JOURNAL OF CARDIOVASCULAR MAGNETIC RESONANCE®
Vol. 6, No. 3, pp. 637–644, 2004

FLOW

Measurement of Aortic Blood Flow by Magnetic Resonance Below and Above the Origin of the Coronary Arteries in Postmenopausal Hormone Replacement Therapy

Morten Beck Sørensen,[1,2,4,*] Thomas Fritz-Hansen,[3] Henrik Halvor Jensen,[1] Anette Tønnes Pedersen,[1] and Bent Ottesen[1]

[1]Department of Obstetrics and Gynecology, [2]Department of Cardiology, and [3]Danish Research Centre for Magnetic Resonance, Hvidovre University Hospital of Copenhagen, Denmark
[4]Cardiovascular MR Unit, Royal Brompton Hospital, London, UK

ABSTRACT

Purpose. Principal blood flow measures might be assessable by velocity-encoded cine magnetic resonance (VENC MR) of aortic blood flow. The feasibility of using VENC MR for clinical research was tested in a contemporary and controversial human model: the effects of 17β-estradiol (E) and cyclic norethisterone acetate (NETA) in postmenopausal women. *Methods.* Sixteen postmenopausal women were included in a crossover study (two 12-week periods, 3-month washout) and randomized to E and NETA or placebo. Aortic blood flow (ABF) was measured eight times by VENC MR below and above the coronary arteries (CA) and was used for estimation of coronary artery blood flow (CABF) and peak flow velocity (Vmax). The calculated value of CABF was negative and was corrected by averaging systolic flow. Data were analyzed in a repeated measurement model including analysis of repeatability (CR) and group variability (CV). *Results.* The CR and CV of ABF were low (11% and 7%) and corresponded at the two levels. Vmax showed similar levels of reproducibility at the two levels. The coronary artery blood flow was less reproducible (39% and 31%). During treatment, ABF above CA was reduced after 12 weeks ($p=0.03$), ABF below CA was unchanged, and CABF was significantly increased without detrimental effects of NETA. Vmax was increased at NETA addition ($p<0.01$). *Conclusions.* Aortic flow quantification by VENC MR is reproducible and useful for assessment of principal

*Correspondence: Dr. Morten Beck Sørensen, Cardiovascular MR Unit, Royal Brompton Hospital, London SW3 6NP, UK; Fax: +44-207-351-8816; E-mail: m.sorensen@rbh.nthames.nhs.uk.

DOI: 10.1081/JCMR-120038084
1097-6647 (Print); 1532-429X (Online)

haemodynamic changes in smaller studies. Derived measurement of CABF lacks precision. Differences in change below and above CA indicate that oral high-dose E and NETA may induce coronary artery dilatation.

Key Words: Cardiac; Blood flow; Magnetic resonance; Estrogen; Menopause.

INTRODUCTION

Cardiovascular magnetic resonance (CMR) is favorable for anatomical and functional cardiovascular imaging and has advantages over other imaging techniques due to robustness, objectivity, and improved repeatability (Bellenger et al., 2000; Sorensen et al., 2002), which supports its use for scientific purposes. Multiple plane short-axis (SA) volumetric CMR is widely recognized as the gold standard for assessment of ventricular volumes and mass (Myerson et al., 2002), which has lately favored its use for assessment of pharmaceutical effects (Gronning et al., 2000). One of the widely used CMR techniques, velocity-encoded cine magnetic resonance (VENC MR), is reliable for both qualitative assessment of blood flow dynamics and direct blood flow quantification (Spilt et al., 2002). Through-plane VENC MR in the ascending aorta for measurement of stroke volume has been directly compared with the SA technique, and the two modalities display excellent agreement (Graves and Dommett, 2000). Precise measurement of aortic blood flow (ABF) not only provides important cardiac function data but might also provide data linked to aortic function and coronary artery blood flow (CABF) (Buonocore, 1994).

Estrogens are potent vasoactive hormones (Mendelsohn and Karas, 1999) capable of inducing acute vasodilatation via plasma membrane-bound receptors (Collins and Webb, 1999). Because central hemodynamic variables such as CABF (Chester et al., 1995; Reis et al., 1994), stroke volume (Pines et al., 1991), and arterial compliance (Rajkumar et al., 1997) have all been shown to be affected by estrogens in postmenopausal women, we chose this important and very topical human model to assess the feasibility of VENC MR in a clinical and pharmacological study of important cardiovascular effects with direct correlation to cardiac morbidity. Secondary purposes of the study were to evaluate the measure of total CABF derived from aortic volume flow (Buonocore, 1994) and also to speculate on hemodynamic effects of clinically used cyclic hormone replacement therapy (HRT).

Table 1. Baseline characteristics of postmenopausal subjects.

Age (yr)	55±0.7
Time since last period (yr)	5.9±1.0
Estradiol (pmol/L)	<40
FSH (IU/L)	80±7.0
Smokers (*n*)	5
Systolic BP (mm Hg)	130±4
Diastolic BP (mm Hg)	74±2
Heart rate (min^{-1})	66±2
BMI ($kg \times m^{-2}$)	27±5

Mean±SE. FSH=follicle-stimulating hormone; BP=blood pressure; BMI=body mass index.

METHODS

Sixteen healthy postmenopausal female volunteers (Table 1) were recruited from the general population. The study was performed in accordance with the Helsinki II declaration and was approved by the local ethics committee. All participants gave written informed consent.

The exclusion criteria were the following: a history of cardiovascular disease, positive exercise test ($\geq$1 mm ST segment deviation during exercise testing with the modified Bruce protocol), hypertension, diabetes or other chronic diseases affecting heart and vessel function, contraindications to MR examinations, contraindications to estrogen and/or progestogen, biochemical screening failure, and intake of estrogens or other vasoactive medication. Aortic valve competence was an inclusion criteria, and this was assessed from CMR cine acquisitions of the left ventricular outflow tract (LVOT) in both coronal and transaxial planes.

A randomized double-blind, crossover study was conduced for two 12 week periods separated by a 3-month washout, as reported previously (Sorensen et al., 2001). The subjects were randomized to intake of either three 28-day HRT cycles [4 mg 17β-estradiol (E) daily for 12 days, 4 mg E plus 1 mg norethisterone acetate (NETA) daily for 10 days and 1 mg E daily for 6 days; Trisequens Forte, Novo Nordisk A/S, Denmark] or matching placebo in the first 12 weeks and the alternating treatment in the second treatment period. The participants were seen at baseline, after

10 days (while only taking E), after 9 weeks (while only taking E, following two full HRT cycles), and after 11 weeks of HRT (while taking combined HRT) in both periods. The effect of adding NETA was calculated as the difference between the latter two visits. Two subjects dropped out of the study: one due to lower back pain (completed placebo) and another because of venous thrombosis (completed placebo).

Subjects were examined in a 1.5-T VISION Magnetom (Siemens, Erlangen, Germany) as they rested in supine position after a 4-hour fast. Blood flow was measured by VENC MR, as previously validated externally (Nayler et al., 1986). We performed an internal validation of the flow quantification in a flow phantom with variable flow velocity and variable cross-sectional area prior to performing the clinical study. True mean velocity correlated very closely to mean phase shift in the region of interest.

Flow Measurements

Through-plane volume blood flow was measured at two aortic levels and slice positions were piloted from coronal LVOT cine acquisitions (Fig. 1): 1) Ascending aorta (AA) blood flow: The slice was positioned perpendicular to aortic flow cross-cutting the pulmonary trunk. 2) Cardiac output or aortic valve (AV) blood flow: The slice was positioned perpendicular to aortic flow in the LVOT below the origin of the coronary arteries and at the level of the aortic valve.

We chose systolic planes for piloting, because the coronary arteries move caudally with the aortic root in systole (Fig. 1) causing underestimation of LV outflow piloted in diastole. Flow acquisitions were always performed in the mentioned order. At the initiation of both flow acquisitions and while the subject was positioned in the scanner, blood pressure and heart rate were measured by an automated blood pressure monitor. Flow data were acquired electrocardiogram (ECG)-gated with an effective repetition time of 24 ms allowing 40–60 measurements of volume flow during the entire cardiac cycle with full RR coverage through gating at every second R wave. Magnetic resonance imaging sequence parameters: TR/TE = 24/5 ms, flip angle (α) = 30°, matrix size = 256 by 256, field of view (FOV) = 300 mm, and velocity encoding = 250 cm/sec. The calculation of flow was performed by the Siemens VISION™ Flow Quantification Module after background correction. Flow quantification was performed blinded and in random order by the same observer at study completion. Stroke volume data have been reported previously (Sorensen et al., 2001).

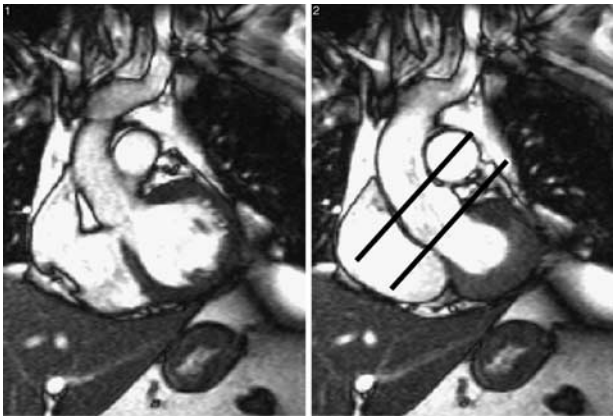

Figure 1. Aortic flow quantification below and above the origin of the coronary arteries. Coronal cine gradient echo images of the left ventricular outflow tract in diastole (left) and systole (right). Imaging planes (lines) were positioned in systole due to the downward motion of the aortic root.

Processing of Flow Data

At valve level, we averaged systolic blood flow over the entire cardiac cycle for quantification of total volume flow after ensuring valve competence. The cardiac cycle length was averaged from flow curves and defined as the interval between two blood flow increments. Systolic volume flow (mL/sec) was also defined from flow curves and both systole and volume flow was measured from the start of blood flow increment to return to baseline. At AA level, total blood flow was measured all through the cardiac cycle, whereas both systolic and diastolic blood flow was calculated as done at valve level. Peak flow velocity was calculated from flow velocity curves.

Statistics

A repeated measurement model was used for statistical analysis, assessing the treatment effects τ_1, τ_2, and τ_3 at the three specified visits during treatment (Senn, 1993). The treatment effects were the calculated residual values after all fixed and stochastic variations had been extracted from the data set. The effects were tested if significantly different from 0 by an analysis of variance. To further elucidate the analysis, the measurement value, y_{it} of subject i on the t point in time, can be expressed by the following equation:

$$y_{it} = \mu + \pi_t + \alpha_{it} + \tau_{it} + w_i + e_{it}$$

where μ is the residual value after all variation has been deducted (intercept), π is the period effect ($\pi_t = 0$

for period 1, $\pi_t = \pi$ for period 2), α is the placebo effect ($\alpha_{it} = \alpha$ at placebo visits, $\alpha_{it} = 0$ at baseline or HRT visits), τ is the treatment effect ($\tau_{it} = 0$ at placebo and baseline visits), w is the person effect, and e is the measurement error. The latter two components are independent, normally distributed stochastic variables. Y_{it} was standardized to body surface area (BSA) for all volume flow data set. A paired two-sided t-test was used for comparison of blood pressure and heart rate during the two flow acquisitions. Differences are regarded significant if the p value is below 0.05.

The treatment effects are in the following presented [listed ± standard error (SE)] and compared to intercept (listed ± SE). We report the repeatability of the variables by calculating the coefficient of repeatability (CR) as SD of the intrasubject difference (interstudy difference) divided by the intercept. The coefficient of variability (CV) is calculated from the SD of the intersubject difference divided by the intercept.

RESULTS

During HRT, serum-estradiol peaked at levels seen during the follicular phase in premenopausal women (Sorensen et al., 2001). During the two-flow acquisition, systolic blood pressure (129 ± 1.7 and 130 ± 1.8 mm Hg, $p = 0.13$) and heart rate (64 ± 0.8 and 64 ± 0.8 min^{-1}, $p = 0.44$) did not change.

Repeatability and Variability of Aortic Blood Measurements by VENC MR

With regard to total volume blood flow measurement, variation between subjects (variability) and variation between measurements in the same subject (repeatability) were both low (Table 2). The reproducibility of the volume flow measurement was similar at the two levels of the ascending aorta. Each of the two measurements of peak flow velocity by VENC MR in the aorta displayed reasonable reproducibility, and changes with HRT at the two levels were similar (Table 3). The derived volume blood flows (diastolic flow and subtracted flows) were considerably less reproducible than absolute volume flow (Table 2).

Effects of Hormone Replacement Therapy

Aortic blood flow measured below the coronary arteries was unaffected by HRT (Table 2), whereas blood flow in the ascending aorta showed a decreasing trend and the change was significant after 12 weeks ($p = 0.03$). Systolic blood flow was unaffected in both positions, whereas diastolic blood flow in the ascending aorta decreased continuously with HRT (at 12 weeks visit $p = 0.0003$). Peak flow velocity increased with HRT with significant augmentation by addition of NETA. The increase was matching at the two levels of the aorta (Table 3).

Table 2. Measurement of aortic blood flow below (AV flow) and above (AA flow) the coronary arteries during cyclic HRT.

	AV blood flow	AA blood flow	Diastolic blood flow	AV−AA	AV−AA (corrected)
$mL \times min^{-1} \times m^{-2}$					
Coefficient of repeatability (%)	11	11	23	39	833
Coefficient of variability (%)	6.4	7.5	20	31	256
Intercept	3120 ± 83	4035 ± 126	918 ± 71	−915 ± 100	4.3 ± 53
Treatment effects					
10th day (estradiol)	+74 ± 105	−229 ± 136	−108 ± 38	+304 ± 111*	+167 ± 78*
10th week (estradiol)	+127 ± 105	−192 ± 136	−135 ± 66*	+322 ± 111*	+158 ± 78*
12th week (combined)	+83 ± 108	−316 ± 140*	−259 ± 68*	+405 ± 114*	+137 ± 80†
NETA addition	−44 ± 125	−124 ± 161	−98 ± 78	+84 ± 131	−21 ± 92

Blood flow reproducibility, intercept (±SE), and treatment effects (±SE on change) corrected for body surface area and the difference for estimation of total coronary artery blood flow (crude difference and corrected difference based on averaged systolic flow).
*$p < 0.05$.
†$p < 0.10$.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Table 3. Peak blood flow velocity in the aortic root and the ascending aorta (AA) during cyclic HRT.

	Vmax (aortic root)	Vmax (ascending aorta)
$cm \times s^{-1}$		
Coefficient of repeatability (%)	18	15
Coefficient of variability (%)	10	17
Intercept	45.1±2.4	50.2±2.2
Treatment effects		
10th day (estradiol)	−3.52±2.9	−0.34±2.1
10th week (estradiol)	+1.28±2.9	+3.99±2.1†
12th week (combined)	+9.38±2.9*	+9.27±2.2*
NETA addition	+8.10±3.4*	+5.27±2.5*

Blood flow velocity reproducibility, intercept (±SE), and treatment effects (±SE on change).
*$p<0.05$ compared to intercept.
†$p<0.10$ compared to intercept.

Subtracted Aortic Blood Flow for Calculation of Coronary Artery Blood Flow

Total volume flow was measured of a higher magnitude in the ascending aorta than in the aortic root; hence, the crude estimate of coronary blood flow was calculated to be negative (Table 2). After correcting the ascending aorta flow for diastolic blood flow by averaging systolic blood flow, as we did at the AV level, the difference regained normal proportions. Both estimates were assessed with very limited reproducibility, and only changes of a large magnitude were detectable. However, both estimates increased continuously and significantly with HRT (Table 2).

DISCUSSION

This is the very first study of VENC MR to assess hemodynamic effects in the central circulation of a pharmaceutical intervention. In a randomized, placebo-controlled and blinded manner, we assessed the principal blood flow effects of an oral HRT preparation commonly used in clinical practice. Despite the findings in numerous studies that estrogens have vasodilator properties (Mendelsohn and Karas, 1999), not many studies have attempted to study in vivo circulatory effects during realistic replacement regimens. In previous studies, coronary artery effects have been assessed at supraphysiological plasma concentrations, either by using higher dosages or by administrating the hormones directly in the target artery. We used higher dosages of HRT than what is normally prescribed at the initiation of treatment; however, the hormone levels we obtained in plasma were within physiological range. There is a lack of evidence of an increase in risk with higher dose HRT. For instance, in the largest study performed to date assessing breast cancer risk during HRT (the Million Women Study), the risk of invasive breast cancer did not show any dose dependency (Million Women Study Collaborators, 2003). However, it needs to be emphasized that any possible cardiovascular benefit of the tested HRT preparation—together with possible reduction in risk of osteoporosis and colon cancer—must be weighted against the possible risks, notably breast cancer and venous thrombosis.

We demonstrated that cardiac output was largely unaffected during HRT and that changes in ABF above the coronary arteries were almost certainly hormone induced, highly reliable, and reproducible. Despite the fact that the calculated difference between blood flow at the two levels was biased and not reliable for the assessment of the absolute values of total CABF, we speculate our findings are being linked to a *relative* increase in CABF. The fact that CABF is almost entirely diastolic and strongly linked to cardiac workload is supportive of an association between our findings and change in CABF. We found that changes in ascending ABF were almost entirely diastolic and were not associated with changes in LV function.

On the basis of prevailing evidence, it appears reasonable to suggest the possible coronary artery effects to be estrogen induced. It is of interest that the addition of the progestogen norethisterone acetate did not modify the flow effects. Despite the fact that NETA is an androgenic progestogen and might adversely affect HDL cholesterol levels, studies of

direct vascular effects have been positive (Glusa et al., 1997). The fact that NETA might not negate the estradiol effect on vessel tone is important because negative vascular effects of other progestogens, especially medroxy progesterone acetate (MPA), have been put forward as explanatory mechanisms for the disappointing outcomes in the two randomized trials of HRT and cardiac outcome (Hulley et al., 1998; Writing Group for the Women's Health Initiative Investigators, 2002). MPA has been shown to directly counteract the beneficial effects of estrogens on arterial function (Miyagawa et al., 1997; Rosano et al., 2000). Our findings support more research of important intermediary variables, and notably clinical cardiac endpoints to encompass possible negative metabolic effects, during HRT with estradiol and norethisterone. The latter hormones were used in WHISP (Collins, 2002), a recently completed UK-based placebo-controlled and randomized HRT study of clinical outcome in women admitted with acute coronary syndromes. The first analysis indicates cardiovascular morbidity benefit of these hormones (Collins et al., 2002). Our findings support a direct coronary artery dilator effect of estradiol, previously observed in human in vitro experiments (Chester et al., 1995). Our findings also concur with a reported increase in basal coronary blood flow and reduced coronary vasomotor resistance with intravenous estrogen, as measured invasively by quantitative coronary angiography (Reis et al., 1994). Also, we showed HRT is directly linked to a flow velocity increase, and we verified this effect as present at both levels of the aorta. This effect has also been demonstrated by Doppler ultrasound and has been suggested to indicate enhanced cardiac contractility with HRT (Pines et al., 1991). It is of interest that the increase in maximum flow velocity was particularly prominent during norethisterone addition, which adds further to the belief that norethisterone is a favorable progestogen with regard to cardiovascular function. In line with the compelling and interesting notion that estradiol and norethisterone appear different from the HRT preparation used in WHI (Writing Group for the Women's Health Initiative Investigators, 2002) and HERS (Hulley et al., 1998), it is not a new observation that different steroidal hormones with similar reproductive effects might have antagonistic properties in the circulation. It has been suggested that diverse biologic effects of different estrogens is linked to different affinity for estrogen receptor alfa and beta (Gruber et al., 2002). We certainly need to study the diversity of sex hormone cardiovascular effects in more detail.

This study tested for the first time the use of VENC MR in important cardiovascular clinical research. Magnetic resonance aortic flow quantification rely on rather basic MR acquisition techniques; it is robust and reproducible and hence ideal for clinical measurement of central haemodynamic data. The technique is indeed applicable to clinical research, and we report data on in vivo reproducibility, which promotes its use in clinical trials in smaller groups of research subjects. Also derived data, such as peak blood flow velocity, is reproducible by MR flow mapping and despite the lower temporal resolution than Doppler echocardiography, it is also favorable as a research tool for assessment of flow velocity. Blood flow velocity measurements at the two levels of the aorta showed good agreement, and changes with therapy were matching. Volume flow quantification near the aortic valve has been suggested to be compromised by turbulence, and we avoided the possible bias of turbulent diastolic flow in the aortic root by calculating aortic flow below the coronary arteries by averaging systolic flow. In view of the versatility and inherent advantages of VENC MR, the technique might be specifically favorable in assessing pharmaceutical effects on cardiovascular function.

One of our intentions with this study was to assess the usefulness of the indirect calculation of total CABF derived from aortic volume flow (Buonocore, 1994). We showed, after testing the algorithm under standardized circumstances by a total of 123 measurements and using the same MR protocol throughout a longitudinal study, that it is compromised by lack of reproducibility. In view of the fact that CABF only represents about 5% of cardiac output and the reliability of the indirect CABF assessment is based on the added precision of two VENC MR acquisitions, the lack of reproducibility is somewhat expected. However, we did show changes with therapy, which are biologically plausible, and it is possible that by optimizing the VENC MR sequences with dual-velocity flow sequences and aortic root tracking techniques for elimination of the bias of the movement of the aortic root (Buonocore and Bogren, 1992; Kozerke et al., 1999), the accuracy of the indirect CABF assessment may be improved.

In conclusion, aortic flow mapping with magnetic resonance is an excellent, noninvasive and reproducible technique for assessment of important circulatory effects in research project in smaller groups of subject. By using the technique it was demonstrated that postmenopausal hormone replacement therapy with estradiol and cyclic norethisterone acetate causes aortic

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

blood flow changes that may be linked to coronary artery dilatation. Norethisterone addition did not reduce the effect of estrogen and may be directly linked to increase in cardiac contractility. Estradiol and norethisterone may possess specific cardiovascular benefit and thus require further clinical testing.

ACKNOWLEDGMENTS

This study was funded by Novo Nordisk A/S, Denmark and The Faculty of Health Sciences, University of Copenhagen Denmark. The statistical consultant, Statcon ApS (www.statcon.dk) is thanked for valuable statistical assistance.

REFERENCES

Bellenger, N. G., Burgess, M. I., Ray, S. G., Lahiri, A., Coats, A. J., Cleland, J. G., Pennell, D. J. (2000). Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance; are they interchangeable? *Eur. Heart J.* 21:1387–1396.

Buonocore, M. H. (1994). Estimation of total coronary artery flow using measurements of flow in the ascending aorta. *Magn. Reson. Med.* 32:602–611.

Buonocore, M. H., Bogren, H. (1992). Factors influencing the accuracy and precision of velocity-encoded phase imaging. *Magn. Reson. Med.* 26:141–154.

Chester, A. H., Jiang, C., Borland, J. A., Yacoub, M. H., Collins, P. (1995). Oestrogen relaxes human epicardial coronary arteries through non-endothelium-dependent mechanisms. *Coron. Artery Dis.* 6:417–422.

Collins, P. (2002). Clinical cardiovascular studies of hormone replacement therapy. *Am. J. Cardiol.* 90:30F–34F.

Collins, P., Webb, C. (1999). Estrogen hits the surface. *Nat. Med.* 5:1130–1131.

Collins, P., Flather, M., Lees, B., Mister, R., Proudler, A., Stevenson, J. (2002). The women hormone intervention secondary prevention trial (WHISP). Late Breaking News, International Congress on the Menopause, Berlin 2002. *Climacteric* 5 (suppl. 1).

Glusa, E., Gräser, T., Wagner, S., Oettel, M. (1997). Mechanism of relaxation of rat aorta in response to progesterone and synthetic progestins. *Maturitas* 28:181–191.

Graves, M. J., Dommett, D. M. (2000). Comparison of cardiac stroke volume measurement determined using stereological analysis of breath-hold cine MRI and phase contrast velocity mapping. *Br. J. Radiol.* 73:825–832.

Gronning, B. A., Nilsson, J. C., Sondergaard, L., Fritz-Hansen, T., Larsson, H. B., Hildebrandt, P. R. (2000). Antiremodeling effects on the left ventricle during beta-blockade with metoprolol in the treatment of chronic heart failure. *J. Am. Coll. Cardiol.* 36:2072–2080.

Gruber, C. J., Tschugguel, W., Schneeberger, C., Huber, J. C. (2002). Production and actions of estrogens. *N. Engl. J. Med.* 346:340–352.

Hulley, S. B., Grady, D., Bush, T., Furberg, C., Herrington, D., Riggs, B., Vittinghoff, E. (1998). Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. *J. Am. Med. Assoc.* 280:605–613.

Kozerke, S., Scheidegger, M. B., Pedersen, E. M., Boesiger, P. (1999). Heart motion adapted cine phase-contrast flow measurements through the aortic valve. *Magn. Reson. Med.* 42:970–978.

Mendelsohn, M. E., Karas, R. H. (1999). The protective effects of estrogen on the cardiovascular system. *N. Engl. J. Med.* 340:1801–1811.

Million Women Study Collaborators. (2003). Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* 362:419–427.

Miyagawa, K., Rosch, J., Stanczyk, F., Hermsmeyer, K. (1997). Medroxyprogesterone interferes with ovarian steroid protection against coronary vasospasm. *Nat. Med.* 3:324–327.

Myerson, S. G., Bellenger, N. G., Pennell, D. J. (2002). Assessment of left ventricular mass by cardiovascular magnetic resonance. *Hypertension* 39:750–755.

Nayler, G. L., Firmin, D. N., Longmore, D. B. (1986). Blood flow imaging by cine magnetic resonance. *J. Comput. Assist. Tomogr.* 10:715–722.

Pines, A., Fisman, E. Z., Levo, Y., Averbuch, M., Lidor, A., Drory, Y., Finkelstein, A., Hetman-Peri, M., Moshkowitz, M., Ben-Ari, E., Ayalon, D. (1991). The effects of hormone replacement therapy in normal postmenopausal women: measurements of Doppler derived parameters of aortic flow. *Am. J. Obstet. Gynecol.* 164:806–812.

Rajkumar, C., Kingwell, B. A., Cameron, J. D., Waddell, T., Mehra, R., Christophidis, N., Komesaroff, P. A.,

McGrath, B., Jennings, G. L., Sudhir, K., Dart, A. M. (1997). Hormonal therapy increases arterial compliance in postmenopausal women. *J. Am. Coll. Cardiol.* 30:350–356.

Reis, S. E., Gloth, S. T., Blumenthal, R. S., Resar, J. R., Zacur, H. A., Gerstenblith, G., Brinker, J. A. (1994). Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. *Circulation* 89:52–60.

Rosano, G. M., Webb, C. M., Chierchia, S., Morgani, G. L., Gabraele, M., Sarrel, P. M., de Ziegler, D., Collins, P. (2000). Natural progesterone, but not medroxyprogesterone acetate, enhances the beneficial effect of estrogen on exercise-induced myocardial ischemia in postmenopausal women. *J. Am. Coll. Cardiol.* 36:2154–2159.

Senn, S. (1993). *Cross-Over Trials in Clinical Research*. Chichester, England: John Wiley and Sons Ltd.

Sorensen, M. B., Fritz-Hansen, T., Jensen, H. H., Pedersen, A. T., Hojgaard, L., Ottesen, B. (2001). Temporal changes in cardiac function and cerebral blood flow during sequential postmenopausal hormone replacement. *Am. J. Obstet. Gynecol.* 184:41–47.

Sorensen, M. B., Collins, P., Ong, P. J., Webb, C. M., Hayward, C. S., Asbury, E. A., Gatehouse, P. D., Elkington, A. G., Yang, G. Z., Kubba, A., Pennell, D. J. (2002). Long-term use of contraceptive depot medroxyprogesterone acetate in young women impairs arterial endothelial function assessed by cardiovascular magnetic resonance. *Circulation* 106:1646–1651.

Spilt, A., Box, F. M., van der Geest, R. J., Reiber, J. H., Kunz, P., Kamper, A. M., Blauw, G. J., van Buchem, M. A. (2002). Reproducibility of total cerebral blood flow measurements using phase contrast magnetic resonance imaging. *J. Magn. Reson. Imaging* 16:1–5.

Writing Group for the Women's Health Initiative Investigators. (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women. *J. Am. Med. Assoc.* 288:321–333.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016