Raloxifene, a selective estrogen receptor modulator, is renoprotective: a *post-hoc* analysis

Michal L. Melamed[1], Terri Blackwell[2], Joel Neugarten[1], Julia H. Arnsten[1], Kristine E. Ensrud[3], Areef Ishani[3], Steven R. Cummings[2] and Sharon R. Silbiger[1]

[1]Department of Medicine, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York, USA; [2]California Pacific Medical Center Research Institute, San Francisco, California, USA and [3]Center for Epidemiology and Clinical Research, Minneapolis VAMC and University of Minnesota, Minneapolis, Minnesota, USA

Estrogens have a protective effect on kidney fibrosis in several animal models. Here, we tested the effect of raloxifene, an estrogen receptor modulator, on the change in serum creatinine or estimated glomerular filtration rate (eGFR) and incident kidney-related adverse events. We performed a *post-hoc* analysis of the multiple outcomes of raloxifene evaluation trial, a double-masked, placebo-controlled randomized clinical trial encompassing 7705 post-menopausal women (aged 31–80 years) with osteoporosis. Participants were randomized to either of two doses of raloxifene, 60 or 120 mg/day, or placebo. Serum creatinine was measured at a central laboratory at baseline and annually. Adverse events were assessed every 6 months and uniformly categorized. Compared with those in the placebo group, participants on raloxifene had a slower yearly rate of increase in creatinine (significant at the low dose) and a significantly slower yearly rate of decrease in eGFR for both doses over 3 years of follow-up. Raloxifene was associated with significantly fewer kidney-related adverse events compared with placebo. Thus, treatment with raloxifene was safe and renoprotective. Clinical trials of raloxifene in post-menopausal women with kidney disease designed to look at kidney outcomes are needed to confirm these findings.

Kidney International (2011) **79,** 241–249; doi:10.1038/ki.2010.378; published online 6 October 2010

KEYWORDS: randomized controlled trials; renal function decline; risk factors

Correspondence: *Michal L. Melamed, Department of Medicine, 1300 Morris Park Avenue, Ullmann 615, Albert Einstein College of Medicine, Bronx, New York 10461, USA. E-mail: mmelamed@aecom.yu.edu*

Received 21 October 2009; revised 23 July 2010; accepted 27 July 2010; published online 6 October 2010

Chronic kidney disease has recently emerged as a public health concern.[1] There are an estimated 15 million people living with kidney disease stage 3,[2] the majority of whom are adults older than 65 years, and over 300,000 patients on dialysis in the United States.[3] Thus, current research is focusing on identification of risk factors for progression of chronic kidney disease. It is currently recognized that women have a slower progression of non-diabetic kidney disease compared with men.[4] Population-based studies from the United States and Norway have shown that women are less likely to progress to end-stage renal disease compared with men.[5,6] However, an observational study of 5845 women in Canada showed that hormone use was associated with a significant loss of kidney function.[7] At present, the mechanism by which sex affects the progression rate of renal disease is unknown, but there is evidence to support gender differences in glomerular hemodynamics, renal size and glomerular number.[8]

In addition, studies in animals support the concept that testosterone is a promoter of renal scarring, whereas estrogen reduces matrix deposition and scarring.[9–11] Selective estrogen receptor modulators (SERMs), such as raloxifene, activate the estrogen receptor and function as estrogen agonists in some tissues, such as bone and vascular tissues, and estrogen antagonists in other tissues, such as breast and endometrium.[12] In the kidney, animal data have suggested a protective effect of SERMs similar to estrogens. In cultured mesangial cells, estradiol and SERMs inhibit the generation of types I and IV collagen, whereas testosterone has no effect.[13–15] In mouse models of diabetes, raloxifene reduces kidney damage.[16,17] In summary, epidemiological studies have shown that women have a slower rate of kidney disease progression compared with men, but that hormone therapy may accelerate the loss of kidney function, and animal studies suggest that female sex hormones may have beneficial effects. We undertook this *post-hoc* analysis of the multiple outcomes of raloxifene evaluation (MORE) trial to test the hypothesis that raloxifene treatment in post-menopausal women with osteoporosis is associated with a slower rise in serum creatinine or fall in estimated glomerular filtration rate (eGFR). Secondary analyses examined whether raloxifene

treatment was associated with lower rates of kidney-related adverse events and lower rates of proteinuria.

RESULTS

Participant characteristics

The three treatment groups were comparable at baseline with the exception of the percentage of women taking anti-diabetic medications and statins at baseline, which was slightly higher in the 60 mg/day raloxifene group and the rate of those with eGFR < 45 ml/min per 1.73 m^2, which was lower in those assigned to 60 mg of raloxifene (Table 1). The mean follow-up time and s.d. was 3.57 (0.99) years for the entire study period, 2.61 (0.62) years for the core treatment phase of 0–3 years. The mean age of the participants was 66.0 years with an s.d. of 7.0. Over 96% of the participants designated themselves to be white. Hypertension was present in 55% of the participants and 4% were diabetic. Half of the women (49.8%) had serum estradiol levels below 1.4 pg/ml. The flow of patients through the study is shown in Figure 1.

Comparison of the core treatment phase to the extension phase

The effect of treatment on the change in serum creatinine and eGFR was found to be significantly different between the core treatment phase (0–3 years) and the extension phase in year 4 ($P < 0.004$). Therefore, all analyses will be limited to the core treatment phase of 3 years of follow-up. The women in our analysis subset who continued to the extension phase ($N = 5244$) were generally healthier than those who did not continue ($N = 1689$). They were significantly younger by a mean of 1.5 years, less likely to be smokers, to have hypertension or proteinuria, less likely to use angiotensin-converting enzyme inhibitors, but were more likely to use non-steroidal anti-inflammatory drugs, and have a lower serum creatinine level at baseline (data not shown). Women in the placebo group were less likely to continue on to the extension (63.47%) compared with those on raloxifene (67.47%, $P < 0.01$). In addition, during year 4, bisphosphonates were used by 160 (8.33%) in the placebo group and 193 (4.88%) of those on raloxifene ($P < 0.0001$). Reported compliance with taking the calcium and vitamin D supplements decreased during the extension phase. The rate of women reporting compliance for calcium and vitamin D supplement use for the period of 2.5–3 years was 96.87 and 98.11%, respectively, but during the 3.5- to 4-year period the rates dropped to 94.74 and 96.00% ($P < 0.0001$).

Serum creatinine and eGFR

The random-effects model results indicate that for both creatinine and eGFR there was no significant difference

Table 1 | Baseline characteristics of the 6933 post-menopausal women in the analysis subset

Characteristic	Placebo (*n*=2323)	Raloxifene 60 mg/day (*n*=2288)	Raloxifene 120 mg/day (*n*=2322)	*P*-value[a]
Age (yrs), mean ± s.d.	66.2 ± 7.0	66.0 ± 6.9	65.8 ± 7.2	0.20
White race, *n* (%)	2250 (96.9)	2217 (96.9)	2237 (96.3)	0.50
Years since menopause, mean ± s.d.	18.8 ± 8.4	18.6 ± 8.4	18.3 ± 8.2	0.11
Diabetes mellitus, *n* (%)	85 (3.7)	100 (4.4)	79 (3.4)	0.21
Hypertension, *n* (%)	1287 (55.4)	1251 (54.7)	1264 (54.4)	0.79
ACE inhibitor use, *n* (%)	118 (5.1)	120 (5.2)	131 (5.6)	0.68
ARB use, *n* (%)	47 (2.0)	45 (2.0)	39 (1.7)	0.65
Statin use, *n* (%)	99 (4.3)	120 (5.2)	86 (3.7)	0.04
NSAID use, *n* (%)	1022 (44.0)	1002 (43.8)	1054 (45.4)	0.49
Diabetic medication use, *n* (%)	29 (1.3)	50 (2.2)	38 (1.6)	0.05
History of hysterectomy, *n* (%)	524 (22.6)	518 (22.6)	499 (21.5)	0.58
Smoking status, n *(%)*				
Never	1369 (59.7)	1352 (59.7)	1350 (59.0)	0.95
Past	538 (23.5)	541 (23.9)	544 (23.8)	
Current	385 (16.8)	371 (16.4)	394 (17.2)	
Body mass index, kg/m^2, mean ± s.d.	25.3 ± 4.0	25.3 ± 4.0	25.3 ± 4.0	0.99
Systolic BP, mm Hg, mean ± s.d.	133.5 ± 19.2	133.5 ± 18.8	133.0 ± 18.8	0.66
Diastolic BP, mm Hg, mean ± s.d.	78.6 ± 10.5	78.2 ± 10.1	78.2 ± 9.9	0.26
Total cholesterol, mg/dl, mean ± s.d.	237.6 ± 41.5	238.4 ± 40.0	236.5 ± 41.0	0.27
HDL cholesterol, mg/dl, mean ± s.d.	60.5 ± 14.7	60.1 ± 14.8	60.2 ± 15.3	0.52
Triglycerides, mg/dl, mean ± s.d.	112.0 ± 62.7	113.0 ± 73.0	111.4 ± 66.4	0.66
Hemoglobin A_{1C}, %, mean ± s.d.	6 ± 1	6 ± 1	6 ± 1	0.29
Estradiol ⩾1.4 pg/ml, *n* (%)	1163 (50.2)	1116 (49.0)	1162 (50.3)	0.61
eGFR < 45 ml/min per 1.73 m^2, *n* (%)	124 (5.34)	78 (3.41)	107 (4.61)	< 0.01
Serum creatinine, mg/dl, mean ± s.d.	1.02 ± 0.14	1.02 ± 0.13	1.02 ± 0.13	0.44
Proteinuria present, *n* (%)	66 (2.9)	54 (2.4)	52 (2.3)	0.93

Abbreviations: ACE inhibitor, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BP, blood pressure;eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; NSAID, nonsteroidal anti-inflammatory drug.
To convert total and HDL cholesterol from mg/dl to mmol/l multiply by 0.0259. To convert triglycerides from mg/dl to mmol/l multiply by 0.0113. To convert estradiol to pmol/l multiply by 3.671. To convert creatinine to μmol/l multiply by 88.4.
[a]*P*-values for continuous variables are from analysis of variance if normally distributed and Kruskal–Wallis test if skewed. *P*-values for categorical variables are from a χ^2-test.

between raloxifene and placebo in baseline levels (β-coefficient for treatment $P \geqslant 0.12$) (first two columns, Tables 2 and 3). The effect of treatment with raloxifene at 60 or 120 mg/day was very similar, as seen in the Figures 2a and 3a. Overall, serum creatinine increased on average by 0.004 mg/dl per year ($P < 0.0001$), with the women on raloxifene increasing at a slower rate over time than those women in the placebo group (60 mg group $P = 0.03$, 120 mg group $P = 0.11$) (last two columns, Table 2, Figure 2). The 3-year mean increase of serum creatinine based on this model was 0.01 mg/dl for the placebo group, 0.0004 mg/dl for those on 60 mg of raloxifene, and 0.01 mg/dl for those on 120 mg of raloxifene. eGFR decreased on average by 0.34 ml/min per 1.73 m^2 per year ($P < 0.0001$) ('Time' column, Table 3), with the women on raloxifene decreasing at a slower rate over time than those women in the placebo group ($P = 0.03$) (last two columns, Table 3, Figure 3). The 3-year mean decrease of eGFR based on this model was 0.98 ml/min per 1.73 m^2 for the placebo group, 0.56 ml/min per 1.73 m^2 for those on 60 mg of raloxifene, and 0.55 ml/min per 1.73 m^2 for those on 120 mg of raloxifene. There were no significant interactions with treatment and diabetes, hypertension, or estradiol level. Results were not substantially altered after further adjustment for baseline diabetic medication use and statin use or adjusting for blood pressure in a time-varying fashion. Estimates of the interaction of time and treatment were similar in magnitude, but lost statistical significance in models adjusting for time-varying low-density lipoprotein cholesterol or hemoglobin A_{1C}, which may be due to the reduced sample size. When including the extension phase in the analysis, there was no significant difference between raloxifene dose or placebo in either serum creatinine change or eGFR decrease ($P \geqslant 0.88$).

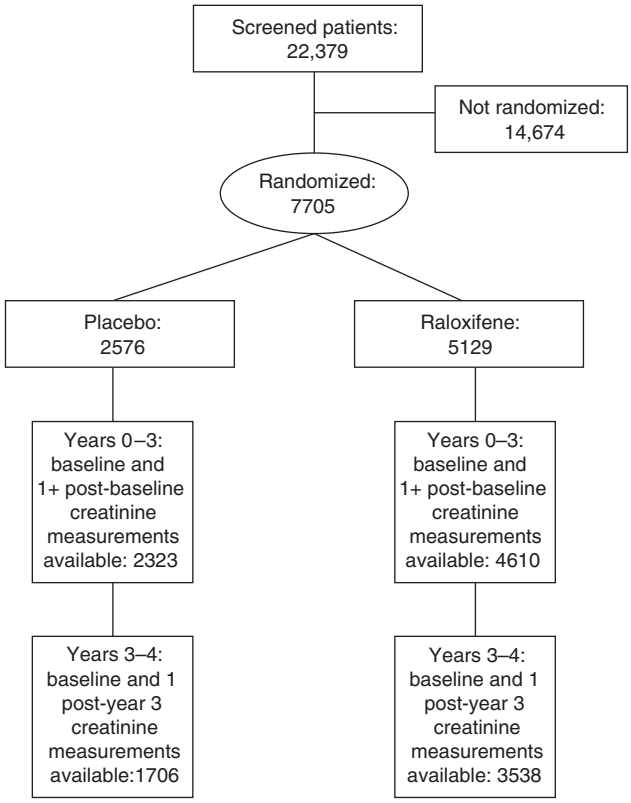

Figure 1 | Study recruitment and follow-up for kidney-specific outcomes in the MORE trial.

Secondary outcomes

A total of 28 (0.6%) participants in the raloxifene group and 29 women (1.1%) in the placebo group had an adverse event related to kidney function, relative hazard (95% (confidence interval) CI 0.50 (0.29–0.85) (Table 4). At baseline there was no difference in the percentage of participants with proteinuria between treatment groups ($P = 0.39$, Table 1).

Table 2 | Association between treatment with raloxifene and change in serum creatinine (mg/dl) over 3 years in 6933 participants of the MORE trial

	Baseline comparison with placebo		Change over time	Rate of change over time compared with placebo group	
Analysis subgroup	RLX 60 β	RLX 120 β	(years) β	RLX60*time β	RLX120*time β
All	−0.006	−0.006	0.004**	−0.002*	−0.002
Diabetics (*n*=265)	−0.028	0.002	0.013	−0.007	0.004
Non-diabetics (*n*=6631)	−0.005	−0.005	0.004**	−0.002	−0.002
P-interaction *P*=0.15					
Hypertensives (*n*=3817)	−0.011	−0.007	0.006**	−0.003	−0.002
Non-hypertensives (*n*=3135)	0.001	−0.004	0.001	−0.002	−0.002
P-interaction *P*=0.22					
Probable kidney disease (*n*=1070)	−0.057**	−0.025	0.005	−0.003	−0.002
No kidney disease (*n*=5767)	0.005	<0.000	0.004**	−0.002*	−0.002
P-interaction *P*<0.001					
Estradiol level <1.4 pg/ml (*n*=3464)	−0.004	−0.006	0.004**	−0.002	−0.001
Estradiol level ⩾1.4 pg/ml (*n*=3442)	−0.007	−0.005	0.004**	−0.003	−0.002
P-interaction *P*=0.84					

Abbreviations: MORE, multiple outcomes of raloxifene evaluation; RLX, raloxifene.
*$P < 0.05$, **$P < 0.01$.
To convert estradiol to pmol/l multiply by 3.671. To convert creatinine to μmol/l multiply by 88.4. Probable kidney disease defined as having one or more of the following: estimated glomerular filtration rate <45 ml/min per 1.73 m^2, diabetes mellitus, proteinuria, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.

Table 3 | Association between treatment with raloxifene and change in eGFR (ml/min per 1.73 m^2) over 3 years in 6933 participants of the MORE trial

Analysis subgroup	Baseline comparison with placebo		Change over time	Rate of change over time compared with placebo group	
	RLX 60 β	RLX 120 β	(years) β	RLX60*time β	RLX120*time β
All	0.235	0.393	−0.336**	0.145*	0.142*
Diabetics (*n*=265)	2.099	−0.277	−0.440	0.117	−0.204
Non-diabetics (*n*=6631)	0.128	0.392	−0.336**	0.143*	0.153*
P-interaction *P*=0.06					
Hypertensives (*n*=3817)	0.450	0.382	−0.419**	0.137	0.141
Non-hypertensives (*n*=3135)	−0.055	0.360	−0.239**	0.148	0.139
P-interaction *P*=0.52					
Probable kidney disease (*n*=1070)	2.757**	1.171	−0.159	−0.075	0.015
No kidney disease (*n*=5767)	−0.277	0.162	−0.382**	0.195**	0.181**
P-interaction *P*<0.001					
Estradiol level <1.4 pg/ml (*n*=3464)	0.150	0.527	−0.374**	0.131	0.144
Estradiol level ⩾1.4 pg/ml (*n*=3442)	0.260	0.234	−0.298**	0.149	0.132
P-interaction *P*=0.66					

Abbreviations: eGFR, estimated glomerular filtration rate; MORE, multiple outcomes of raloxifene evaluation; RLX, raloxifene.
To convert estradiol to pmol/l multiply by 3.671. Probable kidney disease defined as having one or more of the following: estimated glomerular filtration rate <45 ml/min per 1.73 m^2, diabetes mellitus, proteinuria, use of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker.
*P<0.05, **P<0.01.

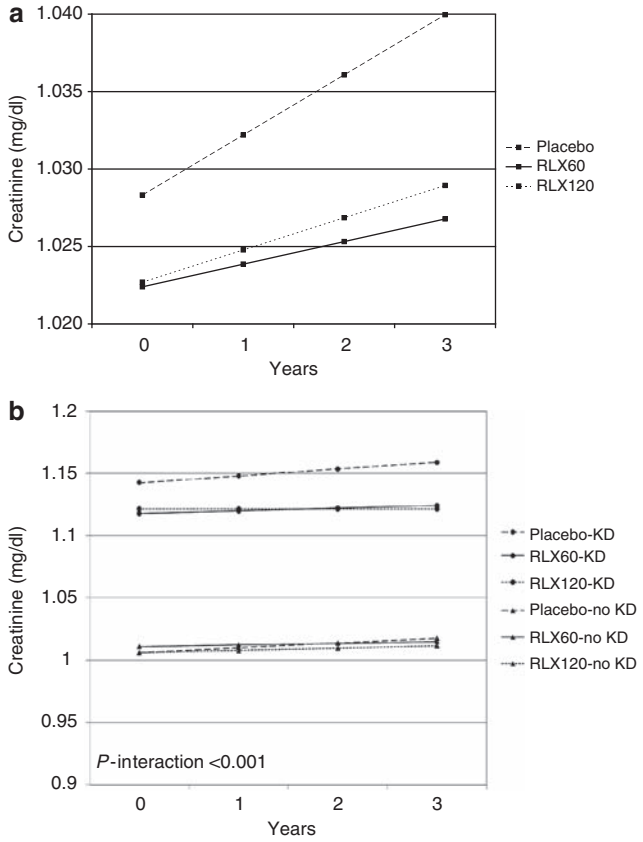

Figure 2 | Creatinine change over 3 years in the MORE trial. (**a**) A 3-year change over time in serum creatinine in 6933 post-menopausal women participating in the MORE trial. (**b**) A 3-year change over time in serum creatinine in 1070 post-menopausal women with probable kidney disease (KD) and 5767 post-menopausal women without kidney disease (no KD) in the MORE trial. MORE, multiple outcomes of raloxifene evaluation.

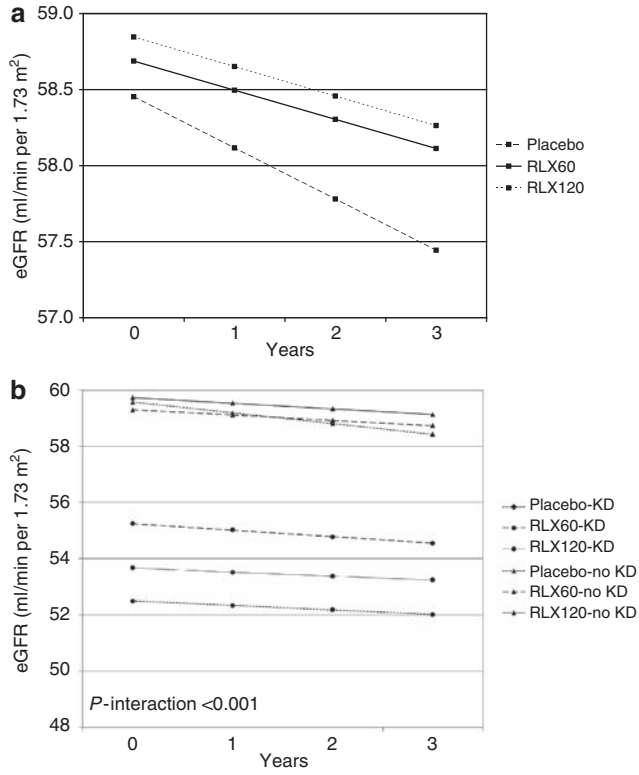

Figure 3 | Estimated glomerular filtration rate (eGFR) change over 3 years in the MORE trial. (**a**) A 3-year change over time in eGFR in 6933 post-menopausal women participating in the MORE trial. (**b**) A 3-year change over time in eGFR in 1070 post-menopausal women with probable kidney disease (KD) and 5767 post-menopausal women without kidney disease (no KD) in the MORE trial. MORE, multiple outcomes of raloxifene evaluation.

Over the 3-year follow-up period, there was no significant change in proteinuria prevalence over time ($P=0.86$), and no significant interaction with treatment and time ($P=0.21$).

Participants with probable kidney disease

There were 1070 participants who were classified as having probable kidney disease based on the presence of baseline eGFR <45 ml/min per 1.73 m^2, diabetes mellitus, proteinuria

Table 4 | Association between treatment with raloxifene and 3-year incidence of adverse events related to kidney disease

Outcome	Placebo N (%)	Raloxifene 60 mg/day N (%)	Raloxifene 60 mg/day Relative hazard (95% CI)[a]	Raloxifene 120 mg/day N (%)	Raloxifene 120 mg/day Relative hazard (95% CI)[a]	Pooled raloxifene N (%)	Pooled raloxifene Relative hazard (95% CI)[a]
Adverse event related to kidney disease	29 (1.13)	10 (0.39)	0.37 (0.18–0.77)	18 (0.70)	0.62 (0.34–1.14)	28 (0.55)	0.50 (0.29–0.85)
Kidney function abnormal	12 (0.47)	4 (0.16)		11 (0.43)		15 (0.29)	
Kidney tubular disorder	1 (0.04)	0		0		0	
Kidney tubular necrosis	0	1 (0.04)		0		1 (0.02)	
Nephritis	2 (0.08)	0		0		0	
Nephrosclerosis	1 (0.04)	0		0		0	
NPN increased	5 (0.19)	3 (0.12)		1 (0.04)		4 (0.08)	
Albuminuria	7 (0.27)	2 (0.08)		1 (0.04)		3 (0.06)	
BUN increased	3 (0.12)	1 (0.04)		0		1 (0.02)	
Anuria	0	0		1 (0.04)		1 (0.02)	
Creatinine clearance decreased	2 (0.08)	0		0		0	
Glomerulitis	0	1 (0.04)		3 (0.12)		4 (0.08)	
Uremia	2 (0.08)	0		1 (0.04)		1 (0.02)	
Uremic coma	0	0		1 (0.04)		1 (0.02)	

Abbreviations: BUN, blood urea nitrogen; CI, confidence interval; NPN, non-protein nitrogen.
To convert creatinine to μmol/l multiply by 88.4.
[a]Reference=placebo group.

or the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. In this sub-group serum creatinine was lower at baseline in the raloxifene 60 mg group. Although it did not reach statistical significance, the difference in magnitude of change over time in creatinine between the placebo group and the treatment groups were slightly larger among those with probable kidney disease at baseline than those without (Table 2 and Figures 2b and 3b). The interaction of treatment and probable kidney disease at baseline is significant for both eGFR and serum creatinine ($P<0.01$). Results from the kidney-related adverse events outcome confirm this association. The risk of incident events related to kidney disease among those with probable kidney disease at baseline was further reduced by treatment with raloxifene (relative hazard (95% CI) 0.27 (0.12–0.59), *P*-interaction 0.09).

DISCUSSION

In this *post-hoc* analysis of a large placebo-controlled, double-masked randomized clinical trial of raloxifene's effect on fractures, we found that participants randomized to raloxifene experienced a slower rise of serum creatinine and slower fall of eGFR compared with the placebo group. Although the changes in serum creatinine and eGFR were very small over the course of the trial, these were generally healthy women who would not be expected to have large decrements in kidney function over the course of 3 years. This is the first large study to test this hypothesis and to show a potential kidney protective effect of raloxifene in humans. In addition, as in a previous analysis of the MORE data, our analysis shows no marked difference in kidney-specific safety for patients with and without kidney disease on raloxifene treatment.[18]

We do not know why there was a difference in treatment effects seen between years 0–3 and year 4. Other analyses of the MORE data for other outcomes did not see a difference between the two phases.[19] One may conjecture that the women who continued to the extension were healthier and, therefore, less likely to experience rises in serum creatinine, and, therefore, it was even less likely an effect could be seen. Alternatively, there may be an interaction between the effect of raloxifene and medications started (such as bisphosphonates) or discontinued (such as calcium and vitamin D) during the extension phase.

There is one small study in humans that suggests that raloxifene may have beneficial effects on kidney function.[20] In this study, 39 post-menopausal women with type 2 diabetes mellitus and albuminuria were randomized to either raloxifene 60 mg or placebo for 6 months. Treatment with raloxifene was one of the main determinants of absolute change in the urinary albumin to creatinine ratio. An analysis of the Nurses' Health Study revealed that women who used post-menopausal hormone therapy for more than 6 years had a lower risk of albuminuria in non-diabetic women.[21] We did not find a difference in the prevalence of dipstick proteinuria, a less accurate measure of proteinuria, over the 3 years of the MORE trial.

There is conflicting evidence about whether synthetic estrogen and estrogen in combination with progesterone itself affect kidney function and proteinuria with some studies showing beneficial results,[22] some studies showing no effect[23] and some showing detrimental effects.[24] However, these studies did not evaluate the effect of estradiol alone, but treated patients with various combinations of hormone therapy. A recent analysis of an administrative database in Alberta Canada followed 5845 women >66 years old for 2 years and found that women on hormone therapy, mostly estrogen or combined estrogen and progesterone were more likely to have a more rapid decline in kidney function, as measured by MDRD eGFR differences.[7] This study differed from ours in several important ways, including the medication studied, raloxifene versus estrogen, the study design, observational versus a randomized controlled clinical trial and most importantly the results. We found that raloxifene

treatment overall was associated with a lower rate of eGFR decline and that in a group with probable kidney disease, there was no difference in eGFR decline between raloxifene and placebo, and also no significant change from baseline eGFRs. The Ahmed analysis found estrogen to be associated with a loss of kidney function. It may be that SERMs function differently in the kidney than estrogens, as they do in other tissues, such as the endometrium.[25] Further research is needed to clarify these very marked differences.

Gender differences in animal models of kidney disease suggest that female sex hormones may have a function in the progression of kidney disease. There are numerous animal models of kidney disease, in which males and post-menopausal females have more severe disease.[9,26] In many rodent models, hormonal manipulation affects the progression of kidney disease. Estrogen deficiency by ovariectomy has been shown to be detrimental and estradiol treatment is protective in these models of disease.[27–31] In a mouse model of diabetes, the db/db mouse, raloxifene has been shown to reduce kidney damage.[16] Studies of cultured mesangial cells show that estradiol, tamoxifen, and raloxifene inhibit the generation of types I and IV collagen,[13–15] whereas testosterone has no effect.[32] In addition to affecting collagen generation, estrogens have been shown to affect the activity of two collagen degrading enzymes, metalloproteinase-2[33] and metalloproteinase-9[34] in cultured kidney cells. One potential mechanism by which estrogen affects collagen generation is by means of TGFβ. In cultured mesangial cells, estradiol inhibits the pro-fibrotic effects of TGFβ by interfering with the activity of casein kinase II[35] and in the TGF-β transgenic mouse, estradiol mitigates the severe glomerulosclerosis seen.[31]

Although raloxifene is a SERM and did not cause increased vaginal bleeding or breast pain during the MORE trial, it was associated with a increased relative risk of venous thromboembolism (relative risk (RR): 3.1; 95% CI, 1.5–6.2).[36] In another clinical trial of 10,101 post-menopausal women, raloxifene use was associated with a hazard ratio of 1.44 (95% CI, 1.06–1.85) for venous thromboembolism and 1.49 (95% CI, 1.00–2.24) for fatal stroke.[37] In that trial, raloxifene use had no effect on the risk of coronary events (hazard ratio, 0.96, 95% CI, 0.84–1.07), but reduced the risk of invasive breast cancer (hazard ratio, 0.56, 95% CI, 0.38–0.83).[37] Although these risks are present, an analysis of Medicaid claims from all 50 states showed that there were over 750,000 yearly prescriptions in 2004.[38] The potential risks of raloxifene use must be weighed against any benefits suggested by this *post-hoc* analysis and the true effects of raloxifene on kidney function and albuminuria need to be tested in a randomized clinical trial evaluating specifically kidney disease outcomes. We found that the differences between the treatment groups and placebo are of modest size and suggest that use of raloxifene is safe from a renal perspective.

There are potential limitations to the current analysis. Urine proteinuria testing was performed by urine dipstick that is not as accurate as a urine albumin to creatinine ratio or a 24 h urine collection and is dependent on urinary concentration. Because it is not a good measure it would tend to bias results towards the null hypothesis. Our measurement of adverse events was limited in that it only consisted of self-reported events that were not confirmed by chart review. In addition, the population in the MORE trial was at very low risk for progression of kidney disease because of low prevalence of diabetes, albuminuria and advanced kidney disease, future studies will need to be adequately powered to evaluate only hard end-points in a population with a higher risk for progression. The limited number of participants with diabetes mellitus, proteinuria and on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers are baseline also potentially limits the generalizability of the results. This is a *post-hoc* analysis and was not part of the original trial hypotheses; therefore, it may be appropriate to apply stricter definitions for statistical significance. However, we present data from a randomized placebo-controlled double-blinded clinical trial of approximately 7000 women followed prospectively for 3 years in 25 countries.

In summary, this *post-hoc* analysis of the MORE trial looking at kidney disease outcomes revealed that women randomized to raloxifene experienced a slower increase in serum creatinine and decline in eGFR compared with their counterparts on placebo. They also had fewer adverse events related to kidney disease. These findings should be confirmed in a large randomized clinical trial in post-menopausal women with advanced kidney disease and albuminuria. However, our data can conclude that the use of raloxifene is safe and is not associated with declines in kidney function.

METHODS

Design, settings, and participants

The MORE study was a multi-center, randomized, double-masked clinical trial designed to test whether treatment with raloxifene reduces the risk of fractures in post-menopausal women with osteoporosis.[36,39] The study, conducted between 1994–1999, enrolled 7705 women at 180 centers in 25 countries.[36] A 1-year extension was added to the 3-year core treatment phase to assess secondary end-points (cardiovascular disease risk, breast cancer risk). Between years 3 and 4 women were allowed to take any medication their own physicians ordered, including bisphosphonates or other bone-active agents, with the exception of oral estrogen or estrogen-progestin therapy. During this extension phase women continued on treatment with raloxifene or placebo as initially assigned.

Exclusion criteria of the MORE trial pertinent to the current analysis included having a creatinine >2.5 mg/dl and active renal lithiasis. There were 722 women without both a baseline and at least one post-baseline measure of creatinine, which is required for the current analysis. Therefore, 6933 women are included in the current analysis (Figure 1). The percentage of participants on placebo and raloxifene was similar among the women in the analysis subset and those 722 women not included in the current analysis ($P=0.68$). The ethical review board at each site approved the study and written informed consent was obtained from all participants.

Randomization and interventions

Women were stratified by site and randomly assigned to receive placebo, raloxifene 60 or 120 mg/day. The sponsor supplied randomly numbered kits containing tablets that were identical in appearance. The women received two tablets daily: two placebo, one placebo and one tablet of 60 mg raloxifene, or two tablets of 60 mg raloxifene. All women in the study also received 500 mg of calcium and 400–600 IU of cholecalciferol daily.[36] Participants, investigators, and laboratory staff were masked to treatment assignment.

Outcomes and follow-up

Serum creatinine was measured in the MORE trial by SciCor (Covance) Central Laboratory Services (Indianapolis, IN) using the modified Jaffe reaction. Participants had creatinine measures done at their original lab at enrollment and annually thereafter. Measures were also made at the termination visit if the participant did not complete the trial. eGFR was estimated using the four-variable standardized Modification of Diet in Renal Disease (MDRD) Study equation for each serum creatinine value.[40] The change over time in serum creatinine level and eGFR were examined as continuous outcomes.

At visits that occurred 3 and 6 months after enrollment and every 6 months thereafter, women were asked whether they had experienced any adverse event since the last visit (any undesirable experience, including any illness or accidental injury occurring during the study, whether or not it is considered to be related to the study drug). These adverse events reported were categorized using the Coding Symbols and Thesaurus for Adverse Reaction Terminology dictionary,[41] but were not independently confirmed by obtaining verification other than participant report. A physician blinded to treatment status (MLM) reviewed all adverse events collected throughout the study and determined those to classify as an adverse kidney event. For this analysis, adverse events related to kidney disease included those coded as, 'Kidney function abnormal, kidney tubular disorder, kidney tubular necrosis, nephritis, nephrosclerosis, non-protein nitrogen increased, blood urea nitrogen increased, albuminuria, anuria, creatinine clearance decreased, glomerulitis, uremia, and uremic coma.' Excluded were any adverse events coded as 'acute renal failure, kidney cortex necrosis, nephrosis, oliguria, polycystic kidney, toxic nephropathy, dehydration, hematuria, and hydronephrosis'. These were excluded as they may represent either acute processes or structural abnormalities, not necessarily associated with progressive kidney disease. Adverse kidney events were compared between the raloxifene and placebo groups as a secondary analysis.

Another secondary outcome was proteinuria, which was measured by semiquantitative urine dipstick using IRIStrips (Roche Diagnostics, Indianapolis, IN). Proteinuria was defined in this analysis as anyone who had a protein dipstick of 1+ or greater. Proteinuria was measured at baseline, annually and at the termination visit if the participant did not complete the trial.

Other measurements

Baseline information regarding age, race, menstrual history, smoking status and health conditions was gathered using a questionnaire. Baseline clinical assessments included height, weight, blood pressure, measurement of serum lipids, hemoglobin A_{1C} and fasting blood glucose. Use of all current prescription and other medication was recorded. Hypertension for this analysis was defined as having a blood pressure $\geqslant$140/90 mm Hg, self-reported hypertensive status or the use of anti-hypertensive medications at baseline. Diabetes mellitus was defined as having the fasting blood glucose of $\geqslant$126 mg/dl, self-reported diabetic status or the use of hypoglycemic medications at baseline (to convert to mmol/l multiply by 0.0555). Serum estradiol was measured at baseline by SciCor (Covance) central laboratory services using a double antibody assay process. Serum estradiol concentrations were categorized as $<$1.4 pg/ml (too low to detect) and $\geqslant$1.4 pg/ml (to convert to pmol/l multiply by 3.671).

Statistical analysis

Baseline participant characteristics were compared across the three treatment groups using the χ^2-test for categorical data, and analysis of variance for normally distributed continuous data and Kruskal–Wallis tests for skewed continuous data.

Characteristics among those women who elected to continue for the year 3–4 extension phase of the trial could be different among treatment and placebo groups resulting from discontinuations. Also, the use of other bone-active agents was allowed during this extension phase. These factors may lead to differing treatment effects on our outcomes during years 0–3 and the year 3–4 extension period (phase of study). The potential differences in characteristics were compared between those who stopped at or before year 3 and those who continued on to the year 3–4 extension using χ^2-tests for categorical data, Student's *t*-tests for normally distributed continuous data and Wilcoxon rank-sum tests for skewed continuous data. For the primary outcomes of creatinine and eGFR, analysis was performed to examine whether there was a significant interaction between treatment and phase of the study. Annualized percent change in creatinine and eGFR were examined using a generalized linear model with independent variables for treatment group and phase, plus the interaction of these two variables. If an interaction between treatment and study phase was significant then the treatment effect on our outcomes during the 1-year extension phase would be considered to be different from the core treatment phase, and subsequent analyses would focus on the core treatment phase.[19]

Random-effects models were used to analyze the association between the treatment effect and the change in serum creatinine level and eGFR. These models account for between-participant variation and within-participant correlation of repeated outcomes.[42] The random effect terms included both the intercept and the slope of the measurements over time, allowing for individual time trends for each participant. Variances and covariances were estimated using the restricted maximum likelihood method. Time was modeled as a continuous covariate, measured as years from baseline. Mean change from baseline to year 3 was calculated based on results from the models.

Any effect of raloxifene on serum creatinine or eGFR may depend on factors, such as endogenous estradiol levels ($\geqslant$1.4 versus $<$1.4 pg/ml) and the presence of hypertension or diabetes. Baseline kidney function may also affect the relationship between raloxifene and change in kidney function. Therefore, we created a category defined as probable kidney disease that included all participants with an eGFR $<$45 ml/min per 1.73 m^2, diabetes mellitus, proteinuria, or use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers at baseline. Secondary analyses examining the effects of raloxifene treatment within strata of these selected baseline factors, was also performed. Additional random-effects models with added terms for the strata plus the interaction of strata and treatment were performed. A model adjusting for any baseline covariates associated with treatment was performed, to see

whether this explained the observed effects of raloxifene. In addition, because blood pressure can affect kidney function and proteinuria, we performed another sensitivity analysis adjusting for achieved blood pressure as a time-varying covariate, to evaluate whether the effects of raloxifene on kidney function may be mediated through effects on blood pressure. Similar analyses were performed with the time-varying covariates of low-density lipoprotein cholesterol and hemoglobin A_{1C}. These analyses were limited to women at the 14 study sites where follow-up measures of low-density lipoprotein cholesterol ($n = 2378$) and hemoglobin A_{1C} ($n = 2615$) were performed.

The secondary outcome of incident kidney-related adverse events was analyzed using a proportional hazards model. The secondary outcome of the presence of proteinuria was analyzed using a longitudinal analysis based on data from all baseline and all post-baseline time points. Generalized estimating equations were preformed with terms for treatment, years from baseline, and an interaction term between the time and treatment to test the hypothesis that the rate of change of proteinuria over time differed among treatment groups.

All statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC). Statistical significance was set at a two-sided P-value of <0.05.

DISCLOSURE

SC receives research support and consultation fees from Eli Lilly. TB receives partial salary support from Eli Lilly. Eli Lilly and the funding agencies did not have any function in the design and conduct of this analysis; collection, management, analysis, and interpretation of the data; and preparation or approval of the paper. A representative from Eli Lilly did review the paper before submission.

ACKNOWLEDGMENTS

We thank the women and participating centers of the MORE trial. MLM and this analysis are supported by an American Heart Association Heritage Foundation Clinically Applied Research Award. This information was presented in abstract form at the 2007 American Society of Nephrology Annual Meeting, San Francisco, California. In addition, MLM is supported by grant number K23 DK078774 from the National Institute of Diabetes, Digestive and Kidney Diseases of the National Institutes of Health. SC receives research support and consultation fees from Eli Lilly. TB receives partial salary support from Eli Lilly. Registered at clinicaltrials.gov #NCT00670319.

REFERENCES

1. Meguid El Nahas A, Bello AK. Chronic kidney disease: the global challenge. *Lancet* 2005; **365**: 331–340.
2. Coresh J, Selvin E, Stevens LA *et al.* Prevalence of chronic kidney disease in the United States. *JAMA* 2007; **298**: 2038–2047.
3. Collins AJ, Kasiske B, Herzog C *et al.* Excerpts from the United States Renal Data System 2004 annual data report: atlas of end-stage renal disease in the United States. *Am J Kidney Dis* 2005; **45**: A5–A7.
4. Neugarten J, Acharya A, Silbiger SR. Effect of gender on the progression of nondiabetic renal disease: a meta-analysis. *J Am Soc Nephrol* 2000; **11**: 319–329.
5. Haroun MK, Jaar BG, Hoffman SC *et al.* Risk factors for chronic kidney disease: a prospective study of 23,534 men and women in Washington County, Maryland. *J Am Soc Nephrol* 2003; **14**: 2934–2941.
6. Eriksen BO, Ingebretsen OC. The progression of chronic kidney disease: a 10-year population-based study of the effects of gender and age. *Kidney Int* 2006; **69**: 375–382.
7. Ahmed SB, Culleton BF, Tonelli M *et al.* Oral estrogen therapy in postmenopausal women is associated with loss of kidney function. *Kidney Int* 2008; **74**: 370–376.
8. Silbiger SR, Neugarten J. The role of gender in the progression of renal disease. *Adv Ren Replace Ther* 2003; **10**: 3–14.
9. Baylis C. Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy. Male gender as a primary risk factor. *J Clin Invest* 1994; **94**: 1823–1829.
10. Sakemi T, Ohtsuka N, Shouno Y *et al.* Effect of ovariectomy on glomerular injury in hypercholesterolemic female Imai rats. *Nephron* 1996; **72**: 72–78.
11. Iams SG, Wexler BC. Inhibition of the development of spontaneous hypertension in SH rats by gonadectomy or estradiol. *J Lab Clin Med* 1979; **94**: 608–616.
12. Khovidhunkit W, Shoback DM. Clinical effects of raloxifene hydrochloride in women. *Ann Intern Med* 1999; **130**: 431–439.
13. Silbiger S, Lei J, Ziyadeh FN *et al.* Estradiol reverses TGF-beta1-stimulated type IV collagen gene transcription in murine mesangial cells. *Am J Physiol* 1998; **274**: F1113–F1118.
14. Silbiger S, Lei J, Neugarten J. Estradiol suppresses type I collagen synthesis in mesangial cells via activation of activator protein-1. *Kidney Int* 1999; **55**: 1268–1276.
15. Neugarten J, Acharya A, Lei J *et al.* Selective estrogen receptor modulators suppress mesangial cell collagen synthesis. *Am J Physiol Renal Physiol* 2000; **279**: F309–F318.
16. Chin M, Isono M, Isshiki K *et al.* Estrogen and raloxifene, a selective estrogen receptor modulator, ameliorate renal damage in db/db mice. *Am J Pathol* 2005; **166**: 1629–1636.
17. Dixon A, Wells CC, Singh S *et al.* Renoprotective effects of a selective estrogen receptor modulator, raloxifene, in an animal model of diabetic nephropathy. *Am J Nephrol* 2007; **27**: 120–128.
18. Ishani A, Blackwell T, Jamal SA *et al.* The effect of raloxifene treatment in postmenopausal women with CKD. *J Am Soc Nephrol* 2008; **19**: 1430–1438.
19. Delmas PD, Ensrud KE, Adachi JD *et al.* Efficacy of raloxifene on vertebral fracture risk reduction in postmenopausal women with osteoporosis: four-year results from a randomized clinical trial. *J Clin Endocrinol Metab* 2002; **87**: 3609–3617.
20. Hadjadj S, Gourdy P, Zaoui P *et al.* Effect of raloxifene – a selective oestrogen receptor modulator – on kidney function in post-menopausal women with Type 2 diabetes: results from a randomized, placebo-controlled pilot trial. *Diabet Med* 2007; **24**: 906–910.
21. Schopick EL, Fisher ND, Lin J *et al.* Post-menopausal hormone use and albuminuria. *Nephrol Dial Transplant* 2009; **24**: 3739–3744.
22. Szekacs B, Vajo Z, Varbiro S *et al.* Postmenopausal hormone replacement improves proteinuria and impaired creatinine clearance in type 2 diabetes mellitus and hypertension. *BJOG* 2000; **107**: 1017–1021.
23. Manning PJ, Sutherland WH, Allum AR *et al.* HRT does not improve urinary albumin excretion in postmenopausal diabetic women. *Diabetes Res Clin Pract* 2003; **60**: 33–39.
24. Ahmed SB, Hovind P, Parving HH *et al.* Oral contraceptives, angiotensin-dependent renal vasoconstriction, and risk of diabetic nephropathy. *Diabetes Care* 2005; **28**: 1988–1994.
25. Black LJ, Sato M, Rowley ER *et al.* Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *J Clin Invest* 1994; **93**: 63–69.
26. Zheng F, Plati AR, Potier M *et al.* Resistance to glomerulosclerosis in B6 mice disappears after menopause. *Am J Pathol* 2003; **162**: 1339–1348.
27. Tofovic SP, Salah EM, Dubey RK *et al.* Estradiol metabolites attenuate renal and cardiovascular injury induced by chronic nitric oxide synthase inhibition. *J Cardiovasc Pharmacol* 2005; **46**: 25–35.
28. Gross ML, Adamczak M, Rabe T *et al.* Beneficial Effects of Estrogens on Indices of Renal Damage in Uninephrectomized SHRsp Rats. *J Am Soc Nephrol* 2004; **15**: 348–358.
29. Tofovic SP, Dubey R, Salah EM *et al.* 2-Hydroxyestradiol attenuates renal disease in chronic puromycin aminonucleoside nephropathy. *J Am Soc Nephrol* 2002; **13**: 2737–2747.
30. Elliot SJ, Karl M, Berho M *et al.* Estrogen deficiency accelerates progression of glomerulosclerosis in susceptible mice. *Am J Pathol* 2003; **162**: 1441–1448.
31. Blush J, Lei J, Ju W *et al.* Estradiol reverses renal injury in Alb/TGF-beta1 transgenic mice. *Kidney Int* 2004; **66**: 2148–2154.
32. Kwan G, Neugarten J, Sherman M *et al.* Effects of sex hormones on mesangial cell proliferation and collagen synthesis. *Kidney Int* 1996; **50**: 1173–1179.
33. Guccione M, Silbiger S, Lei J *et al.* Estradiol upregulates mesangial cell MMP-2 activity via the transcription factor AP-2. *Am J Physiol Renal Physiol* 2002; **282**: F164–F169.

34. Potier M, Elliot SJ, Tack I *et al.* Expression and regulation of estrogen receptors in mesangial cells: influence on matrix metalloproteinase-9. *J Am Soc Nephrol* 2001; **12**: 241–251.
35. Zdunek M, Silbiger S, Lei J *et al.* Protein kinase CK2 mediates TGF-beta1-stimulated type IV collagen gene transcription and its reversal by estradiol. *Kidney Int* 2001; **60**: 2097–2108.
36. Ettinger B, Black DM, Mitlak BH *et al.* Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. *Jama* 1999; **282**: 637–645.
37. Barrett-Connor E, Mosca L, Collins P *et al.* Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. *N Engl J Med* 2006; **355**: 125–137.
38. Udell JA, Fischer MA, Brookhart MA *et al.* Effect of the Women's Health Initiative on osteoporosis therapy and expenditure in Medicaid. *J Bone Miner Res* 2006; **21**: 765–771.
39. Cummings SR, Eckert S, Krueger KA *et al.* The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. *Jama* 1999; **281**: 2189–2197.
40. Levey AS, Coresh J, Balk E *et al.* National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003; **139**: 137–147.
41. National Technical Information Service. *Coding Symbols for a Thesaurus of Adverse Reaction Terms (COSTART)*, 4th edn, 1992. National Technical Information Service: Springfield, VA.
42. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982; **38**: 963–974.