Effects of Manidipine and Delapril in Hypertensive Patients With Type 2 Diabetes Mellitus

The Delapril and Manidipine for Nephroprotection in Diabetes (DEMAND) Randomized Clinical Trial

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See Editorial Commentary, pp XX–XX

Abstract—To assess whether angiotensin-converting enzyme inhibitors and third-generation dihydropyridine calcium channel blockers ameliorate diabetic complications, we compared glomerular filtration rate (GFR; primary outcome), cardiovascular events, retinopathy, and neuropathy in 380 hypertensive type 2 diabetics with albuminuria <200 mg/min included in a multicenter, double-blind, placebo-controlled trial (DEMAND [Delapril and Manidipine for Nephroprotection in Diabetes]) and randomized to 3-year treatment with manidipine/delapril combination (10/30 mg/d; n = 126), delapril (30 mg/d; n = 127), or placebo (n = 127). GFR was centrally measured by iohexol plasma clearance. Median monthly GFR decline (interquartile range [IQR]) was 0.32 mL/min per 1.73 m² (IQR: 0.16–0.50 mL/min per 1.73 m²) on combined therapy, 0.36 mL/min per 1.73 m² (IQR: 0.18–0.53 mL/min per 1.73 m²) on delapril, and 0.30 mL/min per 1.73 m² (IQR: 0.12–0.50 mL/min per 1.73 m²) on placebo (P = 0.87 and P = 0.53 versus combined therapy or delapril, respectively). Similar findings were observed when baseline GFR values were not considered for slope analyses. Albuminuria was stable in the 3 treatment groups. The hazard ratio (95% CI) for major cardiovascular events between combined therapy and placebo was 0.17 (0.04–0.78; P = 0.023). Among 192 subjects without retinopathy at inclusion, the hazard ratio for developing retinopathy between combined therapy and placebo was 0.27 (0.07–0.99; P = 0.048). Among 200 subjects with centralized neurological evaluation, the odds ratios for peripheral neuropathy at 3 years between combined therapy or delapril and placebo were 0.45 (0.24–0.87; P = 0.017) and 0.52 (0.27–0.99; P = 0.048), respectively. Glucose disposal rate decreased from 5.8 ± 2.4 to 5.3 ± 1.9 mg/kg per min on placebo (P = 0.03) but did not change on combined or delapril therapy. Treatment was well tolerated. In hypertensive type 2 diabetic patients, combined manidipine and delapril therapy failed to slow GFR decline but safely ameliorated cardiovascular disease, retinopathy, and neuropathy and stabilized insulin sensitivity. (Hypertension. 2011;58:00-00.) Online Data Supplement

Key Words: ACE inhibitors ■ calcium channel blockers ■ manidipine ■ diabetic nephropathy ■ cardiovascular complications ■ diabetic neuropathy ■ diabetic retinopathy

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Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II antagonists are the antihypertensive agents that more effectively reduce macrovascular disease and limit the onset and progression of nephropathy and retinopathy in subjects with diabetes mellitus. Data suggest that they can be also beneficial in diabetic peripheral neuropathy. Third-generation dihydropyridine calcium channel blockers, such as manidipine, that block T-type calcium channels localized on postglomerular arterioles, reduced albuminuria in hypertensive patients with type 2 diabetes mellitus, even when administered in combination with angiotensin inhibitors. This effect was likely explained by reduced postglomerular resistances and intraglomerular pressures, hemodynamic changes similar to those induced by angiotensin inhibitors.

An unresolved issue is whether combination therapy with a third-generation dihydropyridine calcium channel blocker and an ACE inhibitor is renoprotective in patients without overt renal disease and ameliorates cardiovascular disease and other microvascular complications of diabetes mellitus. Moreover, no trial thus far has evaluated the changes in glomerular filtration rate (GFR) over time and whether and to what extent these changes can be affected by treatment in this population. The multicenter, double-blind, placebo-controlled, randomized trial, DEMAND (Delapril and Manidipine for Nephroprotection in Diabetes), approached the above issues in hypertensive subjects with type 2 diabetes mellitus and normoalbuminuria or microalbuminuria.

Methods

DEMAND was an independent, academic trial conducted by the Mario Negri Institute in cooperation with 7 diabetology units in Italy and 1 in Slovenia. The protocol was in accordance with the Declaration of Helsinki and was approved by the study ethics committee (see Study Organization in the online Data Supplement at http://hyper.ahajournals.org) and the local ethics committees at each center according to Italian and Slovenian regulations. All of the authors had access to all of the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. No medical writer or editor was involved.

Subjects

We enrolled subjects ≥40 years of age with hypertension and known history of type 2 diabetes mellitus (World Health Organization criteria) <25 years of age, with urinary albumin excretion (UAE) <200 μg/min in ≥2 of 3 consecutive, sterile, overnight samples, and serum creatinine ≤1.5 mg/dL. Hypertension was defined as untreated systolic/diastolic blood pressure (BP) >130/85 mm Hg or concomitant antihypertensive therapy. Subjects with hemoglobin (Hb) A1C >11%, ischemic kidney disease, urinary tract obstruction, or urinary abnormalities suggestive for primary glomerular disease, or specific indications or contraindications to ACE inhibitor or calcium channel blocker therapy were excluded. All of the subjects provided written informed consent for study participation.

Study Design

After 12-week washout from angiotensin inhibitors or calcium antagonists and stratification for center and baseline UAE <20 μg/min or ≥20 and <200 μg/min, eligible subjects were randomly assigned to receive manidipine (10 mg/d) plus delapril (30 mg/d), delapril alone (30 mg/d), or placebo. Target BP was 120/80 mm Hg. Additional antihypertensive drugs were allowed to achieve target BP in the following steps: (1) hydrochlorothiazide, indapamide, or furosemide; (2) β- or α-β blockers; and (3) doxazosin, prazosin, clonidine hydrochloride, or α-methyldopa. Rescue therapy with captopril, minoxidil, or other medications had to be discussed with the study coordinator. Potassium-sparing diuretics and angiotensin inhibitors were not allowed. Subjects continued to receive their usual care for diabetes mellitus, including low-sodium (<100 mEq/d) diet. A target HbA1C <7% was recommended for all of the subjects.

Trial Outcomes

Primary efficacy outcome was the rate of GFR decline. Secondary outcomes included a prespecified composite end point of death from cardiovascular causes, sudden death, nonfatal myocardial infarction or stroke, coronary revascularization, amputation, or vascular surgery for peripheral atherosclerotic artery disease; and new onset, progression, or regression of retinopathy and peripheral neuropathy. All of the end points were adjudicated at the blind review by a clinical end point committee unaware of treatment assignment.

Timing of Evaluations

BP was measured at randomization and 1 week, 1 month, and 3 months after randomization, as well as every 3 months thereafter. Blood glucose, serum potassium, sodium, urea, and creatinine levels were assessed at randomization and every 3 months; GFR, UAE, and HbA1C levels, as well as serum lipids and other laboratory parameters were assessed at randomization and every 6 months; renal changes were assessed at randomization and every year. All of the evaluations were repeated after the last randomized subject completed 3 years of treatment and whenever subjects reached an end point (final visit). The total nephropathy score (TNS) was assessed at randomization and 3 years thereafter. The glucose disposal rate (GDR) was measured at randomization and at 1-year follow-up in all of the consenting subjects. Subjects with follow-up UAE ≥200 μg/min in ≥2 of 3 collections confirmed in 2 consecutive visits stopped the study drugs and were treated with an angiotensin II antagonist.

BP, Renal Function, and Glucose Disposal Rate Evaluations

The BP was measured at the brachial artery of the nondominant arm in the morning, before study drug administration and after 5-minute rest in the sitting position, by using a validated oscillometric device (Omron 705IT) and a standard cuff with a bladder of 12×22 cm when the arm circumference was ≤32 cm and of 15×31 cm when the circumference was larger. The mean of 3 measurements taken 2 minutes apart was considered for statistical analyses. Throughout the trial, in each patient the BP was always measured by the same device applied to the same arm by means of equally sized cuffs.

Albuminuria was measured by nephelometry (Beckman Array System) and HbA1C by ion-exchange high-performance liquid chromatography (normal range: 3.53% to 5.21%). GFR was directly measured by the iohexol plasma clearance technique, and iohexol plasma levels were assessed by a modified high-performance liquid chromatography method at the coordinating center. GFR was calculated as the mean of the glucose infusion rate per kilogram of body weight required to maintain steady-state euglycemia during the last 30 minutes of a hyperinsulinemic-euglycemic clamp.

Funduscopy

Fundus changes were assessed at the ophthalmology unit of the Azienda Ospedaliero by 2 ophthalmologists (L.P.I. and M.F.) blinded to study data with indirect binocular ophthalmoscopy performed by an L-0185 slit-lamp biomicroscope (magnification: ×10 and ×16) and hand-held lens (magnification: ×90). Photographs of 4 standard 30° fields of each eye were taken through dilated pupils in stereo pairs (lateral to macula, macula, disc, and nasal) with a Canon CF 60 UV fundus camera (Tokyo, Japan). Retinal involvement was graded from no apparent retinopathy to mild, moderate, or severe preproliferative retinopathy and to proliferative retinopathy. The eye with the higher grade was considered for analysis. New-onset retinopathy was diagnosed when any grade of retinopathy was observed in 2 consecutive evaluations in eyes with no retinopathy at
Neurological Evaluation
Peripheral nerve involvement at inclusion and 3 years was centrally graded by the same examiners blinded to study data (G.L., A.S., R.L., P.P., G.C., and M.L.P.) in the same patients by means of the TNS scale that combines symptoms (sensory, motor, and autonomic), signs (pin and vibration sensibility, strength, and tendon reflexes), vibratory threshold measured by the quantitative sensory testing, and nerve conduction study (sural and peroneal amplitude) findings. Quantitative sensory testing was performed at the first metatarsal phalanx of the big toe using the Computer-Assisted Sensory Examinator IV device (WR Medical Electronics Co, Stillwater, MN). Data were referred to the normative ranges provided by the software. Nerve conduction studies were performed at 25°C to 28°C room temperature and with controlled skin temperature using a Keypoint device (Medtronic, Copenhagen, Denmark). Peroneal nerve compound motor action potential amplitude (normal: >4 mV) and antidromic sural nerve action potential amplitude (peak-to-peak value; normal: >6 μV) were recorded in the dominant leg using surface stimulating and recording electrodes with standard placement.

Neuropathy was defined with TNS >2. Thus, new-onset neuropathy was diagnosed in subjects with TNS ≤2 at inclusion and >2 at 3 years and regression of neuropathy in subjects with TNS >2 at inclusion and ≤2 at 3 years.

Randomization and Blinding
The Chiesi Farmaceutici Statistical Unit created a computer-generated randomization list with a block of 6 patients assigned to each therapy with a 1:1:1 ratio. Randomization numbers were blindly assigned by the treatment assignment secretariat at the Mario Negri Institute (Ranica, Italy). Study treatments were externally nondistinguishable orange round tablets containing either delapril 15 mg plus manidipine 5 mg, delapril 15 mg, or placebo. Patients and investigators were all blinded throughout the study. Individual sealed envelopes containing the randomized treatment code were provided to each center and could be broken for safety reasons after discussion with the study coordinator.

Sample Size
Monthly GFR decline was expected to average 0.33±0.78 mL/min per 1.73 m² in subjects on placebo, whereas a stable GFR was expected in those on combined therapy. Ninety-one patients per group were estimated to provide 80% power to detect the expected difference between groups (unpaired t test, α=0.047). To account for an expected 20% of nonassessable subjects, 342 subjects had to be randomized. Also, those who, at randomization of the 342nd subject, did not meet entry criteria were randomized. This corresponds to a sample of 342 patients to be studied, with 114 patients in each arm.

Statistical Methods
Analyses were by intention to treat. Primary comparison was combined therapy versus placebo and secondary comparison of delapril versus placebo. The primary efficacy variable, GFR decline, was determined in subjects with baseline and ≥2 subsequent measurements. GFR changes over time were initially evaluated by a single slope linear model. However, to account for a possible confounding effect of acute GFR changes after baseline evaluation, additional explorative analyses also considered GFR slope from month 6 to study end without including baseline GFR values. Then a linear mixed model, with random intercept and slope, was used to estimate the rate of GFR loss and to compare serial GFR measurements over time. The model included the following predefined baseline covariates: country (Italy or Slovenia), age, sex, and log-transformed UAE (median of 3 readings). An interaction between treatment and time as fixed effect was considered to test whether the slopes vary according to treatment. In a secondary, exploratory analysis, the slope of decay of GFR was also calculated from the 6-month visit instead of baseline to remove the presence of potential acute glomerular hemodynamic effects. Hazard ratios for time to event and odds ratios for binary outcome variables (with corresponding 95% CI) were estimated by multivariable Cox and logistic regression, respectively. Proportional hazards assumption was checked by Schoenfeld residuals. Exploratory analyses models included baseline variables that, at univariate analyses, were significantly associated with considered outcomes. Mechanisms possibly explaining the observed treatment effects on considered outcomes were explored by including in the models baseline and follow-up HbA1C or mean, systolic, or diastolic BP considered separately. Continuous outcome variables assessed after randomization and groups were compared by a single overall test using longitudinal models, including the baseline value as covariate. Adverse events were classified using the Medical Dictionary for Regulatory Activities system (version 10.1). Multiple reports of the same event were counted only once, and the least favorable report was used. Fatal and nonfatal adverse events were separately reported according to treatment group and overall. Data were gathered by means of an electronic case report form, described elsewhere, and were exported and analyzed by SAS (version 9.1) and Stata (version 10.0) software. Data were presented as numbers and percentages, means (SD), or medians and interquartile ranges (IQR), as appropriate. All of the P values were 2 sided.

Results
Between May 2002 and June 2005, 380 subjects (311 from Italy and 69 from Slovenia) were randomly assigned and followed for a median of 3.8 years (IQR: 3.1–4.7 years; Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org). Patient characteristics (Table 1) and distribution of concomitant medications at randomization (Table S1) and follow-up duration were similar among treatment groups, as well as between subjects with or without slope or GDR data (data not shown).

A total of 258 and 243 subjects consented to centralized funduscopy and neuropathy evaluations, respectively. Their characteristics were similar to those of the whole study population and among treatment groups (Figure S2, Figure S3, Table S2, and Table S3).

BP and Metabolic Control
Throughout the study, systolic, diastolic, and mean BP averaged 137.2±10.0, 80.5±6.2, and 99.4±6.3 mm Hg subjects on combined therapy; 138.9±10.6, 81.2±5.3, and 100.5±5.7 mm Hg in the delapril group, and 139.5±9.9, 82.8±5.8, and 101.7±6.2 mm Hg in the placebo group, respectively (Figure 1). HbA1C levels averaged 6.07±1.31% in subjects on combined therapy; 6.12±1.15% in the delapril group, and 6.18±1.37% in the placebo group (Figure 1). Among the 127 subjects with hyperinsulinemic, euglycemic clamp, the GDR tended to decrease from 5.9±2.8 at randomization to 5.5±2.6 mg/kg per minute (P=0.09) at 1 year. This trend was largely explained by a significant reduction from 5.8±2.4 to 5.3±1.9 mg/kg per minute in the placebo group (P=0.03), whereas the GDR did not change significantly in the combination (5.9±2.9 versus 5.7±2.9 mg/kg per minute; P=0.74) and delapril (5.9±2.3 versus 5.6±2.9 mg/kg per minute; P=0.38) groups.

Outcomes
Kidney Function
In the study group as a whole, the GFR linearly declined by 0.32 mL/min per 1.73 m² (IQR: 0.15–0.52 mL/min per 1.73 m²) per month. The monthly rate of decline was similar on
combined therapy (0.32 mL/min per 1.73 m² [IQR: 0.16–0.50 mL/min per 1.73 m²]), delapril (0.36 mL/min per 1.73 m² [IQR: 0.18–0.53 mL/min per 1.73 m²]), or placebo (0.30 mL/min per 1.73 m² [IQR: 0.12–0.50 mL/min per 1.73 m²]; P < 0.87 and P < 0.53 versus combined therapy or delapril, respectively; Figure 1). GFR decline was similar (0.32 mL/min per 1.73 m² [IQR: 0.15–0.50 mL/min per 1.73 m²] versus 0.36 mL/min per 1.73 m² [IQR: 0.13–0.59 mL/min per 1.73 m²] per month; P < 0.93) between normoalbuminuric and microalbuminuric patients and among treatment arms within these 2 groups considered separately (data not shown). Similar findings were observed when baseline GFR values were not considered for slope analyses (combined therapy: 0.33 mL/min per 1.73 m² [IQR: 0.13–0.50 mL/min per 1.73 m²]; delapril: 0.33 mL/min per 1.73 m² [IQR: 0.15–0.50 mL/min per 1.73 m²]; placebo: 0.33 mL/min per 1.73 m² [IQR: 0.13–0.48 mL/min per 1.73 m²] per month; P = 0.57 and P = 0.95 versus combined therapy and delapril, respectively). No significant changes in albuminuria were observed in the 3 treatment groups throughout the follow-up period compared with baseline (Table S4).

Major Cardiovascular Events

Two (1.6%) of 126 patients on combined therapy had major fatal or nonfatal cardiovascular events compared with 6 (4.7%) of 127 on delapril alone and 10 (7.9%) of 127 on placebo. The hazard ratio between combined therapy and placebo was significant, even after adjustment for smoking habit and baseline and follow-up mean, systolic, or diastolic BP, and HbA1C, whereas the hazard ratio between delapril and placebo was not significant (Figure 2).

Retinopathy

Among the 192 subjects without retinal involvement at inclusion and with funduscopies available on follow-up, 3 (4.5%) on combined therapy developed retinopathy compared with 6 (9.1%) on delapril and 9 (15%) on placebo. The hazard ratio between combined therapy and placebo was significant, even after adjustment for baseline and follow-up mean, systolic, and diastolic BPs. The significance, however, was lost after adjusting for baseline and follow-up HbA1C. The hazard ratios between delapril and placebo were not significant (Figure 2). Among the 45 subjects with retinal involvement at inclusion, 5 had regression of retinopathy without significant differences among groups.

Neuropathy

Two-hundred subjects had TNS evaluation at 3 years. Of the 69 patients on combined therapy, 24 (34.8%) had peripheral
neuropathy versus 26 (41.3%) of the 63 on delapril and 39 (57.4%) of the 68 on placebo. The odds ratios between combined therapy or delapril and placebo were both significant, even after adjustment for baseline TNS (or diabetes mellitus duration); baseline and follow-up mean, systolic, or diastolic BP; and HbA1C (Figure 3). Among the 140 subjects without neuropathy at inclusion, 12 (23.5%) on combined therapy developed neuropathy at 3 years compared with 13 (28.9%) on delapril and 17 (38.6%) on placebo. The odds ratios between combined therapy or delapril and placebo were both significant after adjustment for baseline TNS (or diabetes mellitus duration) and baseline and follow-up mean BPs but were only marginally significant after adjustment for baseline and follow-up HbA1Cs (Figure 3).

Among the 60 subjects with neuropathy at inclusion, 6 (33.3%) on combined therapy had regression of neuropathy at 3 years compared with 5 (27.8%) on delapril and 2 (8.3%) on placebo. The odds ratio between combined therapy and placebo was significant even after adjustment for baseline TNS (or diabetes mellitus duration) and baseline and follow-up HbA1Cs (Figure 3).

**Adverse Events**

There were 2 sudden deaths in the placebo group (Table 2). Two subjects on combined therapy had major nonfatal cardiovascular events compared with 6 on delapril and 8 on placebo. Overall there were 2 subjects on combined therapy with major fatal or nonfatal cardiovascular events compared with 10 on placebo ($P<0.05$). Other events were similarly distributed among groups. No subject was prematurely withdrawn because of treatment-related adverse events.

**Discussion**

We found that, in a large cohort of hypertensive patients with type 2 diabetes mellitus and normoalbuminuria or microalbuminuria, GFR decline over 4 years of treatment by manidipine combined with delapril, delapril alone, or placebo was similar. Changes in UAE during the observation period were also similar among treatment groups. Compared with placebo, however, combined therapy significantly reduced the incidence of a prespecified composite end point of sudden death, death from cardiovascular causes, and nonfatal major cardiovascular events, including myocardial infarction, stroke, or coronary revascularization. Combined therapy also decreased the incidence of new cases of retinopathy, slowed the progression of peripheral neuropathy, and tended to accelerate its regression compared with placebo but did not appreciably affect cardiovascular events and progression of retinopathy. Study treatments were well tolerated.

Despite intensified BP and metabolic control, in the whole study cohort the GFR linearly declined by 0.32 mL/min per month, a rate exceeding by 3 to 6 times the rate reported in healthy subjects.21 This finding was not explained by acute GFR changes after randomization, because a similar rate of GFR loss was observed even when baseline GFR values were
This might explain why delapril therapy reduces incidence of macrovascular events on combined manidipine and delapril therapy was significant also when the analyses were adjusted for baseline and follow-up BP and HbA1C levels corroborates the robustness of the results and suggests that this regimen may have specific cardioprotective effects independent of, or in addition to, improved BP and metabolic control. Based on our present data, we estimated that 16 subjects (95% CI: 8–111 subjects) had to be treated to prevent one major cardiovascular event prevented compared with placebo. These findings formally extend to diabetic subjects evidence from the Avoiding Cardiovascular Events Through Combination Therapy in Patients Living With Systolic Hypertension Study that third-generation calcium channel blockers have specific cardioprotective effects when added on ACE inhibitor therapy in hypertensive subjects at increased cardiovascular risk. This may have clinical implications, because age-adjusted prevalence of cardiovascular mortality is twice as high among subjects with diabetes mellitus as among those without. Moreover, macrovascular complications are a major cause of morbidity in the diabetic population and have an enormous economic burden.

Amelioration in metabolic and, to a lesser extent, BP control appeared to account for at least part of the effect of combined manidipine and delapril therapy on retinopathy and peripheral neuropathy. Because antidiabetic therapy was similar among treatment groups, the observed differences in HbA1C levels could be explained by concomitant differences in insulin sensitivity. Independent of metabolic and BP control, insulin resistance has been suggested to play a major role in the development of diabetic nephropathy.
role in onset and progression of nephropathy, cardiovascular
disease, and other chronic complications of diabetes mellitus.15,22,23 To address whether treatment effect on insulin
sensitivity might have affected the above outcomes, we
directly measured the GDR, taken as a direct indicator of
insulin function. This effect could be explained by different
mechanisms: (1) reduced need for concomitant treatment
with drugs that may worsen insulin sensitivity, such as
diuretics and β-blockers22; (2) direct effects of ACE inhibi-
tion on insulin sensitivity22; and (3) additional effect in the
combined therapy group of manidipine-mediated activation of adipocyte peroxisome proliferator-activated receptor-γ.9

**Limitations and Strengths**

Numbers of patients and events were relatively small. Al-
though prespecified, cardiovascular outcomes were second-
ary efficacy variables and should be considered as hypothesis
generating. On the other hand, time course of chronic
complications of diabetes mellitus was described by using
gold standard techniques, which increased the power of the
analyses. The adequacy of the sample size was supported by
the consistency between observed and predicted GFR decline in controls. Thus, the fast GFR decline observed in our
patients excluded a lower than expected statistical power as a
possible explanation for the failure to detect a treatment effect on this outcome. Actually, virtually identical GFR declines in
the 3 treatment arms were unlikely explained by a type 2 error
and most likely reflected a true ineffectiveness of study
treatment compared with placebo on the considered outcome.
The 95% CI of the hazard ratio for major cardiovascular
events showed that, even in the less optimistic hypothesis, the
risk reduction on combined therapy compared with placebo

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**Table 2. Patients With ≥1 Serious Adverse Event in Each Treatment Group**

<table>
<thead>
<tr>
<th>Serious Adverse Event</th>
<th>Manidipine plus Delapril (N = 126)</th>
<th>Delapril (N = 127)</th>
<th>Placebo (N = 127)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fatal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major cardiovascular event*</td>
<td>0</td>
<td>0</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Cancer</td>
<td>2 (1.6)</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td><strong>Nonfatal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major cardiovascular event*</td>
<td>2 (1.6)</td>
<td>6 (4.7)</td>
<td>8 (6.3)</td>
</tr>
<tr>
<td>Cancer</td>
<td>7 (5.6)</td>
<td>2 (1.6)</td>
<td>8 (6.3)</td>
</tr>
<tr>
<td>Other cardiovascular event</td>
<td>2 (1.6)</td>
<td>2 (1.6)</td>
<td>3 (2.4)</td>
</tr>
<tr>
<td>Other serious adverse event</td>
<td>12 (9.5)</td>
<td>13 (10.2)</td>
<td>8 (6.3)</td>
</tr>
</tbody>
</table>

*P<0.05 for fatal and nonfatal major cardiovascular events in manidipine plus delapril vs placebo.
was clinically relevant (22%). Differences between combined therapy and placebo were consistent for all of the events considered in this study. The results have a large external validity, at least within the white ethnicity, because selection criteria for study participation allowed for identification of a study population with clinical characteristics, such as hypertension and normoalbuminuria or microalbuminuria, that are common for the large majority of subjects with type 2 diabetes mellitus. Moreover, this is the first study formally evaluating GFR decline in a large cohort of patients with normal renal function and no evidence of nephropathy to start with.

Perspectives
In hypertensive type 2 diabetic patients with normoalbuminuria or microalbuminuria, combined manidipine and delapril therapy failed to slow renal function loss but significantly ameliorated cardiovascular disease, retinopathy, peripheral neuropathy, and insulin sensitivity compared with placebo. These findings may provide the background for large and long-term randomized, controlled trials to test the effect of treatment on hard clinical outcomes.

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Disclosures
None.

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Effects of Manidipine and Delapril in Hypertensive Patients with Type 2 Diabetes:
The DEMAND Randomized Clinical Trial

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Short title: DEMAND Trial in hypertensive diabetics

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Appendix

DE.M.A.N.D. STUDY GROUP

Principal investigator - G. Remuzzi (Bergamo - Italy); Study coordinator - Piero Ruggenenti (Bergamo - Italy); Coordinating center - Mario Negri Institute for Pharmacological Research, Clinical Research Center for Rare Diseases Aldo e Cele Daccò (Ranica, Bergamo – Italy); Participating centers – A. Bossi, A. Parvanova Ilieva, I. Petrov Iliev, S. Yakymchuk (Treviglio, Bergamo – Italy); C. Chiurchiu, S. Rota, F. Arnoldi, S. Prandini (Ranica, Bergamo – Italy); A. Kocijancic, J. Zaletel, D. Pongrac, J. Prezelj, T. Gala, M. Kersnik (Ljubljana – Slovenia); G. Trevisan, G. Lepore, A. Fassi, E. Mondo (Bergamo – Italy); F. Inversi, R. Mangili, S. Bruno (Seriate, Bergamo – Italy); A. Bossi, V. Brusegan, A. Parvanova Ilieva, I. Petrov Iliev, V. Lecchi (Romano, Bergamo – Italy); A. Belviso, I. Petrov Iliev (Ponte San Pietro, Bergamo – Italy); S. Genovese (Rozzano, Milano – Italy); Peripheral neuropathy evaluations – G. Lauria, A. Sghirlanzoni, D. Pareyson, R. Lombardi, F. Camozzi, P. Penza (Milano - Italy), G. Cavaletti, M.L. Piatti, L. Marzorati, P. Marmirol, B. Frigeni, L. Mattavelli (Monza – Italy); Funduscopy evaluations – I. Petrov Iliev (Ranica, Bergamo – Italy), M. Filipponi, S. Tadini (Bergamo – Italy); Study monitoring and investigational medicinal product distribution (Mario Negri Institute) – N. Rubis, G. Gherardi, W. Calini, O. Diadei, G. Noris, D. Rossoni, D. Villa (Ranica, Bergamo – Italy); Electronic Case Report Form development and data management – B. Ene-Iordache, S. Carminati, E. Agus, A. Remuzzi (Ranica, Bergamo Italy); Treatment assignment secretariat – G. A. Giuliano (Ranica, Bergamo, Italy); Data analyses – A. Perna, N. Motterlini, M. Ganeva (Ranica, Bergamo – Italy); Laboratory measurements (Mario Negri Institute) – F. Gaspari, A. N. Cannata, F. Carrara, C. Cella, E. Centemeri, S. Ferrari, C. Petrò, E. Savoldelli, N. Stucchi (Ranica, Bergamo – Italy); Administrative and Ethical Committee procedures (Mario Negri Institute) – P. Boccardo (Ranica, Bergamo – Italy); Pharmacovigilance – N. Perico, N. Rubis; Study secretariat – S. Peracchi (Ranica, Bergamo – Italy); Carriers – S. Gelmi (Ranica, Bergamo – Italy); Steering committee – G. Remuzzi (Bergamo – Italy), P. Ruggenenti (Bergamo – Italy), G. Mecca (Bergamo – Italy), A. Perna (Ranica, Bergamo – Italy), A. Sghirlanzoni (Milano – Italy), B. Imbimbo, M. Alberici, F. Gardini (Chiesi Farmaceutici S.p.A., Parma – Italy); Independent Ethics committee – Comitato di Bioetica della Azienda Sanitaria Locale della Provincia di Bergamo (Bergamo – Italy); Clinical endpoint adjudicating committee – Cardiovascular Events: P. Ruggenenti and G. Remuzzi (Bergamo – Italy).
– Italy), Retinopathy: M. Filipponi (Bergamo– Italy) and I. Petrov Iliev (Ranica, Bergamo – Italy),
Neuropathy: A. Sghirlanzoni and G. Lauria (Milano – Italy)
Table S1. Treatments in Patients Randomly Assigned to Study Drugs at Baseline and during Follow-up

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Manidipine + Delapril (N=126)</th>
<th>Delapril (N=127)</th>
<th>Placebo (N=127)</th>
<th>Manidipine + Delapril (N=126)</th>
<th>Delapril (N=126)</th>
<th>Placebo (N=127)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose-lowering regimen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet alone</td>
<td>13 (10.3)</td>
<td>17 (13.4)</td>
<td>14 (11.0)</td>
<td>4 (3.2)</td>
<td>3 (2.4)</td>
<td>4 (3.1)</td>
</tr>
<tr>
<td>Oral hypoglycemic agent alone</td>
<td>87 (69.0)</td>
<td>79 (62.2)</td>
<td>86 (67.0)</td>
<td>49 (38.9)</td>
<td>50 (39.4)</td>
<td>54 (42.5)</td>
</tr>
<tr>
<td>Insulin alone</td>
<td>9 (7.1)</td>
<td>12 (9.4)</td>
<td>13 (10.2)</td>
<td>12 (9.5)</td>
<td>14 (11.0)</td>
<td>17 (13.4)</td>
</tr>
<tr>
<td>Insulin and oral hypoglycemic agent</td>
<td>15 (11.9)</td>
<td>15 (11.8)</td>
<td>10 (7.9)</td>
<td>22 (17.1)</td>
<td>20 (15.7)</td>
<td>23 (18.1)</td>
</tr>
<tr>
<td><strong>Antihypertensive agents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>58 (46.0)</td>
<td>55 (43.3)</td>
<td>55 (43.3)</td>
<td>100 (78.7)†</td>
<td>99 (78.6)‡</td>
<td>114 (89.8)</td>
</tr>
<tr>
<td>Diuretic</td>
<td>29 (23.0)</td>
<td>33 (26.0)</td>
<td>36 (28.3)</td>
<td>63 (49.6)</td>
<td>67 (53.2)</td>
<td>77 (60.6)</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>20 (15.9)</td>
<td>18 (14.2)</td>
<td>24 (18.9)</td>
<td>42 (33.1)</td>
<td>48 (38.1)</td>
<td>57 (44.9)</td>
</tr>
<tr>
<td>Calcium-channel blocker (dihydropyridine)</td>
<td>2 (1.6)</td>
<td>0</td>
<td>1 (0.8)</td>
<td>9 (7.1)</td>
<td>7 (5.6)</td>
<td>9 (7.1)</td>
</tr>
<tr>
<td>Calcium-channel blocker (non dihydropyridine)</td>
<td>1 (0.8)</td>
<td>0</td>
<td>0</td>
<td>3 (2.4)</td>
<td>0</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Sympatholytic agent</td>
<td>31 (24.6)</td>
<td>32 (25.2)</td>
<td>18 (14.2)</td>
<td>69 (54.3)†</td>
<td>66 (52.4)‡</td>
<td>85 (66.9)</td>
</tr>
<tr>
<td><strong>Lipid-lowering agents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>41 (32.5)</td>
<td>38 (29.9)</td>
<td>43 (33.9)</td>
<td>46 (36.5)</td>
<td>40 (31.5)</td>
<td>42 (33.1)</td>
</tr>
<tr>
<td>Statin alone</td>
<td>33 (26.2)</td>
<td>31 (24.4)</td>
<td>32 (25.2)</td>
<td>35 (27.8)</td>
<td>34 (26.8)</td>
<td>32 (25.2)</td>
</tr>
<tr>
<td>Fibrate alone</td>
<td>5 (4.0)</td>
<td>3 (2.4)</td>
<td>6 (4.7)</td>
<td>4 (3.2)§</td>
<td>0</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Statin and fibrate</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Antiplatelet agent</strong></td>
<td>8 (6.3)</td>
<td>23 (18.1)</td>
<td>11 (8.7)</td>
<td>14 (11.1)</td>
<td>15 (11.8)</td>
<td>11 (8.7)</td>
</tr>
</tbody>
</table>

* All differences between the treatment groups and the placebo group, other than those shown, were not significant
† p<0.05 vs Placebo, ‡ p<0.05 vs Placebo, § p<0.05 vs Delapril alone.
## Table S2. Baseline Characteristics of Patients with Funduscopy Evaluations Randomly Assigned to Study Drugs*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Manidipine plus Delapril (N=90)</th>
<th>Delapril (N=81)</th>
<th>Placebo (N=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age - yr</td>
<td>60.1 (7.8)</td>
<td>61.3 (7.6)</td>
<td>60.4 (7.3)</td>
</tr>
<tr>
<td>Male sex – no. (%)</td>
<td>60(66.7)</td>
<td>56(69.1)</td>
<td>66(75.9)</td>
</tr>
<tr>
<td>Body-mass index †</td>
<td>29.6(4.8)</td>
<td>29.6(4.1)</td>
<td>29.2(4.5)</td>
</tr>
<tr>
<td>Known duration of diabetes - yr</td>
<td>5(3-12)</td>
<td>5(3-11)</td>
<td>7(4-12)</td>
</tr>
<tr>
<td>Smoking status – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>36(40.0)</td>
<td>37(45.7)</td>
<td>41(47.1)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>38(42.2)</td>
<td>36(44.4)</td>
<td>36(41.4)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>16(17.8)</td>
<td>8(9.9)</td>
<td>10(11.5)</td>
</tr>
<tr>
<td>Trough blood pressure - mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>149.1(14.4)</td>
<td>146.8(12.2)</td>
<td>147.4(14.5)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>88.5(8.7)</td>
<td>88.1(6.6)</td>
<td>89.1(10.2)</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>108.7(9.3)</td>
<td>107.7(7.3)</td>
<td>108.5(10.1)</td>
</tr>
<tr>
<td>Glycosylated hemoglobin - %‡</td>
<td>5.9(1.6)</td>
<td>5.7(1.2)</td>
<td>5.8(1.4)</td>
</tr>
<tr>
<td>Glucose - mg/dl</td>
<td>158.4(40.7)</td>
<td>161.4(38.7)</td>
<td>174.7(45.6)</td>
</tr>
<tr>
<td>Triglycerides - mg/dl §</td>
<td>134.9(70.8)</td>
<td>119.1(59.9)</td>
<td>134.3(66.2)</td>
</tr>
<tr>
<td>Cholesterol - mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>192.8(34.4)</td>
<td>197.2(33.6)</td>
<td>191.3(32.2)</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>148.5(32.1)</td>
<td>150.4(33.9)</td>
<td>146.6(30.4)</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>44.4(11.8)</td>
<td>47.4(11.8)</td>
<td>44.7(10.2)</td>
</tr>
<tr>
<td>Serum creatinine - mg/dl ¶</td>
<td>0.88(0.16)</td>
<td>0.93(0.21)</td>
<td>0.92(0.16)</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min/1.73m²)</td>
<td>102.6(19.0)</td>
<td>97.3(21.6)</td>
<td>99.6(17.6)</td>
</tr>
<tr>
<td>Urinary albumin excretion - µg/min</td>
<td>5.47 (3.45-12.44)</td>
<td>5.06 (3.61-12.11)</td>
<td>6.62 (4.33-14.57)</td>
</tr>
<tr>
<td>With microalbuminuria - no. (%)</td>
<td>13(14.4)</td>
<td>13(16.05)</td>
<td>12(13.8)</td>
</tr>
<tr>
<td>With retinopathy no. (%)</td>
<td>19(21.1)</td>
<td>9(11.1)</td>
<td>22(25.3)</td>
</tr>
</tbody>
</table>

* Values shown are mean (SD) or median (interquartile range) or number (%)

† The body-mass index is the weight in kilograms divided by the square of the height in meters
‡ Glycosylated hemoglobin was measured by ion-exchange high-performance liquid chromatography (normal range, 3.5 to 5.2 percent).
§ To convert values for triglycerides to millimoles per liter, multiply by 0.01129
|| To convert values for cholesterol to millimoles per liter, multiply by 0.02586
¶ To convert values for serum creatinine to micromoles per liter, multiply by 88.4
**Table S3. Baseline Characteristics of Patients with Available TNS Evaluations Assigned to Study Drugs**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Manidipine plus Delapril (N=81)</th>
<th>Delapril (N=80)</th>
<th>Placebo (N=82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age - yr</td>
<td>59.7 (7.9)</td>
<td>61.6 (7.3)</td>
<td>59.9 (7.2)</td>
</tr>
<tr>
<td>Male sex – no. (%)</td>
<td>51(63.0)</td>
<td>57(71.25)</td>
<td>63(76.8)</td>
</tr>
<tr>
<td>Body-mass index †</td>
<td>29.6(5.2)</td>
<td>29.3(4.1)</td>
<td>29.5(4.8)</td>
</tr>
<tr>
<td>Known duration of diabetes – yr</td>
<td>5(3-11)</td>
<td>5(3-9)</td>
<td>6(4-11)</td>
</tr>
<tr>
<td>Smoking status – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>34(42.0)</td>
<td>40(50.0)</td>
<td>36(43.9)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>34(42.0)</td>
<td>32(40.0)</td>
<td>37(45.1)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>13(16.0)</td>
<td>8(10.0)</td>
<td>9(11.0)</td>
</tr>
<tr>
<td>Trough blood pressure - mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>148.3(14.6)</td>
<td>146.2(14.6)</td>
<td>147.4(14.7)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>88.2(8.7)</td>
<td>88.1(7.7)</td>
<td>89.1(10.1)</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>108.2(9.4)</td>
<td>107.5(8.8)</td>
<td>108.5(10.3)</td>
</tr>
<tr>
<td>Glycosylated hemoglobin - %‡</td>
<td>5.9(1.6)</td>
<td>5.7(1.2)</td>
<td>5.7(1.3)</td>
</tr>
<tr>
<td>Glucose - mg/dl</td>
<td>158.6(40.2)</td>
<td>161.1(40.3)</td>
<td>170.6(43.8)</td>
</tr>
<tr>
<td>Triglycerides - mg/dl §</td>
<td>131.0(73.2)</td>
<td>113.6(52.2)</td>
<td>133.4(64.4)</td>
</tr>
<tr>
<td>Cholesterol - mg/dl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>190.7(34.9)</td>
<td>200.8(34.6)</td>
<td>189.1(32.0)</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>146.9(31.0)</td>
<td>153.7(34.9)</td>
<td>145.7(29.5)</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>43.9(12.1)</td>
<td>47.6(11.4)</td>
<td>43.5(10.0)</td>
</tr>
<tr>
<td>Serum creatinine - mg/dl ¶</td>
<td>0.87(0.15)</td>
<td>0.93(0.20)</td>
<td>0.92(0.16)</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min/1.73m²)</td>
<td>105.4(18.6)</td>
<td>97.4(20.6)</td>
<td>100.3(18.2)</td>
</tr>
<tr>
<td>Urinary albumin excretion - µg/min</td>
<td>5.08 (3.22-10.64)</td>
<td>5.45 (3.63-11.60)</td>
<td>6.73 (4.41-13.96)</td>
</tr>
<tr>
<td>With microalbuminuria - no. (%)</td>
<td>9(11.1)</td>
<td>11(13.8)</td>
<td>11(13.4)</td>
</tr>
<tr>
<td>With neuropathy - no. (%)</td>
<td>21(25.9)</td>
<td>24(30.0)</td>
<td>32(39.0)</td>
</tr>
</tbody>
</table>

* Values shown are mean (SD) or median (interquartile range) or number (%)
† The body-mass index is the weight in kilograms divided by the square of the height in meters
‡ Glycosylated hemoglobin was measured by ion-exchange high-performance liquid chromatography (normal range, 3.5 to 5.2 percent).
§ To convert values for triglycerides to millimoles per liter, multiply by 0.01129
|| To convert values for cholesterol to millimoles per liter, multiply by 0.02586
¶ To convert values for serum creatinine to micromoles per liter, multiply by 88.4
Table S4. Progression to micro/macroalbuminuria and regression to normoalbuminuria

<table>
<thead>
<tr>
<th>Patients</th>
<th>Delapril+Manidipine</th>
<th>Delapril alone</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>progressors s</td>
<td>progressors s</td>
<td>progressors s</td>
</tr>
<tr>
<td>Overall</td>
<td>16 (12.7%)</td>
<td>13 (10.2%)</td>
<td>13 (10.2%)</td>
</tr>
<tr>
<td>Normo-albuminurics</td>
<td>10 (9.3%)</td>
<td>12 (10.9%)</td>
<td>12 (10.9%)</td>
</tr>
<tr>
<td>Micro-albuminurics</td>
<td>6 (33.3%)</td>
<td>1 (5.9%)</td>
<td>7 (41.2%)</td>
</tr>
</tbody>
</table>

*Progressors:* progression to micro/macroalbuminuria  
*Regressors:* regression to normoalbuminuria  
No difference among the three treatment arms was statistically significant
Enrollment

Assessed for eligibility (n=379)

Excluded (n=121)
- Fundus not available (n=121)
- Declined to participate (n=0)
- Other reasons (n=0)

Fundus available (n=258)

Allocation

208 Patients without retinopathy

Allocated to delapril (n=72)
- Received allocated intervention (n=72)
- Did not receive allocated intervention (n=0)

Allocated to placebo (n=65)
- Received allocated intervention (n=65)
- Did not receive allocated intervention (n=0)

Allocated to manidipine plus delapril (n=19)
- Received allocated intervention (n=19)
- Did not receive allocated intervention (n=0)

Allocated to delapril (n=9)
- Received allocated intervention (n=9)
- Did not receive allocated intervention (n=0)

Allocated to placebo (n=22)
- Received allocated intervention (n=22)
- Did not receive allocated intervention (n=0)

50 Patients with retinopathy

Allocated to delapril (n=71)
- Received allocated intervention (n=71)
- Did not receive allocated intervention (n=0)

Allocated to placebo (n=65)
- Received allocated intervention (n=65)
- Did not receive allocated intervention (n=0)

Allocated to manidipine plus delapril (n=19)
- Received allocated intervention (n=19)
- Did not receive allocated intervention (n=0)

Allocated to delapril (n=9)
- Received allocated intervention (n=9)
- Did not receive allocated intervention (n=0)

Allocated to placebo (n=22)
- Received allocated intervention (n=22)
- Did not receive allocated intervention (n=0)

Follow-Up

- Lost to follow-up (n=0)
- Discontinued intervention (n=5)

- Lost to follow-up (n=0)
- Discontinued intervention (n=6)

- Lost to follow-up (n=0)
- Discontinued intervention (n=5)

- Lost to follow-up (n=0)
- Discontinued intervention (n=3)

- Lost to follow-up (n=0)
- Discontinued intervention (n=1)

Analysis

- Analysed (n=66)
- Excluded from analysis (n=5)
- Discontinued intervention (n=5)

- Analysed (n=66)
- Excluded from analysis (n=6)
- Discontinued intervention (n=6)

- Analysed (n=60)
- Excluded from analysis (give reasons) (n=5)
- Discontinued intervention (n=5)

- Analysed (n=16)
- Excluded from analysis (give reasons) (n=1)
- Discontinued intervention (n=1)

- Analysed (n=8)
- Excluded from analysis (give reasons) (n=3)
- Discontinued intervention (n=3)

- Analysed (n=21)
- Excluded from analysis (give reasons) (n=1)
- Discontinued intervention (n=1)

Figure S2 - Flow-chart of the Retinopathy Analysis Cohort
Figure S3 - Flow-chart of the Peripheral Neuropathy Analysis Cohort