ORIGINAL PAPER

IJCP THE INTERNATIONAL JOURNAL OF CLINICAL PRACTICE

Hormone replacement therapy, renal function and heart ultrasonographic parameters in postmenopausal women: an observational study

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SUMMARY

Background and Aim: A certain degree of impaired kidney function is related to an increased cardiovascular risk. The cardiovascular protection exerted in the postmenopausal state by the hormone replacement therapy (HRT) is debated. No studies have so far explored the relationship between menopause, renal function and cardiovascular risk profile in healthy menopausal women in relation with HRT. **Subjects and Methods:** A total of 362 postmenopausal healthy women with normal albumin excretion rate were recruited and divided into two groups (HRT+ and HRT−) according to the presence or absence of HRT. All participants underwent a complete routine biochemical analyses and an echocardiogram. **Results:** Clinical characteristics of the two groups were similar, but HRT+ showed a significantly higher estimated glomerular filtration rate (GFR; by CKD-EPI formula). Regarding the heart ultrasonography, HRT+ had a significantly lower size of the aortic root and left atrium diameter ($p = 0.038$ and $p = 0.012$, respectively); no differences were found in the ejection fraction and Left Ventricular Mass Index (LVMI). In the whole study group, eGFR correlated inversely with LVMI and with the size of the aortic root (both $p < 0.0001$), being GFR the only determinant of the former by a stepwise regression. Dividing the study population according to an eGFR cut-off (> 80 and < 80 ml/min/1.73 m^2); > 80 women, in comparison with < 80, showed a significantly lower LVMI and lower size of aortic bulb, further reduced in the HRT+. **Conclusion:** In a cohort of healthy, drug-naïve, postmenopausal women, HRT seems to positively affect glomerular filtration and is associated with lower values of left ventricular mass and aortic root size, thus offering a further mechanism through female hormones exert cardioprotection.

What's known

- Chronic renal insufficiency is a major risk factor for acute cardiovascular events.
- Menopause accelerates the progression of kidney damage, but the role of estrogens in preserving renal function is debated.
- No information are available on the relationship between hormone replacement therapy (HRT), kidney function and parameters of myocardial function in healthy postmenopausal women.

What's new

In healthy postmenopausal women not carrying the common cardiovascular risk factors, HRT seems:

- to preserve glomerular filtration rate without influencing albumin excretion;
- is associated with more safe values of Left Ventricular Mass Index and size of aortic root, mainly in those with a relatively reduced glomerular filtration.

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Disclosures
None for all the authors.

Introduction

The incidence of cardiovascular disease, the main cause of death among women, increases substantially after menopause (1), possibly because of the changes in the sex hormone environment and the precipitous decrease in plasma oestrogen levels. In the late 1980s, observational studies suggested that hormone replacement therapy (HRT) in the postmenopausal period might reduce the risk of morbidity and mortality of coronary heart disease (2,3); however, this initial enthusiasm was lately reduced by the results of prospective randomised clinical trials for primary and secondary cardiovascular prevention, documenting an increased risk for incidence of breast cancer and adverse effects on the cardiovascular system following HRT (4,5). In the last 5 years, even in the presence of encouraging reports documenting a protective effect of basal HRT in preventing development of breast cancer in approximately 25% of the cases (6) or suggesting vascular benefit of an early initiation of HRT, likely before the existence of advanced atheromatous plaque (7), large epidemiological surveys still raise concerns regarding the opportunity to treat postmenopausal women (8,9) unless the presence of boring symptoms.

Large epidemiological observations, as well as controlled studies, have identified chronic renal damage as a main determinant of cardiovascular morbidity and mortality in the general population and in high-risk cohorts of patients (10,11); interestingly, a continuous relationship seems to occur between risk and clinical hallmarks of chronic renal impairment such as albuminuria and reduced glomerular filtration rate

(GFR) below 60 ml/min/1.73 m^2 (12), and the state of the kidney predicts the occurrence of acute vascular events (13,14) implying the need to adequately identify and manage even a modest decline of kidney function in high-risk subset of patients, as well as in the general population.

The role of oestrogens in preserving kidney function is still matter of debate. Convincing evidence show their direct nephroprotective effects in experimental diabetes and in 5/6 remnant kidney animals (15–17). Beside the strong influence exerted by oestrogens on calcium and phosphate metabolism, the progression of kidney damage may be faster in postmenopausal women (18), and the prospective analysis of the Rancho Bernardo study has recently shown a beneficial effect of HRT on albuminuria development, with no effect on GFR (19). Partially at odds with such traditional view, it has been recently suggested as increased levels of oestradiol may play a role in the development of subclinical kidney damage in both African and white men (20); the topic is made even more complex by partially contradictory results obtained in few interventional studies performed in postmenopausal type 2 diabetic women, with a reduction in proteinuria and a delayed decline of renal function observed in some studies (21) but not in others (22). Scarce available information link heart and renal function in healthy postmenopausal women; to address this issue, we planned this cross-sectional, observational study.

Subjects and methods

Subjects

Three-hundred and seventy-four healthy postmenopausal women, consecutively referring to the 'Donna Cuore' outpatient clinic in the years 2008–2010, were enrolled. Inclusion criteria were absence of diabetes, hypertension or cardiovascular disease or any other chronic major disease, and normal AER (expressed as urine albumin to creatinine ratio) in at least two urine spot samples the 6 months preceding the study. Their menopausal status was confirmed by the centralised measurement of FSH, LH and estradiol, all within the diagnostic range (FSH 41–124 mUI/ml, estradiol 12–30 pg/ml, LH 8.8–55 mUI/ml). All women signed a written informed consent.

In the day of the study, all volunteers underwent a complete physical examination; height and weight were recorded and blood pressure was measured in the sitting position with a mercury sphygmomanometer after a 10-min rest; the mean of two measurements was used for statistical analysis; a fasting venous blood sample was also drawn. A 12-electrocardiogram (ECG) was recorded and analysed by the same cardiologist (M.T.C.). All women also underwent an ultrasonographic evaluation of the heart.

Biochemical parameters

Complete blood count, glucose, HbA1c, serum electrolytes, sGOT, sGPT, uric acid, fibrinogen and high-sensitive C-reactive protein (hsCRP) were determined by standard techniques. Plasma cholesterol and triglycerides concentrations were measured by standard enzymatic assays with commercially available kits. HDL-cholesterol was measured after precipitation of ApoB containing lipoproteins, allowing for the estimate of LDL-C by the Friedewald formula. Serum creatinine was measured by the IDMS traceable method; GFR was estimated using the CKD-EPI equation $(\mathrm{GFR} = 141 \times \min(\mathrm{Scr}/\kappa,1)^{\alpha} \times \max(\mathrm{Scr}/\kappa,1)^{-1.209} \times 0.993^{\mathrm{Age}} \times 1.018$ [if female] $\times 1.159$ [if black]), where Scr is serum creatinine (mg/dl), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1 and max indicates the maximum of Scr/κ or 1 (23). AER was measured on a 24-h urine collection performed the day before the visit.

Cardiac ultrasonography

Transthoracic two-dimensional and Doppler echocardiographic examination was performed with an EnVisor C-Philips ultrasound instrument (Philips, Eindhoven, The Netherlands), equipped with a second-harmonic imaging and a 3.5-MHz transducer, according to the American Society of Echocardiography (24). Relative diastolic wall thickness was estimated as twice the posterior wall thickness divided by left ventricular (LV) end-diastolic dimension. LV volume indices and LV Mass Index (LVMI) were calculated with the use of the Simpson's biplane method, as from De Simone et al. (25). Ejection fraction was calculated using the Simpson formula. Pulsed spectral Doppler was performed to measure mitral inflow parameters, including peak E (early diastolic myocardial) velocity, peak A (late diastolic myocardial) velocity and E/A ratio. The aortic annular diameter was measured perpendicular to the long axis of the vessel between the hinge points of the aortic valve leaflets (inner edge-inner edge). The maximal tricuspid regurgitation velocity was measured by continuous wave Doppler echocardiography in the four-chamber view, and Bernoulli's modified equation was used to calculate the systolic pulmonary pressure. All measurements were taken as the mean of 3 beats.

Statistical analysis

Data are reported as mean $\pm$ SD or median [IQR]. Given the exploratory nature of the study, no prespecified outcome variable was selected, and a

formal power analysis was not carried out. Variables with skewed distribution, including triglyceride and hsCRP, were log transformed for analyses. Comparisons of data between the two groups were performed by *t*-test or ANOVA; linear regression and multivariate analyses were also performed. Data were analysed by using the StatView software package (SAS, Cary, North Carolina, USA). Statistical significance was determined on a probability level of ≤ 0.05.

Results

Among the 374 participants in the study, fasting plasma glucose was diagnostic for type 2 diabetes in 12 (3.2% of women), therefore excluded from the study; the remaining 362 volunteers were divided into two groups according to their hormonal status: women assuming HRT (HRT+, $n = 101$) and women who had never taken or had interrupted since at least 1 year the replacement therapy (HRT−, $n = 261$).

Clinical characteristics and biochemical parameters of the two groups are shown in Table 1. The two subsets of women were well matched for age and BMI, have similar blood pressure levels and did not differ for the main biochemical parameters.

In the whole cohort, mean eGFR was 91.0 ± 17.5 ml/min/1.73 m^2, the median AER was 6.3 mg/24 h [range 2.16–33.9]. When we compared the two groups, no difference emerges in AER (HRT+: 6.45 [2.16–29.10; 8.17]; HRT−: 6.15 [2.70–33.90; 8.25] mg/24 h); the percentage of women having AER in the so called low-albuminuria range (i.e. above 10 mg/24 h) was similar in HRT+ and HRT− (30.7% vs. 33%, p = ns). However, HRT+ were characterised by a significantly higher eGFR (94.3 ± 17.6 vs. 89.8 ± 17.3 ml/min/1.73 m^2, p = 0.026 vs. HRT−).

Electrocardiograms were all evaluated by the same cardiologist. Briefly, none had evidence of lesion waves or major arrhythmias. Table S1 summarises the ECG results; no difference was observed in the distribution of the minor ECG abnormalities between HRT+ and HRT− women.

Table 2 shows the main ultrasonographic cardiac measures. HRT+ were characterised by a significantly lower size of the aortic root and left atrium diameter (p = 0.038 and p = 0.012, respectively); no differences were found in the ejection fraction, height-adjusted LVMI or estimated pulmonary pressure between the two groups.

Table 3 shows the correlations between some ultrasonographic measures and the main metabolic parameters. As expected, LVMI was significantly related with age, BMI and systolic blood pressure and, more intriguingly, with fasting plasma glucose and uric acid; this latter parameter was also directly related with the size of aortic root. In this cohort of healthy postmenopausal women, none of the main anthropometric or metabolic parameters were related with the systolic ejection fraction.

In the whole study group, expected linear inverse correlations emerged between eGFR and age (standard coefficient: −0.314), duration of the menopausal state (standard coefficient: −0.254), and uric acid (standard coefficient: −0.301), all $p < 0.0001$. More interestingly, eGFR correlated inversely with height-adjusted LVMI (standard coefficient: −0.307, $p < 0.0001$) and with the size of the aortic bulb (standard coefficient: −0.238, $p < 0.0001$). We then

Table 1 Clinical characteristics of the two study groups

	HRT+ (*n* = 101)	HRT− (*n* = 261)
Age (years)	54.4 ± 5.0	55.6 ± 5.9
Menopause duration (years)	8.6 ± 6.5	7.1 ± 6.4
BMI (kg/m^2)	25.5 ± 3.6	26.3 ± 4.4
SBP (mmHg)	128 ± 18	131 ± 15
DBP (mmHg)	79 ± 14	81 ± 11
Fasting glucose (mg/dl)	93 ± 11	95 ± 14
HbA1c (%)	5.5 ± 0.9	5.5 ± 0.7
Total cholesterol (mg/dl)	220 ± 40	225 ± 34
LDL-cholesterol (mg/dl)	134 ± 35	138 ± 30
HDL-cholesterol (mg/dl)	64 ± 17	65 ± 16
Triglycerides (mg/dl)	105 ± 53	102 ± 51
Serum creatinine (mg/dl)	0.76 ± 0.12	0.78 ± 0.21
Aspartate transaminase (UI/l)	22 ± 6	21 ± 5
Alanine transaminase (UI/l)	21 ± 12	20 ± 11
K^+ (mEq/l)	3.9 ± 0.7	4.0 ± 0.5
Ca^{++} (mEq/l)	9.2 ± 0.4	9.3 ± 0.8
Uric acid (mg/dl)	4.1 ± 1.0	4.2 ± 1.2
Red cell count (m/mmc)	4.74 ± 0.33	4.70 ± 0.39
Hb (g/dl)	13.9 ± 0.9	13.7 ± 1.0
White cell count (n/mmc)	6369 ± 1589	6282 ± 1605
Platelets ($n \times 10^9$/l)	271 ± 58	273 ± 68
Fibrinogen (mg/dl)	325 ± 60	320 ± 57
hsCRP (mg/l)	0.26 ± 0.43	0.27 ± 0.37

Table 2 Main heart ultrasonographic parameters in the two study groups

	HRT+ (*n* = 101)	HRT− (*n* = 261)
Aortic root (mm)	28.4 ± 3.0*	29.2 ± 3.3
Left atrium diameter (mm)	37.0 ± 2.6*	38.1 ± 3.4
EF (%)	59.1 ± 2.7	58.9 ± 3.3
LVMI/H (g/m^2)	42.2 ± 9.2	43.2 ± 11.3
E/A ratio	0.97 ± 0.24	0.99 ± 0.27
Estimated PAP (mmHg)	30.8 ± 5.4	31.7 ± 5.0

*$p < 0.05$.

Table 3 Matrix of univariate correlations between mean heart ultrasonographic measures and anthropometric and metabolic parameters

	Age	BMI	SBP	Glucose	LDL-cholesterol	HDL-cholesterol	Uric acid	LVMI/H	Aortic root	EF
Age	–									
BMI	0.103	–								
SBP	0.174*	0.129*	–							
Glucose	0.144*	0.307*	0.088	–						
LDL-cholesterol	0.054	0.120*	0.071	0.042	–					
HDL-cholesterol	0.079	−0.316*	0.029	−0.261*	−0.141*	–				
Uric acid	0.121*	0.284*	0.105*	0.210*	−0.016	−0.199*	–			
LVMI/H	0.317*	0.393*	0.164*	0.148*	0.132	−0.110	0.215*	–		
Aortic root	0.026	−0.030	−0.004	0.010	−0.038	0.003	0.146*	0.090	–	
EF	0.081	0.055	0.074	−0.002	0.083	−0.065	−3.3E-4	0.106	−0.100	–

BMI, body mass index; SBP, systolic blood pressure; LVMI/H, height-adjusted Left Ventricular Mass Index; EF, ejection fraction.
*$p < 0.05$.

divided the study population according to an arbitrary eGFR cut-off (above or below 80 ml/min/1.73 m^2: > 80 and < 80, respectively); > 80 women, in comparison with < 80, showed a significantly lower size of aortic bulb and lower LVMI. However, when we analysed only the < 80 subset of women, aortic root size was lower in HRT+ than in HRT−. These data are reported in Figure 1.

By a stepwise regression analysis, in a model including fasting glucose, systolic blood pressure, LDL-cholesterol, GFR and presence/absence of HRT, after adjustment for age and BMI, eGFR (β coefficient: −0.118) was the only determinant of LVMI in the whole study cohort.

Discussion

The main information coming from this cross-sectional, observational study performed in healthy postmenopausal women apparently free from traditional cardiovascular risk factors is a peculiar relationship between HRT and renal function, with a significantly higher estimated GFR in women assuming it. This is somehow in agreement with the cross-sectional analysis of the Rancho Bernardo Study, describing a better GFR in oestrogens user women, even in the absence of any relevant reduction in CKD incidence along the follow-up (19). Among the possible explanations for that, an inhibition of nitric oxide synthase by oestradiol can be hypothesised (26), with clear sexual dimorphism in the NO system, and premenopausal females producing more NO than men; alternatively, a regulation of extracellular matrix production, an attenuation of tubulointerstitial fibrosis (15,16), and even a protective effect on podocytes (27) can be suggested, although these mechanisms are unlikely to be demonstrated in humans. The effect of oestrogen treatment on albumin excretion rate in ageing women are more debated, with not only several large studies documenting a reduction effects (19,21) but also some diverging results (28). The absence of effect of oestrogens on albumin excretion rate in our cohort is likely to be because of the fact that all our volunteers were normoalbuminuric and none of them had diabetes or hypertension, therefore, making very difficult to catch any relevant alteration of albuminuria.

The lack of difference in LVMI and in the ejection fraction between women assuming or not assuming hormone replacement is in agreement with one previous cross-sectional and longitudinal reports (29). However, in our cohort, women on replacement therapy showed a lower size of the aortic root and a lower left atrium diameter; we might speculate a positive effect on aortic distensibility and elastic properties (30) for the former, and a reduction in preload by oestrogens, whose receptors are also located in the great veins (31) for the latter.

A relatively novel finding is the attempt to correlate renal function with cardiac ultrasonographic parameters. It is known that echocardiography in patients with chronic kidney disease frequently shows LV hypertrophy, volume overload, and diastolic and systolic dysfunction, all precursors for the development of heart failure (32), but a few studies have so far addressed similar issues in the absence of any heart disease. LVM increased significantly with increasing subclinical glomerulosclerosis observed in kidney biopsies of healthy kidney donors (33), and serum creatinine is an independent predictor of development of ventricular hypertrophy in neodiagnosed hypertensive individuals (34). Our data inter-

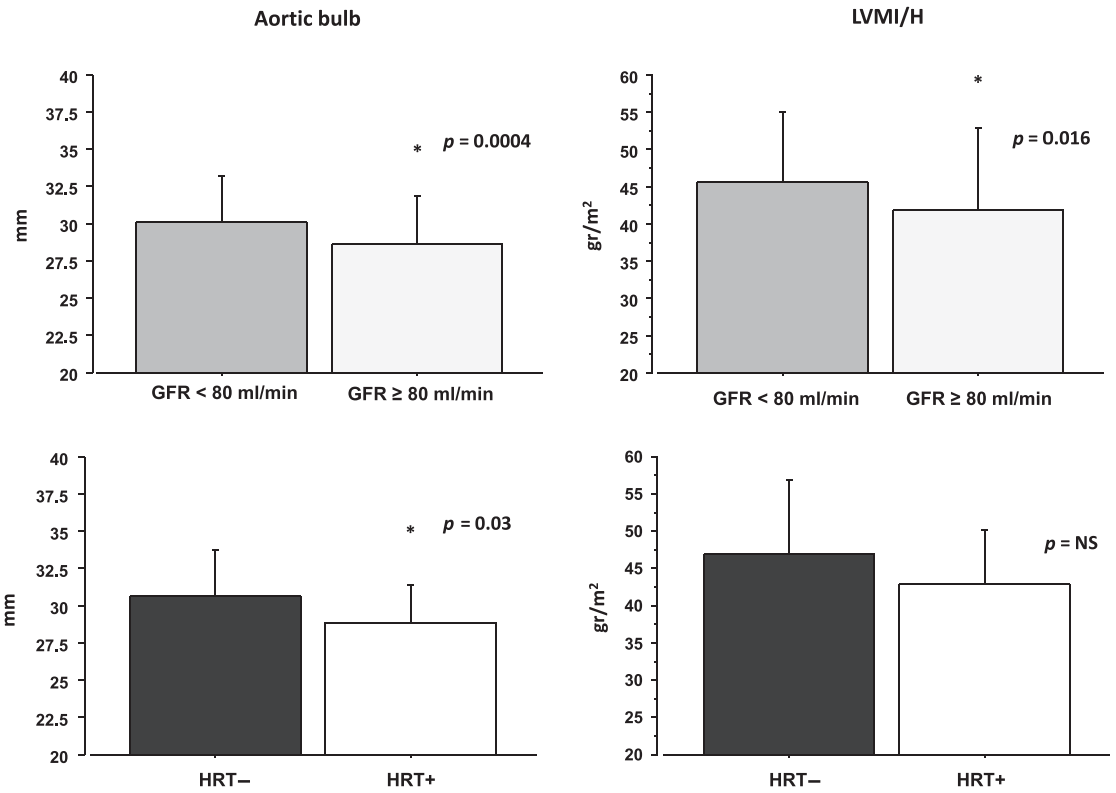

Figure 1 Size of the aortic bulb (left) and height-adjusted Left Ventricular Mass Index (LVMI) in the whole study group (upper panels) according with GFR, and in women with GFR < 80 ml/min/1.73 m^2 (lower panels) according to the presence (HRT+) or absence (HRT−) of hormone replacement therapy

estingly point out an inverse relationship between GFR and LVM even in these women with a fully preserved renal function, again confirming the existence of a continuous relation among renal and heart function, with the latter strictly influenced by the former across a wide range of conditions. To our knowledge, no previous reports have so far related aortic root size with GFR in healthy individuals, although large epidemiological studies had already reported a lower arterial elasticity in subjects with preclinical kidney disease participating in the Multiethnic Study of Atherosclerosis (35). The direct relationship found in our whole cohort of women between uric acid levels and either LVM and size of aortic root somehow supports the hypothesis of a strict cross-talk between the heart and the kidney; an alternative explanation for such relationship recalls the role of uric acid as an independent marker of early cardiovascular impairment, rather than an indicator of the state of the kidney. In agreement with the latter hypothesis, serum uric acid has been inversely related with ejection fraction in patients with chronic heart failure (36) and with aortic root in hypertensive individuals (37), and allopurinol showed the capacity to reduce LV hypertrophy in diabetic patients, likely through a reduction in oxidative stress (38).

Conclusion

Taken together, the results of this observational study performed in real-life conditions reinforce the concept of a strict relationship between GFR, the more reliable index of kidney function, and standardised, quantitative measures of heart performance; the intriguing finding of lower values of LV mass and aortic root in women combining HRT with a higher GFR suggest another putative cardio-protective mechanism exerted by female hormones.

Author contributions

E. Vitolo collected and analysed clinical data and set the database. M. Comassi contributed to collect and analyse clinical data. M.T. Caputo is the cardiologist who performed all the ultrasonographic evaluations. A. Solini designed the study, interpreted the results, wrote the paper.

Acknowledgements

This study was supported by individual grants to E. Vitolo and M. Comassi from the Italian Minister of Health.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Main ECG alterations recorded in HRT+ and HRT− women. Data are reported as *n* (%).

Paper received July 2014, accepted October 2014