Scientific Research Publishing

Open Journal of Nephrology, 2019, 9, 97-114
https://www.scirp.org/journal/ojneph
ISSN Online: 2164-2869
ISSN Print: 2164-2842

Acute Effects of Tolvaptan on Renal Hemodynamics in Autosomal Dominant Polycystic Kidney Disease

—A Randomized, Cross-Over, Double Blind, Placebo-Controlled Study of Renal Plasma Flow and Glomerular Filtration Rate

My Helbo Malmberg[1,2*], Frank Holden Mose[1,2], Rikke Lund Sønderbæk[1,2], June Anita Ejlersen[2,3], Jens Jørgen Jensen[3], Erling Bjerregaard Pedersen[1,2], Jesper Nørgaard Bech[1,2]

[1]University Clinic in Nephrology and Hypertension, Regional Hospital Jutland West, Holstebro, Denmark
[2]University of Aarhus, Aarhus, Denmark
[3]Department of Nuclear Medicine, Regional Hospital Jutland West, Holstebro, Denmark
Email: *mymalmbe@rm.dk

How to cite this paper: Malmberg, M.H., Mose, F.H., Sønderbæk, R.L., Ejlersen, J.A., Jensen, J.J., Pedersen, E.B. and Bech, J.N. (2019) Acute Effects of Tolvaptan on Renal Hemodynamics in Autosomal Dominant Polycystic Kidney Disease. *Open Journal of Nephrology*, **9**, 97-114.
https://doi.org/10.4236/ojneph.2019.94011

Received: September 19, 2019
Accepted: October 20, 2019
Published: October 23, 2019

Abstract

Background: Previous studies have shown that reduced renal plasma flow (RPF) may play a role in progression of renal disease in autosomal dominant polycystic kidney disease (ADPKD). Tolvaptan, a vasopressin 2 antagonist, reduces growth of total kidney volume and slows the decrease in estimated glomerular filtration rate (eGFR) in ADPKD. The purpose of this randomized, cross-over, double-blind, placebo-controlled study was to investigate if acute tolvaptan treatment increases RPF in ADPKD patients. ***Methods***: Eighteen ADPKD patients (chronic kidney disease stages I-III) were investigated twice (min. 10 days apart) after acute treatment with either tolvaptan 60 mg or placebo. Two hours after treatment RPF and GFR were estimated by Technetium-99m diethylenetriamine penta-acetic acid (99-mTc-DTPA) renography. During the examination day, central and brachial blood pressures (BP) were measured using Mobil-O-Graph® PWA. We also measured plasma concentrations of vasopressin (p-AVP), renin (PRC), angiotensin II (p-AngII) and aldosterone (p-Aldo), urine excretion of aquaporin 2 (u-AQP2), urine output (OU), urine osmolality (u-Osm) and fractional excretion of sodium (FE_{Na}). ***Results***: 99-mTc-DTPA renography showed a similar RPF (673 ± 262 ml/min after tolvaptan vs. 650 ± 209 ml/min after placebo, p = 0.571) and GFR (78 ± 26 ml/min after tolvaptan vs. 79 ± 21 ml/min after placebo p = 0.774) after tolvaptan and placebo treatment. P-AVP and UO increased and u-Osm decreased after tolvaptan and remained unchanged during placebo. Systolic BP tended to decrease during renography during tolvaptan. Very

small or insignificant changes were seen in PRC, p-AngII and p-Aldo. ***Conclusions***: Acute tolvaptan treatment did not change renal hemodynamics in ADPKD.

Keywords

Autosomal Dominant Polycystic Kidney Disease, Renal Plasma Flow, Glomerular Filtration Rate, Renography, Brachial Blood Pressure, Central Blood Pressure, Vasopressin, Renin, Angiotensin II, Aldosterone

1. Background

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder, characterized by formation of cysts in the kidneys, causing gradual renal failure [1] [2].

Several factors play a role in the progression of kidney disease in ADPKD patients. Factors worsening the progression include mutation in the polycystin 1 gene (PKD1) compared to mutation in the PKD2 gene, male gender, early hypertension, early and frequent incidence of hematuria, three or more pregnancies, increased total kidney volume (TKV), reduced glomerular filtration rate (GFR) and reduced renal plasma flow (RPF) [3] [4]. Patients with early-stage ADPKD had reduced RPF and increased filtration fraction (FF) [5].

A previous study has shown that tolvaptan reduced TKV growth and induced a decrease in GFR decline, estimated by the CKD-EPI-formula, in ADPKD patients [6] [7]. Tolvaptan, a vasopressin 2 antagonist, dilutes urine via a reduction in adenosine 3',5'-cyclic monophosphate (cAMP) mediated translocation of intracellular aquaporin-2 (AQP2) water channels to the apical plasma membrane of the collecting duct principal cells [8] [9] [10]. Tolvaptan's effect on TKV is thought to be mediated by a reduction in cAMP, *i.e.* the effect should be a reduction in urine concentration ability, which is already reduced in ADPKD patients [11]. Another explanation could be that tolvaptan increased renal plasma flow and thereby improved the glomerulo-tubular balance. The effect of tolvaptan on RPF has not been clarified, but previous studies have suggested that RPF remained unchanged and GFR decreased after a few weeks of treatment [12] [13]. We hypothesized that the beneficial effects of tolvaptan on ADPKD at least partly could be explained by an increase in RPF.

The purpose of this randomized, cross-over, double-blinded, placebo-controlled study of ADPKD was to measure the effect of acute tolvaptan treatment on 1) RPF, GFR and FF, 2) Brachial blood pressure (bBP) and central blood pressure (cBP), and renovascular resistance (RVR), 3) Plasma concentrations of vasopressin (p-AVP), renin (PRC), angiotensin II (p-AngII) and aldosterone (p-Aldo), and 4) Urine excretion of aquaporin 2 (u-AQP2), urine output (OU), urine osmolality (U-Osm), and fractional excretion of sodium (FE_{Na}).

2. Material and Methods

2.1. Study Design

In this randomized, double-blinded, placebo-controlled, crossover study, we studied patients with ADPKD after acute treatment with tolvaptan 60 mg and placebo. Each patient participated in two identical examinations on separate days with an intermediate wash-out period of at least 10 days to eliminate any carryover effects.

2.2. Randomization and Blinding

The Department of Hospital Pharmacy, Regional Hospital Jutland West coated, randomized and distributed the trial medication.

2.3. Recruitment

Eligible ADPKD patients were recruited from the Outpatients' Clinic in University Clinic in Nephrology and Hypertension, Regional Hospital Jutland West and University of Aarhus.

2.4. Subjects

Inclusion Criteria

ADPKD patients meeting the following inclusion criteria were included: Age 18 - 67 years. Both genders. Unfertile women or fertile women using safe contraception throughout the trial period (safe contraception was defined as: birth control pills, spiral, depot injection of progestogen, subdermal implantation, hormonal vaginal ring, transdermal patch, sexual abstinence or sterilization). ADPKD diagnosed by genetic testing for PKD1 and PKD2 mutations. Presence of one of the following ultrasonographic findings in accordance with the classic Ravine criteria [14]: 1) patients with a negative family history of ADPKD with more than 10 cysts in each kidney, and exclusion of other causes of extra renal or renal cyst formations, 2) patients with a family history of ADPKD: 18 - 39 yrs. and 3 cysts or more unilaterally or bilaterally/40 - 59 yrs. and 2 or more cysts in each kidney/≥60 yrs. and at least 4 cysts in each kidney. Kidney function corresponding to chronic kidney disease (CKD) stages I-III (eGFR > 30 mL/min/1.73 m^2).

Exclusion Criteria

Previous kidney transplantation or kidney operation. Neoplastic disease, diabetes mellitus or abnormal liver blood samples (p-alanin transaminase (ALAT), p-bilirubin and p-alkaline phosphatase). Pregnancy, less than 6 months after childbirth or breastfeeding. Withdrawn consent. Tolvaptan intolerance or unaccepted side effects or contraindication for use of tolvaptan, including dehydration, hypovolemic hyponatremia, anuria and hypernatremia. Alcohol or drug abuse in cording to the Danish National Board of health's guidelines. Blood pressure > 170/105 mmHg despite treatment with metoprololsuccinate and/or amlodipine.

Withdrawal Criteria

Development of exclusion criteria, non-compliance or withdrawal of consent.

2.5. Effect Variables

The primary effect variable was RPF. The secondary effect variables were 1) Renal function (GFR, FF UO, FE_{Na}, u-Osm and u-AQP-2), 2) systemic hemodynamics (bBP and cBP), 3) Renovascular resistance (RVR), and 4) vasoactive hormones (PRC, p-AngII, p-Aldo and p- AVP).

2.6. Number of Subjects

With a minimal relevant difference of 77 ml/min in RPF and an estimated standard deviation (SD) of 67 ml/min, 18 subjects were needed using a level of significance of 5% and a statistical power of 90%. Twenty subjects were included to allow for possible drop-outs.

2.7. Study Medications

Tolvaptan (SAMSCA®, Otsuka, Tokyo, Japan) 60 mg and placebo were coated in identical gelatin capsules and were orally administered 2 hours before posterior Technetium-99m diethylenetriamine penta-acetic acid (99-mTc-DTPA) renography.

2.8. Antihypertensive Medications

Antihypertensive medications including diuretics, angiotensin-converting enzyme inhibitors and angiotensin-II inhibitors were discontinued or substituted with metoprolol succinate 25 mg and/or amlodipine 5 mg 14 days prior to each examination day. During the study period, bBP was monitored using a home blood pressure monitor. At a blood pressure higher than 170/105 mmHg, metoprolol succinate 25 mg and/or amlodipine 5 mg was given and increased up to metoprolol succinate 100 mg and/or amlodipine 10 mg. Subjects were withdrawn from the study, if the blood pressure remained higher than 170/105 mmHg despite treatment with metoprolol succinate 100 mg and/or amlodipine 10 mg. The usual antihypertensive treatment was resumed immediately after the 2. examination day. Patients were given the same dose of metoprolol succinate and/or amlodipine 14 days prior to both examination days.

2.9. Ethics

The study was approved by the Regional Committee on Health Research Ethics (case number: 1-10-72-373-14), and the Danish Health and Medicines Authority (EudraCT number: 2015-001903-30). The study was done in agreement with the Declaration of Helsinki and was registered at clinicaltrials.gov (identifier: NCT03803124). Written informed consent was obtained from each subject.

2.10. Diet

ADPKD patients consumed habitual intake of food and beverage during the

study period. Alcohol was prohibited on the day prior to and on the examination days.

2.11. Experimental Procedure

Procedures were identical on the two examination days. Before each examination, a fasting period of 8 hours was required, and a 24-hour urine sample was collected and completed just before medicine intake. The two examination days were conducted at the Department of Nuclear Medicine, Regional Hospital Jutland West, Denmark and on both days tolvaptan/placebo were given orally with 175 ml water before renography. Three hours before the renography, an intravenous catheter was placed in one arm to collect blood samples. Blood pressure was measured every 15 minutes during the examination day. Blood samples were drawn 2 hours before and 1 hour after renography, and were analysed for PRC, p-Aldo, p-AngII, and p-AVP, and for plasma concentrations of sodium, potassium, creatinine and albumin. Urine samples were collected by voiding in standing or sitting position right before medicine intake and 1 hour after renography after blood samples had been collected. Otherwise, patients were kept in a sitting position in a quiet and temperature-controlled room (22˚C - 25˚C). Urine samples were analysed for volume, sodium, albumin, creatinine, osmolality and AQP2. A 24-hour urine sample was collected immediate before trial medicine intake. The followed up period for each examination day were 3 hours after tolvaptan intake.

2.12. Measurements and Calculations

Renal function

Indirect and non-invasive measurements of the kidney parameters were estimated from renographies obtained by a two-headed gamma camera (Phillips Brightview SPECT or Siemens Symbia T16 SPECT/CT) positioned over the lower thoracic/upper abdominal area with the patient in the supine projection. At the time of the bolus injection (6 MBq Tc99m-diethylenetriaminepentaacetic acid (DTPA)/kg bodyweight in a cubital veina dynamic acquisition was commenced: In the first minute, one frame per second and during the rest of the examination, one frame/10 seconds was obtained. Only images from the posterior projections were used for the subsequent analysis. Data processing was performed in the commercial software MEDIC 2000 XP, ver. 5.9.1. As DTPA is handled almost exclusively by free filtration and no reuptake or excretion by the tubular system, the clearance of the tracer approaches GFR. Single kidney GFR was calculated from the uptake index in each kidney using a modification of the Rutland-Patlak method [15].

Single kidney RPF was calculated as the ratio of single kidney GFR determined by the Rutland-Patlak method and FF. The FF was estimated from the bolus passage of DTPA through the kidneys approximately 10 - 45 seconds after the isotope injection. The model estimated the FFiteratively by comparing the

background corrected kidney activity with the calculated activity from the convolution integral, minimizing the sum of squared differences. The analyses were performed by two nuclear medicine physicians blinded to knowledge of whether the patient had received placebo or tolvaptan.

Repeatability of the kidney parameters was calculated from a blinded re-analysis of the renographies after 1 month.

The inter- and intra-assay variation coefficient was calculated as SD/X × 100, where X denotes mean value of GFR, RPF or FF. SD denotes standard deviation, which is calculated as $\sqrt{\left(\sum d^2/2k\right)}$, where d denotes the difference between the duplicates, k denotes number of duplicates. The inter-assay variation coefficient for GFR during tolvaptan and placebo treatment was 9%, for RPF it was 17% during tolvaptan treatment and 16% during placebo treatment. The inter-assay variation coefficient for FF was 12% during tolvaptan treatment and 18% during placebo treatment. The intra-assay variation coefficient was 7.2% for GFR, 7.7% for RPF and 8.9% for FF.

Clearance (C) of substance X was calculated as $C_X = U_X/(P_X \times UO)$, where U_X denotes concentration of x in urine, P_X denotes concentration of x in plasma and UO is urine excretion rate.

FE_{Na} was determined according to the following formula: $FE_{Na} = 100 \times ((uNa \times pCr))/((pNa \times uCr))$.

Kidney volume was estimated by presuming that the kidneys had an ellipsoid volume using the formula: $4/3\pi abc$, where "*a*" denotes the kidney length, "*b*" denotes the kidney's width and "*c*" denotes the kidney's depth. The length and width were estimated by one of the two nuclear medicine physicians, using the posterior renography projection images from both placebo and tolvaptan treatment day. It was assumed that the kidney depth was equal to their width. In order to compare TKV impact on tolvaptan's effect on GFR and RPF, we divided the ADPKD patients into two equally sized groups, the 9 with the largest and the 9 whit the smallest TKV.

Urinary excretion of AQP2

Urine samples were kept frozen at −20˚C until assayed. U-AQP2 were measured by RIA as previously described [16] [17]. The AQP2 antibody was a gift from Professor Soren Nielsen and Professor Robert Fenton, The Water and Salt Centre, Aarhus University, Denmark. Minimal detection level was 32 pg/tube. The coefficients of variation were 11.7% (inter-assay) and 5.9% (intra-assay).

Vasoactive hormones in plasma

Blood samples for measurements of vasoactive hormones were centrifuged for 10 minutes at 2200 G and 4˚C. Plasma was separated from blood cells and kept frozen until assayed. PRC was determined using an immunoradiometric assay from CIS Bio International (Parc Marcel Boiteux, France). The minimal detection level was 1 pg/mL. The coefficients of variation were 4.1% (inter-assay) and 1.8% (intra-assay). P-Aldo was determined by RIA using a kit from Demeditec Diagnostics GmbH (Kiel, Germany). The minimal detection level was 1.44 pg/mL. The coefficients of variation were 17.2% (inter-assay) and 12.6% (in-

tra-assay). P-AngII and p-AVP were extracted from plasma with C_{18} Sep-Pak (Water associates, Milford; MA, USA) and subsequently determined by radioimmunoassay [18] [19]. The antibody against AngII was obtained from the Department of Clinical Physiology (Glostrup Hospital, Denmark). The minimal detection level was 2 pmol/L. The coefficients of variation were 12% for the inter-assay and 8% for the intra-assay. The antibody against AVP was a gift from Jacques Dürr, Miami, Fl, USA. The minimal detection level was 0.5 pmol/L. The coefficients of variation were 13% for the inter-assay and 9% for the intra-assay.

Other biochemical measurements

U-Osm was measured using A_2O Advanced Automated Osmometer (Advanced Instruments, MA, USA). Plasma concentration of sodium, potassium, albumin, hemoglobin, leukocytes, platelets, creatinine, bilirubin, ALAT, alkaline phosphatase, cholesterol, calcium, phosphate, parathyroid hormone (PTH), thyroid-stimulating hormone (TSH) and glycosylated hemoglobin A1c (HbA1c) were measured using routine methods at the Department of Clinical Biochemistry, Regional Hospital Jutland West, Denmark.

Brachial and central blood pressure

BP was measured every fifteen minutes throughout the day. bBP and cBP was measured using an oscillometric device on the participants upper arm (Mobil-O-Graph® PWA).

Renal vascular resistance

Renal vascular resistance (RVR) was estimated by the equation mean arterial pressure (MAP)/RPF. MAP was estimated by the Mobil-O-Graph® PWA during renography.

2.13. Statistics

Statistical analyses were performed using IBM SPSS statistics version 20 (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution. A paired t-test was used for paired comparison between two groups, if data showed normal distribution, otherwise Wilcoxon's signed rank test was used. Correlation was analysed by Spearman's test. Data are given as means ± SD and or as medians with 25% and 75% percentiles in brackets.

3. Results

3.1. Demographics

Twenty ADPKD patients with CKD stage I-III were included in the study. Two of the patients were excluded due to withdrawal of consent. Eighteen patients (11 females and 7 males) with a median age of 51 (range 19 - 67 years), body mass index of 29 ± 7 kg/m^2, eGFR of 82 ± 26 ml/min/1.73 m^2, office systolic bBP of 136 ± 15 mmHg and office diastolic bBP of 83 ± 10 mmHg completed the study. Baseline demographics and clinical characteristics of the eighteen ADPKD patients who completed the study are shown in **Table 1**. P-calcium was 2.38 [2.32; 2.43 mmol/l], p-phosphate 1.00 [0.92; 1.09 mmol/l], PTH6.78 [5.03; 8.53

Table 1. Baseline demographics and clinical characteristics of the eighteen ADPKD patients who completed the study.

Age (years)	49 ± 14
Gender (men/women)	07/11
Body mass index (kg/m^2)	29 ± 7
Office systolic bBP (mmHg)	136 ± 15
Office diastolic bBP (mmHg)	83 ± 10
Heart rate (beats/minute)	74 ± 13
eGFR (ml/min/1.73 m^2)	82 ± 26
u-Albumin (mg/l)	8 [4 - 13]

Values represent n in either group or mean ± SD or median with 25% and 75% percentiles in brackets. eGFR = estimated glomerular filtration rate. bBP = brachial blood pressure.

pmol/l] and b-hemoglobin 8.72 [8.25; 9.18 mmol/l]. There was no anamnestic or clinical signs of heart or lung disease.

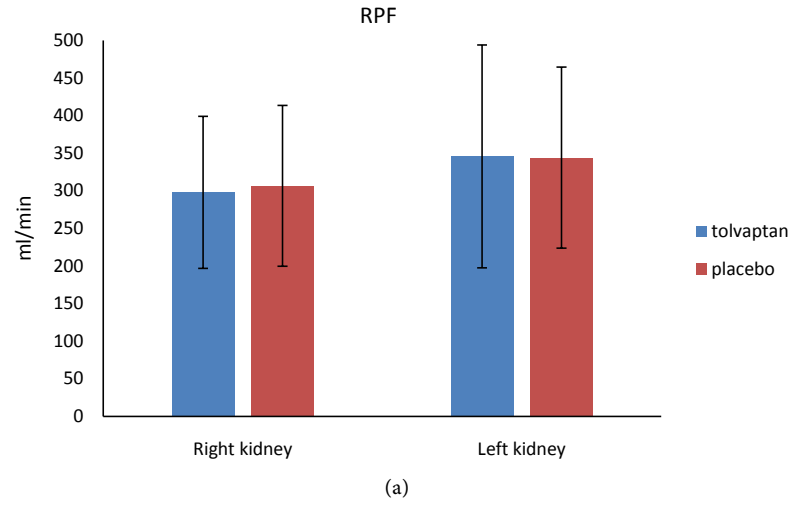
3.2. Renal Hemodynamics

99-mTc-DTPA renography showed a similar total RPF after tolvaptan and placebo treatment (673 ± 262 ml/min vs. 650 ± 209 ml/min p = 0.571, which is a difference of 3.5% NS) **Figure 1(a)** shows single kidney RPF, with no difference between the two kidneys comparing tolvaptan and placebo treatment. GFR estimated by 99-mTc-DTPA renography was also unchanged after tolvaptan (78 ± 26 ml/min after tolvaptan vs. 79 ± 21 ml/min after placebo, p = 0.774, which is a difference of 1.3% NS, **Figure 1(b)**). FF did not change after tolvaptan treatment (13% ± 4% vs. 13% ± 4% p = 0.861, which is a difference of 0% NS, **Figure 1(c)**).

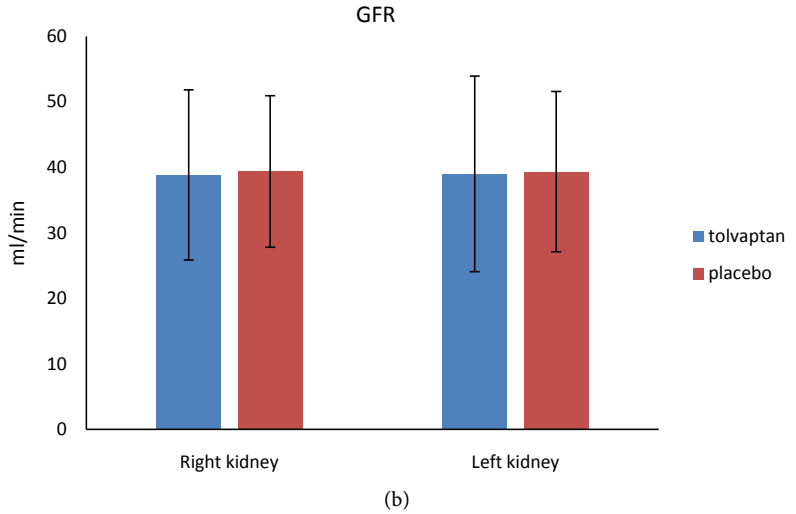
Two groups were created according to kidney size (large TKV 1056 ± 531 cm^3 (n = 9) vs. small TKV 409 ± 110 cm^3 (n = 9), p-value 0.006). A negative correlation between TKV and GFR was detected, *i.e.* ADPKD patients with large kidneys tended to have a lower GFR than ADPKD patients with small kidneys (**Figure 2(a)** and **Figure 2(b)**). No correlation between TKV and RPF was noted (R_s = −0.317, p = 0.200 after tolvaptan treatment and R_s = −0.346, p = 0.160 after placebo treatment). The results are shown in **Table 2** and correspond with the correlation analyses. There were no significant differences in GFR or RPF between treatments.

Blood pressure

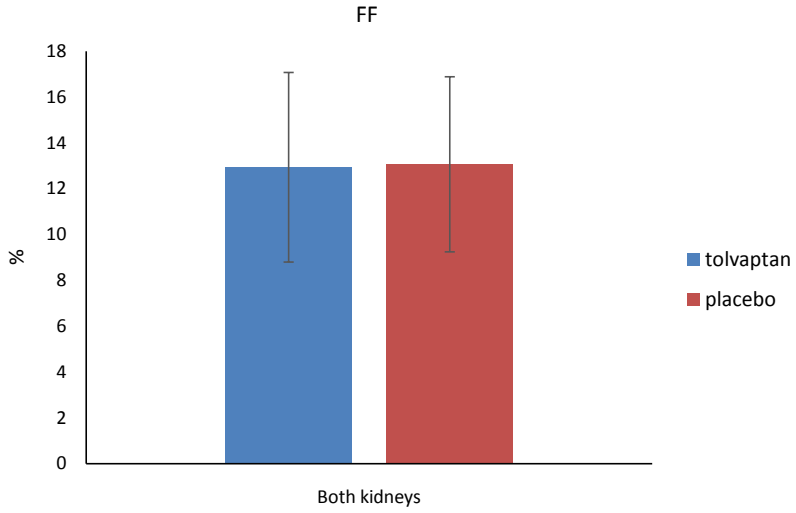
Table 3 shows systolic and diastolic bBP and cBP. We found similar diastolic bBP and cBP throughout the examination days with no significant changes between tolvaptan and placebo. We also found similar systolic bBP during the examination days except during renography, where systolic bBP decreased roughly 6 mmHg after tolvaptan treatment (p = 0.043) but was unchanged after placebo. Systolic cBP also tended to decrease after tolvaptan treatment during renography (p = 0.08), but not during placebo. However, no significant differences were measured, when the changes in systolic bBP and cBP were compared between



(a)



(b)



(c)

Figure 1. (a)-(c) Renal plasma flow (RPF) and glomerular filtration rate (GFR) estimated by renography in right and left kidney after tolvaptan and placebo treatment in eighteen ADPKD patients. Filtration fraction (FF) from both kidneys estimated by renography after tolvaptan and placebo treatment. Values represent mean ± SD.

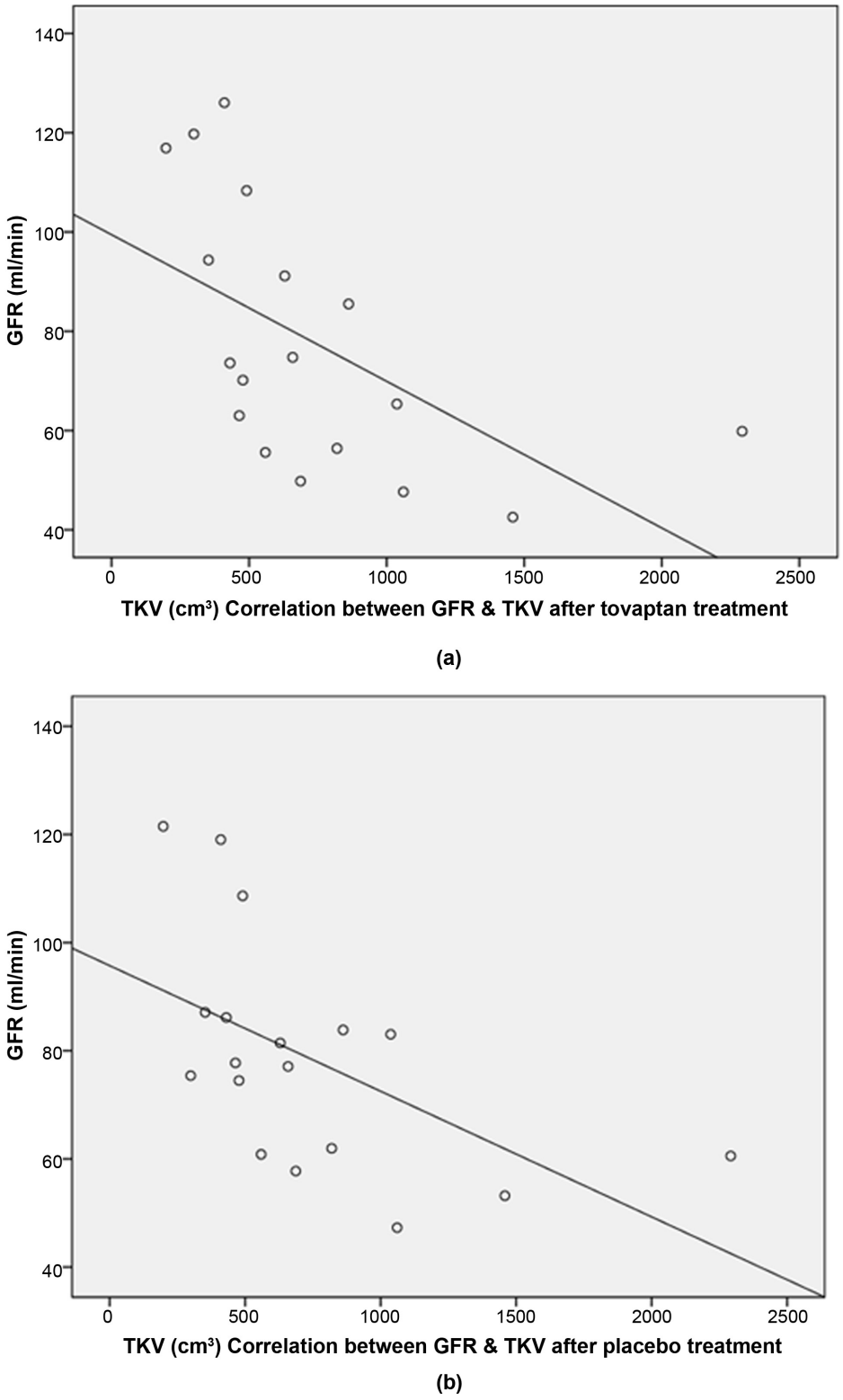


Figure 2. (a) & (b) Correlation between TKV and GFR after tolvaptan ($R_S = -0.728$, $p < 0.05$) and placebo treatment ($R_S = -0.657$, $p < 0.05$). GFR = glomerular filtration rate. TKV = total kidney volume. Kidney volume was estimated by presuming that the kidneys had an ellipsoid volume using the formula: $4/3\pi abc$, where "*a*" denotes the kidney length, "*b*" denotes the kidney's width and "*c*" denotes the kidney's depth.

Table 2. Tolvaptan's effect on RPF, GFR and FF in large vs. small TKV.

		large TKV (n = 9)	small TKV (n = 9)
RPF (ml/min)	- tolvaptan	594 ± 268	713 ± 204
	- placebo	572 ± 217	729 ± 177
GFR (ml/min)	- tolvaptan	64 ± 17	92 ± 27#
	- placebo	67 ± 14	90 ± 21
FF (%)	- tolvaptan	13 ± 5	13 ± 3
	- placebo	13 ± 4	13 ± 4

Values represent mean ± SD. # = p < 0.05 between GFR in large vs. small TKV after tolvaptan treatment. RPF = renal plasma flow. GFR = glomerular filtration rate. FF = filtration fraction. TKV = total kidney volume.

Table 3. Tolvaptan's effect on blood pressure throughout the examination day.

		Trial medicine intake ↓				
Timeline		0 h	1 h	3 h	3.5 h	4 h
		Baseline	Trial medicine treatment	Supine BP during renography	After renography	Changes between baseline and supine BP
Systolic (mmHg)	bBP - tolvaptan	137 ± 12	138 ± 12	135 ± 13*	140 ± 16	2 ± 10
	bBP - placebo	138 ± 18	141 ± 16	141 ± 18	144 ± 21	−3 ± 15
	cBP - tolvaptan	126 ± 11	127 ± 9	125 ± 13	131 ± 14	2 ± 11
	cBP - placebo	128 ± 16	130 ± 15	131 ± 19	134 ± 21	−3 ± 15
Diastolic (mmHg)	bBP - tolvaptan	92 ± 9	94 ± 9	87 ± 10	96 ± 11	5 ± 6
	bBP - placebo	92 ± 10	95 ± 12	89 ± 14	96 ± 16	4 ± 8
	cBP - tolvaptan	93 ± 9	95 ± 9	88 ± 10	98 ± 11	5 ± 7
	cBP - placebo	93 ± 10	97 ± 12	90 ± 13	98 ± 15	5 ± 8

Values represent mean ± SD. * = p <0.05 between tolvaptan and placebo treatment. bBP = brachial blood pressure, cBP = central blood pressure.

tolvaptan and placebo. Thus, the fall in systolic BP during renography seems to be marginal or by chance.

Renal vascular resistance

Estimated RVR during renography was unchanged after tolvaptan (0.40 ± 0.23 mmHg·ml^{-1} min) vs. placebo (0.38 ± 0.17 mmHg ml^{-1} min after placebo treatment, p = 0.743).

3.3. Sodium, Water and Albumin Excretion

UO, u-Osm, FE_{Na} and u-Alb are presented in **Table 4**.

FE_{Na} remained unchanged during the examination day during both treatments with no difference between the two treatments (0.8% after tolvaptan treatment vs. 0.8% after placebo treatment, p = 0.839).

Table 4. Tolvaptan's effect on UO, U-Osm, FE_{Na}, U-Alb, U-AQP2.

		Baseline (24 h urine sample)	After treatment (urine sample collected during a 3-hour period after trial medicine intake)	Changes between Baseline and after Treatment
U-Osm (mosmol/kg)	- tolvaptan	433 ± 166	127 ± 36*	295 ± 146#
	- placebo	424 ± 174	376 ± 139	27 ± 97
U-AQP2 (ng/ml)	- tolvaptan	0.6 ± 0.3	0.2 ± 0.1*	0.5 ± 0.3#
	- placebo	0.7 ± 0.3	0.5 ± 0.3	0.0 ± 0.3
U-AQP2 (ng/mmolCr)	- tolvaptan	107.5 ± 28.7	92.3 ± 21.6*	14.6 ± 20.9#
	- placebo	108.0 ± 34.4	97.7 ± 25.9	9.1 ± 16.1#
U-AQP2 (ng/min)	- tolvaptan	0.5 ± 0.3	0.0 ± 0.0*	0.4 ± 0.3#
	- placebo	0.5 ± 0.3	0.4 ± 0.3	0.1 ± 0.3
FE_{Na} (%)	- tolvaptan	0.9 ± 0.3	0.8 ± 0.4	0.1 ± 0.2
	- placebo	0.8 ± 0.2	0.8 ± 0.3	0.0 ± 0.3
UO (ml/min)	- tolvaptan	1.6 ± 0.5	5.5 ± 0.4*	−3.8 ± 1.5#
	- placebo	1.5 ± 0.4	1.8 ± 0.7	−0.2 ± 0.2
Albuminexcretion rate (mg/min)	- tolvaptan	0.01 [0.00; 0.03]	0.02 [0.01; 0.04]*	−0.01 [−0.01; −0.00]#
	- placebo	0.01 [0.00; 0.02]	0.01 [0.00; 0.02]	−0.00 [−0.00; 0.01]
U-albumin (mg/mmolCr)	- tolvaptan	1.4 [0.5; 3.8]	2.5 [1.4; 4.8]*	−0.9 [−1.7; −0.1]#
	- placebo	1.3 [0.3; 2.7]	1.2 [0.4; 2.9]	−0.1 [−0.4; 0.9]

Values represent mean ± SD or median with 25% and 75% percentiles in brackets * = <0.05 between tolvaptan and placebo treatment. # = $p < 0.05$ between baseline and after treatment. U-Osm = urine osmolality. U-AQP2 = aquaporin-2. FE_{Na} = fractional excretion of sodium. UO = urine flow.

After tolvaptan treatment, UO increased, but was unchanged during placebo treatment (5.5 ml/min after tolvaptan treatment vs. 1.8 ml/min after placebo treatment, $p < 0.001$). After tolvaptan treatment, u-Osm decreased, but did not change after placebo treatment (127 mosmol/kg vs. 376 mosmol/kg, $p < 0.001$).

Albumin excretion increased during tolvaptan treatment but stayed unchanged during placebo treatment and was significant higher during tolvaptan treatment in compare to placebo (0.02 mg/min vs. 0.01 mg/min, $p = 0.001$). Similar results were found after adjusting to creatinine excretion (2.5 mg/mmol Cr during tolvaptan treatment vs. 1.2 mg/mmol Cr after placebo treatment, $p = 0.001$).

3.4. U-AQP2

Table 4 shows no difference at baseline between treatments in u-AQP2 concentration, u-AQP2 excretion rate or u-AQP2 adjusted for creatinine. All three effect variables decreased significantly after tolvaptan but were unchanged after placebo. When changes from baseline to after treatment were compared, the differences between the treatments were maintained for u-AQP2 concentration

and u-AQP2 excretion rate. However, no significant difference was measured for u-AQP2 adjusted to creatinine excretion, which most likely is by change.

3.5. Vasoactive Hormones

Table 5 shows that there were no differences in baseline p-AVP, p-Aldo, PRC or p-AngII. After tolvaptan treatment, there were no changes in p-Aldo, PRC or p-AngII. However, p-AVP increased after tolvaptan treatment, while stayed unchanged during placebo treatment (1.1 pg/ml vs. 0.3 pg/ml, $p < 0.001$). When changes from baseline to after treatment were compared, the change in p-AVP was maintained. Although significant differences were seen between changes in PRC and p-AngII, these changes were extremely small and most likely by chance.

4. Discussion

The present paper reports acute effects of tolvaptan on renal hemodynamics and vasoactive hormones in ADPKD patients. Using 99-mTc-DTPA renography, one dose of tolvaptan did not increase RPF or GFR compared to placebo as hypothesized. However, UO, AVP increased and u-Osm decreased after one dose of tolvaptan treatment as expected.

4.1. Renal Hemodynamics and Glomerular Filtration Rate

We found no change in RPF after acute tolvaptan treatment, which is in agreement with results reported by Irazabal *et al.* and Boertien *et al.* using MRI and PAH clearance to determine RPF [12] [13]. In previous short-term studies, tolvaptan decreased GFR [12] [13], but in contrast to these studies, our results indicate that acute tolvaptan treatment has no immediate effect on GFR. These discrepancies in results could be due to several factors. Firstly, it may be attributed to renal autoregulation, which may obscure a possible effect of tolvaptan

Table 5. Tolvaptan's effect on vasoactive hormones.

		Baseline	3 hours after trial medicine intake	Changes between Baseline and 3 hours after trial medicine intake
P-AVP (pg/ml)	- tolvaptan	0.4 ± 0.2	1.1 ± 0.5*	−0.7 ± 0.5#
	- placebo	0.4 ± 0.2	0.3 ± 0.1	0.1 ± 0.2
P-ANGII (pg/ml)	- tolvaptan	7.8 ± 7.6	6.6 ± 3.8	1.2 ± 5.3
	- placebo	6.6 ± 4.0	5.4 ± 4.1	1.2 ± 1.8#
PRC (pg/ml)	- tolvaptan	7.5 ± 6.1	7.6 ± 6.7	−0.1 ± 1.7
	- placebo	7.5 ± 4.2	6.3 ± 3.1	1.2 ± 1.5#
P-Aldo (pmol/l)	- tolvaptan	258.2 ± 171.7	248.5 ± 137.1	9.7 ± 107.5
	- placebo	253.6 ± 116.1	250.6 ± 151.5	3.0 ± 107.5

Values represent mean ± SD * = $p < 0.05$ between tolvaptan and placebo treatment. # = $p < 0.05$ between baseline and after treatment. P-AVP = plasma vasopressin. P-AngII = plasma angiotensin II. PRC = plasma renin concentration. P-Aldo = plasma aldosterone.

on renal hemodynamics. Thus, in our study, an acute, small decline in brachial systolic blood pressure after tolvaptan treatment did not alter either GFR or RPF. The effect of renal autoregulation on change in GFR and RPF is well known from previous studies during alteration in blood pressure [20]. Secondly, the discrepancies could be due to difference in tolvaptan doses used in different studies, since tolvaptan's effect on GFR may be dose dependent. In the study of Boertien *et al.*, the patients received 90 - 120 mg tolvaptan daily in a period of 3 weeks, while Irazabal *et al.* used 20 mg tolvaptan daily for 7 days [12] [13]. In our study, the patients received one dose of 60 mg tolvaptan, which also was the dose used by Therwani *et al.* [12] [13] [21]. Thirdly, we cannot rule out a type II error in our study, since we used a smaller SD in our power calculation than we observed in the study. However, previous studies had a similar sample sizes (Boertien *et al.* studied 27 ADPKD patients, Irazabal *et al.* studied 20 patients, and Therwani *et al.* studied 18 patients) [12] [13] [21]. Fourthly, it is possible that the disagreements between the studies could be due to difference in the method of estimating GFR. In our study, we used Posterior 99-mTc-DTPA renography, while Therwani *et al.* used ^{51}Cr-EDTA, Boertien *et al.* and Irazabal *et al.* used ^{125}I-iothalamate. Overall, we were unable to falsify the 0-hypothesis of no change in RPF after tolvaptan treatment in ADPKD patients. Our conclusion is that tolvaptan does not change renal hemodynamics when administrated acutely.

4.2. Kidney Size

A previous study suggests that ADPKD patients with large TKV have an added benefit of tolvaptan treatment [6]. *The ERA-EDTA Working Groups on Inherited Kidney Disorders and European Renal Best Practice* has defined rapid progressive ADPKD as patients with large TKV, or rapid increase in TKV, or decrease in GFR at a young age and recommends tolvaptan treatment to these patients [22]. Therefore, we divided our patients into two subgroups, *i.e.* large TKV versus small. Our results showed no difference in effect of tolvaptan on GFR or RPF between the two groups. However, we measured only the immediate and acute effect of tolvaptan, while the previous study investigated the long-term effect of tolvaptan treatment [6].

4.3. Sodium and Water Excretion, u-AQP2 and Vasoactive Hormones

Tolvaptan is known for its aquaretic effects causing p-AVP and UO to increase and u-AQP2 and u-Osm to decrease [12] [23]. Our study showed similar results. Previous studies have shown that ADPKD patients have a high prevalence of hypertension and have a more activated renin-angiotensin-aldosterone system compared to patients with essential hypertension [24] [25]. In our study, the patients were not allowed to take any RAAS-inhibitors or blockers during a 14 days period prior to the examinations. Our results showed very small and similar responses in PRC, p-AngII and p-Aldo after tolvaptan and placebo treatment,

which is in good agreement with a previous study [21]. Thus, it seems unlikely that changes in the RAAS have influenced our results. We found no change in fractional excretion of sodium (FE_{Na}) after tolvaptan treatment, which is consistent with an earlier long-term study [26]. The unchanged FE_{Na} is probably due to the fact that tolvaptan's effects are mainly in the distal part of the nephron, and the effect is primarily on renal water handling and very modest on renal sodium excretion.

4.4. Albumin Excretion Rate

Our results show an increase in albumin excretion rate after acute tolvaptan treatment. In the post hoc analysis of the TEMPO 3:4 trial there was a tendency to increase in albuminuria in the initiation of the treatment [27]. However, after continuation of tolvaptan treatment, albuminuria decreased. The increase in albumin excretion rate may be due to an increase in urine flow per se or a temporary leak in the glomeruli.

4.5. Strengths and Limitations

The study design as a randomized, placebo-controlled, double-blinded, crossover is one of the major strengths of the present study. A proportion of the patients were treated with antihypertensives during the examination period, since complete withdrawal of antihypertensive treatment was not ethically justified. Since the medical treatment was identical during placebo and tolvaptan treatment, a possible difference due to use of antihypertensive therapy is expected to be minimal. We found satisfying inter- and intra-assay variation coefficient using 99-mTc-DTPA renography for estimating RPF and GFR.

5. Conclusion

Our results do not support the hypothesis that the effect of tolvaptan on ADPKD progression is mediated by changes in renal perfusion. Our findings express short-term effects of tolvaptan treatment. A long-term tolvaptan study is needed to clarify whether a long-term effect exists of tolvaptan on renal hemodynamics.

Acknowledgements

The authors thank Otsuka for financial support to the study via an Investigator Sponsored Study Agreement. We thank our laboratory technicians, including Anne Mette Ravn, Henriette Vorup Simonsen and Kirsten Nyggard for their skillful assistance.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Dalgaard, O.Z. (1957) Bilateral Polycystic Disease of the Kidneys; A Follow-up of

284 Patients and Their Families. *Danish Medical Journal*, **4**, 128-133.

[2] Gabow, P.A. (1993) Autosomal Dominant Polycystic Kidney Disease. *The New England Journal of Medicine*, **329**, 332-342. https://doi.org/10.1056/NEJM199307293290508

[3] Schrier, R.W., Brosnahan, G., Cadnapaphornchai, M.A., Chonchol, M., Friend, K., Gitomer, B. and Rossetti, S. (2014) Predictors of Autosomal Dominant Polycystic Kidney Disease Progression. *Journal of the American Society of Nephrology*, **25**, 2399-2418. https://doi.org/10.1681/ASN.2013111184

[4] King, B.F., Torres, V.E., Brummer, M.E., Chapman, A.B., Bae, K.T., Glockner, J.F., Arya, K., Felmlee, J.P., Grantham, J.J., Guay-Woodford, L.M., Bennett, W.M., Klahr, S., Hirschman, G.H., Kimmel, P.L., Thompson, P.A. and Miller, J.P. (2003) Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP): Magnetic Resonance Measurements of Renal Blood Flow as a Marker of Disease Severity in Autosomal-Dominant Polycystic Kidney Disease. *Kidney International*, **64**, 2214-2221. https://doi.org/10.1046/j.1523-1755.2003.00326.x

[5] Meijer, E., Rook, M., Tent, H., Navis, G., van der Jagt, E.J., de Jong, P.E. and Gansevoort, R.T. (2010) Early Renal Abnormalities in Autosomal Dominant Polycystic Kidney Disease. *The Clinical Journal of the American Society of Nephrology*, **5**, 1091-1098. https://doi.org/10.2215/CJN.00360110

[6] Torres, V.E., Chapman, A.B., Devuyst, O., Gansevoort, R.T., Grantham, J.J., Higashihara, E., Perrone, R.D., Krasa, H.B., Ouyang, J. and Czerwiec, F.S. (2012) TEMPO 3:4 Trial Investigators: Tolvaptan in Patients with Autosomal Dominant Polycystic Kidney Disease. *The New England Journal of Medicine*, **367**, 2407-2418. https://doi.org/10.1056/NEJMoa1205511

[7] Torres, V.E., Higashihara, E., Devuyst, O., Chapman, A.B., Gansevoort, R.T., Grantham, J.J., Perrone, R.D., Ouyang, J., Blais, J.D. and Czerwiec, F.S. (2016) TEMPO 3:4 Trial Investigators: Effect of Tolvaptan in Autosomal Dominant Polycystic Kidney Disease by CKD Stage: Results from the TEMPO 3:4 Trial. *The Clinical Journal of the American Society of Nephrology*, **11**, 803-811. https://doi.org/10.2215/CJN.06300615

[8] Knepper, M.A. (1997) Molecular Physiology of Urinary Concentrating Mechanism: Regulation of Aquaporin Water Channels by Vasopressin. *American Journal of Physiology*, **272**, F3-F12. https://doi.org/10.1152/ajprenal.1997.272.1.F3

[9] Marples, D., Knepper, M.A., Christensen, E.I. and Nielsen, S. (1995) Redistribution of Aquaporin-2 Water Channels Induced by Vasopressin in Rat Kidney Inner Medullary Collecting Duct. *American Journal of Physiology*, **269**, C655-C664. https://doi.org/10.1152/ajpcell.1995.269.3.C655

[10] Stefan, E., Wiesner, B., Baillie, G.S., Mollajew, R., Henn, V., Lorenz, D., Furkert, J., Santamaria, K., Nedvetsky, P., Hundsrucker, C., Beyermann, M., Krause, E., Pohl, P., Gall, I., MacIntyre, A.N., Bachmann, S., Houslay, M.D., Rosenthal, W. and Klussmann, E. (2007) Compartmentalization of cAMP-Dependent Signaling by Phosphodiesterase-4D Is Involved in the Regulation of Vasopressin-Mediated Water Reabsorption in Renal Principal Cells. *Journal of the American Society of Nephrology*, **18**, 199-212. https://doi.org/10.1681/ASN.2006020132

[11] Zittema, D., Boertien, W.E., van Beek, A.P., Dullaart, R.P., Franssen, C.F., de Jong, P.E., Meijer, E. and Gansevoort, R.T. (2012) Vasopressin, Copeptin, and Renal Concentrating Capacity in Patients with Autosomal Dominant Polycystic Kidney Disease without Renal Impairment. *The Clinical Journal of the American Society of Nephrology*, **7**, 906-913. https://doi.org/10.2215/CJN.11311111

[12] Irazabal, M.V., Torres, V.E., Hogan, M.C., Glockner, J., King, B.F., Ofstie, T.G., Krasa, H.B., Ouyang, J. and Czerwiec, F.S. (2011) Short-Term Effects of Tolvaptan on Renal Function and Volume in Patients with Autosomal Dominant Polycystic Kidney Disease. *Kidney International*, **80**, 295-301. https://doi.org/10.1038/ki.2011.119

[13] Boertien, W.E., Meijer, E., de Jong, P.E., Bakker, S.J., Czerwiec, F.S., Struck, J., Oberdhan, D., Shoaf, S.E., Krasa, H.B. and Gansevoort, R.T. (2013) Short-Term Renal Hemodynamic Effects of Tolvaptan in Subjects with Autosomal Dominant Polycystic Kidney Disease at Various Stages of Chronic Kidney Disease. *Kidney International*, **84**, 1278-1286. https://doi.org/10.1038/ki.2013.285

[14] Ravine, D., Gibson, R.N., Walker, R.G., Sheffield, L.J., Kincaid-Smith, P. and Danks, D.M. (1994) Evaluation of Ultrasonographic Diagnostic Criteria for Autosomal Dominant Polycystic Kidney Disease. *The Lancet*, **343**, 824-827. https://doi.org/10.1016/S0140-6736(94)92026-5

[15] Petersen, L.J., Petersen, J.R., Talleruphuus, U., Moller, M.L., Ladefoged, S.D., Mehlsen, J. and Jensen, H.A. (1999) Glomerular Filtration Rate Estimated from the Uptake Phase of 99mTc-DTPA Renography in Chronic Renal Failure. *Nephrology Dialysis Transplantation*, **14**, 1673-1678. https://doi.org/10.1093/ndt/14.7.1673

[16] Pedersen, R.S., Bentzen, H., Bech, J.N. and Pedersen, E.B. (2001) Effect of Water Deprivation and Hypertonic Saline Infusion on Urinary AQP2 Excretion in Healthy Humans. *American Journal of Physiology-Renal Physiology*, **280**, F860-F867. https://doi.org/10.1152/ajprenal.2001.280.5.F860

[17] Pedersen, E.B., Thomsen, I.M. and Lauridsen, T.G. (2010) Abnormal Function of the Vasopressin-Cyclic-AMP-Aquaporin2 Axis during Urine Concentrating and Diluting in Patients with Reduced Renal Function. A Case Control Study. *BMC Nephrology*, **11**, 26. https://doi.org/10.1186/1471-2369-11-26

[18] Pedersen, E.B., Danielsen, H. and Spencer, E.S. (1984) Effect of Indapamide on Renal Plasma Flow, Glomerular Filtration Rate and Arginine Vasopressin in Plasma in Essential Hypertension. *European Journal of Clinical Pharmacology*, **26**, 543-547. https://doi.org/10.1007/BF00543482

[19] Pedersen, E.B. (1993) Effect of Captopril on Renal Extraction of Renin, Angiotensin II, Atrial Natriuretic Peptide and Vasopressin, and Renal Vein Renin Ratio in Patients with Arterial Hypertension and Unilateral Renal Artery Disease. *Nephrology, Dialysis, Transplantation*, **8**, 1064-1070.

[20] Carlstrom, M., Wilcox, C.S. and Arendshorst, W.J. (2015) Renal Autoregulation in Health and Disease. *Physiological Reviews*, **95**, 405-511. https://doi.org/10.1152/physrev.00042.2012

[21] Al Therwani, S., Malmberg, M.E.S., Rosenbaek, J.B., Bech, J.N. and Pedersen, E.B. (2017) Effect of Tolvaptan on Renal Handling of Water and Sodium, GFR and Central Hemodynamics in Autosomal Dominant Polycystic Kidney Disease during Inhibition of the Nitric Oxide System: A Randomized, Placebo-Controlled, Double Blind, Crossover Study. *BMC Nephrology*, **18**, 268. https://doi.org/10.1186/s12882-017-0686-3

[22] Gansevoort, R.T. (2016) Recommendations for the Use of Tolvaptan in Autosomal Dominant Polycystic Kidney Disease: A Position Statement on Behalf of the ERA-EDTA Working Groups on Inherited Kidney Disorders and European Renal Best Practice. *Nephrology, Dialysis, Transplantation*, **31**, 337-348. https://doi.org/10.22141/2307-1257.3.17.2016.76538

[23] Tamma, G., Di Mise, A., Ranieri, M., Geller, A., Tamma, R., Zallone, A. and Valenti,

G. (2017) The V2 Receptor Antagonist Tolvaptan Raises Cytosolic Calcium and Prevents AQP2 Trafficking and Function: An *in Vitro* and *in Vivo* Assessment. *Journal of Cellular and Molecular Medicine*, **21**, 1767-1780. https://doi.org/10.1111/jcmm.13098

[24] Chapman, A.B., Johnson, A., Gabow, P.A. and Schrier, R.W. (1990) The Renin-Angiotensin-Aldosterone System and Autosomal Dominant Polycystic Kidney Disease. *The New England Journal of Medicine*, **323**, 1091-1096. https://doi.org/10.1056/NEJM199010183231602

[25] Chapman, A.B., Stepniakowski, K. and Rahbari-Oskoui, F. (2010) Hypertension in Autosomal Dominant Polycystic Kidney Disease. *Advances in Chronic Kidney Disease*, **17**, 153-163. https://doi.org/10.1053/j.ackd.2010.01.001

[26] Muto, S. (2017) Long-Term Safety Profile of Tolvaptan in Autosomal Dominant Polycystic Kidney Disease Patients: TEMPO Extension Japan Trial. *Drug, Healthcare and Patient Safety*, **9**, 93-104. https://doi.org/10.2147/DHPS.S142825

[27] Gansevoort, R.T., Meijer, E., Chapman, A.B., Czerwiec, F.S., Devuyst, O., Grantham, J.J., Higashihara, E., Krasa, H.B., Ouyang, J., Perrone, R.D. and Torres, V.E. (2016) TEMPO 3:4 Investigators: Albuminuria and Tolvaptan in Autosomal-Dominant Polycystic Kidney Disease: Results of the TEMPO 3:4 Trial. *Nephrology Dialysis Transplantation*, **31**, 1887-1894. https://doi.org/10.1093/ndt/gfv422

List of Abbreviations

ADPKD: Autosomal dominant polycystic kidney disease.

AQP2: aquaporin-2.

bBP: brachial blood pressure.

cBP: central blood pressure.

cAMP: adenosine 3',5'-cyclic monophosphate.

CKD: chronic kidney disease. eGFR: estimated glomerular filtration rate.

FE_{Na}: fractional excretion of sodium.

FF: filtration fraction.

P: p-value.

p-Aldo: plasma aldosterone.

p-AngII: plasma angiotensin.

p-AVP: plasma vasopressin.

PKD1: polycystin 1 gene.

RC: plasma renin.

RPF: renal plasma flow.

RVR: renal vascular resistance.

SD: standard deviation.

TKV: total kidney volume.

UO: urine output.

u-Osm: u-osmolality.

6 MBq/kg: 6 megabecquerel per kg body weight.

99-mTc-DTPA: Technetium-99m diethylenetriamine penta-acetic acid.