TKV. We expect to obtain a reduction of the TKV in the group of patients treated by the MAD protocol. TKV was formally qualified by both the FDA and EMA as a prognostic enrichment biomarker for selecting patients at high risk for a progressive decline [30]. TKV is a surrogate marker for progression of disease in ADPKD and it was adopted as a primary outcome in many previous clinical trials [31–33] on ADPKD, including the TEMPO 3:4 study [5] that obtained the EMA authorization of Tolvaptan. We adopted TKV as the primary endpoint due to its higher sensitivity in identifying disease progression in the broad spectrum of patients enrolled in this study (CKD classes from 1 to early stage 4). The sample size of the study was calculated to identify a statistically significant variation of this parameter. Furthermore, the study will evaluate renal function decline as secondary outcome. The identification of a coordinated reduction of TKV and renal function decline in the patients randomized to the MAD compared to those on the BMD will suggest a protective role of the ketogenic diet against disease progression of ADPKD.

2.1.5.2. The Co-primary Endpoint of tolerability and safety. Tolerability

will be assessed by a questionnaire adapted from a previous study [34]. Preliminary data obtained in our feasibility study [24] suggested an excellent tolerability in the three-month period that we believe can be replicated in this extended study. Safety will be assessed by monitoring serious adverse events, classified according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Furthermore, a strategy to correct the state of dyslipidemia will be implemented: The diet will be plant-based and will contain polyunsaturated fats and medium chain triglycerides.

2.1.5.3. The secondary endpoint of efficacy (renal function). will be the comparison of the variation in renal function, expressed as eGFR (mean of the value of eGFR at the end of the study minus baseline eGFR for each subject), according to the CKD-EPI formula between the two randomized arms of the study.

2.1.5.4. The secondary endpoint of biomarker exploration. will evaluate whether the diet has any impact in the variation of exploratory prognostic biomarkers (urinary markers β2MG and MCP-1 [35]).

2.1.6. Participant timeline
Patients will be evaluated in a screening visit to evaluate eligibility criteria and obtain consent to the trial. This screening visit must be scheduled within 30 days before the allocation baseline visit. If during the screening visit the patient is considered eligible and gives consent to the study, an MRI will be programmed to calculate the TKV, which is the primary outcome of the study. At the same time the patient will start the daily assessment of glycemia and ketonemia by digit puncture. In the allocation visit, patients will be randomized to MAD or BND. The treatment will last 12 months. During the treatment period, patients will be evaluated with monthly visits, during which vital signs, weight, and blood pressure will be collected, and laboratory tests will be performed. After the post-allocation treatment time of 12 months, a final visit will be scheduled no later than 14 days after the end of the treatment. No later than 30 days after the end of the treatment the second MRI for TKV calculation will be performed (Table 1).

2.1.7. Sample size
The sample size was calculated on the basis of the primary hypothesis, i.e., the treatment reduces the rate of renal volume increase. Based on data previously collected by our group [36], we observed an increase in the Total Kidney Volume (TKV) of 145 mL per year and a standard deviation of 209 mL in ADPKD patients on standard care (free diet). Under the hypothesis that the diet treatment stops the progression of TKV increase over 1 year, i.e., delta equal to 145 mL and standard deviation equal to 209 mL, setting alpha to 0.05 and beta to 0.20, 34 patients per arm (68 patients total) are necessary to show the statistically significant difference between arms. To allow for a dropout rate of 15 % during the study, an overall population of 90 subjects will be enrolled.

2.1.8. Recruitment
We will recruit 90 patients who will be randomized to MAD or BND. The patients will follow the diet daily for 12 months. Patients will be recruited from the cohort of patients actively following up in our Renal Genetic Disease Clinic [36]. Furthermore, patients enrolled in the Italian Association of Polycystic Kidney (AIRP) will be informed of this trial and will be able to participate on a voluntary basis. About 1100 patients are enrolled in AIRP; a collaboration with AIRP has been formalized.

2.2. Assignments of interventions

2.2.1. Allocation
Patients will be allocated to the two arms by a one-to-one ratio, using a computer-generated list of random numbers that will be

Table 1

Timeline of the procedures of the trial. m = month, d = day. MAD = Modified Atkins Diet, BND = Balanced normocaloric Diet; TKV = Total Kidney Volume; MRI = magnetic resonance image, DST = Dietary Satisfaction Questionnaire.

	Enrolment	Allocation	Post Allocation												Study End
Timepoint	m -1	time 0	m 1	m 2	m 3	m 4	m 5	m 6	m 7	m 8	m 9	m 10	m 11	m 12	+ d14
Enrolment: - Eligibility screening - Informed Consent	X														
- Allocation	X														
		X													
Interventions:- MAD		X	X	X	X	X	X	X	X	X	X	X	X	X	
- BND		X	X	X	X	X	X	X	X	X	X	X	X	X	
Assessments: - Physical exam - Blood Pressure - Lab Exam -	X														
Daily Glycemia and Ketonemia - DST - TKV MRI	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
urinary markers	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
															X
	X														X
	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

centrally generated in the statistical unit. The randomization sequence will be generated by a statistician by using Stata11.0 statistical software and will be stratified by CKD class (1° stratum = CKD 1; 2° stratum = CKD 2 − 3-4) and by Tolvaptan treatment. After the researcher obtains the subject's consent, during the dietetic visit the dietitian will connect to a website dedicated to the study for registration. Then, according to the randomization sequence, the subject will be allocated to a group. Once the patient is assigned to MAD or BND group, the patient, the dietitian and the data analyst will be aware of the treatment allocation. However, the nephrologist and the radiologist will be unaware of the subject allocation during the clinical visit and the TKV assessment, respectively. Patients will be instructed to discuss their diet only with the dietitian and to respect blindness with the other researchers. The statistical unit personnel will independently review the quality of the data.

2.3. Data collection, management analysis

2.3.1. Outcome measures

2.3.1.1. TKV variation. The primary outcome is based on TKV assessment. TKV will be measured by an MRI scan performed by a standardized protocol (Table 2). The volume will be calculated using manual tracing by an expert radiologist blinded in the allocation of patients.

2.3.1.2. Co-primary Endpoint of tolerability and safety. Toxicity (Safety) will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4 on the basis of clinical and

Table 2

MRI acquisition protocol for TKV assessment.

Coil	Centered Over Inferior Costal
Plane	Coronal
FOV	30 − 35 cm
Image Matrix	256 × 256
Slice Thickness	4 mm
Slice Gap	None
Missing Slice	None
T1 Sequence	TR – 4 ms
	TE – 2 ms
	Flip Angle ≤ 15 degrees
	No Fat Saturation
FISP FIESTA Sequence	TR – 7 ms
	TE – 2 ms
	Flip Angle 40–50 degrees
	No Fat Saturation
T2 Sequence	TR Max
	TE ∼ 100 ms
	Fat Saturation
Digital Data	Raw DICOM Images

laboratory data. The risk profile, including the risk of metabolic acidosis induced by the ketogenic diet, will be assessed through monthly scheduled laboratory checks. The lab exams will be centrally performed by the certified laboratory of our Institution. The following anthropometric measurements will be collected: weight (Kg), height (cm), BMI (Kg/cm2), arm circumference, waist-hip ratio, abdominal circumference, skinfold thickness: triceps skinfold, subscapular skinfold. Tolerability will be assessed by the Dietary Satisfaction Questionnaire (Appendix A), which was previously validated [37]. It will be administered by the dietician during the End Study Visit.

2.3.1.3. Secondary endpoint of efficacy (renal function). Renal function will be calculated by the application of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [38].

2.3.1.4. Secondary endpoint of biomarker exploration. β2MG and MCP-1 in the urine will be measured by an ELISA method [35].

2.3.2. Adherence

Participants will be encouraged to adhere to the protocol and complete follow-up through the following strategies:

- Maintaining interest in the study through materials and mailings.
- A website of the study will be updated weekly with information regarding the conduct of the protocol
- Specific Alerts via the phone app KETAPP will remind the patients to perform the daily assessment of glycemia and ketonemia. The data daily uploaded on the data server of the study will help in the identification of the noncompliant patients, who will be contacted by the researchers.
- At each visit the previous month's ketonemia and glycemia data will be reported to the patient to stimulate their adherence to the diet.

Once a patient is enrolled or randomized, we will make every reasonable effort to follow the participant for the entire study period. It is projected that the rate of loss-to-follow-up on an annual basis will be at most 15 %. Study staff are responsible for developing and implementing standard operating procedures to achieve this level of follow-up. Participants may withdraw from the study for any reason at any time. The investigator also may withdraw participants from the study in order to protect their safety and/or if they are unwilling or unable to comply with required study procedures after consultation with the Protocol Chair. Participants also may be withdrawn if the study sponsor or government or regulatory authorities terminate the study prior to its planned end date. Early discontinuation of study diet or switch to the other experimental arm for any reason is not a reason for withdrawal from the study.

All data will be entered electronically. Original study data will be entered and kept on clinic note at the participating site.

The Unit of Statistical and Methodological Support of AOU Policlinico di Modena will be in charge of developing the eCRF, managing the data, randomization and statistical analysis. The personnel involved in this Unit are independent from the clinical Unit. The data will be stored in a server dedicated to RCTs.

2.3.3. Statistical Analyses

Percentages, means (with standard deviations), and medians (with I–III quartile range) will be used for descriptive purposes, as appropriate. For the evaluation of the treatment effect on the primary efficacy endpoint a *t*-test for the comparison of the pre-post diet mean delta calculated as the difference between the kidney volume observed at 12 months minus the volume at baseline will be applied (two-sided α = 0.05). The same parametric approach (*t*-test) will be used for the analysis of secondary endpoints of efficacy while, when this is not appropriate, a non-parametric test (Wilcoxon signed-rank test) will be considered. Statistical analyses will be performed at the Statistics Unit, according to the intention-to-treat principle.

2.4. Monitoring

A Data Monitoring Committee was not set in this study, considering the following elements: there are no a priori reasons for a safety concern, there is no information suggesting the possibility of serious toxicity, the study is not being performed in a potentially fragile or vulnerable population. The study is not being performed in a population at an elevated risk of death or other serious outcomes. The study does not involve a large population; it is not multicenter and is not of long duration. No interim analysis is planned.

2.5. Ethics and dissemination

This protocol and the template informed consent forms will be reviewed and approved by the local ethics committee (Comitato Etico Area Vasta Nord Emilia) with respect to scientific content and compliance with applicable research and human subjects regulations.

Trained Dieticians will introduce the trial to patients, who will also receive information sheets. Patients will then be able to have an informed discussion with the participating medical researcher.

The result of this randomized controlled trial will be summarized in a final report. The study results will be released to the participating physicians, referring physicians, patients, and the general nephrological and medical community. There are no publication restrictions.

3. Discussion

ADPKD guidelines provide few specific suggestions regarding dietary recommendations and mainly refer to advice for the general CKD population [39,40]. Few papers evaluated dietary interventions in ADPKD [41]. In the large MDRD study [37] a low protein diet did not show protective effects in ADPKD patients (a marginal trend not statistically significant toward a beneficial effect of very low protein in advanced renal failure was reported). The suggestion of increasing water intake to induce a secondary inhibition of vasopressin is incorporated in some ADPKD guidelines [42,43] and is related to the clinical success of Tolvaptan [5] and few preclinical studies [44]. However, some discordant results of clinical pilot studies [45–48] and the lack of the data for larger ongoing clinical studies [49] do not allow formulation of a clear position regarding this topic. Salt and caffeine consumption recommendations are incorporated in some guidelines [42,50,51] but they are still based mainly on low quality clinical evidence and/or expert opinion.

To our knowledge, there is no previous clinical experience on the use of the ketogenic diet in patients with ADPKD (with the only

exception of our pilot study [24]). Our protocol represents the first clinical application of recent preclinical acquisitions [23] related to metabolic imbalance of ADPKD [17]. The innovativeness of our study consists of an unprecedented clinical approach to inhibiting the growth of renal cysts. The appearance and progressive enlargement of renal cysts occurs through cell proliferation and accumulation of liquids in the cystic lumen. These processes require significant metabolic energy consumption [10]. Our novel approach is to undermine the energy supply of cystic cells: These cells are completely dependent on glucose for their own energy needs [14]. Cystic cells are not able to supply their energy needs from alternative sources such as lipids and/or amino acids [23]. Furthermore, preclinical data suggest that ketosis can induce cytoplasmic oil droplet accumulation of cyst lining cells, a tissue alteration resembling a state of severe steatosis [23]. These cells are fully capable of taking up circulating fatty acids but appear to be unable to catabolize them, leading to lipotoxicity and cell death [23]. The ketogenic diet, mimicking the clinical situation of prolonged fasting and causing a significant decrease in plasma glucose availability, produces in the patient a metabolic switching that makes ketone bodies available as the main energy resource [52]. Ketone bodies can satisfactorily support the energy demand of non-cystic cells, while submitting the cystic cells to an energy shock and eventually to cell death [53].

The strength of our study lies in a solid preclinical base developed by independent groups on different animal models [14–16,21–23,54–58]. All these scientific findings make up a larger picture that suggests that ADPKD is a metabolic disorder. This constitutes a key of shared and current interpretation, which can be utilized in terms of a possible therapeutic dietetic strategy. Our protocol was built on the previous pivotal experience of a feasibility study conducted on three patients treated for three months [24]. In this study we have accumulated important preliminary data that allowed us to construct a mature and concrete phase II protocol. In particular we have identified the need to apply a more restrictive MAD protocol on the protein prescription to minimize the gluconeogenic escape [59]. We identified a dyslipidemic risk for which we have planned a correction strategy. We have prudently optimized the exclusion criteria to avoid exposing populations inappropriate for a ketogenic treatment (low body mass index subjects, dyslipidemic patients or those with a family history of dyslipidemia, patients with a history of renal stones).

In conclusion because of the scarce clinical experience of ketogenic diet in adult and complete absence of data in ADPKD patients (excluding our feasibility study [24]) we designed a phase II randomized, 12-month, parallel-group superiority study to evaluate the efficacy and consolidate safety of a MAD protocol in ADPKD. This analysis will allow us to evaluate the proof of concept of the therapeutic efficacy of this approach. This exploratory outcome represents the first attempt at evaluating the therapeutic prospect of metabolic interference [53] in the ADPKD clinical setting.

Funding

Author statements

G.B. and R.M. designed the study.

R.D.A. revised the methodological section of the study and performed the power analysis.

R.M, F.F., G.A. and F.T revised the clinical implications of the protocol.

M.M. defined the dietetic regimens of the protocol.

S.G. and G.L. revised the timeline and logistics of the protocol.

F.T., R.M., G.C. and G.B. drafted and revised the paper.

All authors approved the final version of the manuscript.

Declaration of Competing Interest

R.M. and G.C. were involved in the trials of Tolvaptan sponsored by Otsuka Pharmaceutical. R.M. is scientific advisor of Otsuka Italia. All other authors do not declare any conflict of interest.

Appendix A

Dietary Satisfaction Questionnaire for Tolerability Assessment

1 Rate your overall satisfaction with the way you are currently eating

Dislike extremely	1	2	3	4	5	Like very much

2 How often are you hungry?

Often	1	2	3	4	5	Almost never

3 How would you describe your appetite?

Poor	1	2	3	4	5	Excellent

4 In general, are you satisfied with the taste of the food you are currently eating?

Not satisfied	1	2	3	4	5	Very satisfied

5 In general, are you satisfied with the amount of the food you are currently eating?

Not satisfied	1	2	3	4	5	Very satisfied

6 Are you satisfied with the amount of food you eat for BREAKFAST?

Not satisfied	1	2	3	4	5	Very satisfied

7 Are you satisfied with the amount of food you eat for LUNCH?

Not satisfied	1	2	3	4	5	Very satisfied

8 Are you satisfied with the amount of food you eat for DINNER?

Not satisfied	1	2	3	4	5	Very satisfied

9 Are you satisfied with the amount of food or beverage you eat for SNACKS?

Not satisfied	1	2	3	4	5	Very satisfied

10 How different do you feel your eating pattern is from what other people eat?

Very different	1	2	3	4	5	Not different at all

11 How do you feel about other people knowing you will be or are currently changing your eating habits?

It bothers me quite a lot	1	2	3	4	5	Not bothered at all

12 Do other people seem to be bothered by the fact you may eat differently than they do?

Very much	1	2	3	4	5	Not at all

13 Does eating out in restaurants cause you difficulty?

Very much	1	2	3	4	5	Not at all

14 Does eating out at someone else's home cause you difficulty?

Very much	1	2	3	4	5	Not at all

15 How much does how and what you eat interfere with other activities in your life?

Very much	1	2	3	4	5	Not at all

16 How much do you think what you eat affects your health?

Doesn't affect	1	2	3	4	5	Very much

17 To what degree do you feel that making changes in your diet helps to improve how you feel?

Does not help at all	1	2	3	4	5	Helps a lot

18 How difficult do you (or whomever does the shopping) find food shopping?

Very difficult	1	2	3	4	5	Not at all

19 How difficult is it to plan and prepare your meals?

Very difficult	1	2	3	4	5	Not at all

20 How much time, on average, is involved in planning, shopping, and preparing your meals? (hours per day).......
21 Are there any special food which you currently use and enjoy? ………………………………..
22 Are there any special food products you have tried but do not enjoy? ……………………………
23 Are there any specific problems, additional comments or suggestions you would like to make about your current pattern, nutritional supplements, or special food products? ………………………………..
24 How do you enjoy eating now as compared to how you ate in the past (before you joined this diet)?

I liked my previous eating pattern much better	1	2	3	4	5	I liked my present eating pattern much better

25 Did you think you had side effects with this diet?

   Cefalea
   Astenia
   Constipation
   Hypoglycemia

26 How difficult was eat less glycemic food?

Very much	1	2	3	4	5	Not at all

27 Do you think the dietetic assistance was important?

Not at all	1	2	3	4	5	Very much

28 How much did the measurements of ketonemia and glycemia impact on your routine?

Very much	1	2	3	4	5	Not at all

29 How much difficult was the measurements of ketonemia and glycemia

Very much	1	2	3	4	5	Not at all

Appendix B

Reporting checklist for protocol of a clinical trial

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	1
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	1
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	NA
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5−6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5−6
Objectives	#7	Specific objectives or hypotheses	6
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6−7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7−8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8 and Table 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8−9

Methods: Assignment of interventions (for controlled trials)

Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency un-blinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA

Methods: Data collection, management, and analysis

Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	9
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9 − 10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomized analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA

Methods: Monitoring

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	NA
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA

Ethics and dissemination

Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	1
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9 − 10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	NA
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	12
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	NA
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

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