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*Original Article*

# Influence of Current and Past Hormone Replacement Therapy on Bone Mineral Density: A Study of Discordant Postmenopausal Twins

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**Abstract.** There is controversy about the ideal timing of hormone replacement therapy (HRT) and duration of treatment. In this study we have examined intrapair differences in bone mineral density (BMD) in twins who were discordant for HRT use. Twin pairs in which only one co-twin had been exposed to HRT for more than 12 months continuously were selected from 365 postmenopausal monozygotic (MZ) and dizygotic (DZ) pairs recruited as part of the St Thomas' Adult UK Twin Registry of normal volunteers. BMD was measured by dual-energy X-ray absorptiometry at the lumbar spine and femoral neck. Intrapair differences in BMD between HRT users and non-users were compared. A total of 65 HRT-discordant pairs were identified, of which 36 were discordant for current HRT use (mean age: 55.3 years, median duration of HRT use: 36 months) and 29 were discordant for past HRT use (mean age: 60.4 years, median HRT duration: 30 months). Among current users BMD was consistently and significantly higher than in non-users at both sites (lumbar spine mean intrapair difference (IPD%): 12.3%, 95% confidence interval (CI): 7.1%, 17.5%; femoral neck IPD%: 8.6%, 95% CI: 3.4%, 13.7%). The intrapair differences were substantially smaller when past users and non-users were compared (lumbar spine IPD%: 2.4%, 95% CI: –3.7%, 8.6%; femoral neck IPD%: 0.4%, 95% CI: –5.3%, 6.0%). These differences remained little changed after adjusting for the potential confounding effects of the duration of HRT use, and intrapair differences in alcohol and tobacco consumption and physical exercise. The results confirm, in a closely matched design, the findings of other observational research that current use of HRT has a major effect on BMD at the lumbar spine and femoral neck. Past users of HRT do not, however, show the same benefits. The clinical implications of these findings are that HRT needs to be used continuously to influence BMD and that alternative treatments need to be considered in those who discontinue HRT.

**Keywords:** Matched case–control study; Osteoporosis

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## Introduction

Hormone replacement therapy (HRT) is recognized to have beneficial effects on bone density and as a result has an established place in the treatment and prevention of osteoporosis [1]. The ideal timing of use and duration of treatment, however, have not been established. While one study has shown HRT to have a sustained effect in delaying bone loss [2], two prospective studies have shown that the rate of decline in bone mineral density (BMD) after discontinuing HRT is disproportionately high, suggesting there is no prolonged effect [3,4]. A number of retrospective epidemiologic studies have failed to show any influence of past HRT use in preventing fractures [5], again suggesting that benefits are short-lived.

In this report we assess the effect of HRT on BMD by comparing the distribution of BMD in postmenopausal twin pairs in which co-twins differed in their exposure to HRT. We have examined two types of twin pair: (i) pairs in which one member was a current user and their co-twin had never used HRT and (ii) pairs in which one member was a past user and their co-twin had never used HRT. This co-twin case–control twin study design allows an assessment of the influence of current and

past HRT use on BMD in pairs matched for age, genetic factors, and a range of environmental, social and confounding variables both known and unknown. The twin design has an advantage over other published cross-sectional population studies of this association in which it is difficult to take the effects of selection bias and the influence of confounding fully into account.

## Subjects and Methods

### *Study Population*

The subjects were selected from identical (MZ) and non-identical (DZ) twin pairs who had been recruited into the St Thomas' Adult UK Twin Registry by a media campaign as previously described [6]. All were female volunteers broadly representative of the general population. None of the study subjects was aware of the hypothesis under test. Ethics approval was obtained from the hospital ethics committee and full informed written consent was obtained from all subjects at the first visit. Zygosity was determined by a standard questionnaire [7] and confirmed by multiplex DNA fingerprinting.

### *Data Ascertainment*

All subjects had been interviewed by nurses and had completed a detailed questionnaire, with questions designed to ascertain their menopausal history and history of HRT use. Subjects were defined as post-menopausal if amenorrheic for 12 months or greater. Their postmenopausal status was confirmed by measuring serum follicular stimulating hormone (FSH) and estradiol ($E_2$). FSH levels of greater than 43.7 U/l and $E_2$ levels of less than 60 pmol/l were taken as confirming postmenopausal status. Records of smoking, alcohol intake and regular current physical activity were obtained. Regular current physical activity was defined as sporting activity at least once a week in the previous year. Heights were recorded using a wall-mounted stadiometer and weight with an electronic balance. Height and weight were used to calculate the body mass index ($kg/m^2$).

### *BMD Measurements*

BMD was measured at the lumbar spine and hip by dual-energy X-ray absorptiometry (DXA) using a Hologic QDR 2000 (Hologic, Waltham, MA). The coefficient of variation in our hands is 0.6–1.6%. BMD at the lumbar spine was measured at L1–4 and at the femoral neck site at the hip.

### *Subject Stratification*

Individuals were classified as HRT 'users' if they had taken HRT for 12 months or more continuously. These were divided into current users and past users and the duration of use was recorded. Subjects were classified as past users once they had stopped HRT entirely for 6 months or more at the time of the study. 'Never users' had never used HRT. From the entire sample, pairs in which (i) one twin was a 'current user' and the other a 'never user' (C/N pairs) and (ii) one twin was a 'past user' and the other a 'never user' (P/N pairs) were selected. Type of HRT used was determined by asking the twin to select the appropriate type from a list. If more than one preparation had been taken, the preparation used for the longest time was noted.

### *Statistical Analysis*

The influence of exposure to HRT was examined by comparing BMD differences within the C/N and P/N pairs for the lumbar spine and femoral neck. Intrapair BMD differences were expressed as percentages (IPD%) and calculated as the difference in BMD between co-twins ('user' minus 'non-user') divided by the pair's mean BMD and multiplied by 100. Mean IPD% and 95% confidence intervals (CI) were calculated at the lumbar spine and femoral neck for the C/N and P/N groups.

The effect of potential confounding variables on the assessment of the influence of HRT on mean IPD% in each group was calculated using a multiple regression method described in Hopper et al. [8]. In brief, the intrapair differences in BMD (expressed as IPD%) were regressed against the intrapair differences in each confounding variable (user's confounder value minus non-user's confounder value). The adjusted effect of HRT use on IPD% (and its 95% CI) was estimated from the constant of the regression equation. For categorical confounding data, the intrapair differences were included in the regression model as a series of indicator variables representing the direction of the difference within each pair. The confounding variables included in the analysis were intrapair difference in duration of HRT use, tobacco consumption (ever/never), alcohol consumption (ever/never) and regular physical activity (yes/no).

The analysis was carried out using the statistical software package STATA (Stata Corporation, TX).

## Results

Complete data on HRT were available in 365 post-menopausal twin pairs. Of these, 181 were concordant for having never used HRT and 69 were concordant for HRT use ever (either current or past). A total of 49 pairs were identified in whom one member of the pair was a current user of HRT and their co-twin had never used HRT, and 66 pairs were identified in whom one member of the pair was a past user while their co-twin had never used HRT. Of these, 50 pairs were excluded from the analysis because either the HRT user had taken HRT for

**Table 1.** Characteristics of study subjects

| | C/N pairs ($n$=36) | | P/N pairs ($n$=29) | |
|---|---|---|---|---|
| | Current HRT user | Never user | Past HRT user | Never user |
| Age (SD) (years) | 55.3 (4.6) | 55.3 (4.6) | 60.4 (4.5) | 60.4 (4.5) |
| Mean (SD) body mass index (kg/m$^2$) | 24.7 (3.8) | 25.2 (5.1) | 24.4 (3.0) | 24.8 (3.8) |
| Mean age (SD) HRT started (years) | 50.3 (4.4) | – | 49.8 (5.9) | – |
| Median duration (range) of HRT (months) | 36 (12–192) | – | 30 (12–159) | – |
| Mean (SD) age at menopause (years) | 48.9 (5.6) | 49.5 (4.0) | 51.9 (8.8) | 49.5 (6.2) |
| Median (range) time since stopping HRT (months) | – | – | 76 (6–276) | – |
| Smoking ever (%) | 22 (61.1) | 15 (41.7) | 8 (27.6) | 14 (48.3) |
| Alcohol consumption ever (%) | 34 (94.5) | 32 (88.9) | 24 (85.7) | 27 (96.0) |
| Regular physical activity (%) | 17 (38.9) | 14 (30.6) | 13 (44.8) | 16 (55.2) |

less than 12 months continuously, or the past user had discontinued HRT for less than 6 months. The analysis was confined to the remaining 36 current user/never user (C/N) pairs, of whom 8 were MZ and 28 were DZ, and 29 past user/never user (P/N) pairs, of whom 17 were MZ and 12 were DZ. Four current user/past user pairs identified from the initial sample are not included in the analysis as this number is too small for any valid inference.

The characteristics of the study subjects subdivided by HRT status are presented in Table 1. Mean body mass index (BMI), age at menopause and physical activity levels were similar in all groups. The proportion of smokers was highest in current HRT users and alcohol consumption was highest in the co-twin of past HRT users. The median duration of HRT use was slightly higher in current users (36 months) compared with past users (30 months). However, all subjects had, by definition, used HRT for at least 12 months. The median time since stopping HRT in past users was 76 months (range 6–276 months). Of current HRT users, 12 (33.3%) had used estrogen-only preparations, 19 (52.8%) combined estrogen/progesterone, 3 (8.3%) gonadomimetic, and 2 (5.6%) uncategorized or unknown preparations. Of past users, 8 (27.6%) had used estrogen-only preparations, 8 (27.6%) combined estrogen/progesterone, 3 (10.3%) gonadomimetic, and 10 (34.5%) uncategorized or unknown preparations.

An initial analysis of the MZ and DZ pairs separately showed similar results and the data from these groups were pooled for subsequent analysis. The mean intrapair percentage differences in BMD (IPD%) of user versus never user before and after adjustment for potential confounding variables are presented in Table 2. In the C/N pairs, BMD was consistently and significantly higher among users than non-users. A larger IPD% was observed at the lumbar spine, with a difference of 12.3% (95% CI: 7.1%, 17.5%). At the femoral neck this difference was 8.6% (95% CI: 3.4%, 13.7%). In the P/N pairs there was less of an effect: BMD was nonsignificantly higher in past users than non-users. The IPD% in P/N pairs at the lumbar spine was 2.4% (95% CI –3.7%, 8.6%) and at the femoral neck 0.4% (95% CI:–5.3%, 6.0%). At the lumbar spine, the IPD% among C/N pairs was significantly larger than for P/N pairs, as indicated by the exclusion of the mean IPD% of the P/N group from the confidence interval of the C/N group.

**Table 2.** The intra-pair mean percentage difference (and 95% CI) in BMD between users of HRT and never users before and after adjustment

| | Current vs never users $n$ (pairs) = 36 | | Past vs. never users $n$ (pairs) = 29 | |
|---|---|---|---|---|
| | IPD% | 95% CI | IPD% | 95% CI |
| Lumbar spine | 12.3 | 7.1, 17.5 | 2.4 | –3.7, 8.6 |
| Lumbar spine, adusted[a] | 8.8 | 0.9, 16.8 | 2.9 | –7.3, 13.2 |
| Femoral neck | 8.6 | 3.4, 13.7 | 0.4 | –5.3, 6.0 |
| Femoral neck, adjusted[a] | 4.5 | –4.4, 13.4 | 0.8 | –7.8, 9.3 |

IPD%, mean intrapair percentage difference in BMD (defined in text). [a] Adjusted for duration of HRT use and for intrapair discordance for alcohol consumption, tobacco consumption and physical exercise.

Following adjustment for duration of HRT use, intrapair discordance for tobacco and alcohol consumption, and exercise the size of the IPD% in C/N pairs and P/N pairs changed little. The confidence intervals, however, widened (Table 2). The IPD% at the lumbar spine in C/N pairs remained significant; however, the difference at the femoral neck in the C/N pairs, and the differences in size of IPD% when the C/N and the P/N groups were compared at the lumbar spine, were no longer observed to be statistically significant.

## Discussion

The results confirm the well-recognized observation that HRT increases BMD at the lumbar spine and femoral neck in postmenopausal women. However, the increase in BMD was substantially greater for current users of HRT than for past users. The findings support the view that although HRT has a marked protective effect on bone its benefits are quickly lost [5] and that HRT may only be effective if given continuously [9,10]. The BMD

difference of the order of 12% over a 3-year period is larger than expected from trials. However, it is likely that non-HRT users were losing bone during this time interval. Consequently there may have been a divergence in the BMD values between HRT users and non-users.

This co-twin case–control twin study design presents a number of potential advantages over matched case–control studies based on samples drawn from the general population. Twin pairs are naturally matched for age and a range of potential genetic and environmental confounding variables. In the case of MZ twins genetic matching is exact; DZ twins share on average half their genetic material. Both MZ and DZ twins also share a common family upbringing and a range of environmental confounding variables, both measured and unmeasured. Using both MZ and DZ co-twins as controls therefore has the potential to accommodate for the effects of unknown genetic and environmental confounding variables that may influence the association between HRT use and BMD more comprehensively than could be achieved in a conventional matched case–control design.

Although the matched design will have accounted for the major influence of confounders such as age, certain differences remained when users and non-users were compared that could have influenced the interpretation of the results. Of concern was the potential bias resulting from differences in the duration of HRT use between current and past users. While all users had been on HRT for at least 12 months, the median duration of HRT use for current users was 6 months more than for past users. Potential confounding could also have been introduced by intrapair difference in alcohol consumption, smoking and physical exercise. When all these differences were taken into account in the adjusted analysis, the size of the IPD% in both the groups changed little, although, as expected, the confidence intervals widened. This suggests that it is unlikely any of these differences between the C/N and P/N groups was an important source of bias.

HRT may be more effective at different ages, although this point is disputed. In this study the mean age of the P/N pairs was 5 years greater than in C/N pairs. Law et al. [11] suggested in an informal meta-analysis that the effects of estrogen on hip fracture are less in elderly women than at the time of menopause. Conversely, Lindsay and Tohme [12] found the response in bone mass to estrogen was greatest in those furthest from the menopause. In our sample all HRT users started HRT at a similar age. Further, by the nature of the twin study design, both users and non-users are likely to have started with a similar BMD [13], making any age-related bias unlikely.

One limitation of the study was that we were unable to address the issue of whether differences in BMD between the C/N and P/N groups could have arisen through differences in HRT dose and type, because of the relatively small numbers included. In the study design we ensured that all subjects had used HRT for at least 12 months, sufficient time for an effect on BMD to have developed [14]. There is no reason to expect a systematic difference in dose of HRT between current and past users. With reference to the possible influence of type of HRT on BMD, a similar proportion of each group had used estrogen-only preparations but there was a higher proportion of combined estrogen/progesterone users in the current HRT users; however, it was not possible to further subdivide the results for analysis. The study design and the small sample size also limited the power of this analysis to examine in any further detail the dynamics of the changes on bone density after starting and discontinuing HRT. None of these individuals had been followed prospectively.

We had no detailed information on reasons for starting or stopping HRT. This raises a number of methodologic issues. Firstly it may be that HRT was started because of a low BMD in a particular twin. It should be noted, however, that the number of women starting HRT because of a low BMD value is low in the UK. Further, had the reason for starting HRT been a low BMD value, then the results obtained in our analysis may be an underestimate of the true effect of HRT. Secondly, it is possible that HRT users were selected to be healthier and more health conscious, as seen in a number of population studies. Matthews et al. [15] observed, in a prospective study, that women who subsequently used HRT engaged in more leisure-time physical activity, had healthier diets, had lower blood pressure levels and lower weight than subsequent non-users. Similar findings were observed in a large US population cohort [16]. As twins are generally well matched for educational and lifestyle factors, self-selection bias is unlikely to be a major issue in the current study.

Because of the limited numbers of subjects in the present study we were unable to study in more detail the association between the timing of discontinuation of HRT in past users and the rate of subsequent bone loss. The median time since stopping HRT in past HRT users (76 months, range 6–276 months) is, however, similar to that in previous population observational studies of HRT on osteoporosis that have shown no detectable protective effect of HRT stopped 3–5 years previously [5]. Horsman et al. [4] observed a higher rate of bone loss after withdrawal of estrogen therapy compared with the expected rate of loss in untreated postmenopausal women. In addition, Lindsay et al. [3] reported no difference in BMD when patients treated for 4 years followed by withdrawal of estrogen for 4 years were compared with an untreated control group. In relation to estrogen use and fracture, Weiss et al. [17] noted a weaker association between hip and lower forearm fracture and past HRT use than that for current use, although the small numbers precluded any precise assessment of the risk of fracture in past HRT users compared with no previous HRT use. Kiel et al. [18] in a retrospective cohort study observed that past estrogen use more than 2 years previously was less protective than current use for hip fracture. Exogenous estrogen in postmenopausal women reduces bone remodeling to premenopausal levels by decreasing bone resorption

[19], increasing intestinal calcium absorption and reducing renal calcium excretion [20] with a resultant increase in BMD. Exogenous estrogen may also correct the imbalance between the amount of bone resorbed and that formed at each remodeling site, resulting in increasing increments of skeletal mass [21]. These metabolic effects are lost once HRT is withdrawn.

In conclusion, the findings confirm the reports from observational population-based studies that show considerable benefit of current HRT on bone density but no significant effect of past use. The clinical extrapolations of these findings are that HRT needs to be used continuously to influence BMD and therefore fractures. Future studies should examine in detail the optimal timing and duration of HRT and the use of alternative treatment in women who discontinue HRT.

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