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Physiological versus standard sex steroid replacement in young women with premature ovarian failure: effects on bone mass acquisition and turnover

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Physiological versus standard sex steroid replacement in young women with premature ovarian failure: effects on bone mass acquisition and turnover

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Abstract

Background

The aim of this *exploratory* study was to establish whether we could improve skeletal health with a physiological regimen of SSR in young women with premature ovarian failure (POF).

Patients and Methods

In an open-label randomised controlled crossover trial, 34 women with POF were randomised to 4-week cycles of pSSR (transdermal oestradiol, 100µg daily for week 1, 150µg for weeks 2-4; vaginal progesterone, 200mg twice-daily weeks 3-4) or sHRT (oral ethinyloestradiol 30µg and 1.5mg norethisterone daily for weeks 1-3, week 4 "pill-free") for 12 months. Bone mineral density (BMD) was measured by DEXA at study entry and after each 12-month treatment period. Blood samples for hormones and markers of bone formation (bone alkaline phosphatase, BALP, and Type I collagen N-terminal propeptide, PINP) and bone resorption (CrossLaps) were collected pre/post washout and after 3, 6 and 12 months of each treatment.

Results

18 women, mean 27 (range 19-39) years, completed the study. Both regimens caused similar suppression of LH and FSH. Mean baseline lumbar spine BMD z-score was -0.89 (95% CI -1.27 to -0.51) and increased by +0.17 (CI +0.07 to +0.27) in response to pSSR (P=0.003) compared with +0.07 (CI -0.03 to +0.18) during standard HRT (P=0.2). During pSSR, the increment in lumbar spine BMD z-score was related positively to oestradiol (r = +0.49, P =0.04) and inversely to FSH (r = -0.65, P =0.004). Bone formation markers, BALP and P1NP, increased in the pSSR arm (ANOVA P<0.001) but decreased in the sHRT arm (P<0.01). Both treatments suppressed the bone resorption marker, CrossLaps (P <0.001).

Conclusion

We conclude that pSSR over 12 months has a beneficial affect on bone mass acquisition on the lumbar spine in women with POF, mediated by increased bone formation and decreased bone resorption.

Keywords

Ovarian failure, sex hormone replacement, bone mineral density, bone turnover markers.

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Introduction

Osteoporosis, reduced bone mineral density (BMD), and increased risk of fracture have been reported in Turner syndrome (1) and in women with premature ovarian failure (POF) due to other causes, including after successful treatment of childhood cancer. For the survivor of childhood cancer the risk of developing POF depends on the treatment received by the patient (2). The highest risk is to those girls who received radiotherapy to a field that includes the ovary (3,4,5,6). There is some evidence that oestrogen replacement and treatment of short stature with growth hormone will improve bone mass in women with Turner syndrome (7).

The menopause has an adverse affect on bone health that is cumulative over time. A very early menopause has a potentially devastating effect on long-term bone health. There is a large body of research that has evaluated hormone replacement regimens for older women who are menopausal but young women with POF, who will require sex steroid replacement (SSR) therapy for many years, are under researched (8).

*There is no agreed consensus on the optimum oestrogen and progesterone replacement regimens for young women with POF. C*urrently young women with POF are offered combined hormone replacement in the convenient form of the oral contraceptive pill, or hormone replacement therapy designed for older women after the menopause (9). These preparations are not designed to achieve physiological replacement of oestrogen or progesterone either in dosage or in biochemical structure. With these young women looking to a future of 30 or more years of replacement therapy, the optimum mode of SSR to sustain and improve bone health is not known (8).

Oestrogen is a potent stimulator of bone mineral accretion through puberty (10). Following the development of the menopause bone loss proceeds at an increased rate. Achieving an inadequate peak bone mass increases the risk of osteoporosis and bone fractures in later life. In Turner syndrome there is evidence that women who do not achieve optimal peak bone mass have a higher rate of bone fractures (11). There is also evidence that prolonged oral contraceptive use in skeletally immature female young adult monkeys leads to a lower peak bone mass (12).

Physiological SSR (pSSR) achieves sex steroid serum concentrations similar to concentrations in women with normal ovarian function (13) and improves parameters of uterine function (14). Recently we have shown that pSSR in women with POF results in lower blood pressure, better renal function and less activation of the renin-angiotensin system than a standard sex steroid replacement regimen (15). By further study of the same group of young women with POF due to different causes, the aim of this study was to establish whether we could improve skeletal health with a physiological regimen of SSR.

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Methods

Subjects

Forty-two women with POF as a result of Turner syndrome, chemotherapy or radiotherapy treatment for cancer, surgical ovariectomy or unknown cause (idiopathic) were recruited between February 2002 and September 2004 and the trial was completed in November 2006. The detail of the study design and patient demographics has been previously reported (15). POF was defined as onset of biochemically confirmed menopause before the age of 40 years. The study was approved by the local research ethics committee, received Clinical Trial Authorisation by the Medicines and Healthcare products Regulatory Agency (UK), in accordance with the Declaration of Helsinki, and written informed consent was obtained from all subjects.

Study Protocol

This was an open-label randomised controlled crossover trial. At entry, all subjects were receiving a standard non-physiological hormone replacement regimen. After an initial 2-month washout period of no therapy, patients were randomised to receive either pSSR or standard hormone replacement treatment (sHRT). pSSR consisted of transdermal oestradiol 100µg daily for week 1 and 150µg for weeks 2-4 (Estraderm TTS patches, Novartis Pharmaceuticals UK Ltd), with progesterone 200mg twice daily in weeks 3-4 (Cyclogest vaginal pessaries, Actavis UK Ltd). Some subjects used oral progesterone as dydrogesterone 10mg twice daily (Duphaston, Solvay Healthcare Ltd, UK) in preference to vaginal pessaries. sHRT comprised ethinyloestradiol 30µg and norethisterone 1.5mg daily (Loestrin 30, Galen Ltd, UK) for weeks 1-3, followed by 7 “pill-free” days. After the first 12-month treatment period, a second 2-month washout period was completed before subjects crossed over to the alternative treatment.

BMD assessments were made by dual energy X-ray absorptiometry (DEXA) at study entry and after each 12-month treatment period. Blood samples were collected for hormonal measurements and for markers of bone formation, namely bone alkaline phosphatase (ALP) and procollagen type I amino-terminal propeptide, (PINP), and bone resorption, namely the cross-linked C-terminal telopeptide of type I collagen (CrossLaps), before and after each washout period, and at 3, 6 and 12 months of each treatment period. Samples were collected on day 21 of each 4-weekly treatment cycle. Investigators were blinded to treatment allocation until all bone outcome measurements were complete.

DEXA measurements were performed at all scheduled time points. Blood samples were collected at all scheduled time points except for one missing sample after first wash-out in one subject owing to sampling difficulties.

BMD measurements

Bone mineral density (BMD) of the lumbar spine (L1-L4), femoral neck and total hip were measured by a Hologic QDR4500A Dual-Energy X-ray Absorptiometer operated in array mode. The normal data supplied by the manufacturer were used to determine the z-scores (see below); it has previously been established that there is no significant difference between the manufacturer’s normal data and local normal data (J. Hannan, personal communication). The long-term precision at our centre is 2.4%,

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2.7% and 2.3% for lumbar spine, femoral neck and total hip respectively, corresponding to approximately twice the short-term precision quoted by the manufacturer.

Analytical methods

All assays were measured in duplicate, including samples from each subject in a single analytical run to minimise imprecision. LH and FSH were measured by DELFIA time-resolved immunofluorescence assays (Wallac Oy, Turku, Finland), standardised against the second international reference preparations 80/552 and 78/549 respectively. Within- and between-run coefficients of variation were <7%. 17β-oestradiol was measured by a specific radioimmunoassay that does not cross-react with oestrone or ethinyloestradiol (Adaltis Italia S.p.A., Italy) and progesterone by an established in-house radioimmunoassay (16). Within- and between-run coefficients of variation were <10% and <14% respectively. Bone ALP was measured using an enzyme immunoassay specific for bone ALP (Metra™ BAP, Quidel Deutschland GmbH, Marburg, Germany), PINP by RIA (Orion Diagnostica, Espoo, Finland) and serum CrossLaps by ELISA (Nordic Bioscience Diagnostics, Herlev, Denmark). Within- and between run coefficients of variation were <5% for bone ALP, <3% for PINP, and <6% for serum CrossLaps respectively.

Data analysis

BMD was expressed as g/cm^2 and as z-scores (SD scores), calculated by subtracting the mean BMD of the adult age-matched female population from the subject's BMD and dividing by the population standard deviation. Changes in BMD (in g/cm^2 and as z-scores) in response to each treatment arm (pSSR or sHRT) were also calculated by subtracting the BMD at the start of each treatment period from that measured at the end of each treatment period. Changes in bone markers in response to each treatment period were expressed *both* as percentage change in relation to post-washout baseline *and as absolute values*. For each subject, we calculated their mean hormone concentration during pSSR and sHRT treatments as the within-individual average of measurements at 3, 6 and 12 months of each respective treatment period. Data were summarised as mean (reported with 95% confidence interval (CI) of the mean). Comparisons between groups were performed using unpaired t-tests. Paired t-test were used for within-subject comparisons of BMD *and bone marker* responses to pSSR and sHRT. One-way within subject analysis of variance was used for repeated measures of bone markers through time. We used Pearson correlation to explore relations between variables. Multiple linear regression was used to identify independent predictors of BMD response to treatment. To check for period effects and treatment period interactions, we used unpaired t tests to compare the differences and sums respectively of each BMD or mean bone marker response to each treatment for the group who had pSSR first and the group who had it second. Statistical analyses were performed using Analyse-it software (v2.03). Statistical significance was accepted at P <0.05 (two-tailed).

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Results

Of the 42 subjects identified as eligible for the study, 34 women proceeded to randomisation and had baseline investigations and 18 women completed the full study protocol (Figure 1, previously published in ref 15). In general, more women withdrew during the first treatment phase, suggesting lack of tolerance of the intensive nature of the research protocol rather than the intervention. Both treatments were generally well tolerated, although some women reported adverse reactions to the patch adhesive during pSSR.

Table 1 shows the characteristics of the subjects who completed the study protocol compared with those who withdrew. Among those who completed, there were more Turner syndrome patients, more women with pre-pubertal onset of POF and more women randomised to sHRT as first treatment. Those who completed had similar age, height, weight and BMI to those who withdrew. Lumbar spine BMD was lower in the women who completed than in those who withdrew (P =0.03) but bone marker levels at baseline were similar in the two groups.

18 women completed the study (age range 19 – 39 years) of whom 7 had Turner syndrome and 11 had other causes of POF: treatment for cancer (n = 4), bilateral ovariectomy (n = 1) or idiopathic (n = 6). At baseline, women with Turner syndrome were younger and shorter than those with other causes of POF, but did not differ in terms of BMD at any site, bone markers or hormonal status on their pre-study hormonal replacement regimen (Table 2). Similarly, women with pre-pubertal onset of POF were younger than those with post-pubertal onset, but did not differ in terms of BMD, bone markers or hormonal status (Table 2). Regardless of aetiology or age of onset of POF, the lumbar spine was more affected than the femoral neck or total hip.

After first wash-out, LH and FSH were high in all subjects (minimum LH 21.3 U/l, minimum FSH 45.3 U/l), confirming ovarian failure (Table 3). Thereafter, LH and FSH decreased to a similar extent during both pSSR and sHRT, suggesting equivalent sex steroid replacement.

Table 4 shows the changes in BMD in response to pSSR and sHRT. Lumbar spine BMD z-score increased by +0.17 (CI +0.07 to +0.27) from baseline in response to pSSR (P =0.003) whereas there was no significant change in response to sHRT (P =0.2). However, when the two treatments were directly compared in the same subjects, the mean difference in lumbar spine BMD z-score response was +0.09 (95% CI -0.06 to +0.25) which did not reach statistical significance (P = 0.2). There were no significant changes in femoral neck or total hip BMD in response to either treatment. There were no detectable treatment order interactions for any BMD changes in response to the two treatments.

During pSSR, the increment in lumbar spine BMD z-score was positively related to mean within-subject oestradiol (r = +0.49, P =0.04) and inversely related to mean within-subject FSH (r = -0.65, P =0.004) but not LH (r = -0.24, P =0.3) over the same period. Multiple linear regression analysis indicated that both FSH and oestradiol were independent predictors of lumbar spine BMD z-score increments during pSSR (P = 0.005 and 0.05 respectively), explaining 55% of the variance.

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Table 5 shows bone marker data at baseline and after first and second wash-out. Pre-washout markers were similar to levels reported in normal pre-menopausal women, reflecting their pre-study hormone replacement. CrossLaps increased markedly by +86% (CI +38 to +135%) and +73% (+23 to +123%) during first and second wash-outs, whereas bone ALP (+22%, CI +8 to +35%, and +18% , +4 to +31%) and PINP (+24%, -3% to +51% and +17%, -3 to +37%) showed smaller, less consistent changes. There were no significant differences between the markers after second washout compared with after first washout (P =0.5 for CrossLaps, P =0.2 for bone ALP and P =0.5 for PINP), indicating that bone turnover was similar at the start of each treatment period.

All bone markers showed significant changes through time in response to pSSR (*ANOVA* P = 0.003, <0.001 and <0.001 for bone ALP, PINP and CrossLaps respectively) and sHRT (*ANOVA* P <0.001, <0.001 and <0.001). The bone marker responses to pSSR and sHRT, expressed as percentage changes from post wash-out baseline, are compared in Figure 2. Bone ALP and PINP increased in response to pSSR but decreased in response to sHRT (Figure 1A and 1B). *Responses at 3, 6 and 12 months were markedly different between treatments, both in terms of percentage changes versus post wash-out baseline (bone ALP P <0.001 at all time points, PINP P <0.001, <0.001 and 0.03 respectively) and in terms of absolute values (bone ALP P ≤0.001 at all time points, PINP P <0.001, <0.001 and 0.006 respectively).* By contrast, both treatments suppressed CrossLaps, although suppression was less pronounced for pSSR than for sHRT (Figure 1C*, between-treatment differences at 3, 6 and 12 months P 0.01, 0.02 and 0.5 for percentage changes and P 0.01, 0.003 and 0.6 for absolute values respectively).* There were no detectable treatment order interactions for bone marker changes in response to the two treatments.

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Discussion

This is the first prospective *exploratory* study comparing hormone replacement regimens on skeletal health parameters in young women with POF. In this study, pSSR therapy over a 12-month period resulted in a significant improvement in lumbar spine BMD z-scores, whereas sHRT did not. Both regimens suppressed the bone resorption marker, CrossLaps, but only pSSR was associated with an increase in bone formation markers, bone ALP and P1NP. Although our study used different hormone regimens from those in the large randomised HRT trials, we have demonstrated that there was a significant improvement in bone health for women with POF when they received physiological sex steroid replacement therapy, but not when they received standard HRT. These results have important implications for the long-term use of hormone replacement to maximise bone health in women with POF.

Young women with untreated POF are at increased risk of developing osteoporosis, along with cardiovascular disease and cognitive impairment.. *There is no agreed consensus on the optimum oestrogen and progesterone replacement regimens for young women with premature ovarian failure. Factors that influence choice include convenience of preparation and suitability of preparation to achieve optimum skeletal and cardiovascular health. We audited the prescribing habits of all United Kingdom European Society of Paediatric Endocrinology members. A questionnaire was sent to members to enquire as to their choice of hormone replacement in young women with premature ovarian failure. There was a good response to the questionnaire – 42 were sent and 30 (71%) returned. 28 of the 30 respondents had a first preference. The most commonly used is the oral contraceptive pill (64%), Loestrin being the most frequently prescribed OCP (67%). 18% prescribed an "HRT" preparation used for post menopausal women, Prempak C being the only prescribed "HRT". As a result of this small study we chose the oral contraceptive pill as standard HRT for this group of young women (9).* Some women may require hormone replacement for over three decades (17). Despite the widespread use of these hormonal preparations, the long-term continuous use of these conventional doses by women with POF may not provide hormone concentrations that optimise bone health. There is a higher incidence of osteoporotic fractures amongst women with Turner syndrome despite long-term hormone replacement (18). Thus physiological levels of oestradiol may be required to achieve optimum bone mass and rates of bone formation that are not attained through standard, conventional hormone replacement.

It is well documented that bone health significantly deteriorates after biological menopause in young or older women due to reduction of circulating oestrogen (19, 20). Large, randomized trials have shown that HRT in postmenopausal women can dramatically improve BMD and reduce fracture risk (21,22,23). However in these studies, patients were older, had a later onset and shorter duration of menopause and did not have Turner syndrome, nor had they undergone anti-cancer treatment or ovariectomy like some of the patients in our study. Results from these studies cannot therefore be extrapolated to women with POF. Few studies have investigated the effect of HRT in young women with POF, but in Turner syndrome there is evidence to indicate the importance of oestrogen exposure on bone mass acquisition in adolescence (24).

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Our results demonstrate that treatment with pSSR produced a significant increase in lumbar spine BMD z-score whereas sHRT did not. There was no significant change in femoral neck or total hip BMD z-scores in response to either regimen. *However, when the two treatments were directly compared in the same subjects, the mean difference in lumbar spine BMD z-score response did not reach statistical significance. We accept that one year is a short time to see BMD changes in response to any intervention in adults. In clinical practice in postmenopausal women with osteoporosis on treatment, DEXA scans are usually done at not less than 2 year intervals for this reason.* It is well recognised that different parts of the skeleton respond in different ways to oestrogen. The lumbar spine is predominantly trabecular bone, compared to femoral neck which is predominantly cortical, thus oestrogen will affect the BMD of these sites to differing extents. It has been suggested that it is preferable to monitor bone at an area containing a significant proportion of trabecular bone (25). Trabecular bone is more metabolically active, and a greater proportion is undergoing remodelling at any one time compared with cortical bone. *Agents such as oestrogen that affect bone formation and resorption influence it to a much greater degree and more rapidly*. (26) In our study, during pSSR, the increase in lumbar spine BMD was positively related to oestradiol levels and inversely related to FSH, consistent with a direct beneficial effect of oestrogen on net bone mineral accrual at this site.

Results from this study show that whilst both pSSR and sHRT regimens suppressed the bone resorption marker CrossLaps, only pSSR had a positive effect on the bone formation markers bone ALP and PINP, whereas sHRT, in contrast, had a negative effect on bone formation. This indicates that standard doses of HRT may be adequate to suppress osteoclast activity and reduce bone resorption but are insufficient to stimulate bone formation through an increase of osteoblast action. pSSR parenteral administration allows a more consistent, stable physiological level of oestrogen to be achieved since the regimen avoids the hepatic first-pass effect of drug metabolism, which occurs in sHRT therapy. Animal studies of oestrogen receptor knockout mice also suggest that different levels of oestrogen activate different oestrogen receptors and thus exert different actions on bone resorption and formation. At low oestrogen levels, ER β is predominantly activated which is enough to suppress bone resorption. At increased oestrogen levels, both ER α and ER β are activated, stimulating bone formation (27,28). In our study, there was an early rise in bone formation markers at 3 months before a plateau or reduction during subsequent months, suggesting a period of increasing osteoblast proliferation and activity after initial administration, a finding common to another study (29).

An important weakness of our study is the heterogeneous aetiology of the women with POF. Women with Turner syndrome have often been treated with growth hormone during childhood, whereas other women with POF have not, which may affect BMD and body composition (7). BMD measurements by DEXA are also influenced by bone size and may give misleadingly low z-scores in women below the height of 150cm. However, this was not a major factor in our study as only one woman with Turner syndrome who completed the protocol was below 150cm in height (height 149cm). Although Turner syndrome women were generally younger and shorter than non-Turner women, they did not differ in terms of baseline BMD at any site nor in baseline biochemistry. Furthermore, we did not observe any difference in treatment effect between Turner and non-Turner subjects, nor between those with

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pre-pubertal or post-pubertal onset of POF (data not shown). However due to such small sample groups, no firm conclusions can be drawn. It is impossible to generalise treatment benefits for all patients with POF disregarding its aetiology and timing. However, to minimise these potential confounding effects, we chose to use a crossover design in our study to ensure that all subjects with different aetiologies and timing of onset of POF received both treatment regimens to enable each patient to act as their own control.

There was a high dropout rate in this study with only 18 out of 34 subjects (52%) completing the whole protocol, which is a further significant weakness of this study. As more women withdrew during the first phase of treatment, we can attribute these dropouts to difficulty in adhering to study protocol rather than the intervention treatment itself.

Although during the relatively short period of this study, pSSR did not dramatically affect all aspects of skeletal health, it significantly improved BMD z-score at lumbar spine (which was low at baseline) and increased bone formation whereas sHRT did not. These observed differences in treatment effect support the hypothesis that the type and profile of hormone replacement are critical and can have considerable effects on the bone health of women with POF. Long-term randomised studies with larger sample sizes from diagnostically homogeneous populations are needed in order to confirm these results and for an evidence base to be constructed. If evidence continues to grow in support of these skeletal health findings, together with evidence of an improvement in blood pressure and cardiovascular risk previously reported in this group of women (15), the quality of hormone replacement for women with POF could be significantly improved.

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Contributions

Study design: PMC, LEB, PW, HODC, CJHK, WHBW
Sample analysis: NE
Data Analysis: PMC
Initial Writing: PMC, WHBW
Background Research: PMC, TW, HODC, WHBW
Final manuscript approval: Everyone

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Table 1 Baseline characteristics of subjects who completed the study protocol compared with subjects who subsequently withdrew from the study

Characteristic	Completed	Withdrew
	n = 18	n = 16
Aetiology of ovarian failure: Turner Syndrome / Other	7 / 11	2 / 14
Onset of POF: pre / post puberty	12 / 6	4 / 12
Treatment order: pSSR-sHRT / sHRT-pSSR	6 / 12	10 / 6
Age, years	27 (23 – 30)	31 (28 – 34)
Height, cm	160 (156 – 164)	162 (156 – 168)
Weight, kg	71.4 (62.3 – 80.4)	71.2 (61.2 – 81.2)
BMI, kg/m^2	27.7 (24.8 – 30.7)	26.7 (23.9 – 29.7)
Lumbar spine BMD, g/cm^2	0.933 (0.892 – 0.974)	1.041 [a] (0.964 – 1.118)
Lumbar spine BMD z-score	-0.89 (-1.26 to -0.51)	-0.04 [a] (-0.77 to +0.70)
Femoral neck BMD, g/cm^2	0.800 (0.744 – 0.856)	0.833 (0.744 – 0.921)
Femoral neck BMD z-score	-0.38 (-0.88 to +0.12)	-0.10 (-0.89 to +0.69)
Total hip BMD, g/cm^2	0.892 (0.827 – 0.957)	0.947 (0.859 – 1.034)
Total hip BMD z-score	-0.28 (-0.74 to +0.18)	+0.04 (-0.67 to +0.74)
CrossLaps, ng/L	312 (237 – 386)	266 (193 – 338)
Bone ALP, U/L	14.9 (12.1 – 17.8)	15.3 (12.9 – 17.6)
PINP, ug/L	53.1 (42.9 – 63.3)	54.9 (37.3 – 72.5)
LH [b], U/L	12.3 (5.6 – 19.0)	25.4 [a] (15.0 – 35.9)
FSH [b], U/L	21.9 (10.7 – 33.2)	37.4 (22.2 – 52.6)
Oestradiol [b], pmol/L	125 (61 – 190)	315 [a] (109 – 521)

Data are expressed as mean (95% CI mean).
[a] P <0.05 compared with those who completed the study.
[b] Pre-washout, reflecting previous HRT prior to commencing study.

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Table 2: Baseline characteristics of patients who completed the study protocol according to aetiology and age of onset of premature ovarian failure (POF)

	Aetiology		Onset of POF	
	Turner	Other	Pre-puberty	Post-puberty
N	7	11	12	6
Age at study, years	22.9 [a] (20.9 – 24.8)	29.1 (24.1 – 34.1)	24.1 (21.0 – 27.1)	31.8 [c] (24.3 – 39.3)
Height, cm	154 [b] (150 – 159)	164 (159 – 168)	158 (154 – 163)	163 (155 – 171)
Weight, kg	69.4 (56.1 – 82.7)	72.6 (58.6 – 86.6)	72.5 (61.1 – 84.0)	69.1 (48.0 – 90.1)
BMI, kg/m^2	29.0 (24.4 – 33.7)	26.9 (22.5 – 31.4)	28.7 (25.0 – 32.5)	25.8 (19.3 – 32.3)
Lumbar spine BMD, g/cm^2	0.932 (0.834 – 1.031)	0.934 (0.888 – 0.979)	0.921 (0.867 – 0.975)	0.957 (0.877 – 1.037)
Lumbar spine BMD, z-score	-0.91 (-1.84 to +0.01)	-0.87 (-1.28 to -0.46)	-1.01 (-1.51 to -0.51)	-0.65 (-1.37 to +0.07)
Femoral neck BMD, g/cm^2	0.809 (0.713 – 0.905)	0.794 (0.713 – 0.876)	0.775 (0.717 – 0.834)	0.850 (0.703 – 0.996)
Femoral neck BMD, z-score	-0.36 (-1.20 to +0.49)	-0.39 (-1.14 to +0.36)	-0.64 (-1.16 to -0.13)	+0.15 (-1.15 to +1.45)
Total hip BMD, g/cm^2	0.894 (0.754 – 1.033)	0.891 (0.807 – 0.974)	0.868 (0.789 – 0.947)	0.939 (0.790 – 1.087)
Total hip BMD, z-score	-0.16 (-0.91 to +0.60)	-0.36 (-1.04 to +0.32)	-0.45 (-0.96 to +0.06)	+0.05 (-1.14 to +1.24)
CrossLaps, ng/L	393 (280 – 507)	260 (161 – 359)	336 (232 – 440)	263 (142 – 384)
Bone ALP, U/L	15.0 (12.6 – 17.5)	14.9 (10.1 – 19.7)	16.0 (12.4 – 19.6)	12.8 (6.9 – 18.8)
PINP, ug/L	60.3 (52.0 – 68.6)	48.6 (32.0 – 65.1)	58.8 (45.4 – 72.1)	41.8 (25.8 – 57.9)
LH [d], U/L	5.8 (<0.6 – 11.6)	16.5 (6.2 – 26.8)	11.0 (3.6 – 18.4)	14.9 (<0.6 – 33.3)
FSH [d], U/L	13.7 (2.4 – 25.0)	27.2 (9.4 – 45.0)	20.3 (7.9 – 32.6)	25.3 (<1.0 – 56.4)
Oestradiol [d], pmol/L	100 (<36 – 173)	142 (37 – 246)	87 (47 – 127)	203 (<36 – 406)

Data are expressed as mean (95% CI mean).
[a] P <0.05 compared with non-Turner (other).
[b] P <0.01 compared with non-Turner (other).
[c] P <0.05 compared with pre-pubertal onset of ovarian failure
[d] Pre-washout, reflecting previous HRT prior to commencing study.

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Table 3 Hormone levels after first wash-out and during pSSR and sHRT

Hormone	After first washout	pSSR	sHRT
LH (U/L)	38.9 (30.3 – 47.5)	13.5 (7.6 – 19.4)	8.0 (5.0 – 11.1)
FSH (U/L)	85.8 (67.8 – 103.8)	21.0 (13.8 – 28.2)	17.3 (9.9 – 24.7)
Oestradiol (pmol/L)	66 (51 – 81)	406 (280 – 532)	66[a] (50 – 83)
Progesterone (nmol/L)	4.9 (4.2 – 5.6)	5.7 (5.0 – 6.3)	4.8 (4.2 – 5.3)

Hormonal data during pSSR and sHRT are expressed as overall 3 – 12 month mean values (95% CI mean) during each treatment arm respectively.
[a] The assay used for oestradiol does not cross-react with ethinyloestradiol used during sHRT.

Table 4 Changes in BMD in response to pSSR and sHRT

BMD measurement	pSSR	sHRT
Lumbar spine BMD, g/cm^2	+0.019 [a] (+0.008 to +0.029)	+0.01 (-0.002 to +0.022)
Lumbar spine BMD, z-score	+0.17 [a] (+0.07 to +0.27)	+0.07 (-0.03 to +0.18)
Femoral neck BMD, g/cm^2	+0.012 (-0.007 to +0.030)	+0.011 (-0.005 to +0.027)
Femoral neck BMD, z-score	+0.12 (-0.05 to +0.29)	+0.11 (-0.04 to +0.25)
Total Hip BMD, g/cm^2	-0.009 (-0.051 to +0.034)	+0.005 (-0.007 to +0.017)
Total Hip BMD, z-score	-0.04 (-0.16 to +0.08)	+0.03 (-0.08 to +0.13)

Data are expressed as mean (95% CI mean)
[a] P <0.01 versus baseline BMD

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Table 5 Bone markers at baseline, and after first and second wash-out

Bone marker	Baseline	After first wash-out	After second wash-out	Reference range [a]
CrossLaps, ng/L	312 (237 – 386)	487 [b] (405 – 569)	519 (438 – 601)	287 (112 – 738)
Bone ALP, U/L	14.9 (12.1 – 17.8)	17.7 [c] (13.4 – 21.9)	19.0 (14.8 – 23.3)	18.3 (11.6 – 29.6)
PINP, µg/L	53.1 (42.9 – 63.3)	61.8 (50.2 – 73.4)	58.1 (49.6 – 66.5)	46 (19 – 102)

Data are expressed as mean (95% CI mean) in study subjects

[a] Reference data provided by the kit manufacturer for pre-menopausal women, expressed as median (bone ALP) or mean (PINP and CrossLaps) and 2.5th to 97.5th percentile reference ranges.

[b] P <0.001 versus baseline

[c] P <0.05 versus baseline

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Figure legends

Figure 1: Study consort flow chart (ref 15)

Figure 2: Percentage changes in bone markers compared with post wash-out baseline in response to pSSR (solid squares) and sHRT (open squares).
(a) Bone ALP (b) PINP (c) CrossLaps. Data are expressed as mean (95% CI).

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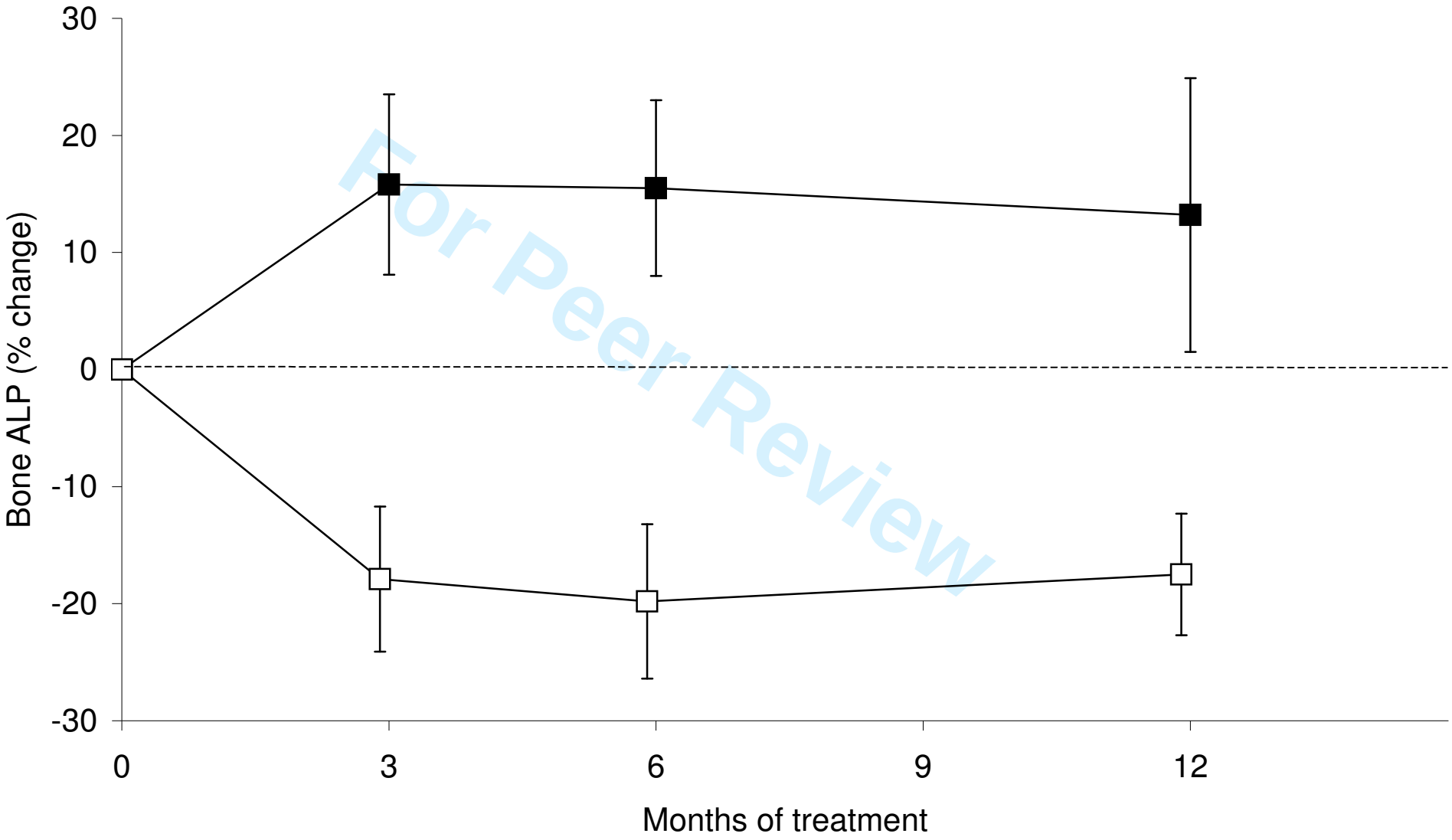

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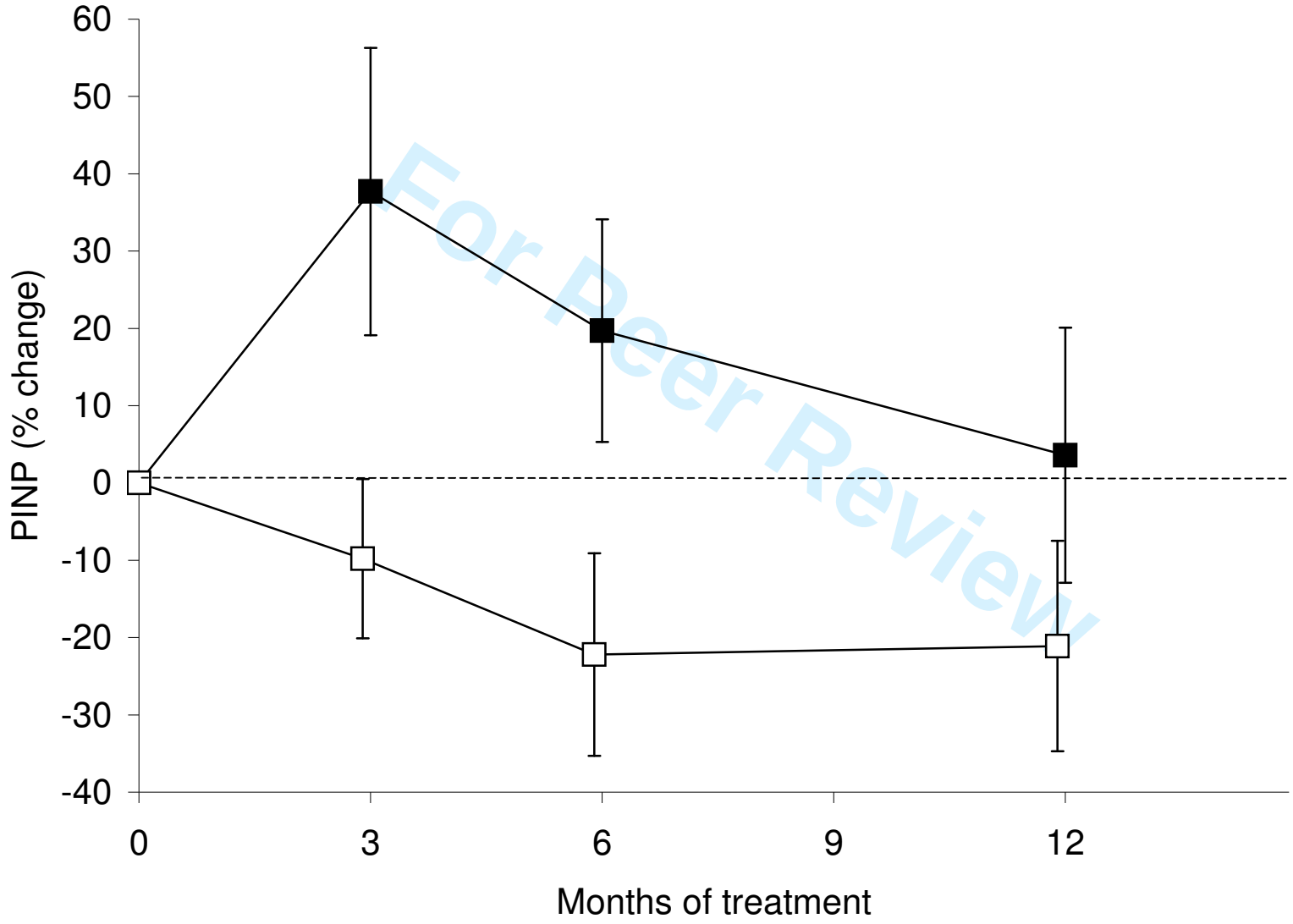

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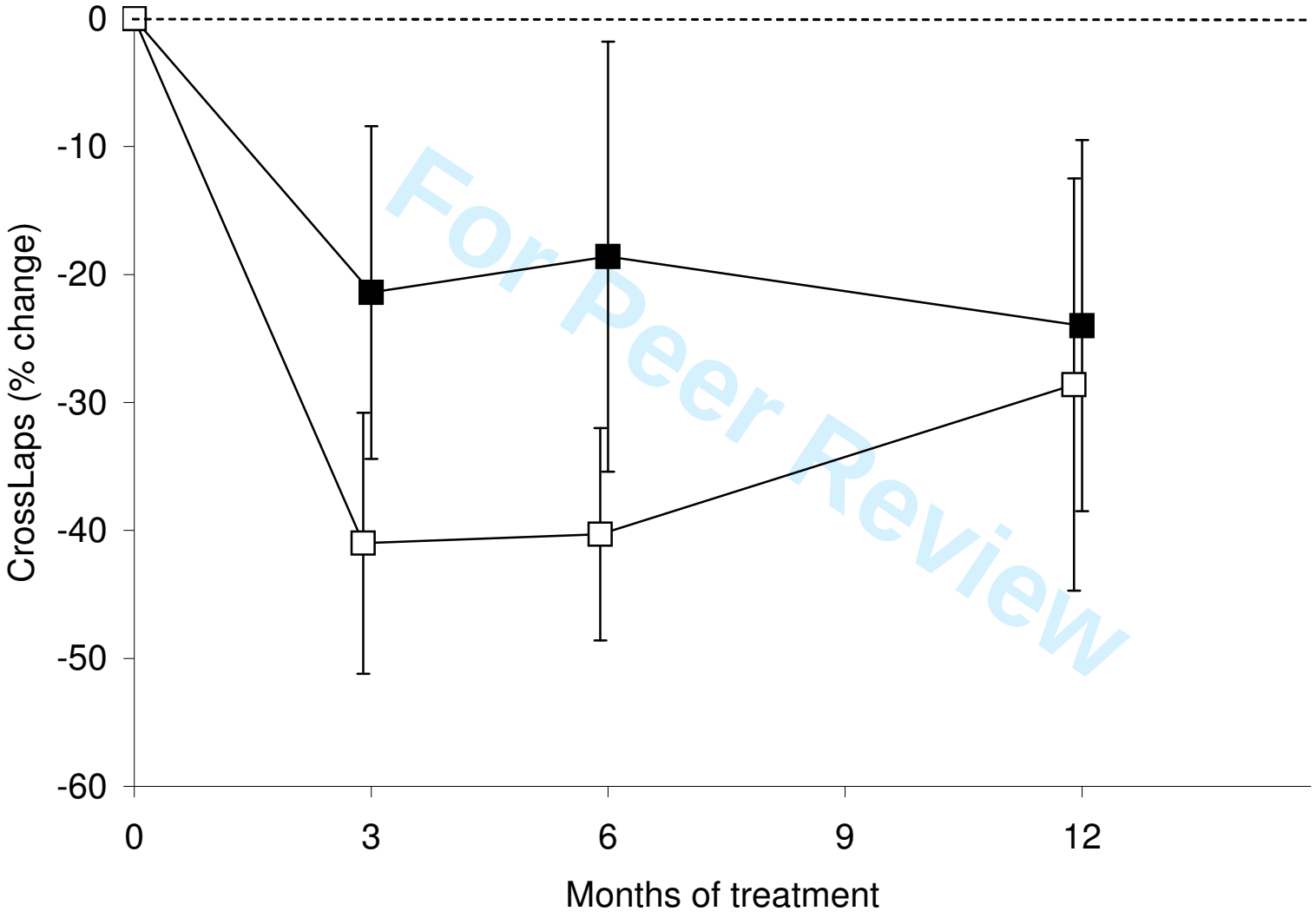

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References

1. Gravholt CH. 2005b. Clinical practice in Turner Syndrome. Nat Clin Pract Endocrinol Metab 1(1): 41-52
2. Wallace WH, Anderson RA, Irving DS. 2005a. Fertility preservation for young patients with cancer. Who is at risk and what can be offered? Lancet Oncol 6(4): 209-18
3. Sanders JE, Pritchard S, Mahoney P, Amos D, Buckner CD, Witherspoon RP, Deeg HJ, Doney KC, Sullivan KM, Appelbaum FR. 1986. Growth and development following marrow transplantation for leukaemia. Blood 68(5): 1129-35
4. Sarafoglou K, Boulad F, Gillio A, Sklar C. 1997. Gonadal function after bone marrow transplantation for acute leukaemia during childhood. J Pediatr 130(2): 210-6
5. Wallace WH, Shalet SM, Hendry JH, Morris-Jones PH, Gattamaneni HR. 1989. Ovarian failure following abdominal irradiation in childhood: the radiosensitivity of the human oocyte. Br J Radiol 62(743): 995-8
6. Wallace WH, Thomson AB, Saran F, Kelsey TW. 2005b. Predicting age of ovarian failure after radiation to a field that includes the ovaries. Int J Radiat Oncol Biol Phys 62(3): 738-44
7. Gravholt CH, Hjerrild BE, Naeraa RW, Engbaek F, Mosekilde L, Christiansen JS. 2005a. Effect of growth hormone and 17β-oestradiol treatment on metabolism and body composition in girls with Turner syndrome. Clin Endocrinol (Oxf) 62(5): 616-22
8. Panay N, Kalu E. 2009. Management of premature ovarian failure.Best Pract Res Clin Obstet Gynaecol 23(1):128-40
9. Bath LE, Critchley HO, Kelnar CJ, Wallace WH. 2001. Choice of hormone therapy in young women with ovarian failure. Clin Endocrinol (Oxf) 55(5): 697-8
10. Slemanda CW, Reister TK, Hui SL, Miller JZ, Christian JC, Johnston CC Jr. 1994. Influences on skeletal mineralization in children and adolescents: evidence for varying effects of sexual maturation and physical activity. J Pediatr 125(2): 201-7
11. Davies MC, Gulekli B, Jacobs HS. 1995. Osteoporosis in Turner's syndrome and other forms of primary amenhorroea. Clin Endocrinol (Oxf) 43(6): 741-6
12. Register TC, Jayo MJ, Jerome CP. 1997 Oral contraceptive treatment inhibits the normal acquisition of bone mineral in skeletally immature young adult female monkeys. Osteoporosis Int 7(4): 348-53
13. Critchley HO, Buckley CH, Anderson DC. 1990. Experience with a 'physiological' steroid replacement regimen for the establishment of a receptive endometrium in women with premature ovarian failure.Br J Obstet Gynaecol 97(9): 804-10
14. Critchley HO, Wallace WH, Shalet SM, Mamtora H, Higginson J, Anderson DC. 1992. Abdominal irradiation in childhood; the potential for pregnancy. Br J Obstet Gynaecol 99(5): 392-4
15. Langrish JP, Mills NL, Bath LE, Warner P, Webb DJ, Kelnar CJ, Critchley HO, Newby DE, Wallace WHB. 2009 Cardiovascular effects of physiological and standard sex steroid replacement regimens in premature ovarian failure. Hypertension 53(5): 805-11
16. Diahanbakch O, Swanson IA, Corrie JE, McNeilly AS. 1981. Prediction of ovulation by progesterone. Lancet 2(8256): 1164-5

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17. Conway GS. 2001. Oestrogen replacement in young women with Turner's syndrome. Commentary. Clin Endocrinol (Oxf) 54(2): 157-8
18. Gravholt CH, Vestergaard P, Hermann AP, Mosekilde L, Brixen K, Christiansen JS. 2003. Increased fracture rates in Turner syndrome: a nationwide questionnaire survey. Clin Endocrinol (Oxf) 59: 89-96
19. Purdie DW. 2004. Hormone replacement therapy and osteoporosis. In: Critchley H, Gebbie A, Beral V, editors. Menopause and hormone replacement. 1st ed. London; RCOG press: 77-86
20. Compston JE. 2001.Sex steroids and bone. Physiol Rev 81(1):419-47
21. Cauley JA, Robbins J, Chen Z, Cummings SR, Jackson RD, LaCroix AZ, LeBoff M, Lewis CE, McGowan J, Neuner J, Pettinger M, Stefanick ML, Wactawski-Wende J, Watts NB. 2003. Women's Health Initiative investigators. Effect of estrogen plus progestin on risk of fracture and bone mineral density: the Women's Health Initiative randomized trial. JAMA 290(13): 1729-38
22. Bush TL, Wells HB, James MK. Writing group for the PEPI. 1996. Effects of hormone therapy on bone mineral density-results from the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial. JAMA. 276(17): 1389-96
23. Wells G, Tugwell P, Shea B, Guyatt G, Peterson J, Zyturak N, Robinson V, Henry D, O'Connell D, Cranney A. Osteoporosis methodology group and the osteoporosis research advisory group. 2002. Meta-analyses of therapies for postmenopausal osteoporosis. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. Endocr Rev. 23(4): 529-39
24. Högler W, Briody J, Moore B, Grnett S, Lu PW, Cowell CT. 2006. Importance of estrogen on bone health in Turner syndrome: a cross-sectional and longitudinal study using dual-energy x-ray absorptiometry. J Clin Endocrinol Metab 89(1): 193-9
25. Hosie CJ, Hart DM, Smith DAS, Al-Azzawi F. 1989. Differential effect of long-term oestrogen therapy on trabecular and cortical bone. Maturitas 11(2): 137-45
26. Miller KL. 1996. Hormone replacement therapy in the elderly. Clin Obstet Gynecol. 39(4): 912-32
27. Sims NA, Dupont S, Kinst A, Clement-LaCroix P, Minet D, Resche-Rigon M, Gaillard-Kelly M, Baron R. 2002. Deletion of estrogen receptors reveals a regulatory role for estrogen receptor-β in bone remodelling in females but not in males. Bone 30(1): 18-25
28. Hall JM, McDonnell DP. 1999. The estrogen receptor beta-isoform (ER beta) of the human estrogen receptor modules ER alpha transcriptional activity and is a key regulator of the cellular responses to estrogens and antiestrogens. Endocrinology 140(12): 5566-7
29. Hannon R, Blumsohn A, Naylor K, Eastell R. 1998. Response of biochemical markers of bone turnover to hormone replacement therapy: impact of biological variability. J Bone Miner Res 13(7): 1124-33

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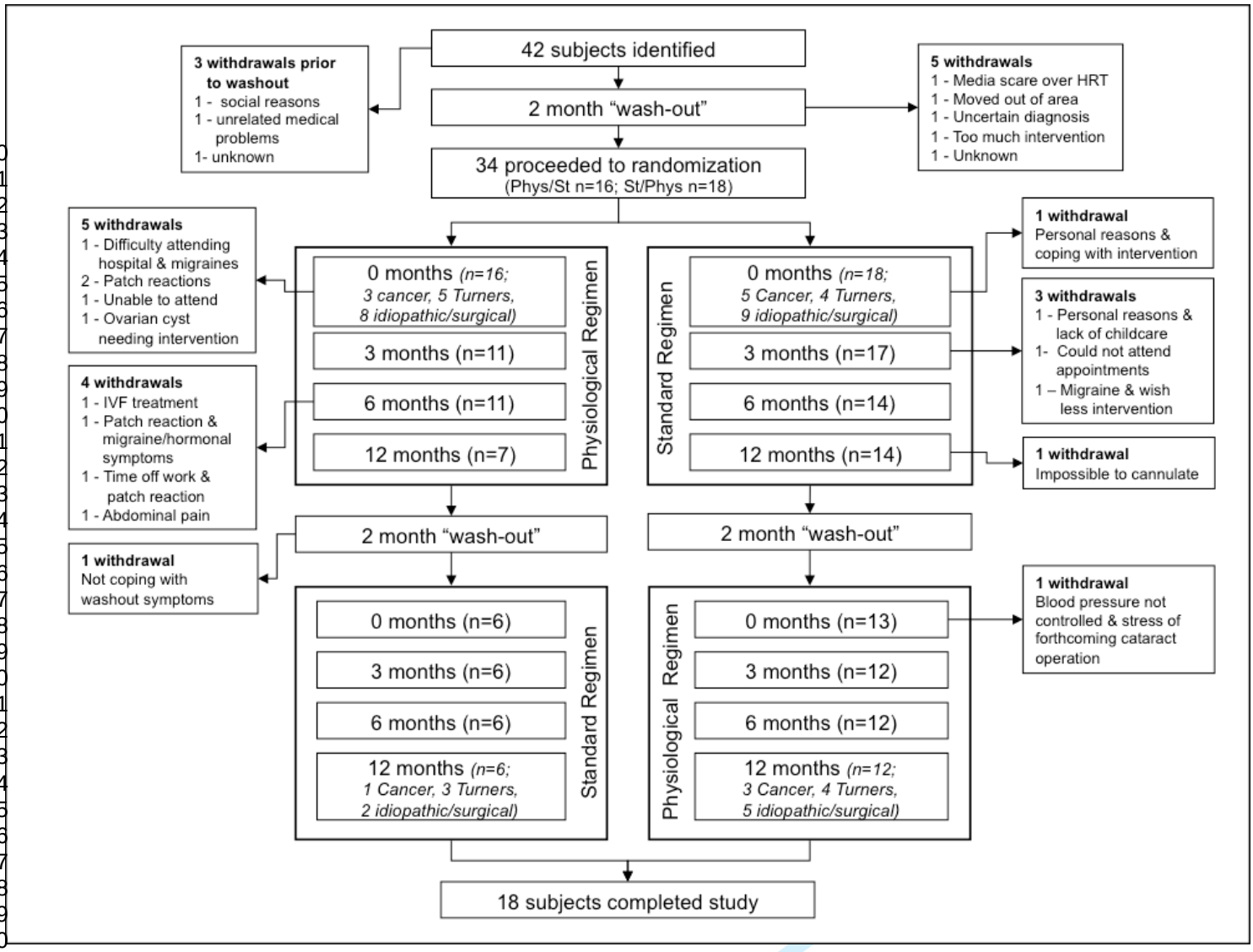
Figure one


42 subjects identified
2 month "wash-out"
34 proceeded to randomization
(Phys/St n=16; St/Phys n=18)
3 withdrawals prior to washout
1 - social reasons
1 - unrelated medical problems
1- unknown
5 withdrawals
1 - Media scare over HRT
1 - Moved out of area
1 - Uncertain diagnosis
1 - Too much intervention
1 - Unknown
5 withdrawals
1 - Difficulty attending hospital & migraines
2 - Patch reactions
1 - Unable to attend
1 - Ovarian cyst needing intervention
4 withdrawals
1 - IVF treatment
1 - Patch reaction & migraine/hormonal symptoms
1 - Time off work & patch reaction
1 - Abdominal pain
1 withdrawal
Not coping with washout symptoms
Physiological Regimen
0 months (n=16; 3 cancer, 5 Turners, 8 idiopathic/surgical)
3 months (n=11)
6 months (n=11)
12 months (n=7)
Standard Regimen
0 months (n=18; 5 Cancer, 4 Turners, 9 idiopathic/surgical)
3 months (n=17)
6 months (n=14)
12 months (n=14)
1 withdrawal
Personal reasons & coping with intervention
3 withdrawals
1 - Personal reasons & lack of childcare
1- Could not attend appointments
1 – Migraine & wish less intervention
1 withdrawal
Impossible to cannulate
2 month "wash-out"
2 month "wash-out"
Standard Regimen
0 months (n=6)
3 months (n=6)
6 months (n=6)
12 months (n=6; 1 Cancer, 3 Turners, 2 idiopathic/surgical)
Physiological Regimen
0 months (n=13)
3 months (n=12)
6 months (n=12)
12 months (n=12; 3 Cancer, 4 Turners, 5 idiopathic/surgical)
1 withdrawal
Blood pressure not controlled & stress of forthcoming cataract operation
18 subjects completed study