

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: The ESCAPE Trial Group. Strict blood-pressure control and progression of renal failure in children. *N Engl J Med* 2009;361:1639-50.

Strict Blood Pressure Control Slows Renal Failure Progression in Children

Supplementary Appendix

METHODS

Additional Information about the Study Protocol

The investigators met every six months from 1998-2007 to evaluate the progress of the study and monitor the achievement of blood pressure targets in each individual patient.

A GCP audit was performed at all participating sites 1 year after the end of the enrollment period.

Additional Information about Patient Characteristics

The children lived in 10 EU countries, Serbia, Switzerland and Turkey and were almost exclusively of Caucasian ethnicity.

Of 468 patients entering the run-in period, 83 turned out unsuitable for randomization due to blood pressure below the 50th percentile without antihypertensive medication (n=3), uncontrolled hypertension (n=1), GFR exceeding 80 ml/min/1.73m² at the end of the run-in period (n=25), unstable renal function with intermittent acute increases of serum creatinine (n=2) or attainment of CKD stage V in the meantime (n=21), poor adherence to medications (n=8), withdrawal of consent (n=5), loss of follow-up (n=7) or other exclusion criteria (n=12) (Fig.1).

Underlying renal disorders in the 385 patients randomized included renal hypo/dysplasia with or without obstructive or reflux nephropathies (n=264), glomerulopathies (n=52; (i.e. FSGS (n=19), CKD following haemolytic uremic syndrome (n=25 (atypical HUS in 7 patients), other (n=8)), and other congenital or hereditary nephropathies (n=69; nephronophthisis (n=22), autosomal-recessive polycystic kidney disease (n=20), cystinosis (n=6), CKD after renal vein thrombosis (n=3), or after exposure to nephrotoxic agents (n=2) and other reasons (n=12)). The distribution of diagnoses did not differ statistically between treatment arms.

Patients with unstable clinical condition who could be expected to develop major intercurrent or permanent changes in renal function due to reasons other than intrinsic CKD progression were excluded a priori. This comprised children with frequent intercurrent infections (UTI or other), active autoimmune disease, relevant urological problems or awaiting urological surgery.

Additional Information on Urine Sampling, Proteinuria and Renal Function

Protein creatinine ratio and 24h proteinuria (expressed as mg/m²/d) were closely correlated ($r=0.93$, $p<0.0001$) without any systematic confounding by patient age or body size, eliminating the need for further standardization.

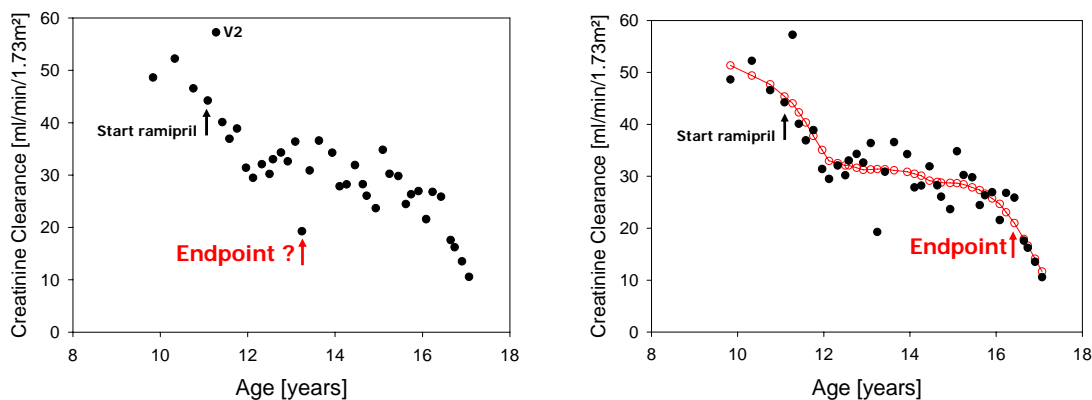
For long-term evaluation of GFR it was decided to follow estimated GFR and apply an advanced smoothing algorithm to reduce bias due to outliers. Parallel assessments of endogenous creatinine clearance were performed in approximately 80 % of patients. In 316 patients at baseline, measured GFR was 46.9 ± 20.4 compared to an estimated GFR of 43.3 ± 29.5 ml/min/1.73m² ($p<0.05$). Measured and estimated GFRs were robustly correlated ($r=0.81$, $p<0.0001$) and the difference between estimated and measured GFR was unrelated to patient age.

Repeated clearance studies using iothalamate or iohexol injections would have caused major issues with Ethics Review Boards in several European countries due to the small but not negligible safety risk associated with these methodologies. Moreover, in preparation of a previous multicenter trial (Wingen et al. Lancet 1997) the group had compared the variability of single-injection inulin clearances with that of serial estimated GFR determinations and had not found a substantial advantage of clearance studies in terms of overall GFR slope variability. Therefore, the consortium opted for high-frequency estimated GFR assessments (central creatinine measurement; 2-month sampling paradigm, i.e. 31 data points in 5 years) followed by smoothing of the individual GFR curves using a curve fitting technique based on local regression (LOESS procedure, SAS 9.0). In this way, high-quality data on longitudinal GFR evolution was obtained. An example of the smoothing procedure is given in Appendix Fig.1.

A k value of 0.55 for the Schwartz formula was validated in the central laboratory and used throughout. Considering the age range studied in this trial, the Schwartz formula foresees to change the k value at age 13 years in boys only. Since this would have introduced an artificial 'step' in the GFR curves, it was decided to use the value of 0.55 throughout the study period also in boys. By this procedure GFR progression might have been slightly overestimated with time in the male adolescents, but this was felt to be a minor inaccuracy as compared to introducing a sudden blip in estimated GFR by abruptly changing the k value at age 13 years. At any rate, the slight underestimation of GFR in pubertal males equally affected both treatment groups.

Appendix Fig. 1 a/ b:

Left panel: Erroneous determination of the endpoint (50% reduction of GFR compared to month 2 (V2)) using original data points for analysis. Right panel: Correct determination of endpoint by analysis of smoothed GFR curve.



Additional Information on Blood Pressure Monitoring

ABPM was performed with Spacelabs 90207 oscillometric devices (Spacelabs Healthcare, Issaquah, WA, USA) at screening, immediately prior to randomization, and every six months during the study period. The ABPM cuff size was chosen according to patient's arm circumference (4 cuff sizes available starting from 12 to 42 cm). Measurements were performed every 15 min during daytime and every 20-30 min during night time as described previously (Wühl et al., *J Hypertens* 2002, 20:1995-2007; Wühl et al., *Kidney Int* 2004, 66:768-776). The lack of normative ABPM data for children younger than 5 years was addressed by extrapolating existing normative values for the first few ABPM profiles of children younger than 5 years of age at start. The fraction of all ABPM profiles for which extrapolation was applied during the study was 35 of 2891 (1.2%).

ABPM data quality was monitored continuously. A minimum profile duration of 24 hours with gaps of less than 2 hours was required for acceptance. Repetition of the ABPM was requested in case of failure to meet the quality standards. The resulting data quality was independent of age. The accuracy of diastolic blood pressure values obtained by oscillometry is a notorious point of controversy. The primary signal picked up by oscillometric measurements is mean arterial pressure (MAP), from which systolic and diastolic values are derived by algorithms which may or may not be accurate across the pediatric age range. To avoid potential bias by discrepancies

between systolic and diastolic BP results, it was decided to directly use MAP as a measure of BP control over time.

The choice to use auscultatory or oscillometric devices for measuring casual office blood pressure was left to the discretion of the participants according to local device availability. This should not have influenced the longitudinal follow up of blood pressure control since the local method applied did not change during the study. Home blood pressure measurements were performed using a single type of device which was distributed to each participating family. While casual and home blood pressure measurements were performed for interim blood pressure monitoring, therapeutic decisions were made exclusively according to the standardized six-monthly ABPM measurements.

Additional Information on Blood Pressure Control with Antihypertensive Co-medication

Additional antihypertensive drugs were prescribed if required to attain blood pressure control within the target range. The following order of escalation was proposed: (1) diuretic, (2) beta-receptor blocker, (3) calcium channel blocker, (4) alpha blocker, (5) centrally acting agent. The preferential use of long-acting drugs with once daily dosing was recommended. Adherence to the escalation scheme was not compulsory since a fraction of patients was on background antihypertensive medication at start of the study, which was decided to be continued unchanged. Moreover, the diversity of the underlying renal diseases (non-applicability of diuretics in polyuric dysplastic kidney disorders) and the variable drug availability in individual countries precluded the uniform administration of the escalation scheme.

Additional Information on Safety Monitoring

Adverse events were reported by the local investigators according to Good Clinical Practice Guidelines, using the following definitions: An adverse event is any untoward medical occurrence whether or not considered related to the intervention. A serious adverse event is any adverse event that is fatal (death), life threatening, results in persistent or significant disability, results in (prolonged) hospitalization or that is medically significant or requires intervention to prevent one or other of the outcomes listed above.

According to the study protocol patients had to be withdrawn from therapy if one of the following occurred:

1. Patient's wish
2. Severe adverse effects
3. Appearance of severe uremia necessitating renal replacement therapy
4. Occurrence of major superimposed disease
5. Obvious noncompliance

Safety parameters (hyperkalemia, acute renal failure, AEs) were followed continuously by the central office.

Additional Information on Statistical Analyses

It was planned in advance to evaluate the influence of the following baseline variables (potential risk factors for renal disease progression) on the primary study outcome: baseline GFR, pre-study progression rate, baseline blood pressure, baseline proteinuria, age, gender, underlying renal disease (glomerulopathy yes/no). Check for normal distribution was planned by Shapiro-Wilk test, normalization for further analysis of Non-Gaussian data by log-transformation. Primary endpoint analysis was planned by Kaplan-Meier life table technique, differences in rates of endpoint attainment should be evaluated by log-rank statistics, and Cox proportional hazard modeling was planned for calculation of effects of potential risk factors. Longitudinal changes of parameters should be evaluated by repeated-measure ANOVA, Pairwise comparisons between baseline and subsequent time points using the CONTRAST option of the GLM procedure. All statistics were performed by SAS (SAS V7 was available at start of study, V9.2 used at end of study). Random center effects were excluded by the GLIMMIX procedure. All reported p-values are 2-sided and not adjusted for multiplicity. Correlation coefficients were calculated using Spearman rank order correlation.

RESULTS

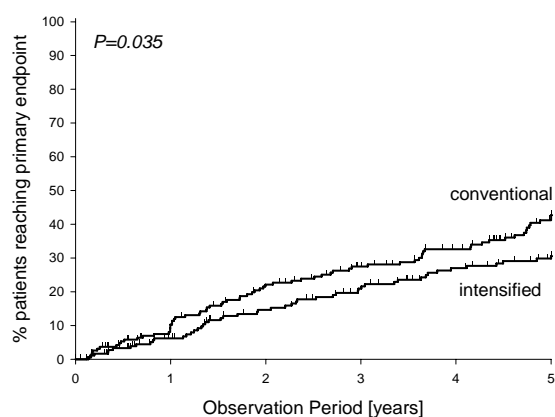
Additional Information on Intervention Efficacy Using Different Starting Points

Different approaches are possible with respect to the choice of the ‘baseline’ time point in this two-stage study. Patients were randomized at month 0. The initial intervention, administration of ramipril at a fixed dose, took full effect at 2 months and was identical in all patients. The actual randomized intervention (intensified vs. conventional BP control) started at 6 months.

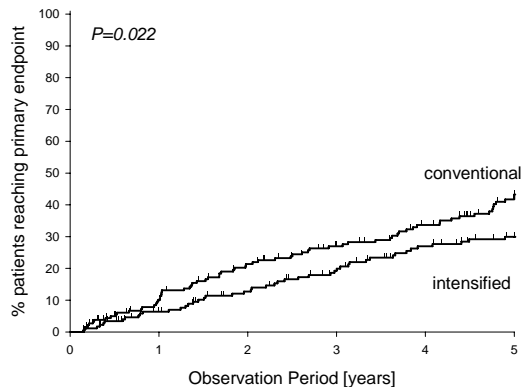
Expectably, a few patients in both randomization arms reached an endpoint during the first 6 and even 2 months. We chose to use month 2 as the starting point of survival analysis, and these data are reported in the main manuscript. In order to complete the picture, we performed additional intention-to-treat analyses starting at the time of randomization (0 months) and at the time of ‘effective’ randomization (6 months). The difference in renal survival between treatment arms was also significant when month 0 ($p<0.03$) or month 6 ($p<0.02$) was used as starting point for the intention-to-treat analysis.

Appendix Fig. 2 a/b/c.

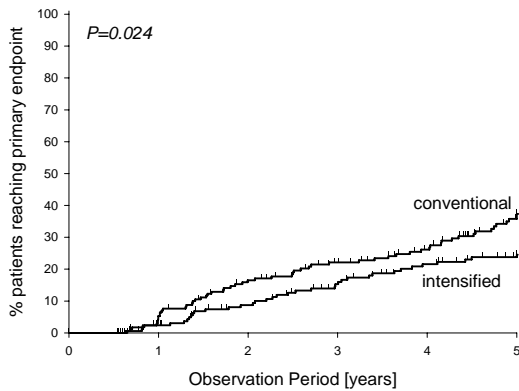
a) Intention to treat analysis using **time of randomization** (month 0) as starting point (initial N conventional group: 196, intensified group: 189)



b) Intention to treat analysis using **start of effective ACE inhibition** (month 2) as starting point (initial N conventional group: 190, intensified group: 182)



c) Intention to treat analysis using **start of randomized intervention** (month 6) as starting point (initial N conventional group: 168, intensified group: 167)



Appendix Table 1a-c: Cox Multivariate Proportional Hazard Analysis

a) Cox proportional hazard analysis starting from **time of randomization** (month 0)

| | Hazard ratio | 95% CI | P value |
|---|--------------|-----------|---------|
| Randomization to intensified blood pressure control | 0.62 | 0.39-0.98 | 0.04 |
| Baseline GFR | 0.93 | 0.92-0.95 | <0.0001 |
| Urinary protein/creatinine ratio | 1.22 | 1.05-1.41 | 0.008 |
| Baseline 24-h mean arterial pressure SDS | 1.13 | 1.01-1.27 | 0.03 |
| Age | 1.06 | 1.01-1.12 | 0.03 |

b) Cox proportional hazard analysis starting from **effective ACE inhibition** (month 2)

| | Hazard ratio | 95% CI | P value |
|---|--------------|-----------|---------|
| Randomization to intensified blood pressure control | 0.59 | 0.38-0.91 | 0.01 |
| Baseline GFR | 0.93 | 0.92-0.95 | <0.0001 |
| Urinary protein/creatinine ratio | 1.42 | 1.13-1.77 | 0.002 |
| Baseline 24-h MAP SDS | 1.15 | 1.04-1.23 | 0.009 |
| Older age | 1.07 | 1.01-1.13 | 0.02 |

c) Cox proportional hazard analysis starting from **time of randomized intervention** (month 6)

| | Hazard ratio | 95% CI | P value |
|---|--------------|-----------|---------|
| Randomization to intensified blood pressure control | 0.59 | 0.36-0.96 | 0.03 |
| Baseline GFR | 0.92 | 0.90-0.94 | <0.0001 |
| Urinary protein/creatinine ratio | 1.16 | 0.98-1.37 | 0.09 |
| Baseline 24-h mean arterial pressure SDS | 1.15 | 1.01-1.32 | 0.04 |
| Age | 1.06 | 1.00-1.12 | 0.05 |

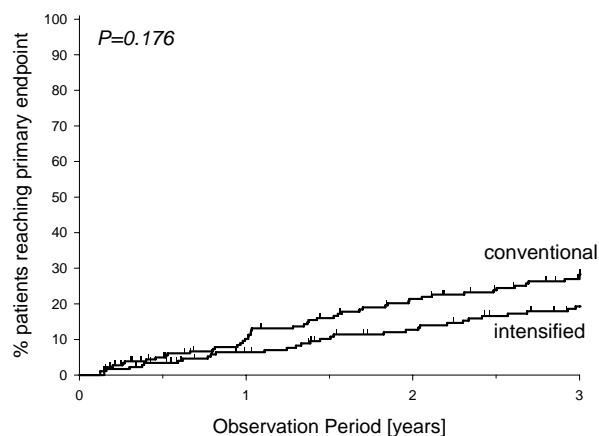
Additional Information on Intervention Efficacy After 3 and 4 Years

Analysing the renal survival using datasets restricted to information obtained after 3 and 4 years of observation evolving trends were visible after 3 and 4 years, which lead to significance after 5 years.

Appendix Fig. 3 a/b.

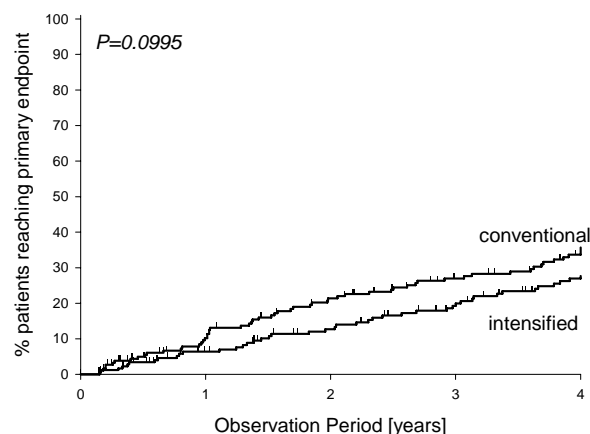
a) Intention to treat analysis restricted to data obtained after 3 years of observation
(initial N conventional group: 196, intensified group: 189)

Renal Survival Analysis at 3 years (log rank: $p=0.176$)



b) Intention to treat analysis restricted to data obtained after 4 years of observation
(initial N conventional group: 196, intensified group: 189)

Renal Survival Analysis at 4 years (log rank: $p=0.099$)



Appendix Table 2a/b: Cox Multivariate Proportional Hazard Analysis

a) Cox proportional hazard analysis restricted to data obtained after 3 years of observation

| Cox Model Analysis at 3 years | Hazard ratio | 95% CI | P value |
|---|--------------|-----------|---------|
| Randomization to intensified blood pressure control | 0.78 | 0.46-1.33 | 0.35 |
| Baseline GFR | 0.93 | 0.91-0.95 | <0.0001 |
| Urinary protein/creatinine ratio | 1.21 | 1.02-1.44 | 0.004 |
| Baseline 24-h mean arterial pressure SDS | 1.10 | 0.96-1.28 | 0.17 |
| Age | 1.06 | 0.99-1.14 | 0.09 |

b) Cox proportional hazard analysis restricted to data obtained after 4 years of observation

| Cox Model Analysis at 4 years | Hazard ratio | 95% CI | P value |
|---|--------------|-----------|---------|
| Randomization to intensified blood pressure control | 0.75 | 0.46-1.22 | 0.25 |
| Baseline GFR | 0.93 | 0.91-0.95 | <0.0001 |
| Urinary protein/creatinine ratio | 1.19 | 1.01-1.39 | 0.03 |
| Baseline 24-h mean arterial pressure SDS | 1.10 | 0.97-1.24 | 0.15 |
| Age | 1.06 | 1.00-1.13 | 0.07 |

Additional Information on Change of GFR During First 2 Ramipril Treatment Months

Mean estimated GFR dropped from 45.6 at baseline to 43.8 ml/min/1.73m² at 2 months. The observed drop by -2.1 ± 6.9 ml/min/1.73m² corresponded to a relative change of -5.3 ± 14.3 % (-46 to +38 %), respectively. No significant correlation was found between the changes in GFR and proteinuria during the first 2 treatment months.

From randomisation (prior to start of ramipril) through the end of the intervention, estimated GFR decreased by -2.8 ± 5.8 ml/year in the total cohort. The change was -2.6 ± 6.2 in the intensified treatment group and -3.1 ± 5.4 ml/year in the conventional treatment group (n.s.).

When only patients without ACE inhibitor pre-treatment during the run-in period were considered, the GFR slope changed from -2.7 ± 8.6 to -1.7 ± 8.3 ml/min/1.73m²/year during the study period (p=0.09).

Additional Information on Subgroup Analyses

The efficacy of the intervention was analyzed in individual subgroups of the study population. Pre-specified variables were pre-study progression rate, underlying renal disease group, gender, age, baseline creatinine, proteinuria and blood pressure level.

The renoprotective efficacy of the intensified blood pressure control was most marked in children with a baseline GFR of <45 ml/min/1.73 m², a pre-treatment GFR loss of >3 ml per year, 24-hour-MAP above the 90th percentile and proteinuria >0.5 mg/mg creatinine (Fig. 3 in the main manuscript). Only those patients in whom blood pressure had not decreased below the 50th percentile upon administration of ramipril alone by month 6 benefited from randomization for intensified treatment.

An initial proteinuria reduction by 50% achieved within the first two months of ramipril administration was highly predictive of renal survival (HR 0.458 (0.266-0.788, p=0.005)). Actuarial five-year survival was 81.1% in patients who achieved >50% initial proteinuria reduction vs. 60.3% in patients with a less marked response (p=0.004).

While patients with glomerulopathies had higher blood pressure both at baseline (2.9 vs. 1.3 SDS, p<0.001) and during the intervention (0.8 vs. -0.1 SDS; p<0.001) and required more add-on agents, the blood pressure change did not differ significantly among renal diagnosis subgroups. Among the different groups of underlying kidney disorders, intensified blood pressure control clearly improved renal survival in patients with glomerulopathies and was also effective in

children with renal hypo/dysplasia (Figures 2B and 3 of main manuscript). No significant effect was observed in patients with other congenital and hereditary nephropathies.

Additional Information on Blood Pressure and Proteinuria on Treatment

Within the group randomized for conventional BP control, 31 % of the patients who ‘inadvertently’ achieved a mean MAP < 50th percentile reached the primary endpoint, as compared to 47% of the patients who achieved a higher MAP (p=0.05). In the intensified BP control arm, the fraction of patients reaching the endpoint among those who achieved BP control < 50th percentile was 21%, i.e. not significantly different from the 31% fraction in the subgroup of patients achieving low-normal BP in the conventional BP control arm.

Appendix Table 3: Post-hoc analysis of the association of different thresholds of achieved blood pressure with 5-year renal survival.

| MAP threshold | 5-year renal survival in % | | |
|---------------|----------------------------|-------|--------|
| | below | above | p |
| 25 pct | 66.3 | 66.8 | 0.63 |
| 50 pct | 73.6 | 57.3 | 0.005 |
| 75 pct | 70.1 | 49.9 | 0.001 |
| 90 pct | 70.7 | 29.2 | <0.001 |
| 95 pct | 71.1 | 16.4 | <0.001 |

Cox Proportional Hazard analysis showed that the risk of attaining the composite endpoint was increased by 15.3 % for each mmHg above the 50th percentile (HR 1.153; CI 1.071-1.241, p < 0.0001), whereas any blood pressure below the 50th percentile did not significantly affect renal risk (HR 1.003, CI 0.898 – 1.119, p=0.96).

Calcium channel blockers were used as first choice antihypertensive co-medication (38%) followed by diuretics (36%) and β -blockers (26%) without differences between the randomization groups.

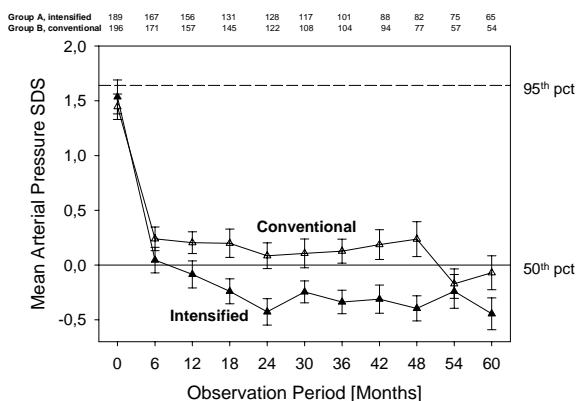
The evolution of blood pressure and proteinuria in the two randomization arms is shown in Appendix Fig. 4a/b. While blood pressure remained stable in both treatment groups, proteinuria increased both in the conventional and in the intensified treatment arm.

The rebound phenomenon was equally observed in patients with ramipril monotherapy and with antihypertensive co-medication. There was no group difference regarding age, diagnosis, blood pressure control at baseline and during intervention, administered ramipril dose, number of concomitant antihypertensive drugs, and the renal failure progression rate at baseline and during intervention.

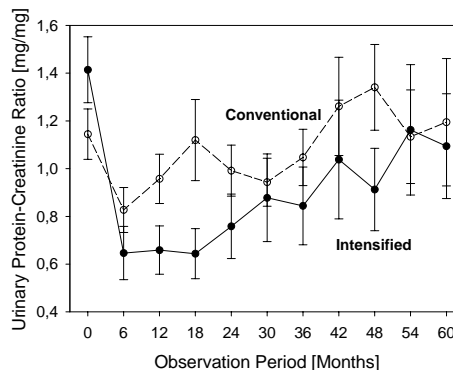
Appendix Fig. 4 a/b

Course of standardized 24h MAP (Fig 4a) and urinary protein excretion (Fig 4b) in patients with conventional and intensified blood pressure control. Data are mean \pm SEM.

a.



b.



Additional Information on Plasma ACE Activity during Intervention

Plasma ACE activity was measured at baseline and after 2, 6, 12, 24, 36 and 48 months of ramipril treatment. Plasma ACE activity was analyzed with temporal delay; patients were not actively withdrawn from the study based on this criterion. Non-adherence would have been suspected by an initial failure of ACE activity to decrease by more than 30% from baseline (3% of patients), or any rebound of ACE activity close to the baseline range during the study period (5%). The mean initial decrease was 78% from baseline. Plasma ACE activity decreased by

more than 50% in 90%, by more than 70% in 76% and by more than 80% in 68% of patients. In 80% of patients mean plasma ACE activity decreased to the normal range (<22.5 U/L).

Additional Safety Information

Effect of ACE inhibition on serum potassium levels

ACE inhibition increased mean serum potassium levels from 4.31 ± 0.52 to 4.71 ± 0.57 mmol/l (mean increase 0.40 ± 0.52 mmol/l; $p < 0.001$). In 348 out of 10,404 observations (3.3% of all tests) the pediatric upper limit of normal (5.6 mmol/l) was exceeded. In all but 5 patients adjustment of diet, addition of a diuretic or prescription of potassium exchange resins resulted in persistent normalization of serum potassium levels.

Effect of ACE inhibition on blood cell count

During the study period hemoglobin level decreased by 0.55 g/dl over time (12.2 ± 1.6 to 11.7 ± 1.6 g/dl; $p < 0.001$), probably due to progression of chronic renal failure. The fraction of patients on erythropoiesis stimulating agents increased from 9.7 % at baseline to 14.0 %.

White blood cells (7.02 ± 1.93 to 6.68 ± 1.65 /pl) and platelets (256 ± 71 to 246 ± 62 /pl; $p < 0.001$) decreased with time on treatment; however these changes were without clinical significance.

There were no significant differences between the randomization groups regarding the incidence or extent of laboratory changes.