Normotensive Women With Type 2 Diabetes and Microalbuminuria Are at High Risk for Macrovascular Disease

ADRIENNE A.M. ZANDBERGEN, MD
ERIC J. SIJBRANDS, MD, PHD
STEVEN W. LAMBERTS, MD, PHD
AART H. BOOTSMA, MD, PHD

OBJECTIVE — The excess risk of macrovascular disease and death associated with diabetes seems higher in women than in men. The pathogenesis for this risk difference has not been fully elucidated. We investigated whether female sex was associated with macrovascular disease and death, independently of known risk factors related to type 2 diabetes, nephropathy, or retinopathy in normotensive patients with type 2 diabetes and microalbuminuria.

RESEARCH DESIGN AND METHODS — We conducted a prospective, prolonged follow-up study of a subgroup of 67 diabetic patients (46 men and 21 women) without established cardiovascular disease who participated in a larger clinical trial. Data were collected on current and past health, medication use, blood pressure, renal function, and HbA_{1c} during the follow-up period of 4.7 ± 0.8 (means ± SE) years. The end point was a composite of death, cardiovascular disease, cerebrovascular events, and peripheral artery disease.

RESULTS — Of the women, eight (38.1%) met the end point compared with six (13.4%) of the men ($P = 0.02$ for difference in event-free survival). The hazard ratio of women relative to men was 3.19 (95% CI 1.11–9.21), which further increased after adjusting for age, systolic blood pressure, BMI, smoking, total-to-HDL cholesterol ratio, urinary albumin excretion, and retinopathy.

CONCLUSIONS — In our study population of normotensive patients with type 2 diabetes and microalbuminuria, female sex was associated with increased risk of fatal and nonfatal cardiovascular disease, independent of the classical cardiovascular risk factors, the severity of nephropathy or presence of retinopathy, or health care utilization.

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Type 2 diabetes, with its rapidly growing prevalence, has become a major public health problem (1). It associates with an increased risk of coronary heart disease, cerebrovascular disease, and peripheral vascular disease (2,3). Strikingly, this excess risk for cardiovascular disease seems relatively higher for female than for male diabetic patients (3–6). Type 2 diabetes substantially contributes to the 500,000 women dying annually of cardiovascular disease in the U.S. It is unclear whether the difference in cardiovascular risk between the sexes is caused by known cardiovascular risk factors (like duration of diabetes and systolic blood pressure) or by lower health care utilization by women compared with men (7,8).

In the present study, we extended the prospective follow-up of normotensive patients with type 2 diabetes and microalbuminuria who participated in a double-blind, randomized, placebo-controlled trial (9). The original study excluded patients with a history of cardiovascular disease, and the prolonged follow-up study removed differences in health care utilization among the participants.

In this study, we investigated whether female sex was associated with cardiovascular disease, cerebrovascular disease, peripheral artery disease, and death, independently of the classical cardiovascular risk factors related to type 2 diabetes, nephropathy, and retinopathy.

RESEARCH DESIGN AND METHODS

— The present study is a prolonged follow-up study of a subgroup of patients ($n = 67$) who participated in a larger clinical trial. The study design, methods, and results of this randomized, double-blind, placebo-controlled multicenter clinical trial have been previously published (9). In the original trial, we investigated the effects of short-term treatment with the angiotensin receptor antagonist losartan in normotensive patients with type 2 diabetes and microalbuminuria. After the 20-week study period, 67 patients who had been recruited from hospitals in the city of Rotterdam and surrounding areas received standard medical care. During follow-up, we collected the data on current and past health, medication use, blood pressure, and laboratory results of assessment of renal function and HbA_{1c} (A1C). The study was performed according to the guidelines of good clinical practice and was approved by the institutional review board. All subjects gave written informed consent before participation.

The eligibility criteria have been described in detail (9). Briefly, patients with type 2 diabetes, urinary albumin excretion from 20 to 200 mg/l, and office measurement of blood pressure ≤150/90 mmHg were randomized. Type 2 diabetes was defined as diabetes diagnosed at age >30 years or controlled by diet or blood glucose–lowering agents for at least 6 months. The current definition of normotension is blood pressure <140/90 mmHg, with a blood pressure–lowering target ≤130/80 mmHg in adults with diabetes (10,11). However, the protocol of this study was designed in 1999, when normotension was defined as blood pressure ≤160/90 mmHg during office measurement. Our criteria resulted in a mean baseline blood pressure of 135/81 mmHg,

From the Department of Internal Medicine, Erasmus University Medical Centre, Rotterdam, the Netherlands.

Address correspondence and reprint requests to Adrienne A.M. Zandbergen, MD, Erasmus University Medical Centre, Department of Internal Medicine, Dr Molewaterplein 40 3015 GD, Rotterdam, Netherlands. E-mail: adrienne_zandbergen@yahoo.com.

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Characteristics of men and women at baseline and after a 5-year follow-up*

	Men	Women	*P* value
n	46	21	
Baseline			
Age (years)	56.0 ± 1.8	54.0 ± 2.8	0.5
Duration of diabetes (years)	14.3 ± 1.5	15.7 ± 1.7	0.5
A1C (%)	8.1 ± 1.3	8.0 ± 1.0	0.7
BMI (kg/m^2)	27.7 ± 0.7	31.6 ± 1.2	0.008
Systolic blood pressure (mmHg)	133.9 ± 1.7	139.2 ± 1.9	0.04
Diastolic blood pressure (mmHg)	79.4 ± 1.1	81.6 ± 1.6	0.3
Total-to-HDL cholesterol ratio	5.38 ± 0.3	4.6 ± 0.3	0.06
Current smoking (%)	30	38	0.6
Serum creatinine (μmol/l)	84.8 ± 2.3	74.0 ± 4.7	0.05
Urinary albumin excretion (mg/l)	70.4 ± 6.1	81.8 ± 11.3	0.3
Retinopathy (%)	27	48	0.1
5-year follow-up			
A1C (%)	8.6 ± 1.4	8.3 ± 1.0	0.4
Systolic blood pressure (mmHg)	139.5 ± 2.6	140.3 ± 3.5	0.9
Diastolic blood pressure (mmHg)	79.9 ± 6.5	77.2 ± 7.8	0.2
Serum creatinine (μmol/l)	89.0 ± 3.8	81.3 ± 4.5	0.2
Urinary albumin excretion (mg/l)	133.7 ± 40.1	124.4 ± 34.2	0.9
Antihypertensive medication (%)	89	100	0.03
Aspirin (%)	23.3	28.6	0.7
Statin use (%)	55	48	0.6

Data are means ± SE.

which can be regarded as normotensive at present.

The main exclusion criteria included a history of myocardial infarction or cerebrovascular events, unstable angina pectoris, and heart failure. Patients with electrocardiogram abnormalities, including left ventricular hypertrophy, acute renal failure, chronic glomerulonephritis, polycystic kidney disease, serum creatinine level >150 μmo/l, A1C >10%, or concomitant use of antihypertensive agents, steroids, or lithium, were excluded as well.

End points

The end point of the present study was a composite of death, acute myocardial infarction, unstable angina pectoris, coronary interventions, heart failure, cerebral ischemic stroke or transient ischemic attack, and peripheral artery disease (peripheral arterial bypass graft, peripheral percutaneous transluminal angioplasty or other percutaneous invasive intervention, or intermittent claudication defined as classical symptoms in combination with at least one unequivocal result of one of the following: ankle/arm index <0.9 or a stenosis [>50%] on an angiogram or duplex scan). Patients with a history of cardiovascular disease were excluded from the original trial, restricting our observations to incident events.

Statistical analyses

Continuous variables are presented as means ± SE and discrete variables as frequencies and percentages. The independent Student's *t* test was used for comparisons between men and women. Cumulative event-free survival was analyzed with the Kaplan-Meier method and the log-rank test, as well as with Cox regression. The 95% CI of the hazard ratio was calculated as the exponent of the regression coefficient and its SE.

As we questioned whether female sex was an independent risk factor for macrovascular outcome in our study population, we used clinical variables, important with regard to macrovascular complications in type 2 diabetes, which were significantly different between the sexes at baseline in our multiple Cox regression models. We also adjusted for other classical cardiovascular risk factors. Two Cox regression models were performed to investigate whether the level of baseline albuminuria or the presence of retinopathy, respectively, could explain for possible sex differences, thereby being able to serve as risk indicators of those patients with the highest cardiovascular risk. Statistical significance was assessed at the 5% level of probability. We used SPSS 12.0.1 for Windows (SPSS, Chicago, IL) for all analyses.

RESULTS — Table 1 shows characteristics according to sex at baseline and after a mean follow-up of 4.7 ± 0.8 years. Women had a higher baseline systolic blood pressure and BMI compared with the men. Age, duration of diabetes, A1C levels, diastolic blood pressure, severity of urinary albumin excretion, frequency of current smoking, and frequency of retinopathy did not differ between men and women. At baseline, 46 (68.7%) patients were treated with an insulin-based regime, the remaining 21 (31.3%) with oral glucose-lowering agents only. As a result of the inclusion criteria, no antihypertensive treatment was used at baseline. At the end of the follow-up period, 93% of the patients used antihypertensive treatment, of which 98% consisted of blockers of the renin-angiotensin aldosterone system, in minority in combination with any of the other classes of antihypertensive medication.

During 85.9 person-years, eight (38.1%) women had a cardiovascular event (cardiovascular disease: three, cerebrovascular disease: two, and peripheral

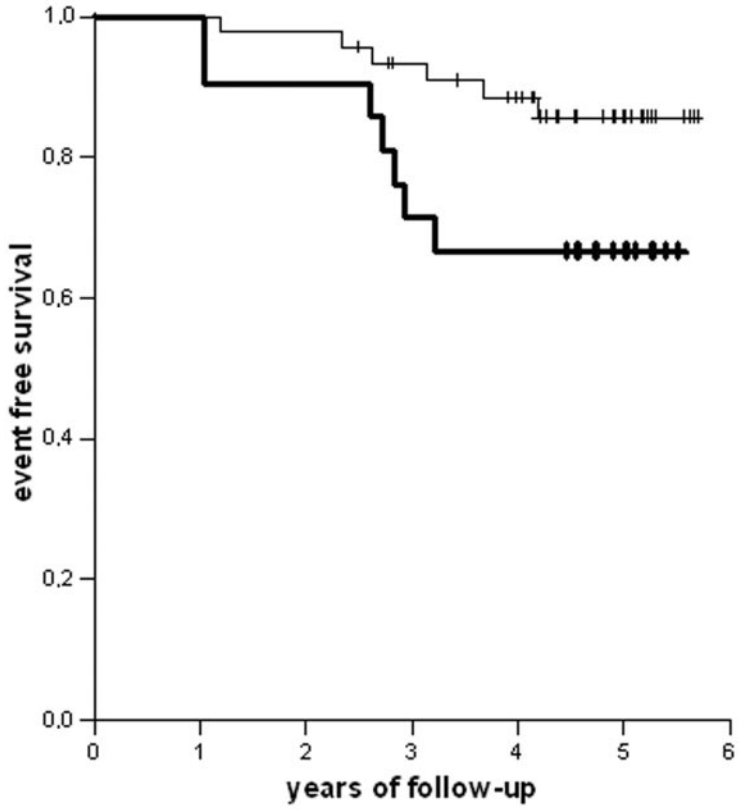

Figure 1— *Kaplan-Meier estimates of event-free survival in women and men with type 2 diabetes. Women had significantly shorter event-free survival compared with men (log-rank test;* P = *0.02).*

artery disease: three, and no deaths were reported), and during 198.5 person-years, six (13.4%) men also met the composite end point (cerebrovascular disease: two; peripheral artery disease: two; death: two [one because of cerebral hemorrhage and one because of cardiac arrest]). One man who first developed peripheral artery disease also developed cerebrovascular disease as well as cardiovascular disease, and one man who first developed cerebrovascular disease also developed peripheral artery disease. The time to the first event in those two men counted for the event-free survival time. The Kaplan-Meier showed a significant difference in event-free survival between women and men ($P = 0.02$) (Fig. 1).

Table 2 shows that women had 3.19 (95% CI 1.11–9.21) times higher risk of cardiovascular disease risk in univariate analysis relative to men. In this study, we questioned whether female sex was an independent risk factor for macrovascular outcome in normotensive patients with type 2 diabetes and microalbuminuria. As demonstrated in Table 1, BMI and systolic blood pressure were significantly different between both men and women. In our multiple Cox regression models, we put in these variables to exclude that the observed increased risk in women is due to higher baseline systolic blood pressure or BMI in women. Furthermore, we adjusted for other classical cardiovascular risk factors, such as age, smoking, and total-to-HDL cholesterol ratio.

We have made two multiple Cox regression models to analyze the effect of nephropathy and retinopathy: models 1 and 2, respectively, in Table 2. In model 1, after adjustment for age, systolic blood pressure, BMI, total-to-HDL cholesterol ratio, smoking, and urinary albumin excretion, the hazard ratio of the women relative to men increased to 6.40 (95% CI 1.24–32.93). In model 2, the point estimate of the cardiovascular risk of women relative to men increased to 8.23 (1.81–37.62).

CONCLUSIONS — In the present prospective follow-up study, we observed that female sex was an independent and important cardiovascular risk factor in normotensive patients with type 2 diabetes and microalbuminuria. Adjustment for the classical cardiovascular risk factors, such as age, systolic blood pressure, BMI, smoking, total-to-HDL cholesterol ratio, and severity of albuminuria or presence of retinopathy, further increased the excess risk of women. Hence, we could not have identified the women at baseline who had an event afterward. Women entered the present study with a significantly higher mean systolic blood pressure than men, although without differences between the sexes in signs of organ damage. During follow-up, significantly more women were treated with antihypertensive drugs, resulting in similar systolic blood pressure in both sexes. This showed that the risk difference between the sexes was also not the result of differences in access to health care.

Previous studies (12) have shown that women with diabetes lose their usual relative protection (compared with men) against cardiovascular disease. Estimates of cardiovascular mortality in diabetic women range from two- to fivefold the rate in nondiabetic women, whereas in diabetic men, estimates vary from one- to threefold the rate in nondiabetic men (13). Data about the reasons for this ex-

Table 2—*Hazard ratios for composite cardiovascular end point of women relative to men*

Variables	Hazard ratio (95% CI)	*P* value
Univariate		
Women relative to men	3.19 (1.11–9.21)	0.03
Multiple Cox regression: model 1		
Women relative to men (adjusted for age, systolic blood pressure, BMI, total-to-HDL cholesterol ratio, current smoking, and urinary albumin excretion)	6.40 (1.24–32.93)	0.02
Multiple Cox regression: model 2		
Women relative to men (adjusted for age, systolic blood pressure, BMI, total-to-HDL cholesterol ratio, current smoking, and retinopathy)	8.23 (1.81–37.62)	0.009

cess risk of cardiovascular disease in diabetic women are conflicting. In a number of studies (7,8,14), differences in the distributions of other major risk factors explained the sex difference in cardiovascular disease risk to a great extent. A recently published meta-analysis established an estimate of the odds ratio for fatal and nonfatal cardiovascular disease due to diabetes in both women and men. The authors concluded that after adjusting for the well-established, modifiable cardiac risk factors, the difference in risk between women and men is modest and not statistically significant (8). To our knowledge, our study is the first prospective follow-up study that investigated normotensive type 2 diabetic patients who had microalbuminuria, without previously established cardiovascular diseases.

The women of our study population had a higher baseline systolic blood pressure as well as a higher BMI compared with the men. However, adjustment for these differences did not change the cardiovascular risk between the sexes. Levels of blood pressure and A1C did not reach the current treatment goals during follow-up but were similar in both men and women and did not change the cardiovascular risk between the sexes in multivariate Cox analysis. Moreover, our results during follow-up indicate that women and men were according to the same protocol, achieving identical treatment targets.

Microalbuminuria has been considered as an indicator of endothelial dysfunction, as well as the first clinical sign of diabetic nephropathy (15,16). It is associated with cardiovascular morbidity and all-cause mortality in both type 1 and type 2 diabetes as well as in nondiabetic individuals (17,18). In the present study, the level of urinary albumin excretion did not explain the sex difference observed in our study population. Moreover, adjustment for the presence of retinopathy at baseline, an important microvascular complication of type 2 diabetes and a sign of arterial atherosclerosis, also did not explain the high risk among women with type 2 diabetes.

The strength of the present study lies in its prospective design and the absence of prevalent cardiovascular disease at baseline, in spite of the presence of microalbuminuria. Moreover, diabetes was equally complicated with retinopathy and nephropathy in women and men. An important limitation of our study is the small sample size. Nonetheless, in concordance with previous studies, we observed statistically significant differences between women and men with regards to cardiovascular risk, which further increased after multivariate regression anlyses. Furthermore, as our patients did not meet the treatment targets, it would be interesting to investigate whether the event rates and sex differences still exist when the current, more aggressive treatment goals for blood pressure lowering as well as A1C are achieved.

In summary, we conclude that female sex is an independent risk factor for macrovascular disease and death in our study population of normotensive patients with type 2 diabetes and microalbuminuria. This excess risk of women relative to men could not be explained by differences in other classical cardiovascular risk factors, the severity of nephropathy or presence of retinopathy, or health care utilization. In contrast to recent studies, our observations suggest that the cardiovascular risk of women with type 2 diabetes can be easily underestimated when additional risk factors are made mandatory. Further research is needed to fully elucidate the pathogenesis of this excessive risk in women with type 2 diabetes because, at this point, we did not find risk indicators that discriminate between women with high and low risk of cardiovascular disease.

APPENDIX

Participating investigators

Dr. R.J.Th. Ouwendijk and Dr. M.G.A. Baggen, Ikazia Hospital; Dr. Th.L.J.M. van der Loos, Rotterdam Eye Hospital; Dr. J.W.F. Elte, Sint Franciscus Hospital; Prof. Dr. S.W.J. Lamberts and Dr. A.H. Bootsma, Erasmus University Medical Centre; Dr. R.J.M. van Leendert, Albert Schweitzer Hospital, Zwijndrecht; Dr. S.G.T. Hulst, Vlietland Hospital, Vlaardingen; Dr. J.W. van der Beek-Boter, Hofpoort Hospital.

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