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FOR CLINICIANS WHO PROVIDE CARE FOR WOMEN

Clinical Implications of the HERS Trial Results



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INTRODUCTION

Recently, the results of the Heart and Estrogen/Progestin Replacement Study (HERS) were published, showing no overall effect of 4.1 years of estrogen plus medroxyprogesterone acetate (MPA) on risk for myocardial infarction (MI) and fatal coronary heart disease (CHD) events in women with established coronary disease. These results have raised important questions concerning the role of hormone replacement for prevention of heart disease in women.¹ Before HERS, it was widely assumed that hormone replacement could cut heart disease risk in half. This was based on the strength and consistency of a large number of observational studies comparing rates of heart disease in users and nonusers of estrogen and an abundance of tissue culture, animal model, and mechanistic data suggesting that estrogen could inhibit the development of atherosclerosis. Indeed, numerous physician practice guidelines already recommend use of estrogen replacement for prevention of heart disease in postmenopausal women.^{2,3} Now it is clear that the proper use of estrogen replacement for prevention of heart disease is more complex than initially assumed. This paper will review the results of the HERS trial in detail, and provide a summary of the implications of the results for clinical practice.

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THE HERS TRIAL DESIGN AND PARTICIPANTS

Women were eligible for the HERS trial if they were postmenopausal (55 to 80 years old, with a uterus) with established coronary artery disease. The presence of coronary disease was documented by history of previous myocardial infarction, mechanical revascularization, or at least 50% occlusion of a major coronary artery. Women were excluded if their cardiac event had occurred within six months of randomization, if they had taken HRT within three months of screening, if they were in another clinical trial, or if they seemed unlikely to take the study drug for the five years of the trial. Importantly, they were also excluded if HRT was contraindicated. These contraindications included history of deep vein thrombosis or pulmonary embolism, a history of breast cancer or suspicious mammogram findings, or endometrial anomalies (history of cancer, hyperplasia, thickening, or abnormal bleeding). Subjects were enrolled at 20 different U.S. academic medical centers.

The subjects were randomized into two treatment groups: a group taking .625 mg conjugated equine estrogens plus 2.5 mg MPA in one tablet daily (n=1,380), and a placebo group (n=1,383). Table 1 shows some demographic characteristics of the study group. Among the 4,830 women who

From the Editor

David F. Archer, M.D.

Dr. Herrington reports on the results of the HERS trial. Twelve percent of women who have established coronary heart disease had a recurrent event during the study (n=172/1380 and n=176/1383 in the hormone vs. placebo group, respectively). Hormone replacement therapy appeared to offer no benefit in reducing cardiac events. The clinician must weight the benefits of HRT for this group of older women whose clinical findings put them at high risk for recurrent cardiac events.

Dr. Thorneycroft highlights the dose of estrogens required for prevention and treatment of osteoporosis. New diagnostic techniques and our aging population have increased our awareness of the prevalence of bone loss, osteoporosis, and osteoporotic fractures. Active, appropriate intervention on the part of the physician equals a successful preventive health strategy.

Drs. Klein and Soules present the dilemma of how to predict the onset of menopause. Current evaluation of follicle stimulating hormone levels can predict reproductive outcome in women of advanced reproductive age, but cannot be relied upon to accurately assess the perimenopause/menopause transition. Serum FSH levels should not be used routinely because of the *high* variability in levels in perimenopausal women. The role of aging in the decline and cessation of ovarian function continues to be an enigma.

Menopausal Medicine

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attended the first screening visit, only 2,763 were ultimately randomized. Nonetheless, the HERS subjects show strong similarities to the general population of postmenopausal women with heart disease in the United States.⁴ The two treatment groups were not statistically different from one another in risk-factor or demographic characteristics. At the trial's end, vital status was documented in 100% of the cohort.

RESULTS OF THE HERS TRIAL CARDIOVASCULAR OUTCOMES

The women in the HERS trial were followed for an average of 4.1 years. In that period, the primary outcomes were the same in each group. In the placebo group, 176 women had non-fatal myocardial infarctions or fatal cardiac events, compared to 172 women in the hormone group (relative hazard 1.24; 95% confidence intervals 0.87-1.75). There were more CHD deaths (71 deaths vs. 58 deaths; relative hazard 1.24, 95% confidence intervals 0.87-1.75) and slightly fewer non-fatal myocardial infarctions (116 vs. 129) in the hormone group compared with the placebo group (relative hazard 0.91; 95% confidence intervals 0.71-1.17). There also were no statistically significant differences in the secondary cardiovascular outcomes (rates of bypass graft or revascularization procedures, unstable angina, congestive heart failure, peripheral arterial disease, or stroke) between the two treatment groups.

These surprising findings occurred despite favorable changes in both low- and high-density lipoprotein cholesterol in the women taking hormones. By the end of the first year of HERS, LDL cholesterol concentrations had decreased 14% in the hormone group and 3% in the placebo group ($p < 0.001$). During the same period of time, HDL cholesterol increased 8% in the hormone group and decreased 2% in the placebo group ($p < 0.001$).

Table 1
Summary of the HERS Participants

N = 2,763 subjects
Average age - 67 years
89% white
60% high school graduates
97% currently or previously married
13% current smokers
90% hypercholesterolemic
59% hypertensive
52% obese
18% diabetic (type I or II)

There was a difference in the timing of adverse CHD events during the trial. More CHD events occurred in the hormone group vs. the placebo group in year one of HERS (57 vs. 38; relative hazard 1.52, 95% confidence intervals 1.01-2.29). On the other hand, by years four and five of the trial, there were fewer CHD events in the hormone group vs. the placebo group (17 vs. 18; relative hazard 0.95, 95% confidence intervals 0.49-1.84). This trend over time is illustrated in Figure 1.

NON-CARDIOVASCULAR SECONDARY OUTCOMES

No statistically significant differences between groups were found in the rates of breast or endometrial cancers, although the numbers of women were too small and the duration of follow-up too short to draw inferences with confidence about the effects of the HERS regimen on these outcomes. There were 32 cases of breast cancer and two cases of endometrial cancer in the estrogen/progestin treatment group, compared to 25 cases of breast cancer and four cases of endometrial cancer in the placebo group (for breast cancer, $p = 0.33$; for endometrial cancer, $p = 0.41$). Cases of other cancers were not statistically different between groups (63 in the active treatment group and 58 in the placebo group; $p = 0.60$), nor were cases of any type of cancer (96 in the active treatment group and 87 in the placebo group; $p = 0.44$).

Overall, 130 fractures of any type occurred in the active treatment group and 138 in the placebo group ($p = 0.70$). Hip fractures occurred at almost the same rate in each group (12 in the active treatment group; 11 in the placebo group). Cases of other fractures were also very similar between groups (119 in the active treatment group; 129 in the placebo group; $p = 0.59$). This was unexpected due to the known benefits of estrogen for osteoporosis in postmenopausal women. But, the HERS trial was not designed with sufficient statistical power to address this scientific issue.

Gallbladder disease occurred more often in the active treatment group compared to the placebo group (84 vs. 62; $p = 0.05$). None of the gallbladder events was fatal. Cholelithiasis was known to be increased by estrogen therapy from previous studies.⁵

WHY THESE RESULTS?

What are we to make of these surprising findings? It would be natural to look for

some flaw in the conduct of the trial to explain this unexpected result. However, there are no obvious flaws in the trial conduct. The baseline variables were comparable in the two treatment groups, there was 100% follow-up for vital status, and the primary outcomes were adjudicated by an independent committee that was blinded to treatment assignment. During the trial, women assigned to receive hormone therapy took their study medication less frequently than women in the placebo group. Conversely, women taking placebo were more frequently started on lipid-lowering therapy. However, adjusting for these differences did not change the outcome. Nor were there any subgroups of women (non-smokers, younger women, women without prior MI, etc.) who appeared to benefit from the HRT regimen.

Some have questioned whether the study was long enough or had enough subjects to rule out a beneficial effect. These concerns would have more merit if the overall relative risk was less than 0.7 or 0.8 but the confidence limits still included 1.0. However, in HERS the relative risk was 0.99, and the confidence limits (0.8-1.2) indicate that it is extremely unlikely that a true beneficial effect of 20% or more was missed. The reason for the overall null effect in HERS is not predominantly because of lack of power, but rather because of the unexpected pattern of early increased risk that offset a later reduction in risk.

Is it possible that the earlier studies relied on to make judgments about the cardiovascular effects of estrogen are wrong, or at least provide incomplete information? The remarkable consistency of the majority of observational studies designed specifically to examine HRT and CHD risk is hard to deny. What is often overlooked,

however, is that most of these studies are not ideally suited to detect an increase in CHD risk early after starting HRT. This is because individuals who suffer morbidity or mortality early after starting a new therapy are less likely to be available for participation in observational studies. It is also worth noting that not every study of estrogen replacement and CHD risk has confirmed a beneficial effect. A recent case-control study from a Kaiser-Permanente cohort⁶ found no reduction in risk for MI in current or past users of HRT. In addition, a recent meta-analysis of 4,124 women in 22 observational studies focusing on HRT and its relation to other non-cardiovascular outcomes observed a trend toward increased risk of cardiovascular events (relative risk 1.39; confidence intervals 0.48-3.95).⁷

Another possibility is that HRT, or at least the estrogen plus MPA regimen used in HERS, has real but opposing effects on CHD risk. Perhaps there is a true pro-thrombotic effect responsible for the early CHD risk that is gradually overcome by the long-term beneficial effects on lipids and progression of atherosclerosis. This hypothesis is supported by the increased risk of venous thrombosis also observed in HERS. It may also be true that this increased risk is limited to a small subset of women who are uniquely susceptible to an adverse effect of the drugs. If this is true, identifying and withholding HRT from this group could allow many other women to enjoy a benefit from HRT.

FUTURE DATA TO CONSIDER

Clearly, more research is needed to clarify the reasons for the unexpected findings in HERS and the proper role of HRT in women with or at risk for heart disease. Several other trials are currently underway

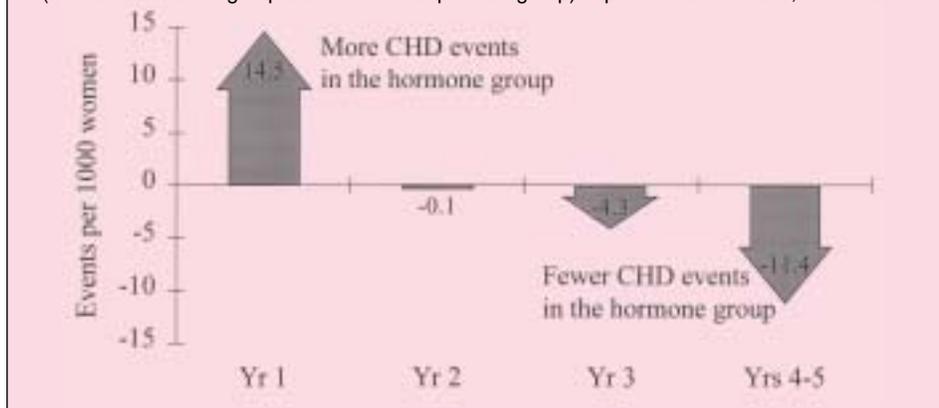
that will provide necessary additional data. Specifically, the NIH-sponsored Estrogen Replacement and Atherosclerosis (ERA) trial is an angiographic endpoint trial examining the effects of estrogen with and without MPA on progression of atherosclerosis. Furthermore, the NIH-sponsored Women's Health Initiative (N = 27,348) and the Medical Research Council-sponsored Women's International Study of Long Duration Oestrogen after the Menopause (WISDOM) (anticipated N = 34,000) will provide data on the effects of estrogen (plus MPA in women with a uterus) on mostly healthy, normal postmenopausal women.

HERS AND CLINICAL PRACTICE

In the meantime, how are clinicians to use the HERS results to guide their decisions about use of HRT? Some things are clear. In women with established coronary disease, estrogen plus MPA should not be started for secondary prevention of heart disease, unless new data emerge that suggest otherwise. On the other hand, women who have safely taken estrogen plus MPA for several years could continue; however, clinicians need to be aware that the HERS data do not yet prove that such a strategy will prevent heart attacks or save lives. It is merely suggested by the time-trend data. In addition, the increased risk of venous thrombosis and gallbladder disease should be considered when weighing the benefits and risks of therapy. There remain other legitimate non-cardiovascular indications for use of estrogen replacement (hot flashes, osteoporosis) in women with heart disease; however, these benefits must be weighed carefully against the prospects of an increased risk of CHD in the first year, as well as the increased risk for venous thrombosis and gallbladder disease.

Based on our current state of knowledge, it is impossible to know if similar results would have occurred had the HERS women taken unopposed estrogen or estrogen with another progestin instead of estrogen plus MPA. In animal studies, MPA attenuates the beneficial effect of unopposed estrogen on atherosclerosis,⁸ while subcutaneous progesterone does not.⁹ MPA also blunts the beneficial effect of estrogen on HDL cholesterol.¹⁰ However, in observational studies of hormone therapy and clinical cardiovascular events, no difference was observed between women using unopposed estrogen and women using estrogen plus MPA.⁶ Thus, until further evidence is available, it will remain uncer-

Figure 1. Comparison of coronary heart disease (CHD) events in the hormone and placebo groups in the HERS trial according to years of follow-up. Arrows indicate the difference in incidence (events in hormone group minus events in placebo group) expressed as events/1,000 women.



tain as to the proper role of unopposed estrogen or estrogen combined with other progestins for secondary prevention of CHD. It is essential to remember that use of aspirin, beta blockers after MI, and appropriate use of lipid-lowering therapy have been proven to reduce cardiovascular morbidity and mortality in women with or at risk for heart disease. These proven forms of therapy should be the centerpiece of any effort to prevent heart disease in women with existing coronary disease.

The role of hormone replacement for prevention of CHD in healthy normal women is less clear. HERS does not provide any specific guidance for this group. Until the results of the Women's Health Initiative are available, clinicians must continue to make the best decisions possible, weighing the currently available evidence and the specific needs of the patient in question.

The author has revealed the following potential conflict of interest: Consultant: Eli Lilly and Co., Pfizer, Inc., Solvay Pharmaceuticals, Wyeth-Ayerst Research.

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Estrogen and Bone Density



Ian H. Thorneycroft, Ph.D., M.D.

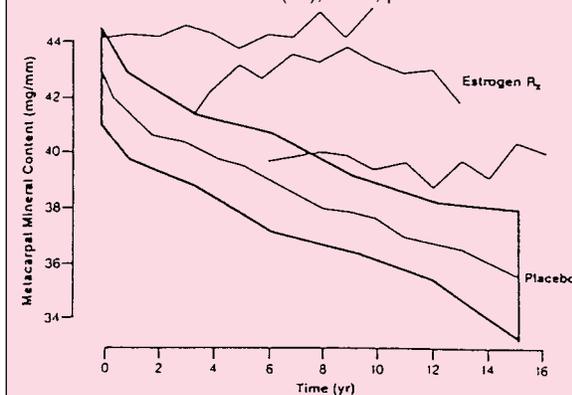
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INTRODUCTION

Women begin to lose bone in their mid 30s and have an accelerated bone loss as they go through menopause. Bone loss also continues through the later decades of life. The lower the bone density, the greater the probability of fracture at all skeletal sites. Hip fractures carry a very high mortality and subsequent disability. Mortality from a hip fracture increases with advancing age. Preventing the bone loss associated with menopause is very desirable to increase a woman's length and quality of life.

The importance of estrogen replacement therapy (ERT) and how it can prevent bone loss after the loss of estrogen has been elegantly described by Lindsay et al.¹ Patients given mestranol 25 µg after ovariectomy maintained their bone density, whereas those not placed on estrogen lost bone. Furthermore, women taking estrogen three and six years later maintained their bone density but did not regain lost bone (Figure 1). In a landmark randomized prospective blinded study by Christiansen et al, menopausal women given an estrogen-progestin combination slightly increased their bone density over the first two years compared to the placebo group who lost bone.² At 24

Figure 1. Bone loss after initiating estrogen replacement therapy at various times after menopause. Reproduced with permission from Lindsay R, Hart DM, Abdalla A, Al-Azzawi F, in Christiansen C (ed), 1987, p. 508.³⁴



months, the subjects were re-randomized to placebo or active drug. Those who continued on estrogen continued to slightly gain bone density; those taken off active drug lost bone in parallel to the original placebo group. The group originally on placebo and placed on estrogen gained a little bone but not equal to the amount that had been lost over the two years while taking placebo. The women continuing to take placebo continued to lose bone (Figure 2). Estrogens given at the right dose clearly prevents menopausal bone loss.

OVERVIEW OF BONE PHYSIOLOGY

Bone is formed by osteoblasts which are derived from preosteoblasts. They secrete the collagen necessary for bone formation, and also alkaline phosphatase and osteocalcin. Periodically, osteoblasts must be replaced by new cells to maintain bone building. The replaced osteoblasts are converted to osteocytes, cells that line the bone surface or undergo apoptotic death. Osteoclasts resorb bone, are multi-nucleated, and are derived from the fusion of many preosteoclast cells which are of hemopoietic cell origin, probably of the CFU-M-derived monocyte-macrophage family. Approximately 10-100 cells may fuse to form the osteoclast. The bone marrow-derived osteoblastic stromal cells play an important role in modulating the differentiation of osteoclast progenitors in two different ways: one is the production of soluble factors, and the other is cell-to-cell recognition between osteoclast progenitors and osteoblastic stromal cells. M-CSF is probably the most important soluble factor, which appears to be necessary for not only proliferation of osteoclast progenitors, but also differentiation into mature osteoclasts and their survival. A number of local factors as well as

systemic hormones induce osteoclast differentiation. They are classified into three categories in terms of the signal transduction: vitamin D receptor-mediated signals [1 alpha,25(OH)2D3]; protein kinase A-mediated signals (PTH, PTHrP, PGE2, and IL-1); and gp130-mediated signals (IL-6, IL-11, oncostatin M, and leukemia inhibitory factor). All of these osteoclast-inducing factors appear to act on osteoblastic cells to induce osteoclast differentiation factor (ODF), which recognizes osteoclast progenitors

and prepares them to differentiate into mature osteoclasts.³

The type I collagen secreted by the osteoblast is secreted as pro-collagen which has specific peptides at both the COOH and the NH₂ terminals. These peptides are removed by specific enzymes. These fragments are excreted and along with osteocalcin and bone-specific alkaline phosphatase can be measured. They indicate the level of bone formation or osteoblastic activity. States of high bone formation are characterized by high levels of these markers.

Collagen I contains high concentrations of hydroxyproline, lysine, and hydroxylysine. The lysines cross link the fibrils of collagen and are eventually converted to pyridinoline and deoxypyridinoline. During bone resorption, collagen is broken down and hydroxyproline, pyridinoline, deoxypyridinoline, and telopeptides with the pyridinolines attached are released. All can be measured in the urine as the free amino acids or the pyridinolines attached to the specific peptides on the collagen molecules. The latter are known as the N-telopeptide or the C-telopeptide. A urinary assay which measures the free pyridinolines is known as Pylinks® and the N-telopeptide as Osteomark®. Hydroxyproline can also be measured but it is also present in skin collagen, whereas deoxypyridinoline and the N-Telopeptide are highly specific to bone collagen. Hydroxyproline is also a metabolite of gelatin requiring a fasting state for its measurement. States of high bone resorption are characterized by the secretion of high levels of these markers.

Menopausal bone loss is characterized by both increased formation and resorption of bone, with resorption dominating. Markers of bone formation and resorption are therefore elevated. Estrogens decrease bone breakdown. Bone breakdown markers decrease within weeks after the administration of estrogen to a menopausal woman. Since bone breakdown and formation are linked, osteoblastic activity is also decreased, and the markers of bone breakdown also decrease after estrogen administration. The increased osteoblastic activity continues longer than the osteoclastic activity after estrogen administration, possibly explaining the increase seen in bone density after estrogen administration. Osteoblasts and osteoclasts have estrogen receptors. ERT induces apoptosis of osteocytes and osteoblasts probably mediated by an effect on osteoblasts.^{4,5} Estrogen stimulates the differentiation of osteoblasts and

inhibits and reverses the increase in osteoclastic hematopoietic stem/progenitor cell population seen after ovariectomy. IL-6 is involved in the osteoclastic resorption following ovariectomy.⁶ The protective effects of estrogen against postmenopausal osteoporosis are mediated by the direct induction of apoptosis of the bone-resorbing osteoclasts by an estrogen receptor-mediated mechanism. ICI164,384 and tamoxifen, as pure and partial antagonists, respectively, completely or partially blocked the effect of E₂ on both inhibition of osteoclastic bone resorption and induction of osteoclast apoptosis.^{5,7}

BONE DENSITY MEASUREMENT

Osteoporosis is defined as the loss of both the mineral and ground substance from bone. The WHO defines osteoporosis as a bone density of 2.5 standard deviations below young normal controls (t-score of <-2.5); whereas the NIH and the National Osteoporosis Foundation define osteoporosis as a bone density of 2 standard deviations below young normal controls (t-score <1.96). It is very clear that the lower the bone density the greater the probability of fracture. Fractures approximately double for each drop in the t-score of 1. Z-scores are the number of standard deviations above or below the mean for a patient compared to age, weight, height, and race matched controls. It differs from a t-score which reference to young normal controls. Z-scores below -2 need further evaluation for the etiology of the osteoporosis such as hyperparathyroidism.

Bone density can be measured by many methods, which are becoming more available. Medicare is now required by law to pay for an every other year screen to detect osteoporosis and a yearly measurement to follow therapy for patients with osteoporosis.

A plain radiograph is not useful to screen for osteoporosis. However, if osteoporosis is diagnosed from a plain radiograph, it is sufficient for diagnosis. A more precise and reproducible method is necessary to follow bone density changes.

Bone density measurements can be divided into central and peripheral measurement. Central machines measure spine, hip, total body, and wrist bone density. They are also capable, with the appropriate software, of measur-

ing body fat and lean body mass. They are referred to as DEXA (Dual Energy X-ray Absorptiometry) machines. Two different energy X-rays are used to allow a computer to subtract the energy absorbed by fat and therefore to calculate bone density. The amount of radiation received from a central DEXA scan is about the level of exposure from a plane trip traveling from New York to San Francisco. These machines measure trabecular bone, which is the bone lost the most after menopause. Peripheral measurements are quicker and less expensive. These machines easily fit into an examination room. Machines are available which measure finger, wrist, and heel bone mineral density (BMD). All but the heel involve ionizing radiation; the heel measurements utilize ultrasound. Only the heel measurement correlates well with hip bone density. Peripheral devices are good for screening but not for measuring response to therapy. A patient with an abnormal BMD needs a central DEXA scan. Wrist bone density was not enhanced by alendronate (Fosamax®) but was by estrogen.⁸ Alendronate, however, protected against hip bone loss and reduced fractures at that site. Computerized tomography (CT) scanning can also determine bone density; however, the radiation dose is very high, and precision is not great enough to follow bone density changes in patients.

Due to the rate of bone loss and the precision of bone density measurements, redetermination of BMD in less than 12 to 18 months is not worthwhile, except in cases of extremely high bone loss.

Who should receive a BMD measurement is a very complex question and can not be answered easily. A bone density

Figure 2. Bone mineral content as a function of time and treatment in 94 (study 1) and 77 (study 11) women soon after menopause. Reproduced with permission from Christiansen C, Christensen MS, Transbol I, 1981, p. 459.2

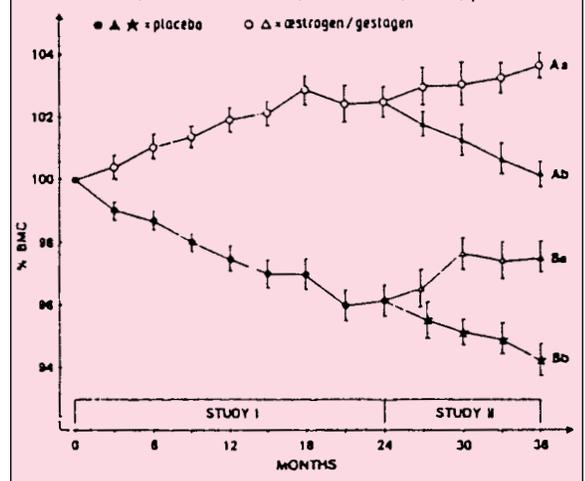
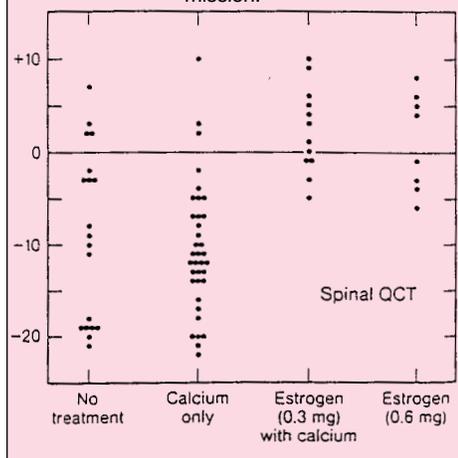


Figure 3 Spinal bone density in patients given Premarm® 0.3 mg, Premarin® 0.6 mg, Calcium or placebo. Reproduced from Ettinger with permission.¹⁹



measurement is more accurate in predicting fractures than a serum cholesterol is in predicting myocardial infarctions. The main problem is cost and whether one would act on the results. Many would argue that the patient will be treated with estrogen regardless, making the measurement superfluous. My preference is to get a baseline in all patients and follow up in two years. If the bone density is normal and maintained, then no further measurements are needed unless the clinical situation changes. Patients who have had a bone density showing osteoporosis are more likely to accept ERT, and the lower the bone density the more likely the patient is to accept ERT. In one study, acceptance of ERT ranged from 20% with normal bone density to 70% with a t-score between -1 and -2.5. In another study, 80% of those with osteoporosis accepted ERT.^{9,10}

Menopausal patients with high turnover bone loss, as determined by high bone formation and breakdown markers, are the most likely to both lose bone and respond to therapy.^{11,12} Unfortunately, although this holds well for groups, the predictive power in individuals is not as good. I believe bone metabolic markers are useful to monitor if: 1) patients are indeed taking their Fosamax® correctly; 2) the minimal dose of estrogen is working; or 3) demonstrating high bone turnover may increase the probability of accepting ERT, as those with low bone density have been shown to do. Bone breakdown markers are not a substitute for bone density measurements; they are complementary techniques.

WHEN TO INITIATE ERT

Bone density decreases continuously after menopause; the slope is greater in women

aged 50-59 than after in women 60 years of age.¹³ Estrogen should be initiated as soon as possible after menopause and continued into late life to achieve the highest bone density. Nevertheless, estrogen initiated after age 60 and continued offers bone-conserving benefit.¹⁴ Use of estrogen in women 65 years or older appears to decrease the risk for fracture in older women.¹⁵ In fact, estrogen has been shown to be most effective in women over age 75 in one study. Contrary to previous thought, estrogen can be initiated late in life and certainly should be for patients with low bone density. The mortality and disability from hip fractures increases with age.

EFFECTIVE/RECOMMENDED DOSES OF VARIOUS ESTROGEN PREPARATIONS FOR PROTECTING BONE

There are few if any side-by-side comparisons between various estrogens and few well conducted dose response studies for individual estrogens. The dose which prevents hip bone loss and/or fractures would be optimum. Unfortunately, few if any studies report the percent of patients responding. Usually, mean bone densities are reported. A significant number of patients could therefore lose bone in spite of the mean for the group being higher than placebo or unchanged from pre-ovariectomy. The recommended minimum dose of estrogens available in the U.S. are listed in Table I.

Fracture data is even harder to come by. This is particularly true for estrogens other than Premarin®, as their use has not been sufficient to give meaningful statistical data in epidemiologic studies. Table II summarizes the epidemiological literature of estrogen and hip fractures. There is about a 50% reduction in hip fractures among patients who use estrogen.

Premarin®: Lindsay and Genant demonstrated that 0.625 mg of Premarin® was the minimal dose for hand and spine protection.^{16,17} Hip bone density is maintained with 0.625 mg.¹⁸ Ettinger has demonstrated that 0.3 mg protected the spine when combined with 1500 mg of calcium.¹⁹ The PEPI study demonstrated that 97% of

patients maintained their hip and spine density when treated with 0.625 mg of Premarin® with or without either MPA or micronized oral progesterone.²⁰ The minimal effective dose of Premarin® therefore appears to be 0.3 mg per day for the spine and 0.625 mg for the hip. Calcium would have to be supplemented to a daily dose of 1500 mg if 0.3 mg was used. The recommended dose is 0.625 mg. Epidemiological studies have clearly shown that patients given Premarin® have reduced fractures at all sites. The most common dose used was 0.625 mg (Table II).^{21,22}

Estratab®: 0.3 mg has been shown to protect the hip and the spine.²³ The 0.625 mg dose was found to be better than the 0.3 mg, and all patients were supplemented with calcium. The minimum dose for the hip and spine would be 0.3mg, and 0.625 mg would be the recommended dose. There are no fracture data, but there is no reason to expect results to be any different than seen with Premarin®.

Ogen®: A dose response curve study demonstrated that the 0.625 tablet protected the spine but the 1.25 tablet was required to protect the hip.²⁴ The minimum dose for the spine appears to be 0.625, and for the hip the 1.25 mg. The recommended dose, based on very little data, would be the 1.25 tablet. No fracture data exists. If the 1.25 dose is equivalent to 0.625 mg of Premarin® (assumed to be the case), then it should protect against spine and hip fractures.

Estrace®: A study with very few patients demonstrated that 0.5 mg prevented bone loss in the spine. Forty percent of those on 0.5 mg lost bone. All were supplemented with calcium.²⁵ Another study compared 1 mg of Estrace® and oral micronized progesterone to 0.625 mg of Premarin® and MPA. Both had equal effect on the spine and hip.²⁶ The minimal dose appears to be 0.5 mg for the spine and 1.0 mg for the hip. The recommended dose given daily therefore is 1.0 mg. These recommendations are based on less than 20 patients per group in the Ettinger study and 16 per group in the comparative study with

Table 1. Recommended and Minimal Doses of Estrogen Available in the USA Based on Published Data

Preparation	Minimum Dose		Recommended Dose	
	Spine	Hip	Spine	Hip
Premarin	0.3 mg	0.625	0.625 mg	0.625 mg
Transdermal	50 µg	50 µg	50 µg	50 µg
Estratab	0.3 mg	0.3 mg	0.625 mg	0.625 mg
Ogen	0.625	1.25	1.25	1.25

Premarin[®].^{25,26} There are no fracture data with Estrace[®]. If the 1.0 mg dose is equivalent to 0.625 mg of Premarin[®], then it should protect against spine and hip fractures.

Transdermal Estrogens: Bone mineral density is maintained in the hip and spine by 50 µg per day.²⁷ The minimum and recommended dose is 50 µg per day. There are no fracture data. For high-risk patients, I use 100 µg per day.

Vaginal Estrogens: There are no data except from the recently introduced silastic vaginal ring. The dose delivered by this preparation is 7.5 µg per day. Absorption has been shown to be negligible after 48 hours. Nevertheless, forearm bone density was increased vs. placebo in very elderly women.²⁸ In my opinion, such therapy should be reserved for the very unusual patient such as one with a contraindication to estrogen. Bone density should be monitored and markers would also be helpful in this case.

Estrogen and Calcium: Virtually all prospective studies comparing placebo, calcium, and estrogen have reported that calcium is basically no better than placebo unless calcium intake without supplement is very low (less than 500 mg per day) in the very elderly patient. A typical study by Ettinger is illustrated in Figure 3.¹⁹ Only a high calcium intake (3% highest percentiles in the studied population) protects

against osteoporosis in Swedish postmenopausal women.²⁹ Ettinger also demonstrated in that study a combination of 0.3 mg of Premarin[®] and a total calcium intake of 1,500 mg per day maintained spinal bone density.¹⁹ Lindsay had previously shown that 0.3 mg of Premarin[®] was not adequate to prevent bone loss. A meta-analysis by Nieves et al demonstrated that adding calcium to ERT potentiates the bone sparing effects of ERT. This allows a lower dose ERT to be utilized or causes increased bone density over ERT alone.³⁰

Combined ERT and Bisphosphonate Therapy: The mechanisms by which bisphosphonates inhibit bone loss are complicated but similar to those of estrogen. The mechanisms involve: a) A direct effect on osteoclast activity and shortening of survival by inducing apoptosis; b) A direct and indirect effect on the osteoclast recruitment (the latter being mediated by cells of the osteoblastic lineage producing an inhibitor of osteoclastic recruitment).³¹ Bisphosphonates preferentially inhibit the later stage of osteoclastogenesis by stimulating osteoblastic cells to secrete inhibitors.³² It is not clear (although the mechanism of action of estrogens and bisphosphonates are similar) that each agent acts at exactly the same sites or stimulates the secretion of the same factors. A combination of the two may therefore be addi-

tive. The combination of ERT plus etidronate bisphosphonate has been shown to be additive in regards to bone density. Women given Premarin[®] and etidronate had a greater bone density than either alone. A four-year randomized study showed an additive effect of etidronate and HRT on hip and spine BMD in postmenopausal women with established osteoporosis.³³ Studies are currently underway comparing Premarin[®], Fosamax[®], and Fosamax[®] plus Premarin[®]. Until more data (including fracture data) are available, the combination of an estrogen and a bisphosphonate should be reserved for those not responding to either agent alone.

Combined ERT/Androgens: Androgens appear to stimulate the osteoblasts as determined by serum alkaline phosphatase bone marker studies.³⁴ Patients given Estratest[®] had higher bone density than patients given Estratab[®] 1.25 mg, suggesting an additive effect of the added androgen.³⁵

HRT: The PEPI study clearly demonstrated that when using a therapeutic dose of Premarin[®] (0.625 mg), the addition of either MPA or oral micronized progesterone did not increase bone density over estrogen alone.³⁶ A lower dose of Premarin[®], 0.3 mg, combined with MPA did preserve bone equally as well as 0.600 mg (two 0.3 mg tablets) of Premarin[®]. MPA may therefore potentiate the effect of ERT or add to

it.³⁷ Osteoporotic women may have a greater response to HRT than ERT.³⁸ At present, there appears to be no advantage to adding a progestin prescription to a patient without a uterus.

HYPER-PARATHYROIDISM
Several studies have demonstrated that menopausal patients with mild hyperparathyroidism can be treated with estrogen with almost equivalent

Table II
Postmenopausal Hormone Use and Hip Fractures: Epidemiology

	<u>Ever Use RR (95% CI)</u>	<u>Current RR(95% CI)</u>	<u>Duration of Use</u> <u>Years of Use (RR95% CI)</u>	
Case-Control				
Hutchinson (1979) ^{a,b,g}	0.2		>5	0.2
Weiss (1980) ^{a,b,d}	0.43 (0.3-0.63)		>10	0.46 (0.30-0.69)
Johnson (1981) ^{a,b,c}	0.67 (0.36-99)			
Paganini-Hill (1981) ^{a,b}	0.79 (0.47-1.36)		>50	0.42 (0.18-0.98)
Oophorectomized	0.14 (0.03-0.7)			
Kreiger (1982) ^{a,b}	0.4 (0.2-0.9)			
Williams (1982) ^{a,f}	0.53 (0.37-0.77)			
Michaëlsson (1998) ^h	0.58 (0.46-0.75)	0.35 (0.24-0.53)	>5	0.27 (0.08-0.94)
Past users	0.76 (0.57-1.01)		>5	1.07 (0.57-2.03)
Kanis (1992) ^h	0.55 (0.31-0.85)			
Jaglal (1993) ^h			>5	0.33 (0.17-0.67)
Alderman (1986) ^f			>5	0.4 (0.2-0.6)
Cohort				
Hammond (1979) ^{a,f}	0.54 (0.33-0.90)			
Ettinger (1985)	Lower but not significant			
Kiel (1987)	0.65 (0.44-0.98)	0.34 (0.12-0.98)		
Naessen (1990)	0.79 (0.68-0.93)			
Estradiol&CEE	0.70 (0.55-0.87)			
Estriol	0.90 (0.72-1.11)			
Paganini-Hill (1991)	1.0 (0.81-1.27)	0.80 (0.53-1.21)	>15	0.88 (0.63-1.24)
Folsom (1995) ^h		0.53 (0.31-0.91)		
Past	0.96 (0.73-1.26)			
Meta-Analyses				
Grady (1992)	0.75 (0.68-0.84)			
Ettinger (1985)	0.40 (0.2-0.5)			

a) Included in Grady meta-analysis; b) Included in Ettinger meta-analysis; c) Oral estrogens; d) Weiss study combined hip and forearm; e) All fractures combined; f) Estimated from the original data in the article; g) Calculated by Grady; h) Not included in meta-analysis by Grady

results compared to parathyroidectomy. This seems a reasonable medical therapy with close monitoring of bone density and bone markers. ERT was not found to reduce serum calcium. Surgery is required for recurrent renal calculi or continued bone loss.³⁹

HYPERTHYROIDISM

Thyroid hormone supplementation reduces bone density. ERT appears to reduce this affect.⁴⁰

CONCLUSION

Menopausal women lose bone at an accelerated rate due to estrogen deficiency. This bone loss continues into late menopause and can be prevented by ERT. Bone loss can be detected by bone density measurements and followed with a combination of bone density measurements and bone markers.

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Clinical Assessment of Relative Reproductive Age



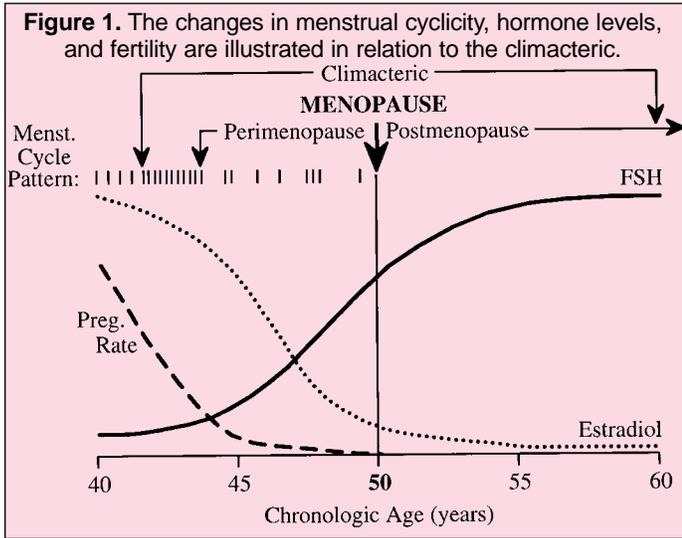
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INTRODUCTION

When considering medical issues in relation to the menopause, it is important to understand the physiologic basis that leads to menopause: the

deterioration in ovarian function. How and when a woman becomes menopausal is relevant to her care after the menopause. While reproductive aging actually begins before birth, the relevant clinical terms relate to the latter aspects of reproductive aging (Figure 1). The climacteric is initiated when it is noted that a woman's menstrual cycles are still regular yet distinctly shorter (e.g., 23 to 24 days) compared to her former cycle length (e.g., 28 days). The climacteric ends several years into the postmenopausal interval when estradiol secretion from the ovary reaches a nadir – a point in time that is not clinically evident. A particular woman experiencing a normal climacteric will have shorter cycles for several years prior to experiencing cycle irregularity that is the hallmark of the



perimenopause. According to Treolar, in a study of over 3,500 women over 46 years and >35,000 years of menstrual experience, the perimenopause tends to last three to four years before final cessation of menses.¹ Because it is difficult to determine prospectively when the last menstrual period has occurred, the established definition of the menopause is 12 months of amenorrhea. The average age of menopause is 50 years; \pm two standard deviations result in a range of 42 to 58 years for a normal menopause. There are many factors that are known to influence the age of menopause for a particular individual. The best historical predictor is the age of menopause in first-degree relatives (mother and sisters).² Many studies have demonstrated that cigarette smoking accelerates the age of menopause by several months to several years in a dose-dependent manner.^{3,4} A prior oophorectomy or significant resection of ovarian tissue can hasten the age of menopause by up to seven years.⁵ Pregnancies and the use of oral contraceptives may delay the age of menopause up to several months, which is statistically but not clinically relevant.^{3,4} The onset of menstrual function (menarche) was thought to influence the age of menopause, but this has subsequently been shown not to be the case.³

Menopause represents the culmination of reproductive aging where ovarian function gradually decreases until the reproductive axis is non-functional. The physiologic basis is ovarian follicular depletion. While it is an axiom that follicles are gradually lost over a woman's lifetime from birth to menopause, the primordial follicle counts that have been cited time and again (e.g., 2 million at birth, 400,000 at menarche, etc.) are only rough estimates based on small

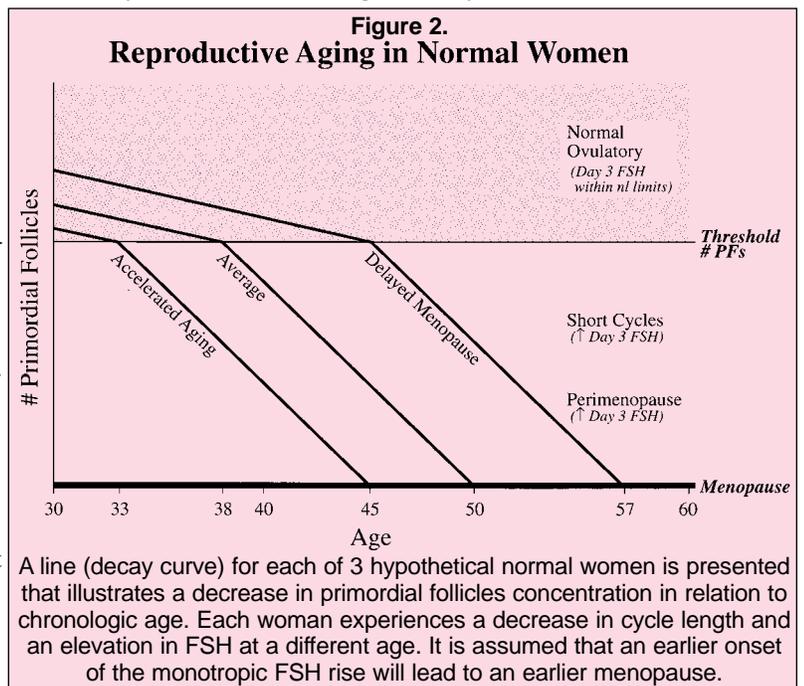
were counted. Each of these investigators used a number of assumptions in regard to counting rules. Only about 100 pairs of ovaries have ever been counted in the world's literature, and many assumptions/extrapolations have been made from this data.⁶⁻⁸ Only one of these studies correlated function (menstrual cyclicity) with follicle counts; it was found that there were significantly greater numbers of primordial follicles in the ovaries of women with regular cycles compared to those who were perimenopausal.⁷ Based on mathematical modeling of the combined data from these studies, it has been proposed (and generally accepted) that there is an acceleration of primordial follicle depletion that begins at about age 38 in the average woman. It is fascinating that age 38 coincides with both the monotropic FSH rise and an abrupt decrease in relative fertility. Current concepts of reproductive aging consider that a woman crosses a threshold of critical remaining primordial follicle numbers late in her fourth decade. Based on the mathematical model, this has been estimated to occur at about 20,000 primordial follicles.⁵ When this threshold

numbers of ovaries using relatively crude counting techniques. Only three investigators have ever attempted to count the number of primordial follicles in the ovaries of normal women at different chronologic ages.⁶⁻⁸ They all used similar techniques: the ovary was serially sectioned (3 to 4,000 sections), every 10th or 20th section was selected, and primordial follicles

is crossed, the accelerated phase of follicular depletion begins. The process may be accelerated by the elevated FSH level in circulation (the monotropic FSH rise) which occurs because there are no longer sufficient numbers of early antral follicles in both ovaries to secrete adequate amounts of inhibin B. This subtle but real FSH rise persists throughout the cycle, but it is most evident in the early follicular phase. This higher FSH level is thought to accelerate dominant follicle development in individual cycles (shorter cycle) as well as to accelerate primordial follicle depletion which eventually leads to the menopause.⁹ Of course, as illustrated in Figure 2, individual women do not all reach this threshold at the same age. Some reach the threshold prior to age 38 and have an earlier (albeit normal) menopause, while others reach the threshold well into their 40s and have a later menopause.

A series of coordinated endocrine and physiologic changes occur when a woman enters the accelerated phase of follicular depletion:

- 1) Accelerated development of the dominant follicle with a shorter follicular phase and decreased total menstrual cycle length.¹⁰ In our studies comparing normal ovulatory women aged 40 to 45 to control women aged 20 to 25, we found the decrease in the follicular phase averaged three days (from 14 to 11 days). However, some women of advanced reproductive age (ARA) can have follicular phase lengths as short as seven to nine days while remaining ovulatory.



2) Dominant follicle development in older ovulatory women remains robust. Estradiol levels in circulation and the follicular fluid are either normal or elevated, while serum progesterone levels in the luteal phase are generally normal.^{10,11} Figure 3 illustrates serum FSH and estradiol levels in normal women aged 20 to 25 (n=12) and 40 to 45 (n=16) beginning with cycle day one (onset of menses). The older group has a subtle but significant FSH elevation throughout the cycle, but this elevation is most evident in the early follicular phase before their elevated estradiol levels partially suppress the FSH and make the rise less evident. This is the basis for obtaining an FSH level on day 3 of the cycle.

3) It is unclear whether earlier follicle development in ARA women is accelerated or just begins earlier in the late luteal phase of the previous cycle. Our studies found an earlier intercycle FSH rise and earlier follicular phase peak in FSH level with earlier onset of follicular development in the ARA woman.¹⁰

4) The basis for the FSH rise is a decrease in inhibin B in the early follicular phase and perhaps a decrease in inhibin A in the luteal phase.^{12,13} Circulating activin A levels may also be elevated and further potentiate the FSH rise.¹⁴

5) In ARA women who remain ovulatory, there are no discernible changes in GnRH secretion (LH pulse pattern),¹⁵ growth hormone secretion,¹⁶ or serum levels of IGF-I or IGF-II.¹¹ Considering that there is no discernible slowing of the GnRH pulse generator, the monotropic FSH rise has now been attributed to an inhibin/activin process as initiated by the ovary. The ovary appears to be the primary determinant of reproductive aging in the human, whereas it is primarily a neuroendocrine-initiated process in the rodent.

6) Once women of ARA reach the perimenopause, the endocrinology becomes even more complex and unpredictable. In general, perimenopausal women have periods of time when they secrete higher levels of estradiol than found in younger women with concomitant suppression of gonadotropins. This pattern can alternate with episodes of high gonadotropin and low estradiol. Even in the presence of high estradiol levels, an LH surge followed by ovulation only occurs intermittently. When ovulation does occur, luteal phase deficiency is common. All of these variations in reproductive hormone secretion result in irregular menstrual cycles.¹⁷

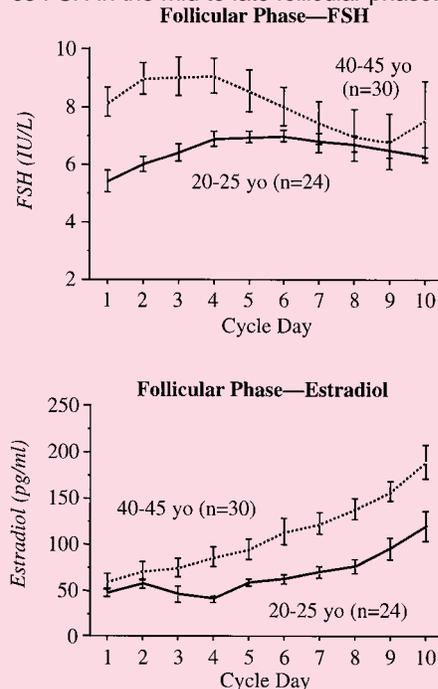
There are several pertinent clinical issues that can arise when a woman is in the latter reproductive years. The irregular bleeding pattern of the perimenopause is a common clinical problem that can be solved with either intermittent progestin or oral contraceptive treatment. Adenomyosis tends to be more prevalent at this time; it can either be followed expectantly or may require a hysterectomy. There is growing evidence that premenstrual syndrome may be more common and symptomatic in ARA women. Standard PMS treatment strategies should be followed. Contraception could be a problem for the sexually active ARA woman who has not chosen sterilization. Low-dose oral contraceptives are an acceptable and popular approach, and an IUD would be reasonable as well. However, the prominent problem that is experienced by ARA women vis-à-vis reproductive aging is infertility. There is ample evidence in historical populations who didn't use contraception and kept good birth records, that there is a precipitous decline in fertility as women age.¹⁸ This decrease in fertility is primarily dependent on the age of the female partner. A woman's peak reproductive efficiency occurs at approximately age 28; there is a gradual decline until age 35 and then a moderate decline from age 35 to 40. After age 40, there is a dramatic decrease in a

woman's relative fertility. A woman is considered to be approximately 50% as fertile at age 40 compared to her peak prior fertility. Not only are ARA women less fertile, but their spontaneous rate of abortion of clinically recognized pregnancies is dramatically increased as well.¹⁹ At age 40 to 45, the spontaneous abortion rate approaches 50%. The oocyte itself appears to be the problem; a majority of oocytes and embryos in ARA women are aneuploid.²⁰ Considering the fact that the baby boom generation has deferred childbearing more than any other generation in history, many women are experiencing infertility and/or reproductive wastage problems secondary to advanced maternal age. When routine infertility tests are applied to these couples, they have no demonstrable increases in any of the diseases known to cause infertility.²¹ On the other hand, they appear to have more unexplained infertility that can often be attributed to advanced maternal age. In consideration of all these clinical issues that apply to the ARA woman, the need for a practical and effective test to determine a woman's relative reproductive age has become very attractive, yet remains rather elusive.

When it comes to assessment of relative reproductive age, both society and clinicians tend to first think of a woman's chronologic age as an indicator of her ovarian function. The relatively wide age range for menopause (42 to 58 years) would indicate that the reproductive axis ages to a state of nonfunction at a vastly different rate dependent upon the individual. Age 40 as a negative milestone in society can only be generally applied to reproductive aging. Whereas it is generally true that most women over 40 have markedly reduced fertility, many women in their 30s may have a significant component of age-related infertility. When it comes to an individual woman, chronologic age can only be used as a rough approximation of a woman's hypothalamic-pituitary-ovarian function. Various clinical tests have been used to try to improve the assessment of relative reproductive age. Over the past decade, various endocrine tests have been used as markers of ovarian reserve. These tests all attempt to serve as indicators of the size of the ovarian pool of primordial follicles. These tests are best performed in the early follicular phase of the cycle, and cycle day 3 has become the standard day to obtain a blood sample for this purpose.²²

The most common tests are looking for

Figure 3. Daily serum levels of FSH & estradiol in normal women beginning with onset of menses (cycle day 1). Older ovulatory women demonstrate accelerated follicular development which partially suppresses FSH in the mid to late follicular phase.



evidence of the monotropic FSH rise, or accelerated follicular development (a day 3 estradiol). A cycle day 3 FSH and estradiol level is our recommendation as the most simple, straightforward assessment of relative reproductive age. We consider an FSH level of ≥ 10 mIU/ml to be significantly elevated based on a mean FSH level of 5.9 ± 1.1 mIU/ml ($x \pm SD$) in 12 normal women age 20 to 25.¹⁰ It must be understood that a woman with decreased ovarian reserve below the critical threshold may have a normal FSH if she is also experiencing accelerated follicular development and the estradiol is elevated on day 3 sufficient to suppress the FSH. We found mean cycle day 3 estradiol levels of 47 (range 22 to 78) pg/ml and 54 (range 20 to 108) pg/ml in 12 younger (age 20 to 25) and 16 older (age 40 to 45) normal women.¹⁰ In the clinical setting, we use 80 pg/ml as our cut-off for a day 3 estradiol. The relevance and meaning of an elevated day 3 FSH or estradiol is the same—decreased ovarian reserve and relatively poor prognosis for future fertility. The medical literature is quite clear that a woman with elevated day 3 FSH or estradiol will: 1) demonstrate decreased response to ovulation induction—a lower estradiol level with fewer follicles and oocytes; 2) require a higher dose of gonadotropin; 3) have a higher assisted reproductive technologies (ART) cycle cancellation rate; and 4) experience a lower pregnancy rate. Certainly the day 3 FSH/estradiol level will vary cycle to cycle in any woman, but only rarely (less than 10% of the time) does a woman vary between the normal and the abnormal range when multiple cycles are examined.

It has been demonstrated that a woman with a prior day 3 elevation of FSH or estradiol will not respond any better to ovulation induction even if stimulation is withheld until there is a cycle in which these levels are subsequently in the normal range.²³ There is a controversy whether day 3 levels of inhibin B are useful in the clinical setting. While a day 3 inhibin B level may help to further define the ovarian reserve, it is not a readily available or useful clinical test at this time. An inhibin B level of 45 pg/ml has been proposed as the critical level.²⁴ However, since the immunoassay (ELISA) for dimeric inhibin B is not readily available outside of research settings, is tricky to run and standardize, and is expensive, we do not currently recommend its routine clinical use.

The clomiphene citrate challenge test

(CCCT) is a common and useful test to assess relative reproductive age.²⁵ It is performed by first doing a day 3 FSH/estradiol level followed by clomiphene citrate 100 mg on cycle days 5 to 9. On cycle day 10, a second serum FSH level is determined. If either of the FSH levels or the estradiol level is elevated beyond the critical cut-off, then the patient is considered to have decreased ovarian reserve. Therefore some patients with decreased ovarian reserve can be identified in spite of a normal day 3 FSH and estradiol. Several publications have indicated that the CCCT approximately doubles the sensitivity of day 3 endocrine testing.^{26,27} The prognosis for a patient with a positive CCCT is the same as the prognosis for a positive day 3 test. Some clinics use the CCCT as their routine for assessment of ovarian reserve, while other clinics feel the additional expense and complexity is not worth the extra effort. If an endocrine marker of decreased ovarian reserve will be used to choose one course of management over another, then the CCCT would be superior since it is more sensitive. It is important to note that a normal early follicular endocrine test of ovarian reserve does not improve the prognosis for fertility that is inherent to a particular woman's chronologic age.

However, all the early follicular endocrine tests suffer from a major flaw: a positive result indicates relatively advanced reproductive age and poor prognosis for fertility treatments. The results are only useful for counseling purposes. Also, there is no available test to predict the onset of the monotropic FSH rise before it has already occurred. A test with only negative predictive value and no effective treatment is always going to be limited. While a positive early follicular phase endocrine test is probably indicative of early menopause, this assertion has never been tested.

It would be ideal to be able to perform a test that would assess current reproductive age and predict the onset of age-related infertility at any time in a woman's reproductive lifespan. No such test is currently available. However, several tests have been proposed that could serve to test ovarian reserve prior to the time it is compromised. Sensitive transvaginal ovarian ultrasound can image small cystic structures in the ovary greater than 2 mm. The pool of early antral follicles (2-10 mm) correlates with the number of primordial follicles remaining in the ovary. It has been

determined by sonography that the early antral follicle pool does not vary across the menstrual cycle. In this study of seven normal women with frequent ultrasounds across the menstrual cycle, it was determined there were three to 11 follicles between 2 and 11 mm in either ovary at any phase of the menstrual cycle.²⁸ Studies have reported that there is a significant correlation between early follicular total ovarian antral follicle count (<10 mm) and chronologic age as well as response to ovulation induction in an ART setting.^{29,30} While this test appears to be simple and straightforward, interobserver variability may limit its application. Whether sonography can be effectively applied across the clinical setting as a routine test of reproductive age needs to be determined. We conjecture that each clinic that attempts this method will need to rigorously establish its own normal data.

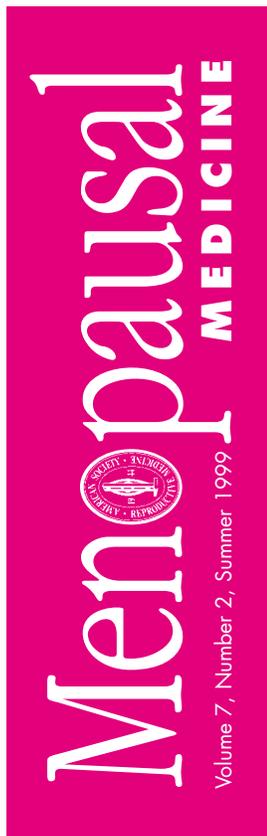
Considering the fact that estimation of the number of primordial follicles remaining in the ovary is the basis for all tests of relative reproductive age, it would be attractive to be able to count the number of primordial follicles from an ovarian biopsy and achieve an accurate estimate of total follicle numbers and relate this to current and future reproductive function. A recent publication from the United Kingdom found a significant negative correlation between the number of primordial follicles on ovarian biopsy and patient age.³¹ A laparoscopy or laparotomy was required for the biopsy and the follicle counts were performed by a relatively inaccurate technique. The critical number of follicles per ovary in relation to ovarian function remains to be determined. Once this is established, then perhaps a biopsy would be sufficient to estimate the total number of follicles and make some useful assessment of future ovarian function. Perhaps in the future when a laparoscopy is performed, a small ovarian biopsy will be routine and subsequent follicle counts will be used in conjunction with a nomogram to provide the patient with an indication of whether she is relatively young or old in terms of her reproductive age. Perhaps we will also be able to accurately predict the onset of menopause.

Clinical issues that relate to relative reproductive age will persist as compelling problems for clinicians. Should a woman continue to delay childbearing or attempt to conceive sooner? Will a particular woman have an early or late menopause?

Accurate answers to questions like these would revolutionize the practice of reproductive medicine. It is our current assessment that endocrine testing will not be able to answer these questions any better than the current tests available. Once we have obtained better knowledge of ovarian function in relation to follicle concentration, then an anatomically based test will have the best potential to provide meaningful answers.

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