Abstract

Background. Higher levels of urinary albumin excretion predict future hypertension and chronic kidney disease. Post-menopausal hormone use may influence the renin–angiotensin system and renal endothelial function, impacting albumin excretion. The association between post-menopausal hormone use and albuminuria is not well defined.

Methods. We explored the cross-sectional association between duration of PMH use and albuminuria in 2445 post-menopausal, non-diabetic women from the Nurses’ Health Study. Women were categorized as hormone non-users, past users or current users grouped by 3-year intervals of duration of use, from ≤3 years to >15 years. The outcome was the top decile of urine albumin/creatinine ratio (ACR). Multivariate logistic regression was used to assess the association between duration of PMH use and risk of being in the top decile.

Results. The mean age was 66.8 years, and 57% were currently using PMH. The median ACR was 2.9 mg/g, and the 90th percentile was 9.2 mg/g. Compared with women with no history of PMH use, the odds ratio for being in the top ACR decile was lower for women with use of >6–9 years, >9–12 years, >12–15 years and >15 years, but there was no dose–response. The overall odds ratio was 0.55 (95% CI: 0.39–0.77) among women with >6 years of current PMH use compared with non-users. Current hormone use of shorter duration and past hormone use were not associated with albumin excretion.

Conclusions. Current PMH use of >6 years is associated with a lower urinary ACR in non-diabetic women.

Keywords: albuminuria; epidemiology; post-menopausal hormones

Introduction

Higher urinary albumin excretion is a predictor of incident hypertension [1] and development of chronic kidney disease [2]. The albumin:creatinine ratio (ACR) is used to estimate urinary albumin excretion [3], and the level that is clinically significant is uncertain. The clinical definition of ‘microalbuminuria’ in women is 25–355 mg/g creatinine (Cr) [4]. However, recent studies have demonstrated an increase in incident hypertension at lower levels of albuminuria. In the Nurses Health Study (NHS), the risk of developing hypertension was 76% higher in women with higher ACR (4.3–24.2 mg/g Cr) compared to lower ACR (<1.7 mg/g Cr) [1]. These studies suggest that factors influencing albumin excretion could have widespread health effects.

One such factor could be post-menopausal hormone (PMH) use. In the United States, 38% of post-menopausal women report using or having used PMH [5]. In 5/6 nephrectomized rats without ovarian function, addition of estradiol attenuated albumin excretion [6,7]. In humans, however, the association between oestrogen supplementation after menopause and albuminuria is ill-defined [8,9]. We therefore examined the association between PMH use and urinary albumin excretion in a cross-sectional study of 2445 post-menopausal participants of the NHS.

Subjects and methods

Study population

The NHS was started in 1976, when 121 700 female nurses aged 30–55 years completed a detailed questionnaire pertaining to health-related information such as illnesses, medications and lifestyle. Since then, these women have completed questionnaires to update their health-related information every 2 years and detailed dietary questionnaires every 4 years.

The NHS participants in this analysis were part of a study of analgesic use and renal function [10]. This subcohort included women who provided an initial blood sample in 1989 (N = 32 826). We wanted to mail a supplementary questionnaire to a subset of these women who were likely to have a high lifetime intake of analgesics and women who were likely to have a low intake. Therefore, we oversampled women who had reported high frequency of analgesic use (≥15 days per month) on biennial questionnaires, and also women who reported no analgesic use on biennial questionnaires. We mailed 4238 analgesic questionnaires in 1999, and 3876 women (91%) responded. Of these women, 3123 also provided a second blood sample plus a urine sample in 2000. Due to financial constraints on our ability to measure renal function markers on all of the blood and urine samples, 2712 women were selected for these analyses, with oversampling of those from the highest levels of lifetime analgesic intake but including women of all levels of lifetime intake including low levels. Women with a history of cardiovascular disease or a history of cancer (except for non-melanoma...
skin cancer) in 1989 were excluded from the initial blood collection since one of the goals of the Nurses’ Health Study was to determine predictors of incident cases of these conditions. Women who developed cardiovascular disease or cancer after 1989 were not excluded from any analyses.

For the current study, we excluded 30 women who were pre-menopausal or had unknown menopausal status and 59 women due to unknown hormone use or duration. Because the ACR distribution was substantially broader for diabetics than that for non-diabetics, the 154 diabetics were excluded. Three women with ACR values >355 mg/g Cr (the threshold for ‘macroalbuminuria’ in women [4]) were also excluded, since it is likely that mechanisms for small amounts of urinary albumin are different than those for larger amounts of urinary albumin [11]. Additionally, 21 women were excluded due to missing information regarding body mass index (BMI) or serum creatinine. After exclusions, 2445 women remained.

Assessment of hormonal status

With each biennial questionnaire, participants reported the number of months during the past 2 years when prescription female hormones were taken, whether these hormones were taken currently and whether the hormone preparation was oestrogen, progesteron or a combination. We calculated the duration of use from sequential questionnaires, accounting for starting and stopping the medications over time. It was impossible to determine whether on the medications were stopped and restarted from the databases. PMH use was categorized as ‘current’, ‘past’ or ‘never’ at the time of the 2000 questionnaire. We wanted to explore whether there was an association with overall PMH use and with the duration of use. Therefore, current use was categorized by duration of use in 3-year intervals, forming six groups: ≤3 years, >3–6 years, >6–9 years, >9–12 years, >12–15 years and >15 years. As there was no prior literature to guide our choice of categories, we chose to break the duration into these six categories to better assess whether there was an inflection point of the association or a dose–response based on longer duration of use.

Assessment of covariates

Age at menopause was the age at which the participant first reported post-menopausal status. BMI, smoking status, physical activity (metabolic equivalent task scores, METS), aspirin, multivitamin and alcohol use were obtained from the 2000 questionnaire. The serum creatinine from the 2000 blood collection was used to calculate the estimated glomerular filtration rate (eGFR), which was measured by the modified Jaffe method. The CV was 10% using blinded split samples. This value was inserted into the four-variable modification of diet in renal disease (MDRD) formula for eGFR: 186 × [creatinine]−1.154 × age−0.203 × 0.742 (1.21 if black) [12]. The creatinine value was not normalized to the Cleveland Clinic standard. History of hypertension, diabetes and cholesterol was derived from self-report on questionnaires up to and including the 2000 questionnaire. Participants taking anti-hypertensive medications in 2000 were also classified as hypertensive.

Assessment of albumin/creatinine ratio

Urine samples from the 2000 urine collection were used to measure the ACR. The ACR was calculated from the urinary albumin and creatinine measurements, and expressed as milligrams of albumin per gram of creatinine (mg/g). Urinary albumin was measured by immunoassay. Urinary creatinine was measured by the modified Jaffe method. The CV for the urinary albumin assay was 8% and for the urinary creatinine assay was 2% using blinded split samples.

Statistical analysis

The distribution of ACR was not normal, and log transformation was also not normal due to the large number of values at or near zero. Therefore, ACR was analysed as a dichotomous variable instead of a continuous variable. Most of the literature looking at PMH and albuminuria dichotomized ACR into microalbuminuria versus no microalbuminuria [8,9,13]. However, based on newer studies showing clinical significance of lower ACR levels [1,14], we defined ‘cases’ as the top decile (top 10%) of ACR values. We chose the top decile based on the ACR distribution of our population, with a median ACR of 2.9 mg/g and an interquartile range of 1.8–4.8 mg/g. The minimum value in the top decile was 9.2 mg/g. We also considered a cutoff of the top quartile, which was 4.8 mg/g, since this had been used in prior studies [1,14]. We ran secondary analyses using the top quartile.

Multivariate logistic regression was used to determine the odds ratio (95% CI) of being a case based on the category of hormonal status. We decided a priori to include age, eGFR, BMI and hypertension status in the multivariate model. The following covariates were examined as additional potential confounders: age at menopause, ACE inhibitor use, calcium-channel blocker use, hypercholesterolaemia, current and past smoking, aspirin use, multivitamin use, physical activity and alcohol intake. If inclusion of one of these covariates changed the odds ratio for PMH use by 10% or more, the covariate was included in the final model. We also looked for an interaction between hypertension status and hormone use in relation to albuminuria.

Secondary analyses were done to explore the association between the different types of hormones and ACR. Although we were able to compare oestrogens, progesterons and combined preparations, we did not have enough variability in types of hormone use to explore the types of oestrogens, and progesterons. We wanted to explore a dose–response relationship, but only 60% of the cohort reported the dose of oestrogen. Of these women, 74% were taking 0.625 mg/day of oestrogen while the other 26% were divided amongst four different dosing categories. We were also unable to explore the route of administration, as 86% of women were using oral PMH and only 7% were using a patch and 7% were using transvaginal hormones. However, we did run analyses excluding non-oral PMH users. A high number of women reported use of unopposed oestrogens, likely due to the fact that 35% of our women reported surgical menopause. We ran secondary analyses adjusting for the type of menopause (surgical versus natural).

Recent data have shown that a first morning urine sample is a more reliable predictor of 24-h urine albumin excretion than a random urine sample [15]. In our population, 94% of women reported that their urine sample was a first morning sample. We did secondary analysis considering only women who submitted a first morning urine sample.

All analyses were performed with SAS statistical software, version 9 (SAS Institute, Inc, Cary, NC, USA).

Results

Demographic, laboratory and other health-related data are presented by the category of hormone use in Table 1. In general, the characteristics did not appear to differ amongst the categories of PMH use, except that women with the longest duration of PMH use had earlier onset of menopause and were older than those with shorter duration of use. In this population, 96% of women were white. The values for urinary albumin, creatinine and ACR are presented by the category of hormone use in Table 2. ACR was weakly inversely correlated with urinary creatinine (r = −0.22, P < 0.001), but was highly correlated with urinary albumin (r = 0.77, P < 0.001). Therefore, ACR values should estimate daily albumin excretion and not creatinine excretion. The median ACR value for current hormone users was 2.6 mg/g and for past users and non-users 3.5 mg/g.

After adjusting for age, women in the categories of current PMH use of ≤3 years and >3–6 years had urinary albumin levels similar to those of non-users. However, women in the four categories of longer duration of use, >6–9 years, >9–12 years, >12–15 years and >15 years, had a lower odds of being in the top decile of urinary ACR when compared with non-users. Further adjustment for BMI, eGFR and hypertension yielded similar results. There was no dose–response with longer duration of PMH use (Table 3). For example, the odds ratio of being in the top decile for 6–9 years of PMH use was 0.41 (95% CI: 0.20–0.82) and that for >15 years 0.63 (95% CI: 0.42–0.94). The combined odds ratio for all women with >6 years of PMH use was 0.26 (95% CI: 0.15–0.46).
Table 1. Age-standardized characteristics by hormone use in 2000

<table>
<thead>
<tr>
<th>Current hormone use in years</th>
<th>No hormone use (n = 499)</th>
<th>Past hormone use (n = 574)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
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<tr>
<td>67.7 (6.6)</td>
<td>68.5 (6.5)</td>
<td>63.1 (7.0)</td>
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<tr>
<td>Age at menopause (years)</td>
<td></td>
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<tr>
<td>49.6 (5.0)</td>
<td>48.1 (6.0)</td>
<td>50.4 (5.7)</td>
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<tr>
<td>eGFR (mL/min/1.73 m²)</td>
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<tr>
<td>76.6 (16.0)</td>
<td>77.0 (16.5)</td>
<td>76.4 (15.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>26.4 (5.6)</td>
<td>26.5 (5.6)</td>
<td>26.5 (4.5)</td>
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<tr>
<td>Hypertension (%)</td>
<td></td>
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<tr>
<td>129.5 (13.6)</td>
<td>129.8 (14.6)</td>
<td>129.7 (13.5)</td>
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<tr>
<td>Diastolic BP (mmHg)a</td>
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<tr>
<td>Ace-I use (%)</td>
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<tr>
<td>CCB use (%)</td>
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<td>High cholesterol (%)</td>
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<td>Current smoking (%)</td>
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<td>Past smoking (%)</td>
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<tr>
<td>ASA (% daily use)</td>
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<tr>
<td>MVI (% daily use)</td>
<td></td>
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<tr>
<td>Met (total/week)b</td>
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<tr>
<td>Alcohol (g/day)b</td>
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<tr>
<td>PMH, estrogen only (%)</td>
<td></td>
<td></td>
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<tr>
<td>PMH, combined estrogen/progesterone (%)</td>
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<td></td>
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<tr>
<td>Participants with ACR values in top decile (%)</td>
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</table>

Values are means (SD) except where noted.

aValues from 1998 because not recorded on 2000 survey.

bValues are medians (IQR); not age standardized.

eGFR, estimated Glomerular Filtration Rate; BMI, body-mass index; ACE-I, angiotensin converting enzyme inhibitor; CCB, calcium-channel blocker; ASA, aspirin; MVI, multivitamin; Mets, metabolic equivalents; PMH, postmenopausal hormones; SD, standard deviation; IQR, interquartile range.

Table 2. Median (IQR) urinary values by hormone use

<table>
<thead>
<tr>
<th>Current hormone use in years</th>
<th>No hormone use (n = 499)</th>
<th>Past hormone use (n = 574)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine albumin (mg/dL)</td>
<td>2.1 (1.2, 3.7)</td>
<td>1.9 (1.3, 3.3)</td>
</tr>
<tr>
<td>Urine creatinine (mg/dL)</td>
<td>61 (43, 88)</td>
<td>61 (42, 89)</td>
</tr>
<tr>
<td>Urine ACR (mgAlb/gCr)</td>
<td>3.5 (2.1, 5.8)</td>
<td>3.5 (2.1, 5.3)</td>
</tr>
<tr>
<td>Participants with ACR values in top decile (%)</td>
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aP-value based on non-parametric Kruskal–Wallis test.

bMedian (IQR) urinary values for the top decile of ACR are 11.4 (5.8, 21.2) for urine albumin (mg/dL), 55 (37, 86) for urine creatinine (mg/dL), and 16.4 (11.7, 33.8) for urine ACR (mgAlb/gCr).

ACR, albumin:creatinine ratio; Alb, albumin; Cr, creatinine; IQR, interquartile range.

use as compared with that for non-users was 0.55 (95% CI: 0.39–0.77). The multivariate-adjusted odds ratio of being in the top decile of ACR for past users compared with women with no PMH use was 0.75 (95% CI: 0.52–1.09). Further adjustment for the other variables in Table 1 did not materially change the results, including adjustment for ACE inhibitors and calcium channel blockers given their known association with albuminuria [16]. We adjusted for current and total lifetime analgesic use, since our study population was derived from an analgesic study cohort. There was no change in results with these analyses. When the analyses were repeated looking at the top quartile of ACR, we found similar associations between ACR and PMH use. There was no interaction with the history of hypertension (P = 0.85).
When looking only at women who provided first morning urine samples, the OR of being in the top ACR decile was 0.50 (95% CI 0.35–0.68, 95% CI 0.30–1.55), their use was infrequent (6.2% of total hormone users). After adjusting for the type of menopause (surgical versus natural), the results were unchanged (OR 0.55; 95% CI 0.39–0.80) for women with ≤6 years of PMH use compared with non-users. In a sub-analysis looking at only women taking oral PMH, the OR for being in the top ACR decile was 0.50 (95% CI 0.35–0.71) for women with >6 years of PMH use compared with non-users. When looking only at women who provided first morning urine samples, the OR of being in the top ACR decile was 0.57 (95% CI: 0.40–0.82) for women with >6 years of PMH use compared with non-users.

### Discussion

Current hormone use of >6 years was associated with a lower odds of being in the top ACR decile in non-diabetic women. This relation was not confounded by any of our candidate covariates except age. Although the majority of ACR values were below the traditional cutpoint for microalbuminuria, recent data suggest that albuminuria, even at low levels, is predictive of cardiovascular disease and hypertension [1,14].

Very few published studies report the relationship between PMH use and albuminuria. One observational study of 491 postmenopausal women found that those taking PMH (36%) had a 19% reduction in ACR over 5 years ($P = 0.008$) [8]. However, the authors did not report the change in ACR in the women not taking PMH. In addition, the odds ratio for developing microalbuminuria was 0.67 (95% CI, 0.43–1.01) in PMH users compared to non-users. Although not statistically significant, this suggests a similar inverse association between PMH use and albuminuria as in our findings [8].

In contrast, a cross-sectional study showed a positive association [9]. Investigators obtained 24-h urine collections from 1518 postmenopausal women. The adjusted OR for microalbuminuria (defined as 30–300 mg albumin/24 h) was 2.15 (95% CI, 1.12–3.77) for users of PMH compared to non-users. The association was stronger among women with >5 years of PMH use (OR 2.56; 95% CI, 1.32–4.97). There are several important differences between that study and ours. Only 10% of the participants were PMH users, compared with 57% of our participants. The mean age of PMH users was 53 years and of non-users was 60 years, while in our study the respective ages were 66 years and 68 years. Although our population was older, the duration of use was longer. In both of the studies, most women began PMH in the peri-menopausal years. Their population was selected specifically so that over 70% of the cohort had >10 mg/day of albumin excretion prior to entering the study. Our study participants were not selected based on albumin excretion. The prevalence of hypertension was 28% in their population and 52% in our population. Although we excluded diabetics and they did not, <5% of their population was diabetic. Despite the study design differences, it is not clear why the studies reached opposite conclusions.

There have been a limited number of randomized trials looking at exogenous hormones and albuminuria [13,17]. In one trial [13], 47 diabetics were randomized to receive PMH or placebo. No difference in ACR was found between the two groups after 6 months. However, despite randomization, the prevalence of ‘clinical microalbuminuria’ (defined as ACR ≥30 mg/g Cr) was 4-fold higher at baseline in the PMH group. The authors did adjust for this in the analyses, but the possibility of selection bias remains. In another trial [17], 41 hypertensive women, half of whom had diabetes, were randomized to transdermal PMH or placebo. There was no difference in the change in 24-h urine protein between the groups over 12 weeks. This study was limited by the short duration, small number of women and transdermal PMH use only, and all of the women being diabetic or hypertensive. In a third trial [18], 60 ‘healthy’ post-menopausal women were randomized to receive either 1 mg of estradiol or placebo for 6 months. At the end of the 6 months, there was no difference in albuminuria as measured by the albumin content in a 12-h overnight urine sample. This study was also limited by the short duration of use. In our study, we found that there was a difference in albumin excretion amongst women with >6 years of PMH use; this study of 6 months in duration is likely insufficiently long to demonstrate this association.
The mechanism linking PMH use and albuminuria is unknown, although it is believed to be due to oestrogen. Albuminuria is often viewed as a glomerular abnormality. However, low levels of albuminuria may be due to a tubular abnormality [19]. Given the low levels of albuminuria, we must consider both glomerular and tubular mechanisms.

There are several theories that involve oestrogen in the regulation of glomerular albuminuria. In rats, oestrogen reduces angiotensin converting enzyme (ACE) mRNA and downregulates ACE transcription and conversion of angiotensin I to angiotensin II [20]. This would decrease the efferent arteriolar pressure, decreasing intraglomerular pressure and therefore decreasing albuminuria [21]. In a study of 330 post-menopausal women, those on oestrogen-based PMH had lower renin levels than those not on therapy ($P = 0.001$) [22]. The renin promoter contains an estrogen-response element that may mediate this effect. Lowering renin would also lower angiotensin II. In contrast to this observational study of PMH and renin levels, most of the mechanistic studies performed in humans have shown up-regulation of the renin–angiotensin system. However, these studies used oral contraceptives, not PMH. Oral contraceptives contain much higher doses of oestrogen [23,24]. Another potential mechanism involves nitric oxide synthetase (NOS). Estrogen administration in rats induces NOS in glomerular endothelial cells. Nitric oxide is a known vasorelaxant, and NOS inhibition in rats causes albuminuria. Thus, induction of renal NOS formation may prevent this from occurring [25,26]. More research is needed in humans to understand the mechanisms of this association between PMH and glomerular albuminuria.

The mechanisms of tubular albuminuria are not as well elucidated. Proximal tubule cells reabsorb filtered albumin through endocytosis, with megalin and cubilin as the critical receptors [19,28]. Little is known about how this process is regulated [19], and therefore it is unknown whether changes in oestrogen levels would influence tubular reabsorption of albumin.

Limitations of our study deserve mention. The cross-sectional design prevents determination of temporal association, and the possibility of residual confounding remains. We only had one urine measurement, and within-person variation in urinary albumin is as high as 14% over 2 days [29]. However, this variability should bias results towards the null. Given the limited amount of information we had regarding the dose of PMH, and the fact that most women were taking a similar dose, we were unable to assess the dose of PMH in relation to our outcome. The participants in this study were primarily white, so the results may not be generalizable. We did not have sufficient power to analyse the diabetic study participants, so our findings may not be generalizable to diabetic women.

In conclusion, long-term PMH use appears to be associated with a lower risk of albuminuria in non-diabetic women. Prospective studies are required to confirm this association.

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Conflict of interest statement. None declared.

References

Kidney function and future risk for adverse pregnancy outcomes: a population-based study from HUNT II, Norway

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Abstract

Background. Current knowledge on preconception reduced kidney function and the risk of adverse pregnancy-related outcomes mainly relies on small studies in selected populations. We aim to investigate whether reduced kidney function is associated with the risk of adverse pregnancy-related outcomes in the general population.

Methods. A population-based study linking all women attending the Second Health Study in Nord-Trøndelag, Norway (1995–97) and subsequent pregnancies registered in the Medical Birth Registry. Multivariable random-effect logistic regression analysis was used to explore the association between renal function and study outcome.

Results. The mean eGFR among 3405 women was 107.6 ± 19.4 ml/min/1.73 m² at baseline; 18.8% and 0.1% had eGFR of 60–89 and <60, respectively. Over the next 11 years, they gave birth to 5655 singletons of whom 885 (17.7%) were complicated with preeclampsia, small for gestational age (SGA) or preterm birth. Women with eGFR 60–89 were not at increased risk for this combined outcome compared to women with eGFR ≥90, although women with eGFR 60–74 tended to have an increased risk. Neither was reduced kidney function a risk factor among women with microalbuminuria, but those with an eGFR of 60–89 plus hypertension had a significantly increased risk: odds ratios for preeclampsia, SGA or preterm birth were 2.58 (95% CI 1.40–4.75, P < 0.001) and 10.09 (95% CI 2.38–42.87, P < 0.001) in hypertensive women with eGFR 75–89 and 60–74, respectively. Relative excess risk due to interaction between reduced kidney function and hypertension was 2.23 (95% CI 1.35–3.10, P < 0.001). Women with a reduced kidney function were not at increased risk for other pregnancy complications like caesarean section, maternal bleeding, dystocia, pre-labour rupture of membranes, Apgar score ≤7, stillbirth or congenital malformations.

Conclusions. Women with eGFR 60–89 ml/min/1.73 m² were not at increased risk for preeclampsia, SGA or preterm birth unless they were also hypertensive.

Keywords: glomerular filtration rate; mild reduced kidney function; population-based cohort study; pregnancy outcome

Introduction

Pregnancy imposes significant stress on the kidneys, resulting in an increased risk for maternal as well as fetal complications in subjects with established moderate-to-serious