

MENOPAUSE

Development of low-dose reproductive hormone therapies in China

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Abstract

A historical account is presented of the development of sex hormone treatment from its beginning at the Peking Union Medical College to its present-day generalization throughout China. The general theme of this work has been to test low-dose hormone regimens. Notable successes include low-dose oral contraception and menopausal hormone treatment. In support of the latter, we present a new clinical study of the effects of low-dose, intermittent, patient-metered hormone replacement therapy (HRT), which shows decreased menopausal symptoms, maintenance of bone health and height, and improved cardiovascular status compared with untreated controls. Cardiovascular testing, included carotid artery ultrasound scanning and computed tomographic coronary angiography, supports a cardioprotective effect of long-term (up to 31 years) low-dose HRT that is begun during the menopausal transition. These results highlight the urgent need for larger, prospective trials of long-term low-dose HRT started during the perimenopausal period.

Keywords: Menopause, estrogen, Women's Health Initiative, Nurses' Health Study, cardioprotection, computed tomographic angiography

Introduction

The human endocrine system maintains homeostasis. Treatment with excess ovarian hormones can interrupt reproduction in normal women or it can maintain homeostasis in estrogen-deficient women. Selection of the proper therapeutic hormone is the first step, which is followed by adjusting the dosage and regimen (e.g. continuous or sequential). While physicians usually follow pharmaceutical recommendations, they may try to adapt the regimen to furnish the lowest doses that suit the needs of individual patients. This report reviews the use of hormone treatment over the past several decades in China, where it has been important to find the lowest therapeutically effective doses that treat while avoiding unwanted side-effects and lowering costs, so that more patients can benefit. Reducing hormone dosage is a simple measure that often increases compliance. This concept of low-dose hormone treatment evolved

over many years and has led to the development of low-dose oral contraception and long-term low-dose hormone replacement therapy (HRT) regimens for menopausal women. The following are some milestones in the development of low-dose hormone treatment regimens in China.

Reduced dosages for oral contraceptives

In the 1960s, the Chinese government implemented a family planning program and many contraceptive compounds (e.g. megestrol, norethisterone and ethinyl estradiol) were utilized for clinical testing. At that time, the accepted mechanisms of oral contraception were: (1) making the cervical mucus hostile to stop the entry of sperm into the uterus; (2) making the uterine endometrium atrophic to prevent implantation; and (3) producing anovulation with pharmacological doses of synthetic sex hormones.

Early clinical trials used megestrol, 4.0 mg, and norethisterone, 2.5 mg, daily and yielded excellent contraceptive results. But there were side-effects and it became apparent that perhaps the dosages of the contraceptives might be reduced while maintaining contraceptive efficacy. Trials confirmed that contraception could be achieved efficiently at one-quarter of the original daily dose (megestrol 1.0 mg and norethisterone 0.625 mg) and these dosages were marketed in China starting in 1967 [1,2]. Worldwide use of low-dosage oral contraception followed 7 years later, the delay being due to the need for testing in all markets before the low doses could be approved for general commercial purposes. It is now widely accepted that reduced dosage has fewer side-effects and complications, leading to better compliance. Moreover, serious problems such as weight gain, chloasma, nausea and vomiting, and arterial thrombosis have all but disappeared. Both the estrogen and progestin have been reduced and new progestins have been introduced. The concept of low-dose oral contraceptives has now been established worldwide.

Reduced doses of mifepristone in termination of early pregnancy

The use of the antiprogestin mifepristone (a single dose of 600 mg) for medical abortion in early pregnancy became legal in China in 1988. Several pharmacokinetic studies of orally administered mifepristone indicated that blood concentrations did not increase linearly with increased dosages. After years of investigation into reducing the dosage of mifepristone, in 1993 a randomized multi-center trial with a total of 4500 subjects was performed. Two main regimens were tested: a single 200 mg dose of mifepristone and a total dose of 150 mg mifepristone given in five consecutive doses (50 mg first, then 25 mg every 12 h for four times) followed on the third day after the last dose of mifepristone with vaginal DL-15-methylprostaglandin F₂-β-methylester (PGO5), 1 mg. The rates of complete abortions were 88.6 and 92.0%, while incomplete abortion rates were 8.1 and 5.8% and failure rates were 1.7 and 0.8%, respectively. In an extension of this study the efficacy of 150 mg mifepristone (administered in multiple small dosages) was close to that of 600 mg mifepristone (91.1%) and significantly better than that of the single dose of 200 mg mifepristone [3]. In 1994, vaginal use of PGO5 was replaced by oral misoprostol, 600 μg. Oral prostaglandin has the benefits of eliciting fewer side-effects while being more convenient to administer, store and transport. It is also economical. The new regimen furnishes the same pregnancy-termination rate as vaginal prostaglandin but is much more patient-friendly, which increases patient use and compliance. Now in China,

the first choice for medical abortion for pregnancies of less than 7 weeks is 150 mg mifepristone in divided doses followed by misoprostol. Thus, low doses of antiprogestone have been proved preferable to high-dose regimens.

Menopausal hormone treatment

Normally, women over the age of 40 years go through the menopausal transition, then menopause. This is followed by a postmenopausal period that may last for more than 40 years, during which time a number of complications will appear as a result of sex hormone deficiency. The most obvious postmenopausal complications are the menopausal syndrome, urogenital tract atrophy, osteoporosis, atherosclerotic vascular diseases and dementia. There are other complications, which affect every organ system and are only now being appreciated [4]. The menopausal syndrome, of hot flushes, sleep disorders, cognitive and affect changes, is usually acute and may be quite distracting; when serious, it should be treated with sex hormones (i.e. HRT) both for symptomatic relief and for prevention of further complications of the adjustment to hormone deficiency. The menopausal syndrome therefore differs from the degenerative processes that appear to start during the menopausal transition, but may only come to light some years after they begin to develop. For prevention of degenerative diseases, HRT should be started early, as prevention, and continued throughout the entire postmenopausal period [5].

Bone dynamics and menopausal hormone treatment

In the 1980s, when work on menopausal hormone treatment (MHT) in China first began, two regimens of HRT to prevent or manage postmenopausal osteoporosis were common: (1) one large dose of long-acting estrogen every 1 to 4 weeks; and (2) low doses of estrogen daily. The treatment continued for several years. Women with intact uteri were also given synthetic progestins to avoid endometrial hyperplasia. Urinary excretion of calcium was measured, which indicated that low-dose estrogen resulted in a low and steady excretion of calcium, whereas one large dose resulted in excretion of more calcium that fluctuated significantly. Therefore, a low-dose regimen, described below, was developed for prevention of postmenopausal bone loss. This HRT regimen was confirmed to be helpful in relieving the menopausal syndrome and urogenital atrophic symptoms, in addition to maintaining bone mass with a reduced risk of fracture [6–9]. In some cases, patients who already had late osteoporosis were also treated with alendronate, calcitonin, etc. which do not interfere with low-dose sex hormone treatment.

Cardiovascular diseases and menopausal hormone treatment

Contrary to the well-accepted benefit of MHT in treating the menopausal syndrome and preventing postmenopausal bone loss, the risk–benefit balance of HRT for the cardiovascular system of postmenopausal women remains controversial [10–12]. On one hand, a large number of *in vitro* and *in vivo* studies and clinical observations uniformly indicate that estrogen benefits the cardiovascular system; while on the other hand, recent controlled prospective studies in older, no-longer symptomatic women with diagnosed or insipid cardiovascular disease (CVD) showed an increased risk of cardiovascular events in HRT users [10]. Furthermore, progestin, which is used in HRT to reduce the risk of endometrial cancer, has been found detrimental to breast and cardiovascular systems [13]. Under these circumstances it is useful to describe our results with a low-dose regimen that maintains the beneficial effects of HRT while maintaining compliance and preventing other menopausal complications.

Basic research on the in vitro effects of hormone treatment on cardiovascular function

Effects of sex hormones on umbilical artery extensibility. We examined the effect of sex hormones (estradiol, progesterone and testosterone) on the extensibility of human umbilical artery rings (HUAR) [14]. We were particularly interested in understanding dose–response relationships between estradiol and vascular extensibility. The extensibility of HUAR with intact endothelium was measured in the presence of 17β -estradiol, testosterone and progesterone (10^{-9} to 10^{-5} M); only 17β -estradiol had a marked extension-increasing effect on HUAR, which was optimal at 10^{-9} and 10^{-7} M. The dose–response was attenuated by super-physiological doses of estradiol with no extension increase detectable at 10^{-5} M (Figure 1A). Progesterone combined with estrogen inhibited the tension-relaxing (dilator) effect of estradiol, but only at high estradiol concentrations (10^{-7} and 10^{-5} M). At 10^{-9} M, the extensibility-increasing effect of estradiol was still apparent when progesterone was present at 10^{-9} and 10^{-7} M (Figure 1B). These results support the idea that lower concentrations (10^{-9} M) of estradiol are more effective in relaxing HUAR tension than are high concentrations.

Effect of estradiol on nitric oxide production by human umbilical vein endothelial cells. Nitric oxide (NO) produced by human umbilical vein endothelial cells (HUVEC) is a potent vasodilator that protects the cardiovascular system against atherosclerosis. We investigated the effect of sex hormones on the

production of NO [15] and found that while estradiol (10^{-9} and 10^{-7} M) increased NO production, progesterone and testosterone at 10^{-9} and 10^{-7} M had no effect, and 10^{-5} M progesterone or testosterone actually decreased NO production (Figure 1C). Moreover, both progesterone and testosterone inhibited the positive effect of estradiol on NO production (Figure 1D). We have no data on the action of other progestins.

Effect of estradiol on homocysteine-induced damage in human umbilical vein endothelial cells. At lower concentration (10^{-9} and 10^{-7} M), estradiol also prevented homocysteine-induced damage and reduction in NO production in cultured HUVEC (Figure 1E), but this effect was not apparent at the higher concentration (10^{-5} M).

Effect of estradiol on angiotensin-induced tissue factor in human umbilical vein endothelial cells. Tissue factor (TF) participates in both blood coagulation and inflammation. TF is key in the formation and progression of atherosclerosis. We investigated the effect of sex hormones on angiotensin II-induced TF expression in cultured HUVEC by measuring TF mRNA and its protein concentration in the presence of individual hormones. Estradiol significantly inhibited the angiotensin II-induced increase in TF expression (Figure 1F) and this effect was observed only at lower concentrations (10^{-9} and 10^{-7} M), not at 10^{-5} M. Progesterone, on the other hand, had no effect at 10^{-9} M and actually enhanced TF expression dose-dependently at 10^{-7} and 10^{-5} M. We did not test other progestins in these studies.

Summary. By all criteria tested (HUAR extensibility; NO production, protection against homocysteine-induced damage and TF production of HUVEC), the best protection by estradiol in *in vitro* cardiovascular models is obtained at lower, physiological concentrations. Progesterone has no protective effect by itself; however, its antagonism against the benefit of estradiol was seen only at 10^{-5} M, and not at 10^{-7} and 10^{-9} M. These findings supported the rationale for treating women with low-dose HRT.

Evaluation of long-term low-dose hormone replacement therapies in postmenopausal women

Reducing the dosage in menopausal hormone treatments. Tibolone was first introduced in China in 1990. Because of our experience with the low-dose oral contraceptives and the above findings, we carried out a 3-year prospective study of tibolone at one-half and one-quarter of the original 2.5 mg dose. Our results showed that even at these reduced dosages, tibolone had the usual effects on bone density, urinary calcium excretion, uterine endometrium, blood

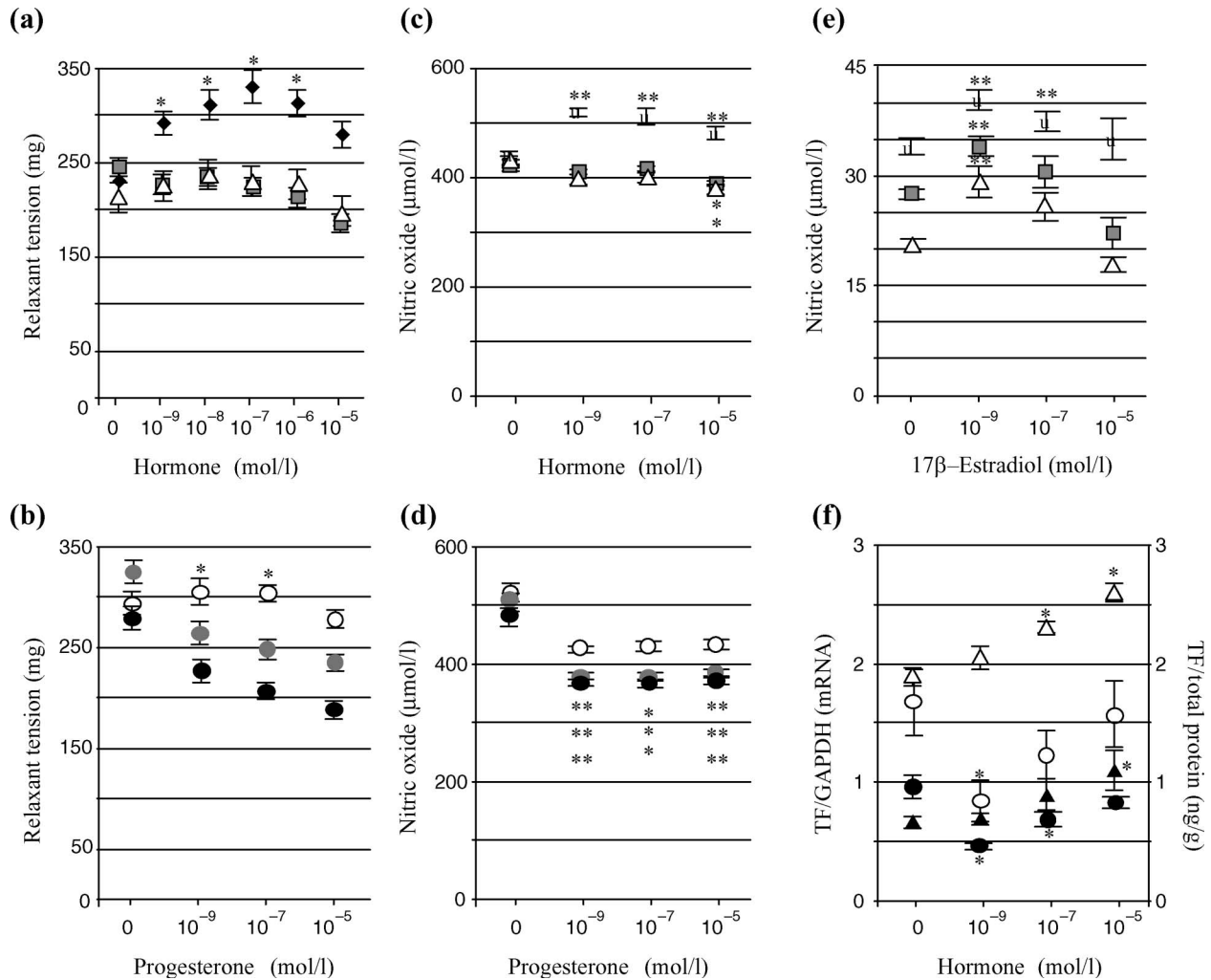


Figure 1. Dose dependence of the effect of sex hormones on the vascular system. (a) Effect of sex hormones on the relaxant tension of human umbilical artery rings. ◆, 17β-estradiol ($n=15$); ■, progesterone ($n=12$); Δ, testosterone ($n=12$). (b) Progesterone inhibits the vascular tension-relaxing action of 17β-estradiol. 17β-Estradiol concentration: ○, 10^{-9} mol/l ($n=15$); ●, 10^{-7} mol/l ($n=6$); ●, 10^{-5} mol/l ($n=6$). (c) Effect of sex hormones on nitric oxide (NO) production by cultured human umbilical vein endothelial cells (HUVEC). Symbols are the same as in A ($n=60$ for each hormone). (d) Progesterone inhibits the stimulatory effect of 17β-estradiol on NO production. Symbols are the same as in B ($n=24$ for each concentration). (e) 17β-Estradiol protects HUVEC from homocysteine-induced damage. Homocysteine concentration: ◆, no homocysteine; ■, 10^{-4} mol/l; Δ, 10^{-5} mol/l ($n=24$ for each concentration). (f) Effect of 17β-estradiol (circles) and progesterone (triangles) on tissue factor (TF) production in HUVEC induced by angiotensin II. Open symbols: the ratio TF mRNA/GAPDH mRNA ($n=20$ for 17β-estradiol, $n=12$ for progesterone); closed symbols, the ratio TF protein (ng)/total protein (g) ($n=16$ for 17β-estradiol, $n=12$ for progesterone). Significant difference: * $p < 0.05$, ** $p < 0.01$. Data from [14] and [15].

lipids, vaginal bleeding and body weight [16]. Based on these studies, use of the one-quarter dose of tibolone (i.e. 0.625 mg) for prevention of osteoporosis in late postmenopausal women was adopted. Other investigators have reached the same conclusion. Bjarnason and colleagues showed in a 2-year study that there was no difference between the half and full dose of tibolone in increasing bone density. The authors also remarked that the dosage might be reduced further [17]. Gallagher and associates published a 2-year clinical study on prevention of bone loss by tibolone at full, one-half, one-quarter and one-eighth doses in 770 postmenopausal women from four centers in the USA and showed that the minimal effective dose was one-quarter (0.625 mg). Side-effects such as vaginal bleeding, breast pain

and weight gain were all reduced with the lower dosage [18].

Intermittent low-dose estradiol replacement therapy. The success of the low-dose approach with tibolone encouraged the development of regimens of patient-regulated, intermittent low-dose conventional HRT formulations. The basic premise of this approach is that menopausal symptoms represent a bioassay of the estrogenic status of the individual woman. Therefore, the patient can meter her HRT to treat her symptoms by maintaining her estrogenicity in a therapeutic range. The objectives of taking the lowest HRT dose are discussed with the patient and she is instructed to use just enough medication to keep her symptoms in check. Side-effects are described

and she is made aware of danger signals, such as untimely vaginal bleeding and leg pain. The actual regimens are as follows. The patient is prescribed a dose of HRT known to be in the lower portion of that compound's effective dose range. The patient uses the medication as needed to control symptoms. For example, she could use the drug daily to control symptoms and then stop until symptoms return, usually within a week. To ensure the best compliance, the only patients given this regimen are physicians and nurses on the medical staff of the Peking Union Medical College Hospital. From its beginning in the mid-1970s the results have been satisfactory in terms of patient acceptance, symptomatic relief and side-effects. However, recent events have prompted a comprehensive study of the long-term effects of our low-dose regimen on CVD and bone health.

The Women's Health Initiative triggers evaluation of possible long-term cardioprotective effects of low-dose, long-term hormone replacement therapy

Buoyed by the many basic and clinical studies indicating the value of long-term disease prevention with HRT [10], the use of low-dose HRT continued without unexpected complications for more than 30 years until, in 2002 and 2004, the US National Institutes of Health published results of the Women's Health Initiative (WHI). The WHI was a study of cardiovascular events in postmenopausal women treated with conjugated equine estrogen (CEE; Premarin[®]), with or without medroxyprogesterone (MPA; Provera[®]) given daily. Despite showing prevention of osteoporosis and back and hip fracture and colon cancer, the WHI was stopped prematurely because it failed to show cardioprotection, as measured by events [10]. It is important to note that the WHI did not study the progress of CVD, just events that occurred indicating the presence of mature CVD, osteoporosis, etc. Moreover, the subjects in this randomized, placebo-controlled prospective trial were chosen solely because they were postmenopausal and were past having menopausal symptoms (by design, only 10% were symptomatic). This combination of requirements made this a study of hormone therapy (HT) rather than HRT [5], and most of the women were 10 years or more beyond the menopause. The doses of estrogen and progestin were the standard ones used at the time the 8-year study started, CEE 0.625 mg plus MPA 2.5 mg, and were administered every day rather than intermittently, as had been the case in the previous observational studies [19]. After the results were calculated, there was no overall improvement in the number of cardiovascular events among the full WHI groups [20]; however, the average age of the WHI subjects at the start of the study exceeded 60 years, the average time since menopause was more than

10 years at the time of starting HT, there was no evaluation of the progress of CVD [10] and the number of women of similar age range in the Nurses' Health Study [21] was too small to have allowed a comparison with the WHI [11].

The WHI caused massive concern and defection from HRT [19–25], even though the apparent conflict between the randomized and observational studies was eventually resolved. Subgroup analyses of the WHI that indicated that WHI women who were in the same age group when they began HT as those in the observational NHS were protected from cardiovascular events to the same extent as were the women in the NHS, i.e. there is a cardioprotective effect of HRT started during the menopausal transition [21]. However, the lack of confidence and the continuing confusion caused by this episode have left an important gap in knowledge of the effects of HRT to be resolved, especially regarding the length of time that HRT may be administered, the doses to be used, and the balance between the beneficial and adverse effects of sex hormone treatment in aging women. We believe that this is a good time to determine the effects of low-dose HRT [10–29]. Regimens must be tested that will retain estrogen's beneficial cardiovascular effects while reducing estrogen-associated risk [24]. Most of the previously reported observational investigations were done in women receiving HRT at dosages for relieving menopausal symptoms that may be higher than necessary for delaying degenerative process (e.g. bone loss, CVD and central nervous system dystrophy) in postmenopausal women. While low-dosage HRT has been increasingly investigated recently [22–24], the effective dose range and regimen remain elusive. Furthermore, even today the risk–benefit ratio of long-term use of low-dosage HRT, which is necessary for delaying postmenopausal degenerative processes, has not been completely assessed. In light of the above and having already used low-dose HRT for up to 30 years, we decided to study bone, cardiovascular and other system effects of women who had been using these regimens for up to 30 years.

The Peking Union Medical College Women's Health Study

With the approval of the Peking Union Medical College authority, from September 1, 2003 to September 30, 2004 we conducted clinical and laboratory testing on patients in Beijing who were treated with HRT in low-dosage regimens and began treatment during the menopausal transition and continued for 5–30 years [30]. All subjects were patients of an HRT monitor group under the supervision of the first author (Q.G.) and had been treated for menopausal symptoms starting at 45 years of age or younger.

Subject population. For this study, 983 (94.6%) of the 1039 female medical staff in Peking Union Medical College Hospital aged than 40 years or older (in service or retired) were interviewed via telephone. Among the interviewees, 714 were menopausal, of whom 255 (35.7%) were receiving HRT under supervision of the monitor group. Eighty-three had received HRT for more than 5 years. This percentage of hormone users is much higher than the 4.4–5.9% of HRT users in the general population of Chinese women with similar financial and educational background. Among the 83 women, 63 had used low-dose HRT for 5–31 years; they were designated the HRT group. They were age-matched with 78 women who had never received HRT (control group). Approximately 75% of the subjects in the HRT group began HRT before the age of 60 years, and more than 70% had been receiving HRT for more than 10 years (Figure 2).

The historical and physical characteristics of the study subjects are shown in Table I. All were physicians or nurses. There were no statistically

significant differences between the HRT and control groups in education, economic and social status, physical exercise, acupuncture and drug usage, including Chinese herbal medicines, calcium, vitamins or lipid-lowering, antihypertensive and hypoglycemic agents ($p > 0.05$, data not shown).

Treatments. Low-dosage HRT was carried out as previously described; depending on the subjects' symptomatology, risk evaluation and availability, different types of preparation, dosage, route of absorption and combinations were used. Because the subjects were medically trained, during the course of HRT they were instructed to self-monitor and adjust the dosage according to their symptoms and specific needs. This resulted in diversified regimens combining low doses of replacement treatments with intermittent dosing. The medications included estradiol valerate, CEE (Premarin[®]), tibolone (Livial[®]) and MPA (Provera[®]) for women who had not undergone hysterectomy. Among the 63 treatment subjects, four used half dosage (two used tibolone 1.25 mg daily and two used CEE 0.3 mg daily), 44 used one-quarter dosage (tibolone 1.25 mg every other day and/or CEE 0.3 mg every other day), and 15 subjects used less than one-quarter dosage of tibolone (1.25 mg twice

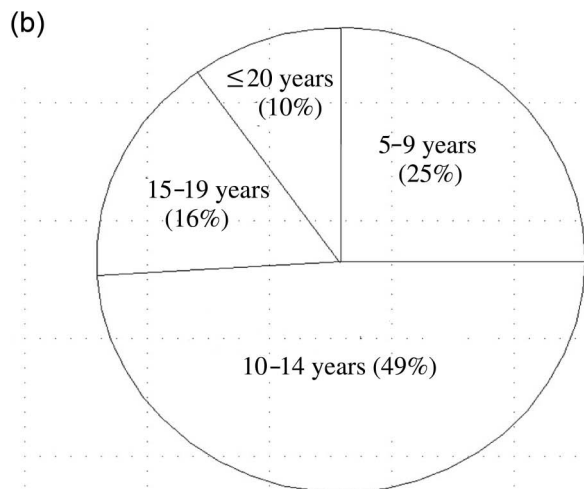
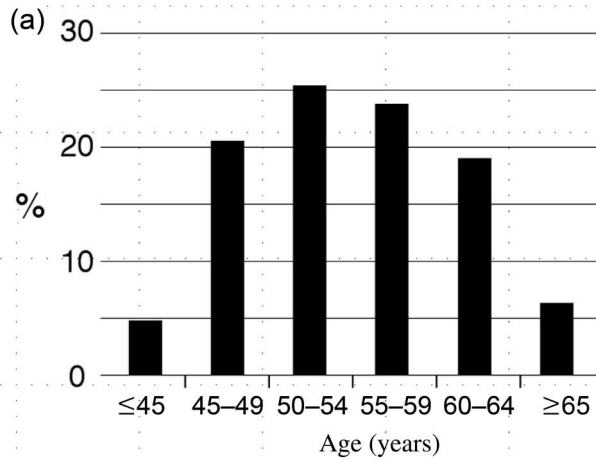


Figure 2. Distribution of (a) age and (b) duration of hormone replacement therapy (HRT) in the HRT group.

Table I. Characteristics of participants.

	Control group (n = 78)	HRT group (n = 63)	p Value
Age (years)	68.4 ± 7.5	63.7 ± 7.0	> 0.05
Body mass (kg)	58.4 ± 8.7	60.6 ± 6.9	> 0.05
Body mass index (kg/m ²)	24.7 ± 3.2	24.6 ± 2.4	> 0.05
Waist circumference (mm)	82.5 ± 8.3	83.5 ± 6.9	> 0.05
Hip circumference (mm)	98.3 ± 7.5	99.4 ± 6.3	> 0.05
Waist/hip ratio	0.84 ± 0.04	0.84 ± 0.05	> 0.05
Smoking	0	0	> 0.05
Alcohol drinking	0	0	> 0.05
Systolic blood pressure (mmHg)	132.5 ± 19.7	126.5 ± 14.4	< 0.05
Diastolic blood pressure (mmHg)	80.0 ± 10.5	79.6 ± 7.9	> 0.05
Estradiol (pg/ml)	29.3 ± 29.2	38.2 ± 24.2	< 0.05
Hypotensive drugs	56/78*	40/63*	> 0.05
Hypolipemic drugs	7/78*	3/63*	> 0.05
Hypoglycemic drugs	2/78*	5/63*	> 0.05
Subclinical ischemia (via ECG)	13/78*	7/63*	< 0.05
Angina	1/78*	1/63*	> 0.05
Myocardial infarction	2/78*	1/63*	> 0.05
Breast cancer	1/78*	0/63*	> 0.05
Lung cancer	1/78*	0/63*	> 0.05

HRT, hormone replacement therapy; ECG, electrocardiogram; values are mean ± standard deviation except for *, which are scores.

weekly). During the course of HRT, they were allowed to stop for as much as a month, but all found that they could not go without HRT and resumed treatment. All of the 63 treatment subjects took less than the manufacturer's recommended monthly dosage.

General findings. Among those female medical staff members who had used low-dose intermittent HRT, 80% of them were still in good health and working energetically in their positions at age 70 years, while among age-matched controls only 9% were working at the same age. Their activity and comfort levels were in keeping with the physical and laboratory results; the HRT group was in better general condition than the controls. There was no complaint of vaginal dryness or genital atrophy symptoms among the treated women although the controls had these complaints. The posture and walking of the HRT group appeared to be better than in the controls, although the small sample and study design did not allow complete evaluation of these physical parameters. Only one of the patients had abandoned treatment. This was due to vaginal bleeding. Because of intolerable symptoms, she returned to HRT after treatment for this benign complication. There were no breast or endometrial cancers reported in either group.

Laboratory tests. After the patient's history of HRT use and an inventory of illnesses and of vaginal bleeding were obtained, cognition was evaluated by integrated neuropsychological tests (Mini-Mental Status Examination [MMSE], World Health Organization California Verbal Learning Test [WHO-CVLT], Logical Memory [LM], Figure Complex [FC], Verbal Fluency [VF], Constructional Praxis [CP], Digit Span [DS]). Levels of plasma estradiol, progesterone and testosterone were measured by immunoassay and lipids, lipoproteins, apolipoproteins in fasting blood serum were assayed using the Olympus (Japan) AU2700 automatic biochemical analyzer. Bone mineral density (BMD) of the lumbar spine and femoral trochanteric region were measured by dual-energy X-ray absorptiometry (DPX-L instrument; Lunar Co., USA). Using standardized geometry, sonography was used to evaluate the thickness of the intima media of the common carotid arteries. Multislice computed tomography (Siemens, Germany) was conducted to assess the degree of calcification and stenosis in the coronary arteries. The volume of the two (bilateral) hippocampuses was assessed using magnetic resonance imaging (Siemens).

Results. The serum estradiol concentration of each age group of HRT users was significantly higher than in the control group ($p < 0.05$), but there was no

Table II. Comparison of blood lipids between the participants.

	Control group (n = 78)	HRT group (n = 63)
TG (mmol/l)	1.68 ± 0.93	1.50 ± 0.80
TC (mmol/l)	5.82 ± 0.93	5.34 ± 0.90**
HDL-C (mmol/l)	1.58 ± 0.30	1.54 ± 0.31
LDL-C (mmol/l)	3.80 ± 0.94	3.55 ± 0.90
TC/HDL-C	3.83 ± 0.93	3.60 ± 0.70*
Apo AI (g/l)	1.56 ± 0.26	1.54 ± 0.28
Lp(a) (mg/l)	223 ± 197	201 ± 144
Apo E (g/l)	0.07 ± 0.02	0.05 ± 0.01**
Apo CIII (g/l)	0.12 ± 0.03	0.11 ± 0.03**
Apo B (g/l)	1.01 ± 0.21	0.93 ± 0.19*

HRT, hormone replacement therapy; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo, apolipoprotein; Lp(a), lipoprotein (a); values are mean ± standard deviation; significant difference compared with the control group: * $p < 0.05$, ** $p < 0.01$.

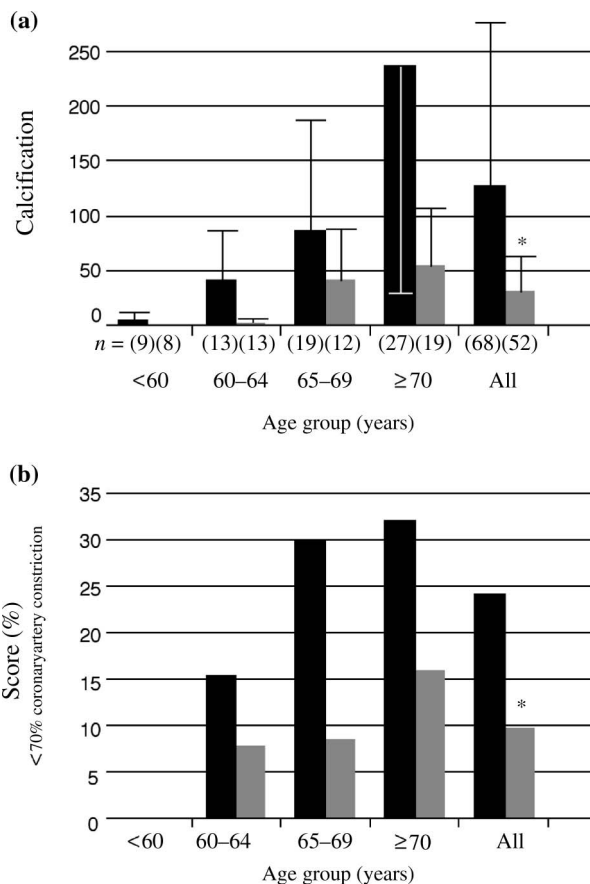


Figure 3. Computed tomographic angiography revealing calcification and stenosis of the coronary cardiovascular system in the control group (■) and the group receiving hormone replacement therapy (▒). (a) Calcification measurements are given in age groups as well as for all subjects; the number of subjects within each age group is indicated in parentheses below each column. Statistical analysis was not performed on individual age groups because of the small sample size. (b) Stenosis of greater than 70% coronary artery constriction was scored against total stenosis found and the ratio expressed as a percentage. Data are given in age groups and in the total sample. Statistical analysis was done only on the total sample, for the same reason as given in (a). Significant difference between groups: * $p < 0.05$.

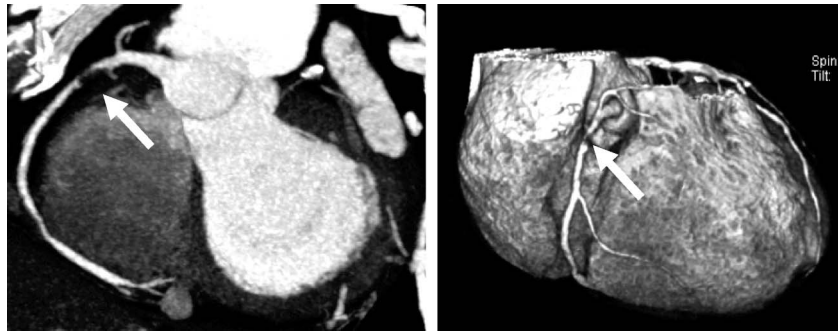


Figure 4. Computed tomographic coronary angiography reconstruction shows a severe stenosis in the right coronary artery (arrow).

group difference in the concentration of progesterone and testosterone (data not shown).

BMD of L2–L4 in the HRT group (1.08 ± 0.14 g/cm²) was significantly higher than that in the control group (0.99 ± 0.15 g/cm², $p < 0.01$). Also, the incidences of osteoarthritic pain and decrease in height were lower in the HRT group than in the control group (71.4 vs. 89.7%, $p = 0.005$ and 93.7 vs. 100.0%, $p = 0.038$, respectively). The standing height of the treated subjects also exceeded that of the controls (data not shown).

Atherosclerosis-associated lipids – total cholesterol, total cholesterol/high-density lipoprotein cholesterol ratio, apolipoprotein E, apolipoprotein CIII and apolipoprotein B – were all significantly lower in the HRT group than in the control group ($p < 0.05$; Table II).

Intima-media thickness (IMT) is an early marker for generalized atherosclerosis. Sonographic examination showed that the IMT of the carotid arteries in the HRT group was significantly less than that of the control group (0.089 ± 0.02 vs. 0.093 ± 0.09 cm, $p < 0.01$).

Sixteen-slice computed tomographic (CT) angiography of the coronary vessels revealed that the average total atherosclerotic plaque in the HRT group was significantly less than in the controls (7.7 vs. 12.7%, $p < 0.05$). The difference was largely due to fewer soft and mixed plaques (which are unstable) in the HRT group than in the control group (1.7 vs. 4.5%, $p < 0.05$ and 3.1 vs. 6.7%, $p < 0.05$, respectively). There was a doubling of the hard plaque score (2.6 vs. 1.3%, $p < 0.05$) and no difference in ulcerative plaque (0.3 vs. 0.2%, $p > 0.05$) in the HRT group compared with the control group.

Calcification measurements taken on CT angiography of the coronary arteries of 52 HRT and 68 control subjects showed that the HRT group had much lower calcification of the coronary artery system ($p < 0.05$; Figure 3A). The calcification level in the two 70-year-old HRT subjects studied was comparable to that of the controls at age 60–64 years.

An example of a $>70\%$ stenotic vessel is shown in Figure 4. Coronary artery stenosis (score threshold set at $\geq 70\%$) appeared to be delayed with age in the HRT group, similar to the results for plaque measurements (Figure 3B). Unfortunately there were only two subjects in the HRT group who were >70 years old, so that statistical analysis was not attempted.

Summary. These results strongly suggest that low-dose HRT treatment postponed age-related atherosclerosis of the cardiovascular system in women treated for 5–31 years. There were also measurable differences favoring the musculoskeletal system and cognition. Although the number of subjects is low, these results support the contention that low-dose, long-term HRT has a dramatic protective effect on the progress of disease in the cardiovascular system in postmenopausal women [22] and furnishes enhanced quality of life.

Conclusions

Over the past four decades it has been possible to develop a philosophy and treatment plan for low-dose hormone use in China. The results in oral contraception, fertility management and MHT rivaled or exceeded reports from elsewhere. This is apparent in the findings in our small pilot cross-sectional study, which indicates that low-dose long-term (5–30 years) HRT maintained genital and musculoskeletal health and, most impressively, prevented the progress of CVD. Judging from our vascular studies, long-term low-dose HRT may have delayed the appearance of atherosclerosis and its complications by 10 or more years. It was also associated with maintenance of hippocampus size, although no cognitive effects could be shown. The low-dose, intermittent HRT regimen did not stimulate breast or endometrium lesions and did not increase body mass or vaginal bleeding.

During the preparation of this report, another group showed that aortic calcium may be reduced in

women studied on average for 9.8 years after starting a 2–3-year course of conventional HRT [27].

Atherosclerosis can be divided into reversible and irreversible phases. Atherosclerotic plaques form during and following injury of the vascular endothelium. Once atherosclerosis reaches the irreversible phase, HRT can no longer help [31]. In this retrospective study, early use of low-dose HRT was associated with protection of the endothelium, delay in the formation of atherosclerotic plaques, and apparent protection of the cardiovascular system. The results of this study emphasize that estrogen should be applied before the formation of atherosclerotic plaques, i.e. at the early stages of menopause. According to our data, calcification and stenosis of blood vessels increased after 60 years of age, suggesting that HRT should be initiated much earlier than age 60 in order to prevent CVD. For example, in the HRT group, 74.6% of subjects began to receive HRT before age 60 years, and only 19.0% of patients began between 60 and 64 years of age. Our results support previous explanations for the failure of HRT to protect against CVD events in the WHI, in which the average age of women beginning HRT was 63 years when the damage due to CVD may already be irreversible.

Although the sample size of this study was small and the HRT was individualized, the results are clear and of importance for future reference and planning of research. At the least, prospective studies using early low-dose and long-term HRT with larger groups of perimenopausal women are warranted, to confirm our results.

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