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Intervertebral disc height in treated and untreated overweight post-menopausal women

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BACKGROUND: The effect of the menopause and HRT on the intervertebral discs has not been investigated. METHODS: One hundred women were recruited, comprising of 44 post-menopausal women on HRT, 33 untreated post-menopausal women and 23 pre-menopausal women. The height of the intervertebral discs between the 12th thoracic vertebra and the 3rd lumbar vertebra was measured by utilizing the bone densitometer height cursors. RESULTS: The untreated menopausal group of women had the lowest total disc height (D1–D3: 1.95 ± 0.31 cm). This was significantly lower than the pre-menopausal group D1–D3: 2.16 ± 0.24 cm) and the hormone-treated group (2.2 ± 0.26 cm) (P > 0.02). The 2nd intervertebral disc consistently maintained a significant difference between the untreated menopausal group (D2: 0.63 ± 0.13) and the other two groups (pre-menopausal group (D2: 0.72 ± 0.09 cm) and treated menopausal group (D2: 0.73 ± 0.12 cm) (P > 0.02). CONCLUSIONS: Estrogen-replete women appear to maintain higher intervertebral discs compared to untreated post-menopausal women. The estrogenic milieu may be relevant because of the significant impact it has on the hydrophilic glycosaminoglycans, the water content, collagen and elastin of the intervertebral discs. The maintenance of adequate disc height may allow the intervertebral discs to retain their discoid shape and viscoelastic function, containing vertical forces which may threaten spinal architecture leading to vertebral body compression fractures.

Key words: estrogen/extracellular matrix/intervertebral discs/menopause/osteoporosis

Introduction

Loss of spinal height in post-menopausal women is mainly attributed to osteoporotic vertebral fractures. In the late 1930s, loss of spinal height due to vertebral fractures was noted in women who had undergone a surgical menopause. Albright *et al.* (1940) demonstrated the increased risk of osteoporotic fractures in oophorectomized women or women who had undergone a premature menopause.

Another important component of the spinal column which may influence spinal height is the intervertebral discs. Intervertebral discs are responsible for 25% of the spinal column height (Williams and Warwick, 1980). To date not much work has been done on intervertebral discs in relation to post-menopausal vertebral fractures (Goh *et al.*, 1999; Muscat Baron, 2002; Urban and Roberts, 2003). Besides allowing flexion, extension and torsion of the spine, the intervertebral discs have important biomechanical properties. One important function is that the intervertebral discs act as shock absorbers, which may be relevant in the pathogenesis of vertebral fracture. Moreover besides the shock absorbing characteristics, the intervertebral discs interact with the central nervous system and the spinal musculature through the presence of mechanoreceptors, which relay information regarding the pressures applied to the discs. This property allows greater distribution of the forces applied to the spinal column (Roberts *et al.*, 1995).

Recent studies utilizing vertebral fractures as the endpoints have further emphasized the importance of the extracellular matrix in the pathogenesis and prevention of vertebral fractures. In the MORE (Multiple Outcomes of Raloxifene Evaluation) study with an increase of only 4% bone mineral density, a 50% reduction in vertebral fractures was noted (Ettinger *et al.*, 1999). The increase in bone mineral density was calculated to be responsible for only 14% of the reduction in vertebral fractures. Similar patterns were noted in the FITS (Fracture Intervention Study) and the VERT (Vertebral Efficacy with Residronate) studies whereby the fracture reduction attributable to the increase in bone mineral density was only 28 and 14% respectively (Cummings *et al.*, 1998; Harris *et al.*, 1999). In all three studies the trend of collagen markers veered towards bone formation rather the bone breakdown. All the above work suggests that besides the bone mineral density, the extracellular matrix of all the components of the spinal column may have a large part to play in the osteoporotic process of the spine.

This study involved the measurement of the intervertebral disc height in three different groups of women: menopausal women on hormone treatment, untreated post-menopausal women and pre-menopausal. The aim of the study was to assess whether intervertebral disc height varied between these three different groups of women possibly due to the influence of their estrogenic status.

Materials and methods

One hundred women were recruited from a bone density directory. Every fifth woman was selected to reduce bias. The woman's information sheet was assessed to segregate the woman according to her menstrual status and if menopausal, to note what treatment she was receiving. Exclusion criteria were women with endocrinopathies, treatment with bisphosphonates, and long term treatment with corticosteroids. Conditions which may affect bone density such as renal disease, thyroid disease and malignancy were also employed as exclusion criteria. A history of tobacco smoking and/or alcohol ingestion of more than two units a day were also considered exclusion criteria. There were no obvious differences in life style between the three different groups of women. If the woman selected was excluded the following woman (sixth) was recruited instead.

The bone density assessments were performed on these women as a form of screening for osteoporosis. The demographic data and bone density results were comparable to those obtained in a larger population utilised in another study indicating that the women recruited in this study were a representative population (Muscat Baron *et al.*, 1999).

Intervertebral disc measurement involved retrieving the image of the selected woman's vertebral bone scan. On obtaining the image, the computer software was adapted so as to apply the screen cursors on to the edges of the vertebrae. Usually these cursors are used to measure the vertebral body height. Five readings were taken of each disc and an average of these readings was taken.

The intervertebral discs measured were those between the 12th thoracic and 4th lumbar vertebra. The discs were assigned the symbol D, so that D1 applied to the disc between the 12th thoracic vertebra and the first lumbar vertebra and the other discs followed suit, i.e. D2, D3, D4. The last intervertebral disc could not be measured consistently and therefore was excluded from analysis. When difficulty arose on measuring the intervertebral disc height due a variety of reasons as in the presence of osteoarthrosis, the grey scale adjacent to the vertebral image was used to increase the accuracy of the disc height measurement.

All measurements were performed by the same operator who was blinded to the menstrual or treatment status of the woman. The error of the method was calculated to be 0.03mm. The coefficient of variation was calculated from the variation from the mean of the total disc height (D1–D3) from the same bone density image. Twenty measurements from the same three discs, on four different occasions were taken giving a coefficient of variation of 2.8%. Ethics approval was obtained from the Malta Medical School Ethics Committee.

Statistical methods

The variables in this study including the demographic characteristics, the intervertebral disc height and T-score were applied to Normality plots to assess their distribution. For distributions of these variables the Normality plots allowed the utilization of the Mann–Whitney *U*-test. $P < 0.05$ was considered significant. Power calculations indicated that a minimum number of 20 patients was required in each group of women to identify a 10% difference in disc height allowing for an alpha value of 5% and power of 80%.

Results

Out of a total of 100 women recruited, 44 women were on hormone replacement therapy for 3 ± 1.5 years, 23 were pre-menopausal women while 33 were untreated menopausal women. Of the women treated with hormone therapy, 15 were on conjugated estrogens (conjugated estrogens 0.625 mg and 1 mg norgestrel), 19 were on different formulations of oral 2 mg estradiol and progestin, six women were on transdermal estrogen and three women were on estradiol implants. The women on estradiol implants and six women on transdermal estrogen had had hysterectomies and bilateral salpingo-oophorectomies and therefore were not given progestins. Both in the hormone treated group and the untreated group there were women who had hysterectomies and salpingo-oophorectomies at an early age, and this may explain the low age of menopause in these groups of women.

The pre-menopausal group was significantly younger than the treated and untreated groups. These pre-menopausal women whose mean age was 49.7 ± 4.0 years, close to the age of the menopause transition, were referred for a bone density assessment in most cases for back pain. There was no significant difference in the age and menopausal age of the treated and untreated menopausal women. The demographic characteristics of the women are shown in Table I.

Significant differences were noted when comparing the intervertebral disc height of the three groups of women. The untreated menopausal group of women had the lowest total disc height (total disc height D1–D3: 1.96 ± 0.31 cm) (Table I). This was significantly lower than that of the pre-menopausal group (2.16 ± 0.23 cm) (Table I) and the hormone-treated group (2.2 ± 0.26 cm) ($P > 0.02$) (Figure 1). The second intervertebral disc (D2) consistently maintained a significant difference between the untreated menopausal group (0.62 ± 0.12) and the other two groups (pre-menopausal group 0.72 ± 0.09 cm and treated menopausal group 0.73 ± 0.12 cm) ($P > 0.02$) (Figure 2). The T-score and women's total height were lowest in the untreated menopausal group, but these differences did not reach statistical significance.

Discussion

The results of this study indicate that the women with adequate estrogen levels had significantly greater disc heights compared

Table I. Demographic characteristics of women recruited in the study

	n	Height (cm)	Weight (kg)	Age (years)	Age at menopause (years)	D1 (cm)	D2 (cm)	D3 (cm)	D1–D3 (cm)	T-Score
Untreated group	33	154.7 ± 6.8	68.6 ± 13.5	54.7 ± 8.1	46.1 ± 5.9	0.68 ± 0.14	0.62 ± 0.12	0.65 ± 0.15	1.96 ± 0.31	–0.8 ± 0.69
Pre-menopausal group	23	156 ± 5.6	66.9 ± 11.3	49.7* ± 4.0	N/A	0.74 ± 0.14	0.72** ± 0.09	0.7 ± 0.12	2.16** ± 0.23	–0.24 ± 1.7
HRT group	44	155 ± 5.7	65.2 ± 12.6	53.2 ± 7.5	46.4 ± 5.7	0.76 ± 0.11	0.73** ± 0.12	0.71 ± 0.13	2.20** ± 0.26	–0.65 ± 1.33

*$P < 0.05$, **$P < 0.02$.
D = disc; N/A = not applicable.

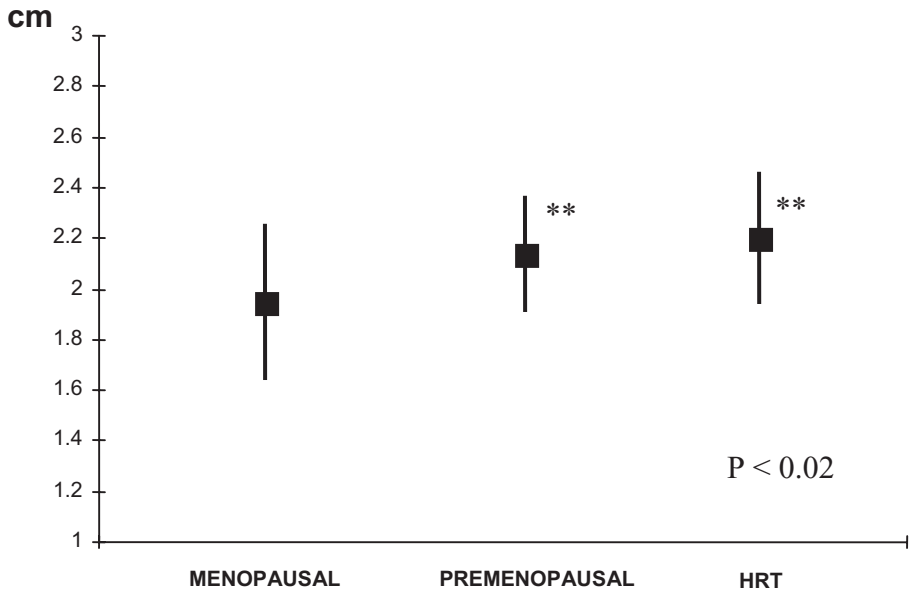

Figure 1. Total intervertebral disc height in different groups of women. D1–D3: total height.

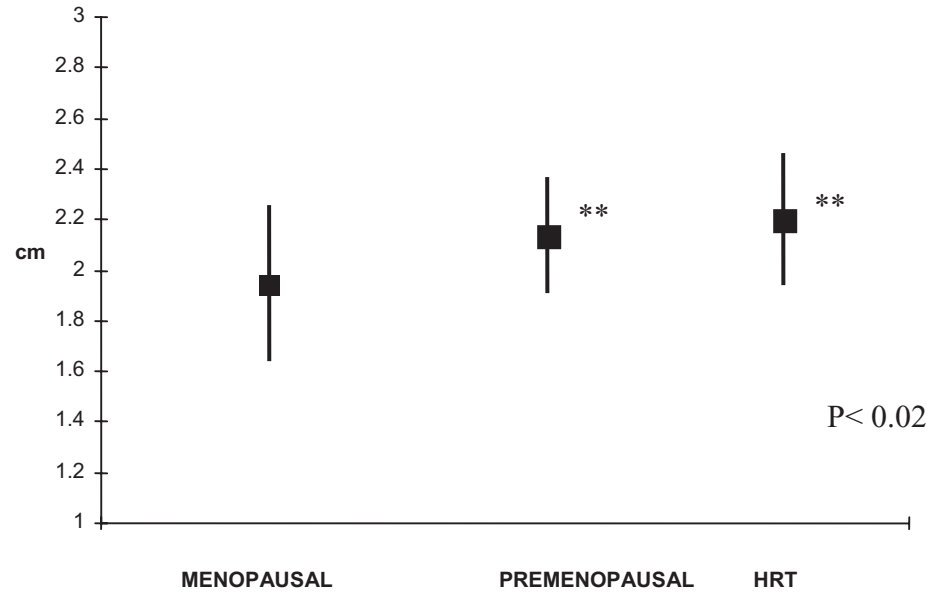

Figure 2. Lumbar disc 2 height in different groups of women.

to untreated menopausal women. The conservation of intervertebral disc height depends on the maintenance of the various components of the disc. In health the two anatomical elements of the disc, the nucleus pulposus and annulus fibrosus, contain a proportionate distribution of collagen types, elastin, hydrophilic glycosaminoglycans and water.

Prior to the third decade the nucleus pulposus contains collagen types IV, II and IX, the major glycosaminoglycan being aggrecan, and more importantly, ~80% of its weight is water (Eyre and Muir, 1977). The aggrecan chains contain a greater chondroitin VI sulphate:chondroitin IV sulphate ratio. Moreover prior to the third decade of life, the chondroitin sulphate VI:keratin sulphate ratio is elevated, maintaining more powerful hydrophilic activity (Johnstone and Bayliss, 1995). The annulus fibrosus has a concentration of type I collagen in its outer layers and type II in the inner lamellae (Nerlich *et al.*, 1997; Boos *et al.*, 2002).

The above anatomical distribution in healthy intervertebral discs provides these structures with efficient viscoelastic properties. These viscoelastic properties allow the disc to sustain heavy loads and to distribute the pressure evenly on to the other components of the spinal column. The cartilaginous endplate contains a number of perforations between the medullary cavity of the vertebra and the intervertebral disc. On compression,

a fluid flux occurs whereby fluid in the intervertebral disc is squeezed out to enter the medullary cavity. On relieving the compression, the hydrophilic nature of the intervertebral disc reabsorbs the fluid previously relinquished, and in doing so the disc acts as a vital tissue pump. Simultaneously nutrition of the disc and removal of waste products occurs (Sagi *et al.*, 2003).

By the end of the third decade the blood supply to the nucleus pulposus is obliterated (Brown and Tsaltas, 1976). This starts a cascade of changes leading to alteration in the collagen content, dehydration of the nucleus pulposus and changes in the glycosaminoglycans (Urban *et al*., 1979). The collagen types I, III and VI increasingly replace the other collagens in both the annulus fibrosus and nucleus pulposus (Nerlich *et al.*, 1997; Boos *et al.*, 2002).

Disc fibrosis, deformity and loss of disc height occur with increasing degeneration. The degenerating disc will be less able to provide stability and will be less efficient at distributing the applied loads evenly. The nucleus pulposus becomes a hard sequestered ball in the middle of the intervertebal disc (Williams and Warwick, 1980).

The intervertebral disc has recently been shown to possess the estrogen β receptor. Estrogen β receptor gene expression was noted in intervertebral disc annulus fibrosis cells. Moreover culture of annulus fibrosus cells in the presence of 10^{-7} mol/l 17β-estradiol significantly increased cellular proliferation (Gruber *et al.*, 2002).

The bone matrix theory expounded by Albright *et al*. (1940) indicated the importance of the connective tissue element in the genesis of post-menopausal osteoporosis. Collagen marker studies elegantly reveal the close connection between bone formation and bone breakdown during the menopause and the influence estrogen replacement therapy has on these biomarkers (Prestwood *et al.*, 1994; Muscat Baron *et al.*, 1997b). Estrogen has been shown to regulate the degradation of organic bone matrix via matrix metalloproteinases and cystein proteinases (Parikka *et al.*, 2001). Carboxyl terminal propeptide of type I collagen (PICP) increases significantly after the application of estradiol to menopausal women (Sands *et al.*, 2000). Skin collagen content has been shown to decrease after the menopause only to be reinstituted with the application of HRT (Brincat *et al.*, 1983).

HRT has also been shown to cause increased skin thickness, presumably due to its effect on the collagen content (Brincat *et al.*, 1985). Alterations in collagen content induced by HRT given to post-menopausal women have also been demonstrated in other organs such as the cardiovascular system (Muscat Baron *et al.*, 1997a, 1998) and the urinary tract (Versi *et al*., 1988).

Animal and human studies have suggested an effect of the menopause on glycosaminoglycan content. Work on rats has suggested that glycosaminoglycan content decreases following castration, only to be regained after treatment with estradiol (Horsfall *et al*., 1994). Hyaluronic acid content was shown to increase sharply with estrogen (Horsfall *et al.*, 1994; Sunil *et al.*, 2000). A consequent linear increase in water content was also noted (Grosman, 1973).

Estradiol has been shown to modulate the inhibiting effect of tissue inhibitory proteinases and proteoglycans on matrix metalloproteinases. These matrix metalloproteinases are responsible for their activity concerning the extracellular matrix-specific gene switching of Colia IV and Colia I genes (Sudhakaran *et al.*, 1999).

In humans, hormonally stimulated menstrual cycles are associated with an increase in proteoglycan concentrations (Carranco *et al.*, 1992). Estradiol significantly increased the synthesis of sulphated glycosaminoglycans in uterine connective tissue of pregnant women (Wiqvist and Linde, 1987). Urine ratio of glycosaminoglycans to creatinine increases markedly in women after the menopause (Larking *et al.*, 1987). Moreover women with post-menopausal osteoporosis had a significant increase in glycosaminoglycan excretion correlating with calcium excretion (Todorova *et al*., 1992).

The above effects of estrogen on basic components of the intervertebral disc may explain the findings in this study. Estrogen-replete women appear to maintain a healthy status of the disc collagen and glycosaminoglycans. As a corollary to the increase in hydrophilic glycosaminoglycans, the water content increases. This may explain the significantly greater height of intervertebral discs in the pre-menopausal women and hormone-treated women compared to the untreated post-menopausal women.

The loss in disc height, especially in untreated menopausal women as shown in this study, has a number of important implications for risk of vertebral compression fracture. The loss in height may lead to decreased compressibility resulting in a diminished distance covered by the fluid pump when under pressure.

The loss in circumferential disc height and increased prominence of the hardened sequestered nucleus pulposus may encourage perforation of the vertebral endplate and subsequent vertebral body compression fracture. Moreover the reduction of the viscoelastic properties of the disc further increase the risk of deformity and eventual osteoporotic vertebral fracture.

A possible confounding variable in the hormone-treated group is the heterogeneity of the hormonal regimens taken by the women recruited. This illustrates the difficulty of performing hormonal treatment studies on post-menopausal women and tailoring the regimen and route of administration to the women's wishes and needs. The large majority of the hormone-treated women were on oral treatment with comparable potency, suggesting a uniform hormonal influence of this group. The smaller component of the treated group with implants possibly equilibrated the lower potency transdermally treated women.

The implant and transdermal groups and some women in the untreated menopausal group had had a surgical menopause at an early age, explaining the low mean age of menopause in both groups of women. In the latter group the long-term estrogen deprivation may have not only led to a lower bone density but may have contributed to a further lowering of intervertebral disc heights. By contrast, the hormone-treated group mantained their disc height.

In conclusion, this study indicated that estrogen-replete women appear to maintain higher and therefore healthier intervertebral discs compared to untreated post-menopausal women. The estrogenic environment may be of such influence

because of the significant impact it has on the extracellular matrix, in particular the glycosaminoglycans and the water content of the intervertebral discs. Adequate disc height may allow the intervertebral discs to retain their discoid anatomy and viscoelastic function, possibly cushioning vertical forces which may threaten osteoporotic spinal architecture leading to vertebral crush fractures.

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