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Premenopausal Reproductive Health Modulates Future Cardiovascular Risk – Comparative Evidence from Monkeys and Women

Jay R. Kaplan, PhD[a,*] and Stephen B. Manuck, PhD[b]

[a]*Departments of Pathology and Obstetrics and Gynecology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC;* [b]*Department of Psychology, University of Pittsburgh, Pittsburgh, PA*

Coronary heart disease (CHD†) remains the major cause of mortality among postmenopausal women living in industrialized countries. Several lines of evidence suggest that ovarian hormones (especially estrogen) protect the coronary arteries of premenopausal women. However, it is also known that women commonly experience disruptions in cyclic hormonal function during their reproductive years. In this perspective, we hypothesize that if regular, cyclic ovarian function affords protection against CHD, ovulatory abnormalities in young women may conversely promote the development of atherosclerosis (the pathobiological process underlying CHD) in the years prior to menopause and thus substantially increase the risk of subsequent heart disease. This hypothesis is supported by evidence from premenopausal nonhuman primates showing that relatively common, subclinical ovarian disruptions – as may be induced by psychosocial stress – are associated with the initiation and acceleration of coronary artery atherosclerosis. If extending to women, these findings would suggest that ovarian dysfunction is an early biomarker for CHD risk and, further, that primary prevention of CHD should begin during the premenopausal phase of life.

INTRODUCTION

Coronary heart disease (CHD) remains the major cause of mortality among postmenopausal women living in industrialized countries [1]. Much evidence suggests that ovarian hormones – especially estrogen – protect the coronary arteries of premenopausal women from atherosclerosis (the pathobiological process underlying CHD), thereby delaying clinical manifestations of this disease until the postmenopausal years [2-8]. However, it is known that ovarian function varies in quality throughout the premenopausal years, with women often experiencing

*To whom all correspondence should be addressed: Jay R. Kaplan, Ph.D., Departments of Pathology and Obstetrics and Gynecology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157; Tel: 336 716-1522, Email: jkaplan@wakehealth.edu.

†Abbreviations: CHD, Coronary Heart Disease; FHA, Functional Hypothalamic Amenorrhea; OC, Oral Contraceptives; CAC, Coronary Artery Calcium; HPA, Hypothalamic Pituitary Adrenocortical.

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disruptions in cyclic hormonal function. Because such disruptions can occur among individuals that continue to cycle spontaneously or who are not actively trying to become pregnant, they are frequently unnoticed and are thus possibly much more common than is generally recognized [9].

To the extent that regular, cyclic ovarian function affords protection against CHD, ovulatory abnormalities in young women – even if relatively mild – might conversely promote the development of atherosclerotic cardiovascular disease during the reproductive years. This perspective reviews evidence from premenopausal nonhuman primates demonstrating that relatively common, subclinical ovarian disruption, as induced by psychosocial stress, accelerates coronary artery atherogenesis. If seen also in women, these findings point to ovarian dysfunction as a possible early marker for CHD risk and suggest that primary prevention of CHD might best begin during the premenopausal phase of life.

The potential relationship between premenopausal ovarian disruption and heightened cardiovascular risk in women has gone largely unrecognized for at least two reasons: 1) young women are thought to be "protected" from atherosclerotic cardiovascular disease during their reproductive years; and 2) substantial logistical challenges impede the assessment of reproductive function, especially subtle deficits, in appropriately large populations of young women. Thus, for example, functional hypothalamic amenorrhea (FHA) comprises a major category of reproductive dysfunction that is well known to contribute to infertility and bone loss, but has not been systematically evaluated with respect to heart disease [9]. As suggested below, evidence from women and nonhuman primates suggests that such an evaluation is warranted and probably overdue.

SEX DIFFERENCES IN CHD AND CORONARY ARTERY ATHEROSCLEROSIS

Coronary heart disease is often thought to be a male malady. Nonetheless, more women than men have died from cardiovascular disease (CHD and stroke) in almost every year since 1984 [1]. Moreover, while it is sometimes suggested that women experience a "female protection" that ends with the menopause [10], incident CHD actually increases monotonically with age among both men and women; there is no upward inflection in women at the time of menopause [2,5,11]. Still, the sexes do differ in that the age-related increase in CHD incidence seen in women lags that observed in men by about ten years as reflected variously in age at first myocardial infarction, the yearly risk of new events, and overall coronary mortality in each decade of life [1,2,11,12].

Atherosclerosis – the accumulation of fibro-fatty plaques (atheromas) within the inner lining of the artery wall – is the primary process underlying the development of CHD. Imaging and autopsy studies show that, as with clinical events and mortality, the progression of coronary artery atherosclerosis in women lags behind that observed in men by about ten years. Despite this lag, there is nonetheless a steady, age-related increase in coronary artery atherosclerosis that begins during the premenopausal years and continues throughout the postmenopausal (> 50 yrs.) phase of life [5,13-18]. The relatively prolonged period of lesion development preceding clinical expressions of this disease suggests that for both women and men the coronary morbidity and mortality observed most appreciably in the fifth decade of life and beyond must arise from arterial damage originating 20 or 30 years previously [13]. For women, this suggests that *postmenopausal CHD has its genesis in the factors that promote progression of atherosclerosis during the premenopausal years*. In fact, CHD is the second most frequent cause of death among women 45 to 54 years of age and is prominent among even younger women, accounting for 12 percent of mortality and comprising the third leading cause of death among 35 to 44-year-olds [1].

NORMAL OVARIAN FUNCTION INHIBITS THE PREMENOPAUSAL DEVELOPMENT OF CHD AND ATHEROSCLEROSIS IN WOMEN

Several lines of evidence indicate estrogen to be cardioprotective during the premenopausal years. First, numerous studies show that premenopausal removal of the ovaries (surgical menopause and resulting hypoestrogenemia) occasions a substantial increase in risk of incident CHD and that this heightened risk may be mitigated by estrogen replacement [19-22]. Moreover, premature ovarian failure (defined as menopause occurring prior to the age of 40) likewise increases coronary disease risk, and estrogen therapy has the same mitigating effects [23-25]. Subtle reproductive insults may also be atherogenic, as one large cohort study related an elevated incidence of anovulation among women 40 to 49 years of age to the clinical appearance of CHD several years later [26]. If premature ovarian failure, anovulation, or oophorectomy increase cardiovascular risk, it might be expected that extended exposure to endogenous hormones would prove cardioprotective. In fact, a number of studies suggest that a greater age at menopause or an increased period of exposure to endogenous sex hormones diminish the risk of CHD in postmenopausal women [27-30].

Finally, and of particular relevance to the potential role of estrogen in modulating CHD risk, are two investigations involving the comparatively rare situation

involving premenopausal women undergoing invasive cardiologic assessment (cardiac catheterization and cineangiography). Although relatively small, both studies associated estrogen deficiency with presence of angiographically confirmed coronary artery disease [31,32]. Notably, the authors of the larger of the studies – containing approximately 100 women – speculated that the relative estrogen deficiency seen in affected study participants was mediated by hypothalamic responses to psychological stress ("hypothalamic hypoestrogenism"), thereby linking adversity in the psychosocial milieu to these individuals' observed coronary disease [31].

ASSESSING OVARIAN DISRUPTION IN MONKEYS AND WOMEN

Evidence cited in the preceding paragraphs suggests that normal ovarian function and estrogen concentrations contribute to cardiovascular health during the premenopausal years and, conversely, that disruptions of ovarian function confer cardiovascular risk. However, systematic evaluation of the relationship between ovarian function and coronary disease in women requires protracted assessment of ovarian hormones to characterize individual differences in ovarian function and to relate these to incident disease or preclinical biomarkers of atherosclerotic risk. In addition to this substantial logistical constraint, ethical considerations limit the use of hormonal manipulations in otherwise healthy individuals, thus precluding rigorous, prospective experiments assessing the effect of estrogen and other ovarian hormones on heart health.

An alternative strategy involves the use of suitable animal models. In this regard, cynomolgus macaques (*Macaca fascicularis*) have proven to be a particularly useful model for studying the role of ovarian hormones and sex differences in the development and expression of CHD [4,33]. When fed an atherogenic diet (containing a similar amount of fat and cholesterol as found typically in the U.S. diet), animals of this species develop atherosclerosis in a pattern similar to that of people and also resemble human beings in the pathologic characteristics of their lesions and in their susceptibility to myocardial infarction [34]. Importantly, females of this species also resemble women in their vulnerability to atherosclerotic cardiovascular disease, including a relative "female protection" in premenopausal individuals [33,35-37]. Furthermore, female cynomolgus monkeys and other Old World anthropoid primates share with women many reproductive characteristics, including not only menstruation and menopause, but as shown below, also a relative susceptibility to reproductive impairments of functional, often psychogenic, origin [4,33,37].

Thus, for example, episodic disruptions of reproductive function are relatively common among women in industrialized societies, with evidence that roughly a quarter of this population experiences infertility at some point in their premenopausal lives [38]. As indicated above, FHA is a primary cause of reproductive disruption in young women. This syndrome is termed "functional" to indicate the absence of an organic cause. Rather, the condition is believed to be induced by psychogenic insults – either alone or in combination with disordered eating and excessive exercise – in individuals otherwise capable of normal reproductive activity [9]. Furthermore, FHA occurs along a continuum from subclinical luteal phase hormonal deficits and anovulation to amenorrhea [39-41].

The hormonal profile of FHA includes estrogen deficiency, gonadotropin levels that are normal or slightly below normal, and normal levels of androgens and prolactin [42]. Moreover, FHA is frequently associated with global hormonal dysregulation, as reflected in part by altered hypothalamic pituitary adrenocortical (HPA) activity and depressed thyroid function [38,42-45]. An elevation in circulating cortisol – the adrenal hormone that orchestrates the metabolic response to stress – is the most prominent and easily assessed neuroendocrine correlate of FHA [46-49]. While psychological traits of individuals may modulate vulnerability to this syndrome, epidemiological research shows a graded relationship between the degree of stress experienced and the extent of reproductive impairment [37,50]. It is perhaps significant that the condition can be ameliorated by behavioral or environmental changes that reduce stress, further emphasizing the importance of the psychosocial environment [50-52].

Notably, assessment of reproductive function through frequent blood sampling and measures of menstrual cyclicity indicates that the subclinical components of FHA – luteal phase hormonal deficits, cyclic irregularity, and anovulation – are relatively common in socially housed monkeys [53]. As in women, these deficits have a stress component. Monkeys naturally align in hierarchies of relative social status and, owing to frequent enforcement of such stratified relationships by high ranking [dominant] animals, it is intrinsically more stressful to hold low [subordinate] rank in the social group. In fact, dominant animals often purposely constrain the behavior of subordinates [54]. Similar to women with FHA, subordinate monkeys tend to be both hypercortisolemic (one indicator of biological stress) and ovarian impaired (i.e., deficient in both estrogen and progesterone) relative to their dominant counterparts [53,55].

The often hesitant, constrained behavior of subordinate female monkeys living in small social groups is seemingly analogous to the "lack of control" said to characterize women diagnosed with FHA [4,37,51]. Also comparable to women, the ovarian dysfunction observed in psychosocially stressed monkeys reportedly increases

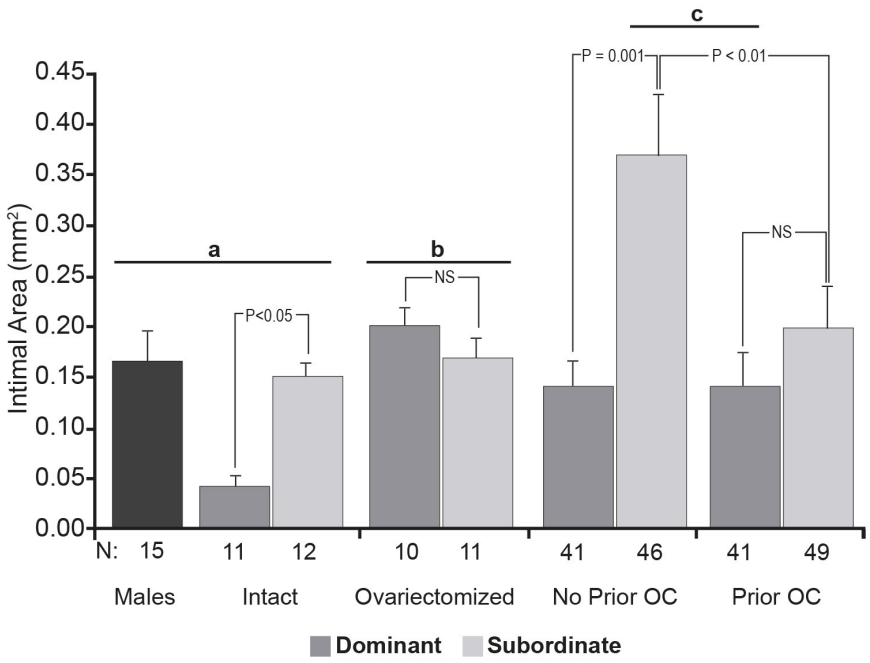

Figure 1. **Coronary artery atherosclerosis extent in socially housed monkeys consuming an atherogenic diet**. **a**. Males and reproductively intact females above (dominant) or below (subordinate) the median in social status following two years of diet consumption (data from [33]); **b**. Dominant and subordinate females following ovariectomy and two years of diet consumption (data from [58]); **c**. Dominant and subordinate females that had either been treated or not with OCs for two years premenopausally, followed by ovariectomy and three more years of diet consumption (data from [69]). The three primary findings are that: 1) dominant females were protected relative to subordinate females and males; 2) ovariectomy eliminated the atheroprotection typical of dominant individuals; and 3) dominant social status or OC exposure lowered the premenopausal trajectory of atherosclerosis progression thereby reducing the extent of lesions observed following ovariectomy – an outcome that was independent of any post-ovariectomy estrogen treatment.

in severity when these animals are concomitantly subjected to caloric restriction and treadmill exercise [56]. Finally, monkeys resemble women in that these functional reproductive deficits are reversible; subordinate animals that became dominant over the course of a study regain normal ovarian function, whereas dominant females losing rank experience subsequent reproductive impairment [53,57]. The striking parallels between women and female monkeys with respect to functional reproductive deficits are discussed in detail elsewhere [4,37].

STUDIES OF ATHEROSCLEROSIS IN SOCIALLY HOUSED MONKEYS: PREMENOPAUSAL IMPLICATIONS

Research employing socially housed cynomolgus macaques fed an atherogenic (i.e., moderately high fat and cholesterol) diet provides unique insights into the relationship between reproductive function, psychosocial factors, and the initiation and progression of atherosclerosis during the reproductive years. These studies also illustrate the potential impact of premenopausal conditions on postmenopausal disease. Lessons learned from this research are first summarized and then briefly commented upon in the following paragraphs.

Female "Protection" from Coronary Artery Atherosclerosis Requires Normal Ovarian Function

An initial investigation evaluated reproductively intact, socially housed females fed an atherogenic diet for two years [33]. In addition to the females, there was a comparison group of socially housed adult males. As expected, the females developed only about one-third as much coronary artery atherosclerosis as did similarly treated males, a significant effect. However, this was true only for those females that were dominant in their social groups; the atherosclerosis of subordinate females was indistinguishable from that of males (Figure 1a). As described above, subordinate monkeys were also characterized by a relatively high incidence of luteal phase progesterone deficiency and anovulation and were relatively hypoestrogenic [53]. The data also indicate that plasma

high density lipoprotein cholesterol concentrations – a protective factor in human atherosclerosis – were positively associated with estrogen status. That is, dominant, ovarian sufficient individuals had higher "good cholesterol" concentrations than their subordinate, ovarian deficient counterparts [58].

A companion study involving the same diet for the same duration used animals that had had their ovaries removed as a means of modeling menopause. This surgical manipulation is necessary because natural menopause in monkeys typically occurs toward the end of life rather than at midlife as in women [59]. Thus, using a surgical approach provides the opportunity to study mid-life ovariectomized monkeys as a model for mid-life menopausal women. Notably, the ovariectomized dominant and subordinate monkeys in this study did not differ from each other in atherosclerosis extent or plasma cholesterol concentrations. Rather, lesion development in both was approximately equivalent to that observed in reproductively intact subordinates and in males, suggesting that ovariectomy eliminates the "female protection" from atherosclerosis typically exhibited by dominant individuals (Figure 1b) [60]. Finally, a third study in this series again used the same diet for the same duration, but here exposed the females to males, allowing the females to become serially pregnant. In this experiment, the exacerbation of coronary artery atherosclerosis previously observed in reproductively intact subordinate females was eliminated by pregnancy, a hyperestrogenic state. That is, coronary artery atherosclerosis extent in this experiment was equivalently inhibited in the subordinate and dominant monkeys (data not shown) [61].

Several additional studies offered the opportunity to confirm the observation that atherosclerosis is typically exacerbated among subordinate relative to dominant, non-pregnant premenopausal monkeys consuming an atherogenic diet [62]. In fact, a meta-analysis comprising five studies and 200 females indicated that subordinate animals reliably developed atherosclerosis that was more than twice as extensive as that observed in their dominant counterparts.

It is worth considering the potential mechanisms that could have mediated the observed pattern of atherosclerosis in this series of investigations. Numerous investigators have suggested that the stress hormone cortisol, which is elevated in FHA, may contribute to the development of coronary artery disease [63,64]. In the studies described above, subordinate animals were hypercortisolemic relative to their dominant counterparts, irrespective of reproductive status, indicating that it was in fact stressful to occupy a subordinate position in these social groups [65]. However, only among the reproductively intact females did elevated cortisol concentrations co-occur with exacerbated atherosclerosis. In the ovariectomized cohort, dominant and subordinate animals had equivalently extensive atherosclerosis but only the subordinates had elevated cortisols [66]. Similarly, atherosclerosis was equivalently inhibited among pregnant animals, regardless of status and cortisol. The significant association across all experimental treatments between ovarian hormones – but not cortisol – and atherosclerosis suggests to us that reproductive hormones underlie the relationship between status and atherosclerosis in these female monkeys [4].

A reasonable, although parenthetic, question would be to ask why behaviorally induced ovarian dysfunction should be so robust in these small groups of monkeys and whether the biological relevance extends beyond the laboratory. Although a full discussion of this topic is beyond the scope of this perspective, we and others have suggested that stress-associated ovarian impairment is possibly adaptive, as it allows long-lived female mammals experiencing a stressful environment to delay an energetically expensive pregnancy until safer, more propitious circumstances prevail. A more extensive and complete discussion of evolutionary considerations and the health implications of ovarian suppression in primates and other mammals may be found in a series of reviews [e.g., 37,67].

Exogenous Estrogen Selectively Protects Reproductively Intact Monkeys that are Subordinate in their Social Groups

The precocious acceleration of atherosclerosis associated with ovarian impairment in the reproductively intact cohort of the prior study suggests that exogenous estrogen might prove protective, especially among monkeys predisposed by estrogen deficiency – namely socially subordinate animals. In a test of this hypothesis, premenopausal monkeys were randomized to consume a diet relatively high in fat and cholesterol, which for half of individuals also contained a triphasic oral contraceptive (OC) [68]. Following two years of diet and OC treatment, atherosclerosis was measured in an iliac artery biopsy, selected because we had previously demonstrated that atherosclerosis at this site correlates significantly with atherosclerosis measured at the same time in the coronary arteries [68].

The premenopausal biopsy data showed that OC treatment was protective, but selectively so for the at-risk individuals – i.e., subordinate animals. Thus, dominant individuals developed little atherosclerosis over the course of study, whether or not they were treated with contraceptive steroid. On the other hand, while untreated subordinates developed extensive atherosclerosis as expected from previous studies (i.e., Figure 1a and [62]), OC administration inhibited lesion development in otherwise identically treated subordinates; in fact, lesion

extent in the OC-treated subordinates was indistinguishable from that in dominant monkeys. As in earlier investigations, the untreated subordinates experienced more frequent anovulation and greater luteal phase deficiency than dominants, consistent with the hypothesis that the ovarian impairment accompanying social subordination accelerates atherogenesis.

Premenopausal Hormonal Conditions Predict Postmenopausal Atherosclerosis Extent

Following collection of the iliac artery biopsy, all monkeys in the prior experiment were ovariectomized and subsequently studied postmenopausally for three years. During this period, some monkeys received hormone treatment in the form of conjugated equine estrogens (CEE). Data from the postmenopausal phase of this life course study provided a unique opportunity to estimate the extent to which premenopausal hormonal status influenced postmenopausal atherosclerosis [69]. Remarkably, the cardioprotective effects of treatment with OCs seen in iliac artery biopsies from subordinate monkeys at the end of the premenopausal phase of the experiment – as well as the absence of such protection in the untreated subordinates – persisted all the way to the postmenopausal evaluation of coronary artery atherosclerosis three years later in these same animals. That is, the postmenopausal pattern of coronary artery atherosclerosis was essentially indistinguishable from the pattern observed in the iliac arteries of the same monkeys at the end of the premenopausal phase [68]; the significant inhibitory effect of premenopausal dominant social status and OC treatment determined the overall pattern of postmenopausal atherosclerosis and did so irrespective of any intervening postmenopausal treatments (i.e., hormone replacement) (Figure 1c).

In summary, monkeys at high risk premenopausally [i.e., subordinate animals without estrogen supplementation] continued to be at high risk postmenopausally, whereas atherogenesis was inhibited in OC-treated subordinates and in dominants with or without administered OCs. *That subordinates' heightened atherosclerotic risk and its reversal by estrogen supplementation persisted postmenopausally and unmodified by postmenopausal hormone replacement underscores the importance of early events in the development of coronary artery atherosclerosis*. These results also confirm the important contribution that longitudinal investigations using appropriate animal models can make to an understanding of the pathogenesis of diseases like CHD, which emerge over a period of decades and are affected by environmental factors such as diet and the social milieu.

CONCORDANT OBSERVATIONS: OCS AND CHD IN WOMEN AND MONKEYS

Two studies of CHD and premenopausal OC use in women are relevant to the observations reported here on the relationship between OC exposure and atherosclerosis in monkeys. In one, angiographically confirmed coronary artery disease among over 900 postmenopausal women was less severe in those who reported prior use of OCs than in their counterparts who did not use OCs [70]. The second study assessed the impact of OC use among diabetic and healthy premenopausal women on coronary artery calcium (CAC), a constituent of advanced atherosclerotic plaque and prominent biomarker of risk for incident CHD [71]. Here, diabetic women had substantially greater CAC than non-diabetic counterparts, a result that is perhaps not surprising given the relatively high incidence of cardiovascular mortality observed in diabetic women relative to those without diabetes and in comparison to diabetic men [72,73]. In this study, OC use was associated with retarded progression of CAC across all cohorts [diabetic and normal controls], but with the greatest inhibition observed in diabetic women and those reporting taking OCs for the longest period of time. Hence, OCs may be cardioprotective in premenopausal women, and especially so in the most susceptible individuals, in this instance diabetics. In the current context, it is perhaps relevant that diabetic women share with subordinate monkeys – which also receive the greatest benefit from OCs – evidence of more prevalent ovarian dysfunction, including hypoestrogenemia, anovulation, and menstrual irregularity [e.g., 71,74].

Admittedly, estrogenic compounds, including OCs, are well known to be promote blood clotting and their use has been associated with an increased risk for venous thromboembolism, thrombotic stroke, or myocardial infarction in numerous – though not all – studies [e.g., 70,75]. Such risks are magnified in women over 35 years of age and in the presence of smoking and pre-existing cardiovascular risk factors such as hypertension and hyperlipidemia. For purposes of this perspective, the observations with respect to CAC and coronary occlusion are presented to establish the biological plausibility that estrogens may inhibit the premenopausal progression of atherosclerosis irrespective of any prothrombotic effects that may be observed in some women using estrogen-containing OCs [70].

A PROPOSAL FOR WOMEN'S HEALTH RESEARCH

Whether behaviorally induced reproductive deficits of protracted duration (akin to those seen in monkeys) also increase cardiovascular risk is women is not yet

known. Hence, their study in relation to relevant pathologic processes poses a potentially fruitful area of further research. It may be asked, for instance, whether subtle but chronic disruptions of normal menstrual cycling can account, in part, for associations of known behavioral risk factors with non-invasive measurements of preclinical atherosclerosis, or whether long-term use of OCs such as those cited above preferentially retards atherosclerosis in women having an adverse psychosocial or sociodemographic risk profile (e.g., history of depression, social isolation, or socioeconomic disadvantage).

If the results of such research align with the cardiovascular effects of behaviorally entrained ovarian dysfunction discovered in cynomolgus monkeys, the additional effort required to target clinical endpoints (e.g., heart attack and stroke) in population-based epidemiologic cohorts would be well-justified. Finally, the reversibility of stress-induced ovarian deficits using behavioral interventions suggests a potential approach to the premenopausal prevention of atherosclerotic cardiovascular disease in susceptible women.

CONCLUSIONS AND OUTLOOK

The foregoing sections suggest that the outcome of appropriately designed studies using monkeys can inform our understanding of the way that behavioral and hormonal factors may interact to affect CHD risk in women. Perhaps the three most important insights gained from these investigations are that: 1) the quality of premenopausal ovarian function influences the development of atherosclerosis during the premenopausal years, with stress-induced ovarian impairment associated with precocious acceleration of lesions; 2) even the subclinical expression of FHA (i.e., luteal phase hormone deficiencies, anovulation, and cyclic irregularity) is sufficient to potentiate the exacerbation of coronary artery atherosclerosis; and 3) the extent of atherosclerosis that develops during the premenopausal years establishes the trajectory of postmenopausal risk.

The translational relevance of the observations in monkeys seems clear: If the reproductive years represent a vulnerable period during which estrogen inhibits the initial development of atherosclerosis or the progression of small uncomplicated lesions to large plaques, the application of appropriate psychological or hormonal interventions to women with FHA could shift the trajectory of CHD in a downward (beneficial) direction. To be effective however, any such strategies must be initiated sufficiently early. Moreover, efforts at prevention should be focused on the most vulnerable populations including women with FHA and extending perhaps to other subgroups that may be characterized by ovarian impairment, including diabetics and those young women predisposed to diabetes.

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