Ovarian Dysfunction, Stress, and Disease: A Primate Continuum

Jay R. Kaplan and Stephen B. Manuck

Abstract

Menopause is recognized as a period of increased risk for coronary heart disease (CHD) and osteoporosis. Vulnerability to these conditions is often attributed to the naturally occurring estrogen deficiency characteristic of this part of the life cycle. Premenopausal reductions in endogenous estrogen occasioned by functional ovarian abnormalities or failure are hypothesized to be similarly pathogenic and to accelerate development of CHD and osteoporosis prematurely, thereby increasing the health burden of older women. These functional abnormalities, which occur along a continuum from mild, luteal phase progesterone deficiency to amenorrhea, are relatively common and are often attributed to psychogenic factors (stress, anxiety, depression, or other emotional disturbance), exercise, or energy imbalance. Although numerous investigators have commented on these functional deficits, the abnormalities can be difficult to diagnose and are generally unappreciated for the contribution they may make to postmenopausal disease. Studies in nonhuman primates confirm that these deficits are easily induced by psychological stress and exercise, and that they accelerate the development of cardiovascular disease and perhaps bone loss in the presence of a typical North American diet. However, functional reproductive deficits are also reversible and are thus potentially amenable to environmental or behavioral intervention. Data from both women and nonhuman primates support the hypothesis that functional reproductive deficits are adaptive when triggered appropriately but are detrimental when activated in an environment (e.g., sedentary lifestyle, high-fat diet) permissive to the development of chronic disease.

Key Words: amenorrhea; anovulation; CHD; evolution; monkey; osteoporosis; reproduction; stress

Introduction

Menopause is a prominent event in the lives of women, because it both signals reproductive senescence and marks a period of increased vulnerability to chronic disease. In fact, the menopausal loss of cyclic ovarian function is believed to contribute significantly to two diseases comprising a major portion of the health burden of older women—coronary heart disease (CHD[1]) and osteoporosis. Although both conditions are relatively rare in premenopausal women, CHD is currently the largest single cause of death among women over 50 yr of age, and fully half of all postmenopausal women will experience an osteoporosis-related fracture and associated morbidity (Anderson 2001; Melton et al. 1989).

These disease-related sequelae of menopause have been the focus of extensive investigation and, in many respects, are now well understood. It is largely unappreciated however, that ovarian function not only differs distinctly between discretely differentiated "active" and "inactive" periods of the life cycle (i.e., pre- and postmenopause), but also varies appreciably in quality between individuals and throughout the course of women's reproductive lives. In this article, we propose that to the extent cyclic ovarian function affords protection against CHD and osteoporosis, any reduction in endogenous estrogen occasioned by ovulatory abnormalities or failure in young women will similarly accelerate development of these two diseases of aging. Although affected individuals might not manifest the aforementioned diseases clinically during their premenopausal years, they may be at greatly increased risk postmenopausally. Supporting this suggestion is the observation that the "protection" against CHD and osteoporosis seemingly enjoyed by premenopausal women is largely eliminated if the ovaries are surgically removed and the resulting estrogen deficiency is not corrected (Rosenberg et al. 1981; Sowers et al. 1998a). It might be speculated that other tissues dependent on estrogenic stimulation (e.g., the central nervous system) would also be affected adversely by premenopausal ovarian dysfunction (Berga 2001).

At present, our understanding of the causes, extent, and pathobiological consequences of premenopausal ovarian dysfunction is uneven and incomplete. Among the causes, results of numerous studies suggest that exercise and dieting can induce reproductive abnormalities. Psychogenic factors

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[1]Abbreviations used in this article: ACTH, adrenocorticotropin hormone; CHD, coronary heart disease; CRH, corticotropin-releasing hormone; FHA, functional hypothalamic amenorrhea; FSH, follicle-stimulating hormone; GABA, gamma aminobutyric acid; GnRH, gonadotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenocortical; LH, luteinizing hormone; LPD, luteal phase deficiency; OC, oral contraceptive; PCOS, polycystic ovarian syndrome; T3, triiodothyronine; WISE, Women's Ischemia Syndrome Evaluation.

(e.g., "stress," anxiety, or depression) also contribute substantially, although their role remains somewhat controversial owing to difficulties in defining and quantifying such phenomena. Moreover, exercise, dieting, and psychogenic factors often coincide, making it almost impossible to distinguish their independent contributions to any particular episode of ovarian abnormality. Regarding extent of occurrence, emerging evidence suggests that premenopausal ovarian dysfunction—manifested along a continuum from mild deficits in the luteal phase of the menstrual cycle to anovulation and amenorrhea (sustained interruption of menses)—is much more common than is generally appreciated by either women or their clinicians. Furthermore, although it is recognized that severe premenopausal ovarian dysfunction (such as amenorrhea) may accelerate bone loss and impair fertility, the pathobiological consequences of the more frequent mild deficits are largely unknown.

Research employing animal models, especially nonhuman primates, has long provided a means of untangling the complexities of human reproduction. The relation between ovulation and menstruation in women, for example, was somewhat of a mystery even as late as 1920. It was not until this time that G. W. Corner, using rhesus monkeys, first combined anatomical investigation with systematic observations of menstrual cyclicity (Corner 1923, 1927). That strategy, used also in the classic studies of C. W. Hartman (1932) and S. Zuckerman (1930), helped clarify the sequence and timing of ovulation, corpus luteum formation, and menstruation as applied to both women and monkeys. Experimental investigations relating to human reproduction increased greatly in number through the rest of the 20th century. Importantly, such studies have now expanded to consider the role of ovarian hormones in chronic diseases such as osteoporosis and atherosclerosis (the pathological process underlying CHD). The latter investigations provide important new insights into the influence of premenopausal ovarian function on the health trajectories of women moving through the pre- and postmenopausal periods of life.

The following review begins by considering what is currently known about normal and abnormal reproductive function in women, with particular emphasis on abnormalities that are environmentally induced and thus not secondary to organic conditions (e.g., tumors, congenital abnormalities, ovarian enzymatic dysregulation). Data derived from studies of nonhuman primates are then used to address three related issues relevant to women's health in the United States: (1) the effect of common stressors—primarily psychogenic factors, but also exercise and dieting—on premenopausal ovarian function; (2) the extent and severity of the resulting ovarian deficits; and (3) the influence of such deficits (mild as well as more severe) on pre- and postmenopausal health, with special reference to osteoporosis and CHD. Finally, the nature of individual differences in response to psychogenic stress and the possible adaptive origin of stress-induced ovarian deficiency is explored.

Normal Reproductive Function in Women

The monthly reproductive cycle of women and other Old World anthropoid primates is like a well-choreographed dance, dependent not only on the proper timing of hormonal events, but also on an appropriate contribution from each of the components. These components include the hypothalamus, anterior pituitary gland, ovaries, and genital tract, the actions of which are coordinated by neuroendocrines and ovarian hormones. Several classic references (Ferin et al. 1993; Hotchkiss and Knobil 1994; Knobil 1988) provide much of the material for the following, abbreviated description of the normal primate reproductive cycle. This cycle has three ovarian phases: (1) follicular: maturation of a single, ovum-containing follicle; (2) ovulatory: the rupture of the follicle and ejection of the ovum; and (3) luteal: transformation of the follicle into a hormone-producing corpus luteum. The follicular phase of the menstrual cycle coincides with proliferation in the endometrium (inner lining of the uterus), which becomes secretory during the luteal phase. In the nonpregnant state, the luteal phase is followed by the sloughing of endometrial cells and, hence, menstruation.

In Figure 1, the major components of the human and anthropoid reproductive cycle are illustrated, and the source and major targets of the following primary hormones that coordinate and regulate the cycle are identified: luteinizing hormone (LH[1]), follicle-stimulating hormone (FSH[1]), estra-

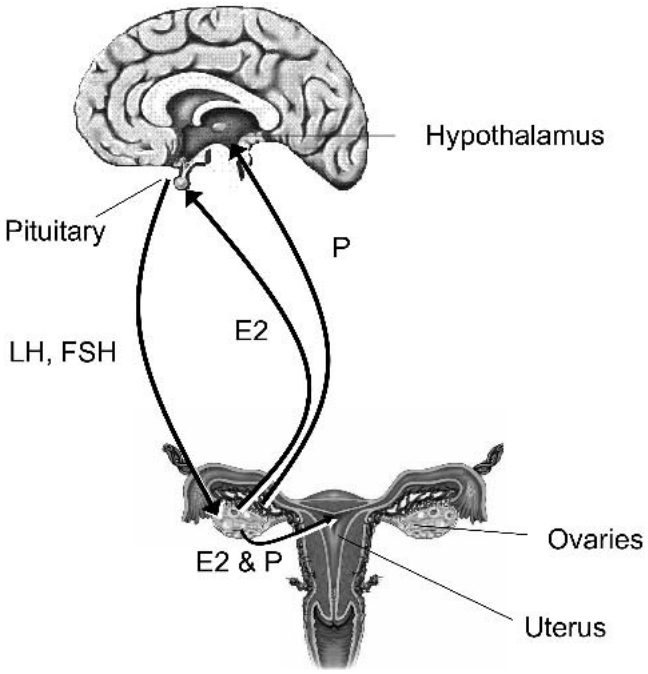

Figure 1 Simplified depiction of hormonal links among central and peripheral components of the primate reproductive system. LH, luteinizing hormone; FSH, follicle-stimulating hormone; E2, estradiol; P, progesterone.

diol, and progesterone. LH and FSH are termed gonadotropins, because they originate in the anterior pituitary and function to stimulate the female gonads (ovaries).

One additional neuroendocrine factor central to cyclic regulation is gonadotropin-releasing hormone (GnRH[1]), a hypothalamic peptide that conditions the release of LH and FSH from the pituitary. The hormonal, ovarian, and endometrial events are shown in Figure 2 in the sequence in which they would normally occur. By convention, the first day of menstruation is considered day 1 of the menstrual cycle.

At the start of the menstrual cycle, the timely increase in FSH above a given threshold promotes further follicular growth beyond the gonadotropin-independent stage and leads ultimately to the selection of the dominant follicle. The growth of follicles is heralded by a geometric increase in estradiol, whereas the selection of the dominant follicle typically manifests as an exponential increase in estradiol that triggers the LH surge. Paradoxically, it is the secretion of estradiol from the dominant follicle that suppresses FSH and prevents multifollicular development. The LH surge fosters ovulation by setting in motion a cascade of intraovarian events that allow for rupture of the follicle wall and the transformation of granulosa cells into the progesterone-secreting corpus luteum. During the luteal phase, the large quantities of estradiol and progesterone made by the corpus luteum suppress FSH and LH and prevent further gonadotropin-dependent folliculogenesis until the corpus luteum involutes.

As noted above, the follicular release of estradiol not only affects the pituitary, it also causes the endometrium to proliferate. The luteal release of progesterone then transforms the proliferated endometrium into a well-vascularized secretory gland prepared to accept and support implantation of a fertilized ovum. In the absence of fertilization, the withdrawal of hormonal support from the corpus luteum results in ischemia and necrosis of the endometrium, followed by menstrual bleeding.

The remaining major component of the primate reproductive system is the group of hypothalamic cells that produce GnRH. These cells, located in the arcuate nucleus of the mediobasal hypothalamus, are synchronized to release GnRH in a pulsatile manner. This set of cells thus comprises what is termed the GnRH pulse generator, a functional unit that receives inputs from other parts of the hypothalamus, higher brain centers such as the cortex, and peripheral organs, including the ovaries and adrenal glands. Such inputs influence the GnRH pulses, which vary in amplitude and frequency across the cycle (e.g., the relatively rapid pulsing of the follicular phase slows in response to the increased

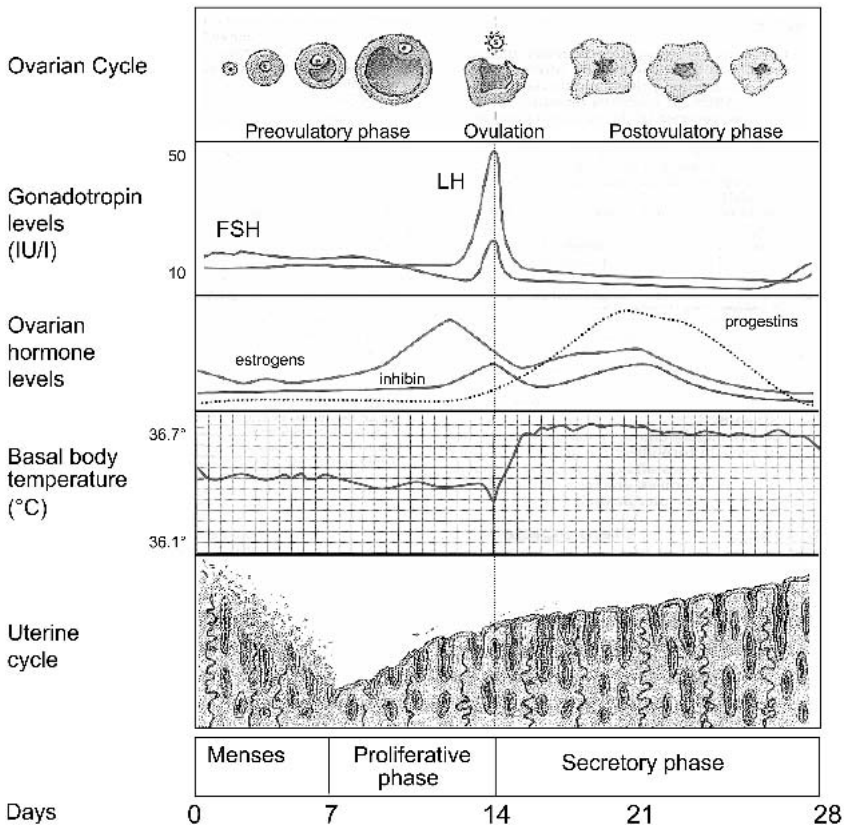

Figure 2 Physiological characteristics of the primate human menstrual cycle, synchronized to the first day of menses and the day of ovulation. FSH, follicle-stimulating hormone; LH, luteinizing hormone.

amount of progesterone released during the luteal phase). These pulses, in turn, permit the anterior pituitary to respond to ovarian stimulation by secreting appropriate amounts of LH and FSH in the follicular, ovulatory, and luteal phases of the cycle. The GnRH pulse generator is the final common pathway controlling the ovarian cycle, notable in that it integrates signals originating from both the internal and external milieus.

The preceding description only begins to hint at the complexity of the reproductive cycle and our incomplete knowledge concerning its control. For example, although the late luteal elevation in FSH induces the growth of several primordial follicles, only one continues to develop—presumably because estradiol (and probably the hormone inhibin) from the most developed follicle inhibits the further release of FSH, which in turn causes the remaining follicles to undergo atresia (i.e., degeneration). However, the factors leading to the choice of any particular follicle for development are largely unknown. Moreover, the mechanisms causing estradiol negative feedback on gonadotropin release early in the follicular phase (presumably by desensitizing the pituitary to GnRH stimulation), but positive feedback just before follicular maturation and ovulation (by increasing sensitivity to GnRH stimulation), are similarly unknown (e.g., Ferin et al. 1993). Among the greatest of mysteries is how the numerous factors known to affect the GnRH pulse generator are integrated to result in (or disrupt) the pulsatile release of GnRH. Noradrenergic and dopaminergic neurons colocalize with the GnRH neurons and no doubt influence their activity. GnRH release is also affected by activation of the opioidergic and GABAergic systems, which in turn are influenced by the activity of the hypothalamic-pituitary-adrenocortical (HPA[1]) axis.

Functional Reproductive Deficits

Etiology and Epidemiology Overview

The normal reproductive cycle requires the contributions of the hypothalamus, pituitary gland, and ovaries to be properly timed and titered, suggesting numerous opportunities for disruption. Among the most important disruptions epidemiologically are those induced by psychogenic and metabolic insults (psychological stress, exercise, diet) in individuals otherwise capable of normal reproductive activity. Such deficits are termed "functional" to reflect the absence of impairment in the reproductive organs and to suggest that removal of the insult should reverse the deficit. In women, the primary functional impairment induced by psychogenic and metabolic factors is hypothalamic in origin, involving alterations in the activity of the GnRH pulse generator. The most obvious clinical sign of this abnormality is the ceasing of menses (amenorrhea) in an individual who had been cycling regularly; the resulting condition is referred to as functional hypothalamic amenorrhea (FHA[1]).

Although FHA represents the single largest cause of amenorrhea (Hirvonen 1977; Reindollar et al. 1986), it is often a diagnosis of exclusion after elimination of the possible organic causes of amenorrhea (Warren 1996; Warren and Fried 2001). Primary among the other potential causes of amenorrhea in previously menstruating women is polycystic ovarian syndrome (PCOS[1]), a heterogeneous pathological condition generally associated with abnormal elevations in androgens, LH, and insulin, as well as hirsutism and obesity (Ferin et al. 1993; Laven et al. 2002; Loucks et al. 2000). The etiology of PCOS is uncertain, although it may originate with dysregulation of the interaction between adrenal and ovarian function after puberty (Ferin et al. 1993). In contrast, the etiology of FHA is more clearly environmental, associated with psychophysiological and behavioral responses to life events. The hormonal profile of FHA is also distinct from that of PCOS and includes estrogen deficiency, gonadotropin levels that are normal or slightly below normal, and normal levels of androgens and prolactin (Berga et al. 1989). The primary deficit is a diminished or inappropriately pulsed release of GnRH, which in turn reduces the amount of LH and FSH available to support follicle development and ovulation. The undeveloped follicles fail to produce estradiol or a functional corpus luteum, resulting in a quiescent endometrium as well as estrogen deficiency.

Psychogenic Factors

Psychological stress and emotional disturbances have always figured prominently in descriptions of FHA (Gregory 1957; Kroger and Freed 1956). Early writings on the topic relate the ceasing of menses to sudden fright or grief, mental and physical demands of the workplace or school, and psychiatric illness, especially depression and psychosis (Novak 1931; Ripley and Papanicolaou 1942). Klinefelter was among the first to attribute the disorder to a hypothalamic deficit, naming it "hypothalamic hypoestrinism" (Klinefelter et al. 1943). However, Reifenstein (1946) is generally credited with the classic elucidation of FHA, a syndrome he termed "psychogenic" amenorrhea in recognition of the etiological role of psychological disturbances. Reifenstein speculated that psychic trauma induced a defect in the release of nerve impulses from the hypothalamus, resulting in a failure of the pituitary to release adequate amounts of LH. He also speculated that counseling or improvement of an adverse environment would ameliorate the syndrome. To a great extent, these three factors (the importance of psychogenic stimuli in etiology, the hypothalamic origin of the defect, and the potential reversibility of the syndrome) remain central features in our understanding of FHA (e.g., Berga et al. 2003).

The prevalence of FHA varies considerably, depending on the population under consideration. In industrialized countries, for example, the syndrome is estimated to affect between 2 and 4% of randomly selected women less than 40 yr of age (Drew 1961; Fries et al. 1974; Gregory 1957;

Münster et al. 1992); this figure may increase to as much as 7% in women younger than 25 (Münster et al. 1992). A classic review by Drew (1961) documents the rate to be even higher in certain subgroups, such as women going away to college for the first time and those joining the armed forces. It is also high among women with depression, regardless of age (Bisaga et al. 2002; Harlow et al. 2003). Even more substantial than the variation observed across groups under normal living conditions are the effects associated with wartime experiences, whether in war factories, war-related work on farms, or internment camps.

In such circumstances, FHA prevalence, which may surpass 70%, increases proportionately with the degree of stress (Drew 1961). Wartime data suggest two additional, fundamental characteristics of FHA: (1) onset of the syndrome appears to coincide with the exposure to stress and to precede any malnutrition or dietary restriction (Whitacre and Barrera 1944); and (2) as observed in virtually all populations, there is considerable individual variation in response to stress.

Although environments characterized by lack of personal control and social separation are most potent in disrupting reproductive function, some women—those who are perfectionists, "eager to please," and have difficulty coping—are particularly vulnerable to such effects (Berga and Girton 1989; Giles and Berga 1993; Marcus et al. 2001). Unfortunately, most investigations compare women with established reproductive dysfunction against controls, potentially obscuring the initial contributing factors. However, a prospective observational study of college students before and after beginning work on an Israeli kibbutz revealed that the girls who later became amenorrheic were more anxious, stubborn, and perfectionist in their approach to life than were girls who retained normal function (Shanan et al. 1965).

Exercise, Diet, and Energy Balance

The preceding paragraphs emphasize the role of psychogenic factors in triggering FHA. However, there is considerable evidence that excessive exercise and disordered eating also disrupt the GnRH pulse generator and induce FHA (Hirvonen 1977; Perkins et al. 2001; Warren and Fried 2001). Among athletes, for example, the prevalence of amenorrhea is typically 25% or more, especially when the women engage in weight-bearing sports (e.g., ballet, running) that emphasize lean body mass (reviewed by Warren and Fried 2001). As with psychogenic amenorrhea, there is considerable individual variation in the reproductive response to physical activity, even among elite athletes; and some individuals are resistant to cyclic alterations regardless of exercise level (Loucks and Thuma 2003; Loucks et al. 1989).

Although most data on athletes are observational, Bullen and colleagues (1985) evaluated menstrual disorders in untrained women exposed to a progressive program of strenuous exercise. This landmark study showed menstrual irregularities to emerge on initiation of the exercise regimen and to increase in severity with increasing exercise demands. By the end of the 2-mo exercise, 13 of 28 women had experienced delayed menses and thus were becoming amenorrheic; within 6 mo after completing the experiment, all women regained normal cycles, again demonstrating the reversibility of the syndrome.

An important result in the aforementioned Bullen study was that the ovarian deficits were more severe and prolonged in the subset of women who were also subjected to mild dietary restriction. Starvation, of course, has long been linked to cessation of reproductive function (e.g., Keys et al. 1950). Furthermore, anorexia nervosa—the psychiatric disorder leading to maintenance of abnormally low body weight—is generally associated with prolonged amenorrhea (Ferin et al. 1993). In addition, women with FHA are frequently leaner than age-matched controls (Warren et al. 1999). However, the transition to amenorrhea is not merely secondary to loss of a specific amount of body fat, as suggested by Frisch and McArthur (1974). Rather, reproductive abnormalities begin developing in advance of changes in body composition (Bonen 1994). In fact, emerging evidence suggests that disordered eating patterns, even those not causing significant weight loss, contribute substantially to FHA (Pirke et al. 1985; Warren et al. 1999). For example, women with FHA consume less fat and fewer carbohydrates than controls and are physically more active, even if not engaging in formal exercise (Miller et al. 1998; Warren and Fried 2001). The combination of altered eating patterns and increased physical activity may lead to a state of net negative energy balance that markedly increases vulnerability to reproductive deficits.

A prospective experiment conducted on normally cycling women whose physical activity (caloric expenditure) remained constant while caloric intake was altered provides additional evidence concerning the pivotal role of energy balance in the conversion of a normal to an abnormal reproductive state (Loucks and Thuma 2003). In this investigation, LH pulsatility was interrupted abruptly when daily energy intake was restricted by more than 33% (Loucks and Thuma 2003). Although this outcome reflects a relatively intense caloric challenge, the data highlight the role of dietary intake in the maintenance of normal reproductive function.

The foregoing observations have led some investigators to hypothesize that energy imbalance is the primary mediator of FHA, even when psychogenic stress or exercise are also present (Couzinet et al. 1999; Warren and Fried 2001; Warren et al. 1999). Such a conclusion, of course, is unsatisfying because it does not identify the underlying factors initiating the changes in diet and physical activity that result in negative energy balance. There is evidence, for example, that psychological stress can induce disordered eating and thus the metabolic alterations capable of destabilizing reproduction (Berga 1996; Brown et al. 1983). Furthermore, because psychological stress and excessive exercise are fre-

quently accompanied by disordered eating, it is nearly impossible to disentangle their independent contributions to the natural history of FHA.

Clinical Versus Subclinical Manifestations

The prior discussion focused on amenorrhea, the most obvious sign of reproductive dysfunction. By the late 1940s, however, it had become apparent that functional reproductive deficits sufficient to cause infertility or recurrent abortion could occur in women who appeared to be having regular menstrual cycles (Jones 1949). The subtlest of these abnormalities is a deficit in progesterone secretion during the luteal phase of the cycle ("luteal phase deficiency" [LPD[1]]), initially discovered through careful daily measurement of temperature and urinary pregnanediol (a metabolite of progesterone) (Ginszberg 1992; Jones 1949; McNeely and Soules 1988). Later studies revealed that the corpus luteum in such cases is characterized by multiple hormone deficits, including low estradiol and inhibin concentrations (Soules et al. 1989a,b). The absence of an increase in basal body temperature during the luteal phase is another sign of LPD.

A more significant reproductive deficit observed in women appearing to menstruate regularly is anovulation, indicated by either markedly suppressed luteal progesterone concentrations or the absence of an LH surge at midcycle (Berga 1996; De Souza et al. 1998). Technically, anovulation occurs when no follicle fully matures or when a follicle matures and then dies. Depending on the degree and timing of follicular maturation, there may be fluctuations in estradiol secretion that intermittently stimulate and then destabilize the endometrium and result in vaginal bleeding that is interpreted as normal menses (Berga 1996; Ferenczy 2003; Guyton and Hall 2000). Variant anovulatory conditions, such as luteinized unruptured follicle syndrome, may also be accompanied by cyclical hormone fluctuations capable of producing menstruation (LeMaire 1987; Yen 1991). Finally, anovulation is also sometimes used (inaccurately) to refer to release of an immature egg from an incompletely developed Graafian follicle (Vaitakaitus 1997). Fertility would thus be grossly impaired, although affected women may still menstruate as if cycles were normal.

Because they are subclinical entities, LPD and anovulation are inherently difficult to study. These syndromes can only be detected through repeated hormonal evaluation or accurate daily temperature readings, requirements that generally preclude investigation in large populations. As a result, knowledge of the epidemiology of LPD and anovulation is somewhat limited. The studies of De Souza and colleagues (De Souza et al. 1998, 2003) suggest that under some conditions (among women engaged in recreational exercise, for example), these defects are quite common. In one of these investigations, the reproductive hormones of 24 recreational runners were compared with those of 11 sedentary controls across three consecutive menstrual cycles by means of daily urine samples. All individuals were cycling regularly. Notably, fully 57% of cycles in runners were abnormal (45% LPD, 12% anovulatory), an outcome related to blunted FSH elevation in the luteal-follicular transition. A follow-up investigation compared 20 recreational runners and 10 sedentary controls with respect to reproductive and metabolic hormones across three consecutive menstrual cycles. Again, more than half of cycles in exercising women were abnormal, with evidence that the abnormalities were induced by intermittent negative energy balance.

A study by Prior and colleagues (1990) also suggests an unexpectedly high incidence of subclinical ovarian deficits. These investigators assessed menstrual cyclicity in 66 women known to have had two consecutive ovulatory cycles immediately before entering the study. Approximately one third of the women were elite exercisers (training for a marathon), one third ran regularly but less intensely, and the remainder was relatively sedentary. Menstrual function was evaluated by daily temperature readings across a 1-yr period, and abnormalities were defined by anovulation or brevity of the luteal phase. This study revealed a relatively high incidence of ovulatory disturbances; approximately 30% of all cycles were either anovulatory or evidenced LPD. However, unlike the investigations cited above, menstrual cycles of marathon runners did not differ from those of the other two groups in the study, perhaps because all were preselected for good cycle quality. In the absence of an effect of exercise, it is tempting to suggest that variation in cycle quality in this study may have been related to stress or disordered eating, in much the way these factors contribute to FHA.

Studies such as those by Prior and colleagues and De Souza and coworkers are too limited in scope to allow an accurate estimate of the incidence of subclinical ovarian deficits in the general population. However, they do suggest that these disorders are more common than might be suspected by either women or their physicians (Ginsburg 1992). Assessment of reproductive status in rural and agricultural populations in South America, Africa, and Asia provide additional insight into the potential prevalence of subclinical ovulatory disturbance (Ellison et al. 1993). In these investigations, women show significant variability in ovulatory function across seasons, tending to become anovulatory (although still cycling) when workload is high or energy intake reduced, and regaining normal ovulatory function when conditions improve (Ellison et al. 1993, 1989). Heavy subsistence workloads and poor diet often co-occur, making it difficult to identify the primary cause of abnormality in these instances. In one study of Polish agricultural and domestic workers, however, ovulatory function was impaired during parts of the year when workload was heavy, even though the increased energy expenditure was balanced by greater caloric intake. The authors interpret these data as indicating that a heavy workload can independently disturb ovulation.

Other factors may also be at work. For example, the increase in luteal phase basal metabolic rate may tip the balance from normal to impaired ovarian function, even when usual energy expenditure appears to be balanced by caloric intake (Strassman 1996). Psychogenic factors also may contribute to reproductive deficits under subsistence conditions, as work-related physical activity itself is known to be psychologically stressful (Nordstrom et al. 2003; Rothenbacher et al. 2003). The higher incidence of anovulatory cycles among poor Bolivian women compared with their wealthier counterparts (Vitzthum et al. 2002) could similarly indicate a psychogenic effect because low socioeconomic status is often considered a form of psychological stress (Marmot 1999). Although ecological studies such as these have design and measurement limitations, they nonetheless demonstrate that the human female reproductive system is sensitive to environmental perturbation and, importantly, that ensuing deficits are reversible.

Amenorrhea, then, is like the tip of an iceberg, its incidence signaling the likely presence of a far greater number of individuals having reproductive deficits that are not easily discerned, but that nonetheless are indicative of potentially significant physiological disruption. In this context, it is probably useful to consider the environmentally induced functional reproductive conditions described thus far as representing points along a continuum, from relatively common but subtle expressions of reproductive deficit (e.g., LPD), to more significant but less frequent manifestations (anovulation and amenorrhea). It is also likely that amenorrhea itself is preceded by abnormalities in bleeding patterns, such as frequent, irregular bleeding and long intervals between bleeding episodes (oligomenorrhea) (Berga 1996). As environmental stress increases and physiological or psychological coping mechanisms fail, women may move along this continuum from less to more significant dysfunction, even though considerable individual variation in resistance to disruption will characterize most populations (Kelley et al. 1954; Liu 1990).

Mechanisms

Despite decades of study, the physiological causes of functional reproductive deficits are still imperfectly known (Warren 1996). Ignorance in this arena derives in part from difficulty in distinguishing causal neuroendocrine changes from those that occur concomitantly with, or result from, reproductive dysfunction (Liu 1990). Furthermore, virtually all studies compare women with established functional deficits (mostly FHA because this syndrome is more easily recognized than LPD or anovulation) against normal controls. This strategy obscures the temporal order of physiological events and thus represents a significant limitation, because there is no evidence that factors maintaining reduced GnRH drive are the same as those that played a role in the initiation of the disruption (Berga et al. 2000).

An additional complication is that women with FHA and their normal controls are not differentiated only by reproductive characteristics. Rather, FHA is frequently associated with global hormonal dysregulation, as reflected in HPA activation and associated increases in opioidergic tone, depressed thyroid function, reduced concentrations of the peptide leptin (released by fat cells), reductions in the concentration of the neurosteroid allopregnanolone (a metabolite of progesterone), and altered activity of the inhibitory gamma aminobutyric acid-a ($GABAa^1$) receptor system (Berga 1996; Berga et al. 1989; Dominguez et al. 1997; Laatikainen 1991; Suh et al. 1988). Furthermore, although women with FHA often have a body mass index that falls within the normal range, fat-to-lean mass ratios are generally lower that those observed in normal controls. Studies with anovulatory or LPD women indicate similar, albeit less pronounced, physiological alterations (e.g., Loucks and Thuma 2003). Finally, it should be noted that different types of stressors or challenges may occasion a slightly different balance of neurohumoral aberrations, resulting in a presentation that is not uniform across women or situations (Berga 1996; Berga et al. 1997; Warren 1996).

Perhaps the most prominent and easily assessed neuroendocrine correlate of FHA is HPA activation, as indicated by increased blood concentrations of cortisol, the adrenal hormone that orchestrates the body's metabolic response to stress (e.g., Berga et al. 1997; Liu 1990; Tsigos and Chrousos 2002). This increase in cortisol is initiated by a stress-induced release of corticotropin-releasing hormone (CRH^1) from the hypothalamus and subsequent pituitary secretion of adrenocorticotropin hormone ($ACTH^1$). Importantly, a large body of experimental and observational data shows CRH and cortisol capable of disrupting the reproductive axis (Chrousos 2000; Chrousos et al. 1998; Loucks and Thuma 2003; Tisigos and Chrousos 2002). Furthermore, metabolic deficits (calorie restriction), strenuous exercise, and psychological stress—each of which is associated with FHA—can stimulate HPA activation. In addition, when FHA reverses, HPA function generally becomes normal before any improvement in reproductive condition (Berga et al. 1997). Based on these observations, numerous investigators have suggested that functional reproductive deficits result primarily from direct actions of the HPA hormones, probably modulated by a CRH-stimulated increase in the opioid beta-endorphin (Ferin 1999) (Figure 3).

Research conducted since the early 1990s suggests that the causal train of events is likely to be considerably more complicated than that depicted in Figure 3. First, studies of women with FHA, compared with controls, reveal no difference in either CRH or beta-endorphin (as measured in cerebrospinal fluid), despite a significant elevation in cortisol (Berga et al. 2000). This observation indicates that there is increased sensitivity at the level of the adrenal gland or dysregulation in glucocorticoid negative feedback to the hypothalamus rather than simply an increase in central drive (Berga et al. 2000; Genazzani et al. 2001). Moreover, it

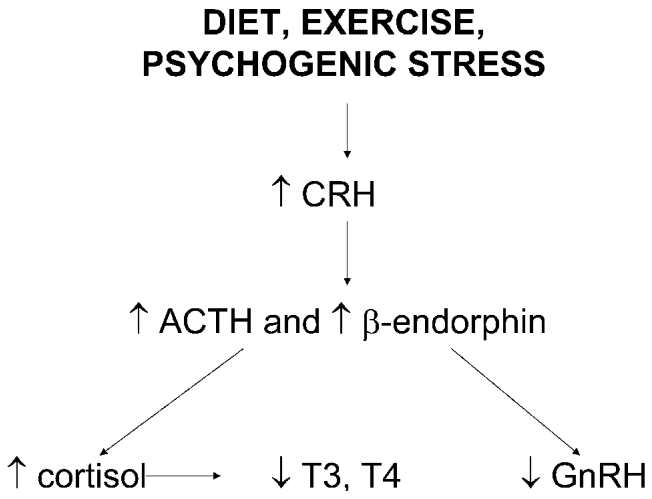

Figure 3 Traditional view of the pathways mediating the disruption of reproductive function by environmental stressors. CRH, corticotropin-releasing hormone; GnRH, gonadotropin-releasing hormone.

remains uncertain whether cortisol is directly responsible for gonadal dysfunction in vivo or is a marker for another agent, since exogenous administration in a dose approximating that elicited by significant stress does not acutely impair reproductive drive (Samuels et al. 1994). Nonetheless, reproductive function is apparently an early casualty of HPA activation, or its concomitants, because gonadal suppression is present in the context of relatively modest cortisol increases (e.g., FHA), as well as in association with conditions characterized by more substantial elevations (anorexia nervosa and melancholic depression) (Gold and Chrousos 2002; Gwirtsman et al. 1989).

Uncertainties regarding the direct role of the HPA axis in reproductive suppression have resulted in attempts to identify other factors that may be involved. Among these is the neuropeptide arginine vasopressin, which can act synergistically with CRH to influence both cortisol secretion and beta-endorphin activity under conditions of stress (Ferin 1999; Chrousos et al. 1998). Moreover, stimulation of GABAa receptors (e.g., by the agonist alprazolam) can normalize LH pulsatility in FHA women. This effect implies a role for GABAergic tone in the maintenance of FHA through its effects on CRH release, GnRH release, or both (Berga et al. 2000; Judd et al. 1995). The recognition that mild, intermittent energy imbalance contributes to the expression of functional reproductive deficits has also focused much interest on leptin, the hormone that promotes satiety but that also may act to suppress the HPA axis and potentiate activity of the GnRH neurons (Barash et al. 1996; Chrousos et al. 1998; Cunningham et al. 1999; Hilton and Loucks 2000; Miller et al. 1998). Notably, leptin is significantly reduced in women with FHA.

As if the picture were not sufficiently complicated, there are yet more substances that may be acting centrally, either to affect GnRH activity directly or to modulate the effect of the aforementioned hormones. The most prominent of these include allopregnanolone (which can be a GABAa agonist) (Genazzani et al. 2002; Meczekalski et al. 2000; Robel and Baulieu 1995), neuropeptide Y (Chrousos et al. 1998; Falsetti et al. 2002; Hilton and Loucks 2000), and the monoamine neurotransmitters dopamine (Liu 1990), norepinephrine (Herbison 1997), and serotonin (Berga et al. 1991). Clearly, a large number of factors potentially interact within the central nervous system, either to initiate or to maintain suppression of GnRH pulsatility. It is perhaps not surprising that a more complete understanding of this phenomenon has thus far eluded investigators.

Public Health Implications

It is often suggested that the physiological alterations accompanying FHA, LPD, and anovulation are adaptive, in that they maintain homeostasis while shifting energy from reproductive activities to those that are necessary to combat life-threatening emergencies (Berga 1996; Chrousos et al. 1998; Dobson et al. 2003; Warren and Fried 2001). For the most part, however, the environments that give rise to FHA and other functional reproductive deficits in industrialized societies do not comprise emergencies. Rather, they represent everyday challenges, in response to which physical and psychological coping mechanisms activate central neural processes sufficient to disrupt ovarian cyclicity in a proportion of individuals.

Emerging evidence suggests that the shift of metabolic resources away from reproduction is not necessarily benign. Individuals may benefit in the short run, particularly in extreme circumstances. However, this otherwise adaptive response could have harmful consequences if prolonged, frequent, or activated inappropriately. Most obviously, conservation of reproductive energy reduces fertility either by preventing fertilization or by impairing implantation of a fertilized ovum (Berga 1996; Ferin 1999). Perhaps less well appreciated, ovarian hormones target the vasculature, skeletal system, and brain in addition to the reproductive tract. It is now suspected that these systems may also be affected adversely by recurrent or sustained deficiencies in reproductive hormones (Berga 2001; Khosla and Bilezikian 2003). An additional consequence is that the neuroendocrine adjustments accompanying functional reproductive deficits may themselves cause damage if they are exaggerated or protracted. Sustained elevations in cortisol secretion, for example, have long been implicated in the exacerbation of cardiovascular disease, osteoporosis, and damage to the central nervous system (Berga 2001; McEwen 2001; Moses et al. 2000; Reid 1997; Troxler et al. 1977).

In fact, considerable data support the view that functional reproductive deficits accelerate the development of chronic disease, thereby adding to the postmenopausal health burden. For example, osteoporotic fracture is a major

cause of morbidity in older women. The prevention or delay of fracture depends on the development and maintenance of adequate bone density. In turn, the development of peak bone density in women is a process that begins peripubertally and persists late into the third decade of life (Bachrach 2001; Bailey 1997; Ott 1990). That this skeletal development is an estrogen-dependent process is evidenced by ontogenetic and experimental data, and by the significant positive correlation that exists between bone density and circulating estradiol in premenopausal women (Dhuper et al. 1990; Riggs et al. 2002; Sowers et al. 1998b; Warren et al. 1991). Moreover, there is a significant decline in bone density after surgical removal of the ovaries (Sowers et al. 1998a). Clinical trials as well as observational studies show lower bone density to be associated with an increased risk of fracture, and estrogen replacement after either surgical or natural menopause inhibits adverse changes in bone density and prevents fractures (e.g., Writing Group for the Women's Health Initiative Investigators 2002).

Of substantial relevance in this context are the numerous studies linking FHA to reductions in premenopausal bone density, particularly among athletes (e.g., Cann et al. 1984; Drinkwater et al. 1984) and ballet dancers (Kaufman et al. 2002; Warren et al. 1986, 1991, 2002). These individuals are at risk of developing a syndrome known as the "female athlete triad," a clinically recognized entity composed of disordered eating, amenorrhea, and osteoporosis (Beals and Manore 2002; Golden 2002). Such abnormalities are not limited only to athletes, because bone density is also reduced in women with FHA that is not of athletic origin (Biller et al. 1991). Notably, the reduction in bone density associated with the female athlete triad and other types of amenorrhea is significant enough to increase the risk of premenopausal fracture (Davies et al. 1990; Warren et al. 1986), which is also associated with increased risk of postmenopausal fracture (Hosmer et al. 2002). Such observations have alarmed pediatricians, who have noted a marked increase in the incidence of functional reproductive impairments among their adolescent and young adult patients (Dhuper et al. 1990; Gordon 2000).

Although there is general agreement that FHA is associated with a significant reduction in bone density, the risks posed by subclinical functional deficiencies—LPD and anovulation—are less clear. For example, in the Prior et al. (1990) premenopausal study described above, loss of bone density at the end of 1 and 5 yr of follow-up correlated positively with the extent of LPD observed during the initial year of evaluation. However, two subsequent studies failed to confirm an association between subclinical, functional reproductive deficits, and bone loss (De Souza et al. 1997; Waller et al. 1996). The numerous differences in study design and population among these investigations could explain the different outcomes. Hence, the aforementioned De Souza et al. study examined women a decade younger than those assessed by Prior and colleagues, and evaluated these women for only 3 mo. In the Waller study, baseline bone density determinations and assessment of menstrual characteristics were not contemporaneous, and LPD was observed in only 5% of cycles, compared with 30% in the Prior investigation. Perhaps the most reasonable conclusion from these studies is that the current data are still inadequate to determine whether subtle ovarian deficits are associated with premenopausal bone loss.

Coronary heart disease comprises a second major portion of the postmenopausal health burden, because it is the largest single cause of death in older women (Anderson 2001). This statistic may be somewhat surprising in view of the commonly held belief that women enjoy some degree of protection against CHD compared with men. In truth, this protection is expressed as a relative, approximately 10-yr delay in disease onset (Higgins and Thom 1993; McGill and Stern 1979). Consequently, CHD incidence is relatively low in premenopausal women, but increases steadily after menopause. The various vascular and metabolic effects of endogenous estrogen are believed to account for most of the sex difference in the rate of development of CHD (Manson 1994; Mendelsohn and Karas 1999). That removal of the ovaries is accompanied by a dramatic increase in the risk of CHD, which can be mitigated by estrogen replacement, provides additional evidence that estrogen is protective premenopausally (Colditz et al. 1987; Rosenberg et al. 1981; Stampfer et al. 1990).

Irrespective of the relative delay in disease onset, CHD accounts for significant morbidity and mortality in women over 60 yr of age. The underlying cause of CHD is atherosclerosis, the accumulation of fibro-fatty plaques (atheromas) within the artery wall. These lesions develop as a result of inflammatory and immune reactions provoked by risk factors such as elevated plasma cholesterol and hypertension (e.g., Greenland et al. 2003; Hansson 2001; Khot et al. 2003; Libby 2002). Stress-induced activation of the autonomic nervous system and the HPA axis may also contribute to this process (Bairey Merz et al. 2002; Julius 1995; Knox 2001; Troxler et al. 1997). Inasmuch as the underlying lesions of atherosclerosis progress over decades, it is likely that the clinical events occurring postmenopausally have their beginnings in the premenopausal years. This conclusion is supported by the most recent report of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study, an autopsy assessment showing that by 34 yr of age, at least one third of all women (regardless of race) have raised lesions in their coronary arteries (Strong et al. 1999). In vivo arterial imaging also demonstrates relatively extensive focal atherosclerosis in premenopausal women (Sutton-Tyrrell et al. 1998; Tuzcu et al. 2001). We hypothesize that by reducing endogenous estrogen, functional reproductive deficits may be responsible for the accelerated atherosclerosis observed in some premenopausal women, predisposing affected individuals to the clinical manifestations of CHD in later years.

Substantial impediments challenge the testing of an hypothesis that FHA or related deficits hasten the development

of atherosclerosis in young women and thus put them at increased postmenopausal risk. Perhaps the biggest challenge for any study is the difficulty in assessing ovarian status (especially LPD and anovulation) of women in a group large enough to serve as the basis for a cardiovascular investigation. Another issue relates to the choice of cardiovascular endpoint. Although it might be ideal to study the relation between premenopausal ovarian status and the occurrence of postmenopausal clinical events (e.g., anginal pain, myocardial infarction, and sudden coronary death), such investigation would require decades to accomplish. Direct measures of atherosclerosis extent, as by coronary angiography or intravascular ultrasound, are also problematic, because they are invasive and would thus be limited to the relatively small number of premenopausal women referred for diagnostic evaluation due to symptoms consistent with myocardial ischemia. Alternatively, surface ultrasound could be used to evaluate atherosclerosis noninvasively in the carotid arteries (as a surrogate for the coronary vessels). This approach was used in the Healthy Women Study to assess atherosclerosis across the menopausal transition, beginning when women were between 42 and 50 yr of age (Matthews et al. 2001). To be useful for the purposes described here, however, it would be necessary for such measurements to begin at about 25 or 30 yr of age, occur in combination with reproductive hormone assessments, and persist for at least 25 yr.

In view of the foregoing difficulties, it is perhaps not surprising that there are only a few studies that shed light on the relation between premenopausal hormonal status and cardiovascular risk. Three of these investigations evaluated cardiovascular endpoints in relation to a history of menstrual irregularity. By far the largest of these was the Nurses Health Study, which for 14 yr followed 121,700 nurses aged 30-55 yr at intake. Women reporting usually irregular or very irregular cycles had significantly increased risk for fatal or nonfatal CHD compared with their regularly cycling counterparts (Solomon et al. 2002). Similar findings were made in two smaller studies. The first of these reported that 202 premenopausal women with confirmed myocardial infarction had a significantly higher lifelong incidence of irregular menstrual cycles than was observed in 374 controls (La Vecchia et al. 1987). A later, smaller investigation documented an association between menstrual irregularity and atherosclerosis extent in the uterine artery of 64 women undergoing hysterectomy (Punnonen et al. 1997). Unfortunately, the interpretation of these studies is uncertain, because all were based on patient recall of menstrual history rather than on hormonal determinations. As a result, it is unclear whether the menstrual irregularity was secondary to a functional hypothalamic deficit and thus reflected estrogen deficiency, or was due to some other abnormality such as PCOS, a condition characterized by elevated androgens rather than reduced estradiol. Of the two, PCOS is generally thought to be more prominent as a cause of the kind of irregularity reported in studies using patient recall.

The final and most intriguing evidence in this arena is derived from two investigations that focused on the relatively rare situation in which premenopausal women underwent invasive cardiological assessment (cardiac catheterization and cineangiography) owing to suspected CHD. Albeit small, both studies evaluated estrogen levels with respect to angiographically confirmed coronary disease. In one of these investigations, the Women's Ischemia Syndrome Evaluation (WISE[1]), the estrogen status of 13 premenopausal women with documented coronary disease was compared with that of their 83 premenopausal counterparts in whom angiographic evaluation was negative (Bairey Merz et al. 2003b). Notably, the women with coronary disease had significantly lower estradiol, estrone, and FSH levels than controls. This association between estrogen deficiency and the presence of coronary atherosclerosis was independent of other risk factors. The authors speculated that stress-induced, hypothalamic disruption caused the relative estrogen deficiency and contributed to the observed coronary disease in affected women. Data from the same study also revealed that postmenopausal coronary artery disease was less severe among women who previously had used oral contraceptives than among those who had not, providing additional evidence that premenopausal estrogen exposure is cardioprotective (Bairey Merz et al. 2003a).

The second study compared 14 women who had confirmed CHD with 14 controls who had not been subjected to angiography (Hanke et al. 1997). Again, the women with CHD were estrogen deficient relative to controls, leading the investigators to suggest that decreased circulating estrogen increases risk of cardiovascular disease in premenopausal women. It is perhaps worth noting in this context that lack of control over the environment—a predisposing factor for FHA—has also been identified as a primary behavioral risk factor for CHD in women (Haynes and Feinleib 1980). The foregoing studies thus provide clinical evidence supporting the hypothesis that premenopausal estrogen deficiency, perhaps induced by psychological stress, accelerates atherogenesis.

Unanswered Questions

Taken together, the available data suggest that ovarian impairment secondary to psychosocial, metabolic, or exercise stress may accelerate premenopausal bone loss and atherosclerosis. However, existing studies leave several clinically relevant questions unanswered:

- How prevalent are subclinical reproductive deficits? The public health significance of these conditions depends, in part, on the number of women affected. Unfortunately, there are no studies determining the incidence of LPD and anovulation in a reference population of normal women (Ginsburg 1992). The sub-

groups typically evaluated (i.e., women experiencing infertility or habitual abortion, athletes, and recreational exercisers) exhibit an incidence ranging from 10 to 50%.

- How much do FHA, anovulation, and LPD contribute to the development of postmenopausal osteoporosis and CHD? Osteoporosis and atherosclerosis develop relatively slowly. Yet, studies of bone loss in women have generally been limited to comparisons of bone density in reproductively impaired, premenopausal women, relative to controls; these largely retrospective comparisons are done either at a single point in time or over a relatively short period. Studies of heart disease are similarly limited, either by use of imprecise measures of ovarian function, or by retrospective comparison of estrogen status in disease-free controls with estrogen in a small number of premenopausal women with confirmed disease. The impact of functional reproductive deficits (premenopausal) on the risk of postmenopausal osteoporosis or CHD is thus largely unknown.
- Do psychogenic factors and exercise contribute independently to the etiology of functional reproductive disorders, or is negative energy balance the necessary and sufficient condition? With few exceptions, relevant studies have been limited to those comparing the dietary, behavioral, and physiological characteristics of women with functional deficits against controls; etiology has generally not been examined. Where such an evaluation has been done (starting with habitually sedentary, nondieting individuals), substantial dietary restriction was required to induce a change in LH pulsatility (Loucks and Thuma 2003).
- Why are some women vulnerable to ovarian disruption while others subjected to the same environmental conditions are resistant? Numerous studies of women engaged in strenuous exercise illustrate this phenomenon particularly well: Some individuals become amenorrheic rapidly, others develop LPD but continue to menstruate, and the remainder seems unaffected. Identifying factors responsible for vulnerability and resistance could greatly facilitate intervention or prevention.

The foregoing questions are unlikely to be addressed in human studies due to logistical challenges and ethical constraints. The use of appropriate animal models offers a potential alternative methodology that allows investigators to exert experimental control over relevant environmental variables, manipulate suspected risk factors, employ invasive techniques of measurement, and evaluate prospectively the development of health-related consequences of functional reproductive deficits. The following sections review contributions made by such studies. These sections focus on nonhuman primates, which resemble humans in reproductive characteristics, behavior, and disease susceptibility, and thus have been particularly useful in such investigations.

Use of Nonhuman Primates to Model the Etiology and Sequelae of Functional Reproductive Deficits

Substantial reproductive differences exist among the numerous orders of mammals, making some more useful than others as human surrogates for investigating the causes and consequences of reproductive dysfunction. For example, ovulation in response to mating behavior ("reflex ovulation") occurs in species as diverse as rabbits, mink, ferrets, voles, domestic cats, and camels. In contrast, the surge in gonadotropins resulting in ovulation originates internally in rats, pigs, sheep, cows, and human and nonhuman primates, leading to their designation as "spontaneous ovulators" (Ferin et al. 1993). The development of the corpus luteum also varies; it may either be induced by stimulation of the cervix (rats, mice, hamsters) or occur spontaneously irrespective of sexual stimulation (sheep, cows, primates). Menstruation—the monthly reproductive cycle distinguished by endometrial destruction and several days of uterine hemorrhage, and persisting in the absence of pregnancy—is perhaps the most taxonomically constrained mammalian reproductive characteristic, because it is limited to Old World monkeys, apes, and women (Zuckerman 1930; Zuckerman and Parks 1932). A considerable body of research, especially in the genus *Macaca,* demonstrates that the menstrual cycles of women and other Old World anthropoid primates are remarkably similar in overall plan, timing of constituent events, and hormonal profile (Corner 1923; Knobil 1988; Zuckerman 1930).

In view of their many shared reproductive similarities, it is perhaps not surprising that the same kinds of functional menstrual deficits observed in women occur also in monkeys. In fact, what appears to be the first description of reversible hypothalamic anovulation was published with reference to rhesus monkeys, not women (Corner 1927; Corner et al. 1945). Subsequent studies make clear that monkeys, like women, experience deficits ranging from LPD and anovulation to oligomenorrhea and amenorrhea (Wilks et al. 1976, 1977; Williams and Hodgen 1982). As in women, disruption of gonadotropin release is thought to be the usual proximate cause of these reproductive abnormalities (Wilks et al. 1976). Taken together, existing evidence suggests that macaque monkeys are well suited to model reproductive phenomena in relation to risk of chronic disease. Most such studies have been performed in either rhesus (*Macaca mulatta*) or cynomolgus (*Macaca fascicularis*) macaques, which closely resemble each other in reproductive characteristics (Williams and Hodgen 1982).

Importantly, female rhesus and cynomolgus monkeys also have been used extensively to model the development of both osteoporosis and atherosclerosis. As in women, bone development accelerates peripubertally in these monkeys, with final closure of the epiphyses and attainment of peak bone mass in both rhesus and cynomolgus monkeys occurring by about 10 yr of age (Cerroni et al. 2000; Hotchkiss et al. 2001). At this point in development, the monkeys

are probably equivalent to 30-yr-old women. After monkeys have reached peak bone mass, surgical menopause (bilateral ovariectomy) induces a rapid decline in bone density (Hotchkiss et al. 2001; Jerome 1998). This loss can be prevented by estrogen treatment, a result similar to that observed in postmenopausal women (Hotchkiss et al. 2001; Wells et al. 2002).

Rhesus and cynomolgus monkeys are useful in the investigation of atherogenesis, because when fed diets that elevate blood lipids, they develop lesions that are similar in morphological characteristics and location to those seen in humans (Kaplan et al. 1985). Moreover, lesion development progresses in macaques through the same stages and occurs in the same pattern as in humans. Thus, atherosclerosis develops first in the aorta and the proximal portions of the main branch coronary arteries and later in the common and internal carotid arteries. Notably, studies in cynomolgus macaques also reveal that males develop more extensive atherosclerosis than do reproductively intact (i.e., premenopausal) females (Hamm et al. 1983), and males experience myocardial infarction at a rate similar to that seen in their human counterparts (Bond et al. 1980). The smaller size and greater commercial availability of the cynomolgus monkey make it somewhat more useful than the rhesus, and it has become the species of choice in cardiovascular studies.

Conceptually, the behavioral homologies between people and monkeys are as relevant to understanding the disease consequences of functional ovarian deficits as are the reproductive and pathobiological similarities described in the previous paragraphs. This relevance is based on the fact that anthropoid primates are obligate social animals, which reference their behavior to other members of their social community rather than to a specific location or environmental resource. As a result, information concerning quality of life is not derived solely from the physical environment, but instead, is also filtered through the constant social communication that marks the existence of every individual (e.g., Dittus 1977). Macaque social groups, for example, are characterized by elaborate patterns of positive social interaction, including generation-spanning networks of affiliation, alliance, and mutual support, as well as prominent and well-defined social status ("dominance") relationships and hierarchies (Kaplan 1987). Notably, the macaques (and closely related baboons) are unique among nonhuman primates in the breadth of environments they occupy (ranging from tropical lowland to desert to temperate montane), an ecological aggressiveness made possible in part by an elaborate group-dwelling social adaptation characterized by intense conflict and confrontation (Kaplan 1977; Rowell 1971). This confrontational social adaptation, in turn, subsumes behaviors (competitiveness and aggressiveness vs. lack of control and submission) analogous to those thought to confer increased risk for CHD in humans (Kaplan and Manuck 1999; Kaplan et al. 1985). To the extent that these behaviors and associated social environments disrupt menstrual cyclicity in some individuals, they similarly may be associated with an increased risk of osteoporosis (Gordon 2000).

Etiology of Functional Reproductive Deficits Observed Experimentally in Monkeys

Role of Metabolic and Exercise Stress

Severe nutritional restriction (e.g., starvation) and conditions like anorexia nervosa suppress reproduction in monkeys as well as women (Drew 1961; Dubey et al. 1986; Keys et al. 1950). However, the functional reproductive deficits occurring most frequently in women reflect relatively mild patterns of disordered eating, because adverse changes in reproductive function occur well in advance of changes in body mass or composition (e.g., Laughlin et al. 1998; Loucks et al. 1992). In male rhesus monkeys, a significant reduction in LH pulsatility occurs after a single missed meal, indicating that even a mild change in energy availability can have reproductive consequences for primates (Cameron 1996; Cameron et al. 1993). Interestingly, women in the follicular phase of the menstrual cycle and ovariectomized monkeys are more resistant than males to the effects of short-term dietary restriction (Cameron 1996). This resistance appears to extend also to reproductively intact female rhesus monkeys subjected to chronic dietary stress (Roberts et al. 2001). The most extensive published study assessed the effects of a 30% energy restriction (compared with controls) maintained for 6 yr in animals averaging 14 yr of age (Lane et al. 2001). This research revealed that although body weight and fat mass were substantially lower in the energy-restricted animals, there was no obvious effect of dietary restriction on bone density, blood markers of bone metabolism, or follicular phase FSH, LH, estradiol, or progesterone. Furthermore, the number and length of menstrual cycles were not altered by the dietary manipulation. However, it is not known whether this degree of energy restriction can induce mild LPD, because luteal phase hormones were not assessed and follicular hormones were evaluated only on day 5 of each cycle.

Among women, the effect of disordered eating on the reproductive system is most prominent in individuals engaged in recreational or competitive sports. The studies of Williams and colleagues are particularly informative in this regard, because they prospectively investigated the impact of strenuous physical activity (running) on reproductive function among initially normally cycling cynomolgus monkeys consuming a constant number of calories daily (Williams et al. 2001a). The investigators trained eight monkeys to run on a treadmill for 2 hr per day, 7 days per week; when fully trained, the animals were running approximately 12 km/day. Blood samples for the determination of reproductive hormones were collected from the runners and their eight sedentary controls every other day while animals were briefly restrained without anesthesia. Animals were housed individually, which precluded usual patterns of social interaction. Amenorrhea occurred in all exercising animals (but no sedentary controls), although the onset varied from 7 to 24 mo after the start of training. There were no

significant differences in cycle characteristics between running and sedentary animals until two cycles before the occurrence of amenorrhea. At that time, the runners exhibited lengthened cycles that tended to be anovulatory and deficient in luteal progesterone, changes that appeared to be secondary to suppression of gonadotropin release. The three notable findings in this study were that (1) the transition to amenorrhea was abrupt; (2) there was considerable variability in the onset of reproductive dysfunction; and (3) the running monkeys did not lose weight relative to controls despite consuming the same number of calories, implying a metabolic adaptation to exercise.

In a follow-up study, the investigators tested the hypothesis that the observed exercise-induced amenorrhea was due to low energy availability (Williams et al. 2001b). To accomplish the test, they assessed the effect of supplementary feeding in four of the amenorrheic runners from the previous investigation. All of the animals had been amenorrheic for at least three cycles before the provision of supplemental food, which was offered in the form of "treats" (granola bars, dried and fresh fruit) and commercial monkey chow. The animals continued exercising throughout the study. Body weight increased significantly, and recovery of reproductive function occurred in all animals, although the rate of recovery was bimodal—two of the monkeys recovered within about 2 wk although the remaining two did not revert to cyclic hormonal activity for almost 2 mo. Of additional interest, the exercising monkeys experienced a significant decrease in circulating triiodothyronine ($T3^1$), a hormone linked to metabolic rate. T3 subsequently increased as diets were supplemented and animals regained cyclic ovarian function. Together, the findings of these two investigations implicate low energy availability as a primary signal that impairs reproductive function in the context of exercise. The reduction in T3 and maintenance of stable body weight in association with running-induced amenorrhea suggest further that strenuous exercise occasions a metabolic shift consistent with energy conservation, at least in monkeys randomly assigned to a running regimen.

Psychosocial Stress

Despite the frequent suggestion that psychogenic factors (including stressful life circumstances, inappropriate coping mechanisms, and excessive anxiety or depression) play a role in the etiology of FHA and related deficits, prospective studies demonstrating that such factors contribute to reproductive dysfunction in women are almost entirely lacking. In part, the paucity of direct evidence reflects the difficulty in determining the initiating cause of reproductive dysfunction once it is established. An additional problem is that stress is not easily defined or quantified. Finally, ethical constraints preclude prospective assignment of women to stressful procedures of sufficient length and intensity to cause reproductive deficits.

Studies in nonhuman primates have investigated some of the factors described above. In a study in which moderate stress was the scientific object of the study and that involved 11 normally cycling, adult rhesus monkeys, Xiao and colleagues (2002) evaluated stressor effects on animals that were housed in individual cages and accustomed to being restrained daily without anesthesia for vaginal swabbing and blood collection. The animals had undergone a brief surgical procedure, and then had experienced stress consisting of physical restraint by a head cap and tethering system that in each instance lasted 12 days. The procedure involved attachment and removal of the head cap, and placement in a new housing room occupied by social strangers. The animals were divided into two groups, with one exposed to a stressor during the follicular phase of the menstrual cycle while monkeys in the other group were stressed in the luteal phase. Reproductive hormones, cycle length, body weight, and cortisol from two prior normal cycles were compared with data collected during the stress period and two subsequent menstrual cycles.

In this investigation, stress imposed in the follicular phase induced a decline in LH and progesterone concentrations in the luteal phase of the same cycle. Exposure to stress during the luteal phase led to an immediate reduction in luteal LH and progesterone that persisted into the next menstrual cycle. Across all animals, serum cortisol rose on the first day of stress and remained elevated for 2 wk after the cessation of stress. There appeared to be no change in energy availability, because body weight and food consumption remained constant. The authors interpret their results as demonstrating that stress rapidly induces reproductive dysfunction similar to LPD. Notably, recovery from stress was not immediate, because effects persisted at least into the next cycle. These data provide initial evidence that psychogenic factors can induce reproductive dysfunction independently of caloric restriction or alterations in physical activity.

Intriguingly, Cameron showed that normally cycling, moderately exercising cynomolgus macaques subjected to mild dietary restriction became anovulatory after exposure to social strangers (analogous to the stressor used by Xiao et al. [2002]). In contrast, neither dietary restriction nor movement to a novel location independently induced reproductive impairments in these exercising monkeys (Cameron 2003). This latter observation seems particularly salient inasmuch as exercise, dietary restraint, and psychogenic factors often occur simultaneously in women.

The studies described above evaluated female macaques maintained under controlled conditions that did not allow for species-typical social interaction. However, there is considerable evidence that certain naturally occurring social behavior, expressed during everyday encounters, suppresses reproductive function in some individuals. Most frequently, females that are subordinate in their groups or genealogies are at the greatest disadvantage (Abbott 1987; Harcourt 1987). In corral-housed rhesus monkeys, for example, subordinate animals have a lower total percentage of ovulatory cycles than do dominants (Pope et al. 1986; Walker et al.

1983). Observations on captive baboons suggest that the menstrual dysfunction observed in subordinate females may relate specifically to extended bouts of harassment received from dominant individuals (Rowell 1970).

Long-term assessment of menstrual cyclicity in several studies of captive cynomolgus macaques housed in social groups each containing four to six animals also illustrates the occurrence of reproductive dysfunction among subordinate monkeys (Kaplan et al. 1996). In one such investigation, luteal phase progesterone concentrations evaluated during every cycle in a 2-yr period among 23 individuals documented that subordinate females had five times as many anovulatory menstrual cycles and three times as many menstrual cycles characterized by luteal phase progesterone deficiencies as their dominant counterparts; moreover, the cycles of subordinates tended to be relatively estrogen deficient (Adams et al. 1985b; Kaplan et al. 1996). The syndrome appeared reversible—similar to what has been observed in women—because the few subordinate animals that became dominant over the course of the study regained normal ovarian function, whereas dominant females losing rank experienced subsequent reproductive impairment. Notably, the occurrence of amenorrhea was equally uncommon in subordinates and dominants.

The observation that subordinates experienced a high incidence of "subclinical" functional deficits and minimal amenorrhea was replicated in several later studies (Kaplan et al. 2002a; Shively et al. 1997; Williams et al. 1994). Relative to dominants, subordinate females also exhibit an exaggerated cortisol response to adrenocorticotropin challenge after dexamethasone suppression, and on necropsy are found to have enlarged adrenal glands (Adams et al. 1985a; Kaplan et al. 1986; Shively and Kaplan 1984). The latter observation suggests that subordinate animals experience substantial stress (e.g., Abbott et al. 2003), which likely reflects the fact that dominance relationships strongly canalize patterns of social behavior in captivity, with animals of high social status typically monopolizing access to food, space, and preferred social partners (Kaplan et al. 2002b).

In studies of captive cynomolgus macaques, subordinate animals housed in small social groups thus suffer reduced control over their environment, become hypercortisolemic, and tend to be (reversibly) reproductively impaired. This triad of circumstances closely models the conditions characterizing functional reproductive deficits of women as originally described by Klinefelter and Reifenstein and later elaborated on by numerous other investigators (e.g., review by Berga 1996). Use of this animal model under controlled experimental conditions therefore affords an opportunity to evaluate potential pathogenic effects of a syndrome analogous to the psychosocially related menstrual deficits affecting women. In contrast to human studies, which link functional ovarian deficits most clearly to bone loss, the majority of investigations using monkeys have focused on the development of atherosclerosis. In the text below, studies on coronary atherogenesis are considered first, followed by a description of preliminary findings relating to bone loss.

Stress, Functional Reproductive Deficits, and Atherosclerosis in Cynomolgus Monkeys

Commonalities of Study Design

The studies described in the ensuing sections share the following investigational strategies relating to diet, housing, reproductive manipulations, and the assessment of social status and atherosclerosis:

- The experimental diet mimics that consumed in industrialized societies—the "typical North American diet." In response to such a diet, monkeys develop substantial atherosclerotic lesions in approximately 2 yr, a duration that provides a compressed time frame within which to observe the pathogenic effects of behavioral and hormonal risk factors.
- Monkeys are housed in numerous small social groups, each containing four to six individuals. Thus maintained, the animals exhibit the species-typical range of competitive and affiliative behaviors. The most prominent feature of these groups is the dominance hierarchy or pecking order, which is generally linear and transitive in structure and which strongly influences the patterning of most social interactions.
- Studies evaluating menopausal effects on disease use surgical removal of the ovaries to produce a hormone-deficient state. This procedure is necessary because the majority of macaques die before reaching natural menopause (Gilardi et al. 1997). Use of the surgical approach also allows the effects of hormonal deprivation to be evaluated apart from aging.
- The social status (dominant or subordinate) of each animal relative to the others in her social group is based on the outcome of competitive encounters, which are highly asymmetric in this species and yield clear winners and losers as judged by specific facial expressions, postures, and vocalizations (Sade 1973).
- Atherosclerosis extent is quantified morphometrically in the coronary, carotid, cerebral, and iliac arteries and in the aorta, after necropsy.

Male/Female Differences: Influence of Social and Hormonal Status on Atherosclerosis

An initial investigation showed that premenopausal cynomolgus females, as a group, had less atherosclerosis than similarly aged male monkeys when fed fat-containing diets for an equivalent interval (Hamm et al. 1983). Notably, females identified as "subordinate" (i.e., those falling below the median in winning competitive encounters) had signifi-

cantly more atherosclerosis than their "dominant" (above the median) counterparts. A subsequent study was designed to evaluate the potential hormonal correlates of lesion development (and its retardation) in females, and to attempt to replicate the previously observed association between subordinate social status and atherosclerosis (Kaplan et al. 1984). Here, 23 reproductively intact females were housed together in groups of four or five animals each and fed the North American-like diet for 2 yr. Social behavior and gonadal hormones were monitored across this period. In addition to the females, there was a comparison group of 15 socially housed, adult males. As expected, the females developed significantly less coronary artery atherosclerosis than similarly treated males. However, this was true only for dominant females; the atherosclerosis of subordinate females and males was indistinguishable (Figure 4), thus confirming our initial observation that female "protection" does not extend to animals of subordinate status.

The effects of status on atherosclerosis could not be explained by concomitant variation in blood lipids. As described above, however, subordinate monkeys had fewer ovulatory cycles, a greater percentage of cycles with LPD, and reduced circulating concentrations of estradiol compared with dominants (Adams et al. 1985b). Subordinates also had larger and more responsive adrenal glands than dominants (Kaplan et al. 1986). These observations raised the possibility that either the relative ovarian impairment or adrenal hyperactivity of subordinate animals might explain their loss of "female protection."

Relevant in this regard is evidence from two related studies of animals consuming the same diet for the same duration. In one study, ovariectomized, dominant monkeys were found not to differ from subordinates in end-of-study atherosclerosis. Instead, lesion extent in both was approximately equivalent to that observed in reproductively intact subordinates (Figure 5). This finding suggests that ovariectomy eliminates the "protection" against diet-induced atherogenesis typically experienced by dominants (Adams et al. 1985a). As depicted in Figure 5, we also observed that repeated pregnancy—a hyperestrogenic state—almost completely inhibited the development of atherosclerosis, irrespective of social status (Adams et al. 1987). The provision of exogenous estrogen to ovariectomized monkeys similarly inhibits the development of lesions in both dominant and subordinate individuals (Adams et al. 1990).

Variation in social and hormonal status is also associated with functional changes in arteries, as measured by cineangiography. In a study of premenopausal animals consuming the North American diet for over 2 yr, for instance, the coronary arteries of subordinate monkeys showed an impaired ability to dilate in response to a vascular challenge compared with dominants. Moreover, circulating estradiol correlated inversely with arterial impairment, further implicating estrogen deficiency as a major culprit in the premenopausal acceleration of vascular disease (Williams et al. 1994).

Influence of Premenopausal Behavioral and Hormonal Conditions on Pre- and Postmenopausal Atherosclerosis

If ovarian impairment accelerates atherogenesis in premenopausal females, one might hypothesize that exogenous estrogen would prove protective, especially among monkeys predisposed by estrogen deficiency—namely socially

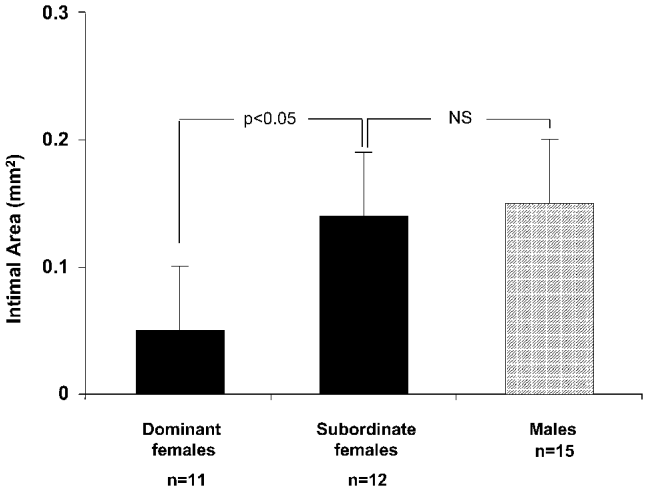

Figure 4 Coronary artery atherosclerosis extent in socially housed male and female cynomolgus monkeys consuming an atherogenic diet for 2 yr. Animals habitually and predictably winning the majority of their competitive interactions were labeled "dominant." The others were "subordinate." NS, not significant.

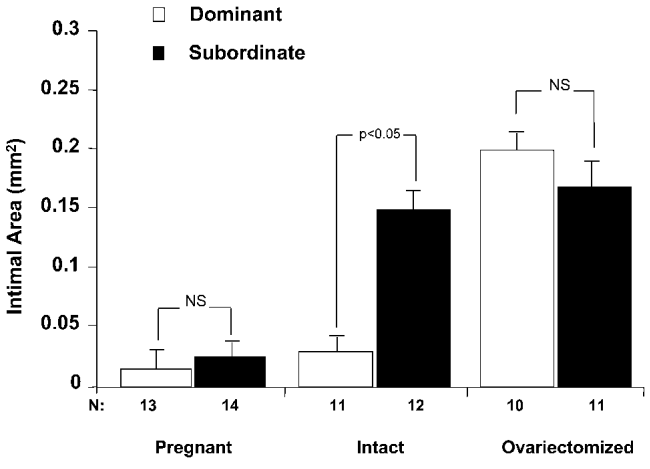

Figure 5 Coronary artery atherosclerosis extent in socially housed female cynomolgus monkeys that for 2 yr consumed an atherogenic diet and were repeatedly pregnant, reproductively intact, or ovariectomized. Animals habitually and predictably winning the majority of their competitive interactions were labeled "dominant." The others were "subordinate." NS, not significant.

subordinate animals. In a test of this hypothesis, 177 premenopausal animals were randomized to consume either the typical North American diet, or the same diet containing an oral contraceptive (OC[1]) (Kaplan et al. 1995). After 2 yr of diet and OC treatment, atherosclerosis was measured in an iliac artery biopsy. Monkeys were then ovariectomized and subsequently studied postmenopausally for 3 yr, during which time one third of the animals received estrogen replacement (conjugated equine estrogens). The premenopausal biopsy data provided in Figure 6 reveal that OC treatment was protective, but selectively so for the subordinate animals. Untreated subordinates developed considerably more atherosclerosis than did dominants, whereas treated and untreated dominants did not differ. As in previous studies, the subordinates experienced more frequent anovulation and greater LPD than dominants, consistent with the hypothesis that social subordination may potentiate atherogenesis via accompanying ovarian impairment.

Data from the postmenopausal phase of the above study provided an opportunity to estimate whether premenopausal behavioral or hormonal status predicts postmenopausal atherosclerosis, and whether any such effect is altered by premenopausal OC exposure or postmenopausal hormone replacement (Kaplan et al. 2002a). Of particular interest was the fate of animals at "high risk" (i.e., subordinates not treated with OC) compared with those at low risk (all others). Indeed, coronary atherosclerosis in postmenopausal monkeys was again predicted by the interaction of social status and OC treatment, just as it had predicted iliac atherosclerosis in the biopsies taken from the same monkeys premenopausally (Figure 7; compare with Figure 6). In other words, the animals at high risk premenopausally (the non-OC subordinates) continued to be at high risk; in contrast, atherosclerosis was inhibited in the low-risk animals (dominants with or without OC treatment, OC-treated subordinates). Furthermore, this effect occurred *irrespective* of the various postmenopausal interventions. The persistence of the premenopausal effects despite a prolonged period of postmenopausal hormone replacement underscores the potential importance of early events in the development of coronary artery atherosclerosis.

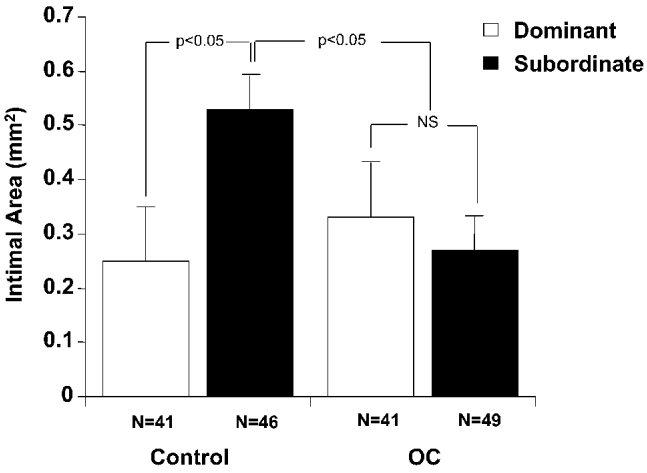

Figure 6 Iliac artery atherosclerosis extent in reproductively intact, socially housed female cynomolgus monkeys that for 2 yr consumed the typical North American diet that for half of animals also contained an oral contraceptive. Animals habitually and predictably winning the majority of their competitive interactions were labeled "dominant." The others were "subordinate." OC, oral contraceptive. NS, not significant.

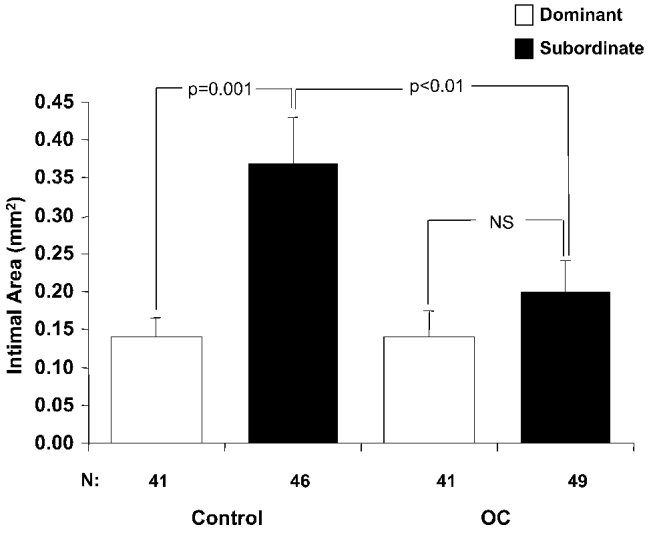

Figure 7 Coronary artery atherosclerosis in surgically postmenopausal cynomolgus monkeys stratified by premenopausal social status and oral contraceptive exposure. Animals habitually and predictably winning the majority of their competitive interactions were labeled "dominant." The others were "subordinate." OC, oral contraceptive. NS, not significant.

The foregoing results support three conclusions: (1) Social subordination places premenopausal females at high risk for development of atherosclerosis; (2) exogenous estrogen (OC) provides selective protection to high-risk monkeys, suggesting that estrogen deficiency mediates the increased risk experienced by these animals; and (3) the heightened risk associated with social subordination and the selective protection offered by estrogen treatment both persist into the postmenopausal period— a persistence that occurs despite the absence of continued OC treatment and the likelihood that social status is no longer influencing atherogenesis (because after ovariectomy, dominants and subordinates are equally devoid of endogenous estradiol).

Two caveats condition the interpretation of the monkey studies. First, although most speculation regarding mechanism has focused on estrogen deficiency, a contribution by other factors (e.g., excessive HPA activation or its concomitants) to the acceleration of atherosclerosis in subordinate, "high-risk" individuals cannot be ruled out. This condition is especially true in light of the observation that subordinates have larger adrenal glands than dominants and frequently show an exaggerated cortisol response to adre-

nocortical stimulation (Kaplan et al. 1986; Wood et al. 2003). Furthermore, the protective effect of pre-and early postmenopausal estrogen suggested by these observations must be reconciled with the results of recent studies conducted on postmenopausal women, which indicate that estrogen has neutral or adverse effects on atherosclerosis in that age group (Writing Group for the Women's Health Initiative Investigators 2002). In this regard, it has been suggested that estrogen inhibits the early (pre- and perimenopausal) development of atherosclerosis, but is ineffectual or even detrimental in the presence of established disease (Hodis et al. 2003; Karas and Clarkson 2003).

Influence of Social and Hormonal Status on Pre- and Postmenopausal Bone Density

As in humans, aging and menopausal status affect bone density (and thus risk of fracture) in macaques (e.g., Colman et al. 1999). However, there are substantial barriers to investigating the effects of premenopausal behavioral and hormonal factors on the establishment and maintenance of postmenopausal bone density, even in nonhuman primates. First, animals must be evaluated over long periods of time to detect significant changes in bone density. Furthermore, a decision must be made whether to conduct studies in the context of natural or surgical menopause. As stated previously, natural menopause in a macaque does not occur until late in life (≈25 yr of age) (Gilardi et al. 1997). A study involving animals in this age range would pose significant logistic, husbandry, and statistical challenges, obstacles that would be amplified by the death of many individuals before or immediately after menopause.

An alternative strategy involves surgical removal of the ovaries. Even this approach is not without problems, because macaques continue accumulating bone until they are 9 or 10 yr of age. It would be difficult to detect effects, especially subtle ones, if surgical menopause were induced in younger animals while bone mass was still increasing (Hotchkiss et al. 2001; Jerome 1998). Ideally, premenopausal assessment would encompass the period after attainment of peak bone mass. Animals could then be ovariectomized and followed for at least 1 or 2 yr, allowing the effects of both pre- and postmenopausal conditions to manifest fully.

Given the numerous challenges, it is perhaps not surprising that the ideal prospective study has not been conducted. Although problematic, in part because many animals had not yet reached full skeletal maturation at the start of the investigation and because there was no initial randomization for bone density, the pre- and postmenopausal "life course" study described above provides initial data for assessing the effect of premenopausal conditions on postmenopausal outcome. Toward this end, spinal bone mineral density was evaluated on several occasions using dual energy, X-ray absorptiometry. An exploratory analysis covaried body mass and considered bone density measured at the end of the postmenopausal phase of the study in relation to premenopausal social status and OC exposure.

The results of this analysis, shown in Figure 8, indicate less bone mineral density in subordinates than dominants, irrespective of OC exposure. This finding was not unexpected, at least with respect to the untreated subordinates, because these animals were estrogen deficient relative to dominants and thus may have failed to achieve maximum bone density before ovariectomy. Such an explanation, however, does not suffice for the subordinates that were exposed to exogenous estrogen in the form of OCs, a treatment that retarded atherogenesis. It may be that the osteopenic effects of the stress-induced hypercortisolemia that typically characterizes subordinate monkeys offset any benefits from OC exposure. Interestingly, OC treatment also fails to increase bone density in women with anorexia nervosa, a condition that similarly involves hypercortisolemia and reproductive suppression (Klibanski et al. 1995).

Assessment and Implications of Nonhuman Primate Studies

Prevalence of Subclinical Deficits

As stated above, the prevalence of subclinical deficits has not been assessed to date in a reference population of North Americans. However, the incidence of LPD and anovulation increases to a surprisingly high level (more than 50%) among one selected subset of women—those who exercise recreationally (De Souza et al. 1998). Although there are no published studies of monkeys exercising "recreationally," cynomolgus macaques running strenuously without an in-

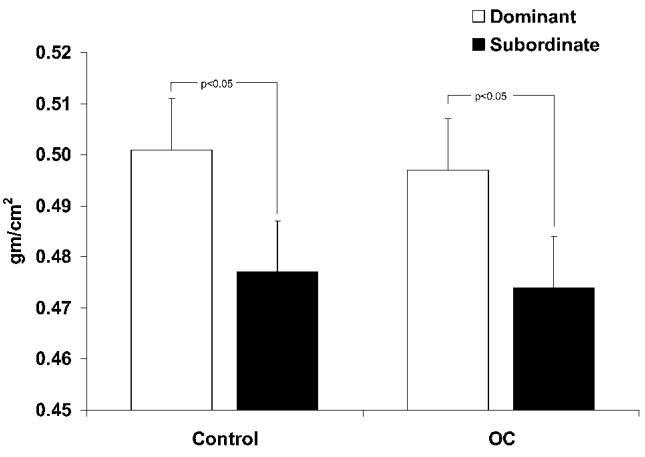

Figure 8 Spinal bone mineral density assessed postmenopausally in cynomolgus monkeys stratified by premenopausal social status and oral contraceptive exposure. Animals habitually and predictably winning the majority of their competitive interactions were labeled "dominant." The others were "subordinate." OC, oral contraceptive.

crease in caloric intake ultimately become amenorrheic (Williams et al. 2001a). First, however, they pass through a stage of LPD and anovulation similar to that observed among women engaged in recreational running. Moreover, there is considerable variability in the time course of this impairment; some monkeys retain normal cyclicity for up to 2 yr whereas others become abnormal in as few as 5 mo. Perhaps monkeys allowed to run at a reduced pace would exhibit subclinical reproductive deficits (like women running recreationally), rather than amenorrhea.

Most North American women do not engage in recreational exercise, even to the extent described above by De Souza and colleagues, and hence are not accurately modeled by strenuously trained monkeys. Cynomolgus monkeys housed in small social groups and consuming a typical North American diet without restriction may better approximate the broad population of nonexercising women. Under such conditions of housing, the incidence of LPD and anovulation is 30%. This average is deceptive, however, because social status influences menstrual cyclicity in these monkeys. Hence, the incidence of subclinical deficits is about 50% among socially subordinate animals, compared with 10% among their dominant counterparts. Interestingly, only 4% of animals—regardless of social status—exhibit FHA, approximating the incidence in randomly assessed populations of women (Drew 1961).

Extending the current observations in monkeys and selected subsets of women to a reference population suggests that the overall incidence of subclinical reproductive deficits is approximately 30%, with deviation from this average related in large part to environmental conditions. Individuals at relatively higher risk would include those engaged in even modest amounts of regular exercise (particularly when associated with any degree of disordered eating) and those at the higher end of exposure to the stresses of daily life. Chronic undernutrition is another factor that greatly increases the incidence of subclinical reproductive impairment, although the impact of this condition is likely greatest in nonindustrialized countries (Ellison et al. 1989, 1993). Interestingly, the 30% incidence of subclinical ovarian deficits estimated from the monkey studies approximates the 12-mo incidence of abnormality among women thought to be cycling normally and selected to represent all levels of physical activity (Prior et al. 1990).

Contribution of Functional Reproductive Deficits to the Development of Postmenopausal Disease

The incidence of heart attacks among women doubles between the ages of 55 and 65, representing the largest single cause of death in this age range (AHA 2002). Epidemiological and autopsy studies reveal that the underlying atherosclerosis likely originates during the premenopausal years (Strong et al. 1978, 1999; Sutton-Tyrrell et al. 1998; Tuzco et al. 2001). Notably, two clinical studies provide evidence that functional reproductive deficits and associated estrogen deficiency contribute to the premenopausal development of disease (Bairey Merz et al. 2003b; Hanke et al. 1997), and a third study indicates that premenopausal OC exposure reduces the severity of postmenopausal coronary disease (Bairey Merz et al. 2003a).

The studies on female monkeys extend these observations by demonstrating experimentally that estrogen deficiency (induced endogenously by social subordination or exogenously by removal of the ovaries) accelerates atherogenesis among premenopausal animals consuming the equivalent of a typical North American diet (reviewed in Wagner et al. 2002). Furthermore, as among the women in the WISE study (Bairey Merz et al. 2003b), atherosclerosis is reduced in postmenopausal monkeys treated with OCs premenopausally (Kaplan et al. 2002a).

Perhaps most importantly, these findings suggest that subclinical, functional reproductive deficits (LPD, anovulation) contribute to the postmenopausal CHD burden of women. More generally, the results suggest that premenopausal estrogen deficiency, *regardless of etiology,* is potentially atherogenic. Of particular concern in this regard is the period immediately before menopause (the "perimenopause," approximately 45-50 yr of age), which affects all women and is marked by follicular exhaustion and abnormalities in the production of estrogen and progesterone (e.g., Burger et al. 2002; O'Connor et al. 1998 and 2001; Recker et al. 2000). It might be suspected that women experience a general increase in vulnerability to atherosclerosis during this period; the risk might be even greater for those who also experienced functional reproductive deficits during their 20s and 30s.

The significance of subclinical reproductive deficits is less clear for bone health than for CHD. Existing studies in women are mixed (e.g., De Souza et al. 1997; Prior et al. 1990), and the data from monkeys are suggestive rather than definitive. As noted above, the life course monkey study does not resolve this issue, because it was confounded by the skeletal immaturity of animals at surgical menopause and by a lack of randomization for this endpoint. It should be noted, however, that women experience substantial bone loss during the perimenopausal years, a phenomenon directly correlated with the increasing estrogen deficiency occurring at the same time (Recker et al. 2000). This observation supports the hypothesis that estrogen deficiency experienced in conjunction with subclinical reproductive deficits may impair skeletal health and thus heighten risk of postmenopausal fracture.

Taken together, the data from randomized studies of nonhuman primates and observational investigations of women raise concern that stress-induced reproductive impairment represents much more than an isolated loss in fertility. It may also confer increased risk of future cardiovascular disease and fracture, and by implication adversely affect other targets of estrogenic activity, including the central nervous system. As a result of their high incidence and potential health consequences, the benefits of

detecting and correcting subclinical ovarian impairments could be substantial. However, detection of such deficits is difficult. Moreover, treatment is not likely to be straightforward, as evidenced by the failure of OC exposure to reverse bone loss in stressed monkeys and anorexic women. Recent studies suggest that a more global approach, including changes in lifestyle or even cognitive behavioral therapy, may be necessary to correct the underlying stress and reverse its neuroendocrine and endocrine concomitants (Berga et al. 2003).

Independent Role of Exercise and Psychological Stress in the Etiology of Functional Reproductive Deficits

The possibility that exercise or psychological stress might induce reproductive deficits in the absence of caloric restriction, disordered eating, or negative energy balance remains controversial (e.g., Berga 1996; Couzinet et al. 1999; Jasienska and Ellison 1998; Loucks and Thuma 2003; Warren and Fried 2001). In monkeys trained to run on a treadmill and consuming a constant number of calories, the resulting amenorrhea can be reversed by dietary supplementation (Williams et al. 2001a,b). This observation reinforces the suggestion that negative energy balance is a necessary component of exercise-associated, functional reproductive deficits. However, these monkeys were forced to run every day, accumulating mileage equivalent to two marathons per week. Methodologically, it is difficult to control for the possibility that psychological stress and negative energy balance may interact to influence the observed reproductive deficits and their reversal. A subsequent report tends to confirm this view, because mild caloric restriction was found to induce anovulation in moderately exercising monkeys, but only after exposure to stress (Cameron 2003). It is tempting to speculate that psychological stress reduces the threshold at which the LH pulse generator is disrupted by dietary restriction or exercise.

Although negative energy balance may mediate reproductive deficits in exercising monkeys, it does not appear to contribute to the subclinical reproductive deficits that occur in relation to social subordination among sedentary animals. Numerous studies have shown that stress secondary to social subordination or physical restraint results in LPD and anovulation in the absence of caloric restriction or weight loss (Adams et al. 1985b; Kaplan et al. 2002a; Shively et al. 1997; Xiao et al. 2002). Furthermore, spontaneous reversals of social status are followed by reversals of ovarian status in adequately fed cynomolgus monkeys (Adams et al. 1985b). These data provide evidence that psychosocial stress can induce subclinical reproductive deficits independently of other factors. However, caloric intake in such studies is generally not well controlled, leaving open the possibility that stressed monkeys subtly alter their dietary intake and physical activity in ways that affect energy balance and thus neuroendocrine function. Hence, although stress may be a prime cause of functional reproductive deficit, it might well interact with disordered eating and increased physical activity in women and other primates to initiate or maintain hypoestrogenism.

Individual Differences in Susceptibility and Resistance to Functional Reproductive Deficits

Women and monkeys exposed to psychological, caloric, or exercise challenges display considerable individual variation in reproductive response. Some exercising women retain normal cyclicity, some progress to LPD or anovulation and maintain that status, and others rapidly become amenorrheic (e.g., De Souza et al. 1998 and 2003; Loucks et al. 1989; Warren and Fried 2001). Although monkeys that exercise strenuously all become amenorrheic, some are resistant for years whereas others succumb within a few months (Williams et al. 2001a). Similarly, women respond variably to the stresses of everyday life as well as the more extreme circumstances associated with wartime experiences (Drew 1961). Finally, the studies reviewed above reveal that reproductive function among socially housed monkeys is influenced by psychosocial factors, with prominent ovarian impairment experienced by animals of subordinate social status (Kaplan et al. 1996).

It remains unclear why situations that induce functional deficits in some individuals leave others unscathed. Some investigators have speculated that personality characteristics such as perfectionism and eagerness to please render women especially prone to engage in coping behaviors (e.g., dieting and exercise) that increase their vulnerability to reproductive deficits (Berga et al. 1991; Marcus et al. 2001). For this reason, counseling or cognitive behavioral therapy is sometimes recommended as a first response to reproductive dysfunction (Berga 1996; Berga et al. 2003; Domar et al. 1990). Among subordinate monkeys, a persistent lack of control over access to social partners, space, and food may suffice to disrupt reproductive function, irrespective of personality traits.

Interestingly, depressed women and monkeys resemble their counterparts with functional reproductive deficits, particularly with respect to chronic HPA activation (Chrousos 2000; Holsboer 2001; Shively et al. 1997; Young et al. 2000). Such activation directly or indirectly may induce concomitant reproductive disruption (Chrousos et al. 1998). It is notable in this regard that a premenopausal history of depression is an atherosclerosis risk factor in perimenopausal women (Jones et al. 2003), and a predictor of pre- and postmenopausal bone loss (e.g., Cizza et al. 2001; Michelson et al. 1996; Yazici et al. 2003). Depression, then, or its concomitant traits of temperament, may represent an additional marker of vulnerability to ovarian dysfunction. Like functional reproductive deficits themselves, individual differences in susceptibility to ovarian disruption probably have a multifactorial origin, varying by situation. Although perhaps not insoluble, the determinants of these differences

remain poorly understood and provide fertile ground for future investigation.

Evolutionary Considerations

Biological phenomena are typically explained either by referencing immediate physiological/developmental precursors ("proximate cause") or their adaptive value and, thus, evolutionary origins ("ultimate cause") (Mayr 1982). This review has thus far focused on proximate factors—status and stress, negative energy balance, exercise—which result in functional reproductive deficits. In this section, we address the possibility that these deficits represent an adaptive response to environmental challenge that reversibly shifts energy away from reproduction and toward maintenance and survival (e.g., Berga 1996; Warren and Fried 2001).

Female primates might gain much from such an adaptation in a temporarily inauspicious environment, because reproduction is costly in terms of energy requirements and the health risks connected with parturition. Furthermore, Old World anthropoid females give birth only once a year, almost always to a single offspring. For this reason, substantial foregone alternatives exist in terms of survival and successful future reproduction associated with an unsuccessful birth event or infant rearing experience. Individuals who could respond facultatively to poor environmental conditions by delaying reproduction until more favorable circumstances obtain would have a selective advantage over those lacking this ability. The adaptive mechanism hypothesized to encompass such responses to changing environmental conditions in humans and other primates has been referred to variously as "reproductive suppression," "reproductive filtering," and "flexible responsiveness" (Vitzthum 2001; Wasser and Barash 1983; Wasser and Place 2001).

A variety of stimuli theoretically could signal the presence of an environment unfavorable to reproduction. Investigators who study nonindustrialized societies often argue that the quantity and quality of consumed food alerts the central nervous system to respond adaptively by either permitting or disrupting reproductive activity (e.g., Ellison 2003; Vitzthum 2001). In contrast, scientists working with nonhuman primates tend to view social interactions and the psychosocial environment as comprising the primary stimuli that activate the neural circuits controlling reproduction (e.g., Wasser and Place 2001). This difference in emphasis may relate in part to the relative ease with which food consumption and psychosocial factors can be quantified in populations of humans and nonhuman primates. Thus, caloric intake can be estimated by questionnaire or interview in people but is refractory to approximation in freely interacting monkeys or apes with unrestricted access to food. However, researchers often document the type, frequency, and pattern of social interaction occurring in entire groups of nonhuman primates; such data can be used to quantify social environments in ways that are difficult to replicate directly in human studies.

Although there may be disciplinary bias in attributing cause, current evidence suggests that both diet and psychosocial conditions influence primate reproduction. Under some circumstances, however, the psychosocial environment might comprise a more efficient signaling system than caloric intake, because it can provide information about environmental quality that presages sustained changes in food availability. In a monkey population, for example, a deteriorating environment might be incapable of supplying sufficient energy to support successful completion of pregnancy and infant rearing by all resident females. In such situations, aggressive reinforcement of dominance relationships would disrupt menstrual cyclicity and thus prevent pregnancy in subordinate individuals. Although temporarily incapable of reproducing, subordinates would gain an immediate benefit to the extent that their energy requirements would be minimized and their need to compete with pregnant, dominant animals in direct contests over food would be reduced. These subordinates would also gain a long-term benefit by avoiding likely reproductive failure and the squandering of resources that could support future reproduction on the return of more propitious circumstances. A strategy that suppresses the energy needs and reproductive output of subordinates also benefits dominants, in that it assures their infants of adequate food and increased representation in the next generation.

Observational data support the existence of such an adaptive mechanism. As reviewed previously, dominant females in macaque and baboon groups aggressively target their subordinate counterparts and thereby disrupt ovarian function (Adams et al. 1985b; Rowell 1970). Furthermore, such targeting is more intense in the context of a worsening food supply (Dittus 1977). However, dominant females attack subordinates even in the absence of direct competition over food, possibly as part of a strategy of harassment designed to generate a low, albeit persistent, level of stress in their victims (Silk 2002). Consequently, reproductive performance is often suppressed in subordinates, as indicated by reduced fecundity and infant survival, lengthened interbirth intervals, and delayed maturation of female offspring; furthermore, such suppression occurs regardless of whether individuals live in groups subject to natural fluctuations in food supplies or those that are artificially provisioned (e.g., Altmann et al. 1988; Dittus 1977, 1980; Drickamer 1974; Harcourt 1987; Sade et al. 1976; Wasser 1999). In this regard, it has been suggested that competitive and antagonistic interactions initiated by women toward their peers reflect the high value that they, like their monkey counterparts, place on the acquisition and defense of resources that secure their own and their offsprings' survival (Campbell 1995). Perhaps this self-interested investment strategy also explains why females are less likely to reconcile after fights than males (e.g., De Waal 1989). Male social relationships are relatively transient and serve the moment, whereas in females, status is for the duration of offspring rearing.

The evolution and persistence of a mechanism that reproductively disadvantages a predictable subset of the popu-

lation (e.g., subordinates) would seem to require that, over time, environments vary in quality, or that individuals experience a change in status that alters their access to resources. Only in such instances would a strategy of deferred reproduction be worthwhile. Long-term studies of macaques and baboons suggest that both conditions are met. Hence, the natural environments of these species often vary radically within the course of a generation (Altmann and Alberts 2003; Dittus 1977; Rhine et al. 2000). Furthermore, female social status may also change, either through group fission and dispersal or spontaneous reversal of pairs or entire genealogies (Chepko-Sade and Sade 1979; Chikazawa et al. 1979). Such changes are not surprising, given the evidence that social status reflects relationships among animals cohabiting in a specific environment rather than an unvarying trait of an individual (Kaplan et al. 2002b). Importantly, changes in reproductive function follow changes in status; hence, a decline in status is followed by ovarian dysfunction and vice versa (Adams et al. 1985b; Shively and Clarkson 1994). The foregoing observations are consistent with the hypothesis that reproductive suppression represents an adaptive mechanism allowing females, dominant and subordinate, to maximize their long-term reproductive output.

Women who live under a variety of ecological conditions undergo similar reproductive suppression and reversal in response to environmental change, although they are perhaps driven by a more variable set of proximate signals. In nonindustrialized, agricultural settings, menstrual cycle quality changes in relation to the seasonal availability of food. Reproduction may be deferred over even longer periods in nomadic societies in which women must carry their immature children over great distances (Lee 1984). As suggested above, investigators typically relate such alterations in reproductive condition to caloric intake rather than psychogenic factors, which may be more difficult to quantify. Moreover, these studies generally focus on the reproductive characteristics of populations rather than on individual differences within populations (Ellison 2003). Notably, in one instance in which individual differences were considered, anovulation was noted to be more frequent in women of low (vs. high) socioeconomic status (Vitzthum 2001). In industrialized countries, where food is normally available in abundance throughout the year, research interest centers on subsets of individuals with functional reproductive deficits; such deficits are usually attributed to stress and other behavioral factors, including disordered eating and recreational exercise (Berga 1996).

Interestingly, progesterone concentrations in all phases of the menstrual cycle are significantly lower among agriculturalists living in Africa, Asia, and South America than in North American women (Ellison et al. 1993). Ovulation and pregnancy occur in these populations at gonadal hormone levels that would impair reproductive function in North American women. This observation has led some investigators to speculate that early exposure to adverse environmental conditions (e.g., as experienced among agriculturalists) alters hormonal setpoints and may even result in a "live fast, die young" life strategy that favors short-term reproductive investment at the cost of long-term survival (Promislow and Harvey 1990; Vitzthum 2001). The foregoing hypothesis is somewhat at odds with data from nonhuman primates, which indicate that disadvantaged juveniles are slower to mature and are less fecund than their advantaged counterparts (Altmann et al. 1995; Drickamer 1974; Harcourt 1987; Sade et al. 1976). Although perhaps not representing an intractable problem, it is ultimately necessary to explain interpopulation variability in human gonadal hormone characteristics in a manner consistent with the known reproductive biology of other primates.

Although the life strategy consequences of reproductive suppression manifest in people and other primates are still a matter of some debate, the hypothesized adaptive mechanism appears to operate similarly across many anthropoid species. Hence, proximate stimuli that either directly or indirectly signal environmental quality induce reversible changes in ovarian function consistent with an hypothesis postulating "conservation of energy." The widespread taxonomic distribution of this phenomenon, in turn, suggests an evolutionary history that predates the appearance of modern humans and associated developments such as the domestication of plants and animals. It might be posited that the physiological concomitants of reproductive suppression—including temporary estrogen deficiency—would be most advantageously expressed in environments analogous to those that conditioned its evolution. For humans, this environment might include situations in which individuals are subjected to significant periodic shortfalls in energy availability, either in relation to seasonal variation or as a consequence of an unpredictable condition like warfare. However, reproductive suppression may also result in an unanticipated increase in vulnerability to chronic disease, particularly when provoked by psychogenic factors and experienced in combination with a sedentary lifestyle and excessive exposure to a fat-laden diet.

The idea that mechanisms evolving in response to one environment may have maladaptive health consequences in another has been applied to many phenomena other than reproductive suppression. The defense reaction, for example, involves activation of numerous bodily systems in preparation for fight or flight as a response to perceived external threats. Although adaptive in circumstances requiring explosive bursts of energy for survival, it has been speculated that accompanying increases in blood pressure and heart rate can promote hypertension and vascular damage when triggered inappropriately by everyday psychosocial stressors (Curtis and O'Keefe 2002; Julius 1995). Similarly, the capacity for insulin resistance, which may have evolved in response to the high protein/low carbohydrate diet that characterized the Pleistocene environment of early humans, contributes to the development of the metabolic syndrome (a major cardiovascular risk factor) when individuals are exposed to a modern diet high in simple carbohydrates (Colagiuri and Miller 2002). Finally, there is emerging appre-

ciation that the inflammatory and immune responses critical for surviving trauma and warding off infection under primitive conditions accelerate the development of atherosclerosis in an environment where individuals live longer and consume large amounts of fats susceptible to oxidation (Ridker 2002). However, although these other presumably adaptive phenomena can adversely affect women and men, reproductive suppression represents an additional and unique potential health burden for women.

Conclusion

This article highlights the significance of premenopausal reproductive dysfunction, social status, and psychological stress in relation to the risk of postmenopausal diseases such as CHD and osteoporosis. Although numerous investigators have commented on functional reproductive deficits, these abnormalities can be difficult to diagnose and are generally unappreciated for the contribution they may make to the health burden of postmenopausal women. Studies in nonhuman primates reveal these deficits to be both common and pathogenic to organ systems targeted by ovarian hormones, especially the vasculature and skeletal systems. However, such investigations are still in their infancy, leaving many questions unanswered. The primary areas of uncertainty include the following:

- Identification of the neuroendocrine mechanisms leading to the initial disruption of the GnRH pulse generator, as well as those maintaining reproductive abnormalities;
- Comparison of the pathways mediating the reproductive effects of psychological stress, caloric restriction, and excessive physical activity;
- Evaluation of the reproductive effects of psychological stress, caloric restriction, and excessive physical activity, in combination and at varying levels of insult; and
- Determination of the extent to which functional reproductive deficits—clinical and subclinical—adversely affect the vascular and skeletal systems and whether such effects extend also to the central nervous or immune systems.

Old World female monkeys are well suited to such investigations, because they so closely resemble women in behavior and biology and because prior research in these species demonstrates that psychosocial conditions, physical activity, and caloric intake can be successfully manipulated to model human situations.

Finally, functional reproductive deficits are also reversible and are thus potentially amenable to intervention by alteration of lifestyle, a fact that has been known for at least 200 yr. Late in the 18th century, for example, the prescription for a woman suffering from ovarian dysfunction in response to "grief, sudden fear, anxiety or any of the passions which tend to obstruct the menstrual flux" was to ". . . place her in a situation where she can enjoy the benefit of free air and agreeable company. There let her eat wholesome food, take sufficient exercise, and amuse herself in the most agreeable manner; and we have little reason to fear, but Nature, thus assisted, will do her proper work" (Buchan 1785).

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References

Abbott DH. 1987. Behaviourally mediated suppression of reproduction in female primates. Zool Soc Lond 213:455-470.

Abbott DH, Keverne EB, Bercovitch FB, Shively CA, Mendoza SP, Saltzman W, Snowdon CT, Ziegler TE, Banjecvic M, Garland T Jr, Sapolsky RM. 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Horm Behav 43:67-82.

Adams MR, Kaplan JR, Clarkson TB, Koritnik DR. 1985a. Ovariectomy, social status, and atherosclerosis in cynomolgus monkeys. Arteriosclerosis 5:192-200.

Adams MR, Kaplan JR, Koritnik DR. 1985b. Psychosocial influences on ovarian endocrine and ovulatory function in *Macaca fascicularis*. Physiol Behav 35:935-940.

Adams MR, Kaplan JR, Koritnik DR, Clarkson TB. 1987. Pregnancy-associated inhibition of coronary artery atherosclerosis in monkeys. Evidence of a relationship with endogenous estrogen. Arteriosclerosis 7:378-384.

Adams MR, Kaplan JR, Manuck SB, Koritnik DR, Parks JS, Wolfe MS, Clarkson TB. 1990. Inhibition of coronary artery atherosclerosis by 17-beta estradiol in ovariectomized monkeys. Lack of an effect of added progesterone. Arteriosclerosis 10:1051-1057.

AHA [American Heart Association]. 2002. Heart Disease and Stroke Statistics—2003 Update. Dallas: American Heart Association.

Altmann J, Alberts SC. 2003. Variability in reproductive success viewed from a life-history perspective in baboons. Am J Hum Biol 15:401-409.

Altmann J, Hausfater G, Altmann SA. 1988. Determinants of reproductive success in savannah baboons, *Papio cynocephalus*. In: Clutton-Brock TH, ed. Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems. Chicago: University of Chicago Press. p 403-418.

Altmann J, Sapolsky R, Licht P. 1995. Baboon fertility and social status. Nature 377:688-690.

Anderson RN. 2001. Deaths: Leading causes for 1999. Natl Vital Stat Rep 49:88.

Bachrach LK. 2001. Acquisition of optimal bone mass in childhood and adolescence. Trends Endocrinol Metab 12:22-27.

Bailey DA. 1997. The Saskatchewan pediatric bone mineral accrual study: Bone mineral acquisition during the growing years. Int J Sports Med 18(Suppl 3):S191-S194.

Bairey Merz CN, Dwyer J, Nordstrom CK, Walton KG, Salerno JW, Schneider RH. 2002. Psychosocial stress and cardiovascular disease: Pathophysiological links. Behav Med 27:141-147.

Bairey Merz CN, Johnson BD, Sharaf B, Berga S, Braunstein G, Bittner V, Reis S, Pepine CJ, Mankad S, Pohost G, Sopko G, Kelsey SF. 2003a. Prior oral contraceptive use and coronary artery disease (CAD): Data

from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) Study (Abstract). 14th Annual Meeting of The North American Menopause Society P-51, p 87.

Bairey Merz CN, Johnson BD, Sharaf BL, Bittner V, Berga SL, Braunstein GD, Hodgson TK, Matthews KA, Pepine CJ, Reis SE, Reichek N, Rogers WJ, Pohost GM, Kelsey SF, Sopko G. 2003b. Hypoestrogenemia of hypothalamic origin and coronary artery disease in premenopausal women: A report from the NHLBI-sponsored WISE study. J Am Coll Cardiol 41:413-419.

Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA. 1996. Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144-3147.

Beals KA, Manore MM. 2002. Disorders of the female athlete triad among collegiate athletes. Int J Sport Nutr Exerc Metab 12:281-293.

Berga SL. 1996. Functional hypothalamic chronic anovulation. In: Adashi EY, Rock JA, Rosenwaks Z, eds. Reproductive Endocrinology, Surgery, and Technology. Vol 1. Philadelphia: Lippencott-Raven. p 1061-1075.

Berga SL. 2001. Systemic benefits of cyclic ovarian function. J Soc Gynecol Invest 8:S3-S6.

Berga SL, Daniels TL, Giles DE. 1997. Women with functional hypothalamic amenorrhea but not other forms of anovulation display amplified cortisol concentrations. Fertil Steril 67:1024-1030.

Berga SL, Girton LG. 1989. The psychoneuroendocrinology of functional hypothalamic amenorrhea. Psychiatr Clin N Am 12:105-116.

Berga SL, Loucks AB, Rossmanith WG, Kettel LM, Laughlin GA, Yen SSC. 1991. Acceleration of luteinizing hormone pulse frequency in functional hypothalamic amenorrhea by dopaminergic blockade. J Clin Endocrinol Metab 72:151-156.

Berga SL, Loucks-Daniels TL, Adler LJ, Chrousos GP, Cameron JL, Matthews KA, Marcus MD. 2000. Cerebrospinal fluid levels of corticotropin-releasing hormone in women with functional hypothalamic amenorrhea. Am J Obstet Gynecol 182:776-784.

Berga SL, Marcus MD, Loucks TL, Hlastala S, Ringham R, Krohn MA. 2003. Recovery of ovarian activity in women with functional hypothalamic amenorrhea who were treated with cognitive behavior therapy. Fertil Steril 80:976-981.

Berga SL, Mortola JF, Girton L, Suh B, Laughlin G, Pham P, Yen SSC. 1989. Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 68:301-308.

Biller BMK, Coughlin JF, Saxe V, Schoenfeld D, Spratt DI, Klibanski A. 1991. Osteopenia in women with hypothalamic amenorrhea: A prospective study. Obstet Gynecol 78:996-1001.

Bisaga K, Petkova E, Cheng J, Davies M, Feldman JF, Whitaker AH. 2002. Menstrual functioning and psychopathology in a county-wide population of high school girls. J Am Acad Child Adolesc Psychiatry 41: 1197-1204.

Bond MG, Bullock BC, Bellinger DA, Hamm TE. 1980. Myocardial infarction in a large colony of nonhuman primates with coronary artery atherosclerosis. Am J Pathol 101:675-692.

Bonen A. 1994. Exercise-induced menstrual cycle changes. A functional, temporary adaptation to metabolic stress. Sports Med 17:373-392.

Brown E, Bain J, Lerner P, Shaul D. 1983. Psychological, hormonal, and weight disturbances in functional amenorrhea. Can J Psychiatry 28: 624-628.

Buchan W. 1785. Domestic Medicine. William Buchan's 1785 Home Medical Guide for the Treatment and Prevention of Disease in the 18th Century, 2nd ed. http://www.americanrevolution.org/medicine.html

Bullen BA, Skrinar GS, Beitins IZ, von Mering G, Turnbull BA, McArthur JW. 1985. Induction of menstrual disorders by strenuous exercise in untrained women. N Engl J Med 312:1349-1353.

Burger HG, Dudley EC, Robertson DM, Dennerstein L. 2002. Hormonal changes in the menopause transition. Recent Prog Horm Res 57:257-275.

Cameron JL. 1996. Regulation of reproductive hormone secretion in primates by short-term changes in nutrition. Rev Reprod 1:117-126.

Cameron JL. 2003. Exercise and amenorrhea. An oral presentation at the Menstrual Cycle and Bone Health Meeting, NIH Campus, Bethesda, MD: May 22, 2003.

Cameron JL, Helmreich DL, Schreihofer DA. 1993. Modulation of reproductive hormone secretion by nutritional intake: Stress signals versus metabolic signals. Hum Reprod 8(Suppl 2):162-167.

Campbell A. 1995. A few good men: Evolutionary psychology and female adolescent aggression. Ethol Sociobiol 16:99-123.

Cann CE, Martin MC, Genant HK, Jaffe RB. 1984. Decreased spinal mineral content in amenorrheic women. JAMA 251:626-629.

Cerroni AM, Tomlinson GA, Turnquist JE, Grynpas MD. 2000. Bone mineral density, osteopenia, and osteoporosis in the rhesus macaques of Cayo Santiago. Am J Phys Anthropol 113:389-410.

Chepko-Sade BD, Sade DS. 1979. Patterns of group splitting within matrilineal kinship groups: A study of social group structure in *Macaca mulatta* (Cercopithecidae: Primates). Behav Ecol Sociobiol 5:67-86.

Chikazawa D, Gordon TP, Bean CA, Bernstein IS. 1979. Mother-daughter dominance reversals in rhesus monkeys (*Macaca mulatta*). Primates 20:301-305.

Chrousos GP. 2000. The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: Neuroendocrine and target tissue-related causes. Int J Obes 24(Suppl 2):S50-S55.

Chrousos GP, Torpy DJ, Gold PW. 1998. Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: Clinical implications. Ann Intern Med 129:229-240.

Cizza G, Ravn P, Chrousos GP, Gold PW. 2001. Depression: A major, unrecognized risk factor for osteoporosis? Trends Endocrinol Metab 12:198-203.

Colagiuri S, Miller JB. 2002. The "carnivore connection"—Evolutionary aspects of insulin resistance. Eur J Clin Nutr 56(Suppl 1):S30-S35.

Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. 1987. Menopause and the risk of coronary heart disease in women. N Engl J Med 316:1105-1110.

Colman RJ, Kemnitz JW, Lane MA, Abbott DH, Binkley N. 1999. Skeletal effects of aging and menopausal status in female rhesus macaques. J Clin Endocrinol Metab 84:4144-4148.

Corner GW. 1923. Ovulation and menstruation in Macacus rhesus. Contrib Embryol 75:77-101. Washington DC: Carnegie Institute (publication no. 32).

Corner GW. 1927. The relation between menstruation and ovulation in the monkey. JAMA 89:1838-1840.

Corner GW, Hartman CG, Bartelmez GW. 1945. Development, organization, and breakdown of the corpus luteum in the rhesus monkey. Contrib Embryol 204:119-146, plus plates.

Couzinet B, Young J, Brailly S, Le Bouc Y, Chanson P, Schaison G. 1999. Functional hypothalamic amenorrhoea: A partial and reversible gonadotrophin deficiency of nutritional origin. Clin Endocrinol 50:229-235.

Cunningham MJ, Clifton DK, Steiner RA. 1999. Leptin's actions on the reproductive axis: Perspectives and mechanisms. Biol Reprod 60:216-222.

Curtis BM, O'Keefe JH Jr. 2002. Autonomic tone as a cardiovascular risk factor: The dangers of chronic fight or flight. Mayo Clin Proc 77:45-54.

Davies MC, Hall ML, Jacobs HS. 1990. Bone mineral loss in young women with amenorrhoea. Br Med J 301:790-793.

De Souza MJ, Miller BE, Loucks AB, Luciano AA, Pescatello LS, Campbell CG, Lasley BL. 1998. High frequency of luteal phase deficiency and anovulation in recreational women runners: Blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. J Clin Endocrinol Metab 83:4220-4232.

De Souza MJ, Miller BE, Sequenzia LC, Luciano AA, Ulreich S, Stier S, Prestwood K, Lasley BL. 1997. Bone health is not affected by luteal phase abnormalities and decreased ovarian progesterone production in female runners. J Clin Endocrinol Metab 82:2867-2876.

De Souza MJ, Van Heest J, Demers LM, Lasley BL. 2003. Luteal phase deficiency in recreational runners: Evidence for a hypometabolic state. J Clin Endocrinol Metab 88:337-346.

De Waal F.1989. Peacemaking among Primates. Cambridge MA: Harvard University Press.

Dhuper S, Warren MP, Brooks-Gunn J, Fox R. 1990. Effects of hormonal status on bone density in adolescent girls. J Clin Endocrinol Metab 71:1083-1088.

Dittus WPJ. 1977. The social regulation of population density and age-sex distribution in the toque monkey. Behaviour 63:281-322.

Dittus WPJ. 1980. The social regulation of primate populations: A synthesis. In: Lindburg DG, ed. The Macaques: Studies in Ecology, Behavior and Evolution. New York: Van Nostrand Reinhold Co. p 263-286.

Dobson H, Ghuman S, Prabhakar S, Smith R. 2003. A conceptual model of the influence of stress on female reproduction. Reproduction 125:151-163.

Domar AD, Seibel MM, Benson H. 1990. The mind/body program for infertility: A new behavioral treatment approach for women with infertility. Fertil Steril 53:246-249.

Dominguez CE, Laughlin GA, Nelson JC, Yen SSC. 1997. Altered binding of serum thyroid hormone to thyroxine-binding globulin in women with functional hypothalamic amenorrhea. Fertil Steril 68:992-996.

Drew FL. 1961. Epidemiology: The epidemiology of secondary amenorrhea. J Chron Dis 14:396-407.

Drickamer LC. 1974. A ten-year summary of reproductive data for free-ranging *Macaca mulatta*. Folia Primatol 21:61-80.

Drinkwater BL, Nilson K, Chestnut CH, Bremner WJ, Shainholtz S, Southworth MB. 1984. Bone mineral content of amenorrheic and eumenorrheic athletes. N Engl J Med 311:277-281.

Dubey AK, Cameron JL, Steiner RA, Plant TM. 1986. Inhibition of gonadotropin secretion in castrated male rhesus monkeys (*Macaca mulatta*) induced by dietary restriction: Analogy with the prepubertal hiatus of gonadotropin release. Endocrinology 118:518-525.

Ellison PT. 2003. Energetics and reproductive effort. Am J Hum Biol 15:342-351.

Ellison PT, Panter-Brick C, Lipson SF, O'Rourke MT. 1993. The ecological context of human ovarian function. Hum Reprod 8:2248-2258.

Ellison PT, Peacock NR, Lager C. 1989. Ecology and ovarian function among Lese women of the Ituri Forest, Zaire. Am J Phys Anthropol 78:519-526.

Falsetti L, Gambera A, Barbetti L, Specchia C. 2002. Long-term follow-up of functional hypothalamic amenorrhea and prognostic factors. J Clin Endocrinol Metab 87:500-505.

Ferenczy A. 2003. Pathophysiology of endometrial bleeding. Maturitas 45:1-14.

Ferin M. 1999. Clinical review 105: Stress and the reproductive cycle. J Clin Endocrinol Metab 84:1768-1774.

Ferin M, Jewelewicz R, Warren M, eds. 1993. The Menstrual Cycle. Physiology, Reproductive Disorders, and Infertility. New York: Oxford University Press.

Fries H, Nillius SJ, Pettersson F. 1974. Epidemiology of secondary amenorrhea: A retrospective evaluation of etiology with special regard to psychogenic factors and weight loss. Am J Obstet Gynecol 118:473-479.

Frisch RE, McArthur JW. 1974. Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. Science 185:949-951.

Genazzani AD, Bersi C, Luisi S, Fruzzetti F, Malavasi B, Luisi M, Petraglia F, Genazzani AR. 2001. Increased adrenal steroid secretion in response to CRF in women with hypothalamic amenorrhea. J Steroid Biochem Mol Biol 78:247-252.

Genazzani AD, Luisi M, Malavasi B, Strucchi C., Luisi S, Casarosa E, Bernardi F, Genazzani AR, Petraglia F. 2002. Pulsatile secretory characteristics of allopregnanolone, a neuroactive steroid, during the menstrual cycle and in amenorrheic subjects. Eur J Endocrinol 146:347-356.

Gilardi KV, Shideler SE, Valverde CR, Roberts JA, Lasley BL. 1997. Characterization of the onset of menopause in the rhesus macaque. Biol Reprod 57:335-340.

Giles DE, Berga SL. 1993. Cognitive and psychiatric correlates of functional hypothalamic amenorrhea: A controlled comparison. Fertil Steril 60:486-492.

Ginsburg KA. 1992. Luteal phase defect: Etiology, diagnosis, and management. Endocrinol Metab Clin N Am 21:85-104.

Gold PW, Chrousos GP. 2002. Organization of the stress system and its dysregulation in melancholic and atypical depression: High vs low CRH/NE states. Mol Psychiatry 7:254-275.

Golden NH. 2002. A review of the female athlete triad (amenorrhea, osteoporosis and disordered eating). Int J Adolesc Med Health 14:9-17.

Gordon CM. 2000. Bone density issues in the adolescent gynecology patient. J Pediatr Adolesc Gynecol 13:157-161.

Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, Garside DB, Wilson PW. 2003. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 290:891-897.

Gregory BAJC. 1957. The menstrual cycle and its disorders in psychiatric patients. J Psychosom Res 2:61-79.

Guyton AC, Hall JE, eds. 2000. Textbook of Medical Physiology. 10th ed. Philadelphia: WB Saunders Company.

Gwirtsman HE, Kaye WH, George DT, Jimerson DC, Ebert MH, Gold PW. 1989. Central and peripheral ACTH and cortisol levels in anorexia nervosa and bulimia. Arch Gen Psychiatry 46:61-69.

Hamm TE Jr, Kaplan JR, Clarkson TB, Bullock BC. 1983. Effects of gender and social behavior on the development of coronary artery atherosclerosis in cynomolgus macaques. Atherosclerosis 48:221-233.

Hanke H, Hanke S, Ickrath O, Lange K, Bruck B, Mück AO, Seeger H, Zwirner M, Voisard R, Haasis R, Hombach V. 1997. Estradiol concentrations in premenopausal women with coronary heart disease. Coron Artery Dis 8:511-515.

Hansson GK. 2001. Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol 21:1876-1890.

Harcourt AH. 1987. Dominance and fertility among female primates. Zool Soc Lond 213:471-487.

Harlow BL, Wise LA, Otto MW, Soares CN, Cohen LS. 2003. Depression and its influence on reproductive endocrine and menstrual cycle markers associated with perimenopause. Arch Gen Psychiatry 60:29-36.

Hartman CG. 1932. Studies in the reproduction of the monkey, *Macacus* (*Pithecus*) rhesus, with special reference to menstruation and pregnancy. Contrib Embryol 23:1-161. Washington DC: Carnegie Institute (publication no. 525).

Haynes SG, Feinleib M. 1980. Women, work and coronary heart disease: Prospective findings from the Framingham heart study. Am J Public Health 70:133-141.

Herbison AE. 1997. Noradrenergic regulation of cyclic GnRH secretion. Rev Reprod 2:1-6.

Higgins M, Thom T. 1993. Cardiovascular disease in women as a public health problem. In: Wenger NK, Speroff L, Packard B, eds. Cardiovascular Health and Disease in Women. Greenwich CT: LeJacq Communications Inc. p 15-19.

Hilton LK, Loucks AB. 2000. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am J Physiol Endocrinol Metab 278:E43-E49.

Hirvonen E. 1977. Etiology, clinical features and prognosis in secondary amenorrhea. Int J Fertil 22:69-76.

Hodis NH, Mack WJ, Lobo R. 2003. What is the cardioprotective role of hormone replacement therapy? Curr Atheroscler Rep 5:56-66.

Holsboer F. 2001. Stress, hypercortisolism and corticosteroid receptors in depression: Implications for therapy. J Affect Disord 62:77-91.

Hosmer WD, Genant HK, Browner WS. 2002. Fractures before menopause: A red flag for physicians. Osteoporos Int 13:337-341.

Hotchkiss J, Knobil E. 1994. The menstrual cycle and its neuroendocrine control. In: Knobil E, Neill JD, eds. The Physiology of Reproduction. 2nd ed. New York: Raven Press Ltd. p 711-749.

Hotchkiss CE, Stavisky R, Nowak J, Brommage R, Lees CJ, Kaplan J. 2001. Levormeloxifene prevents increased bone turnover and vertebral bone loss following ovariectomy in cynomolgus monkeys. Bone 29:7-15.

Jasienska G, Ellison PT. 1998. Physical work causes suppression of ovarian function in women. Proc R Soc Lond B Biol Sci 265:1847-1851.

Jerome CP. 1998. Primate models of osteoporosis. Lab Anim Sci 48:618-622.
Jones GES. 1949. Some newer aspects of the management of infertility. JAMA 141:1123-1129.
Jones DJ, Bromberger JT, Sutton-Tyrrell K, Matthews KA. 2003. Lifetime history of depression and carotid atherosclerosis in middle-aged women. Arch Gen Psychiatry 60:153-160.
Judd SJ, Wong J, Saloniklis S, Maiden M, Yeap B, Filmer S, Michailov L. 1995. The effect of alprazolam on serum cortisol and luteinizing hormone pulsatility in normal women and in women with stress-related anovulation. J Clin Endocrinol Metab 80:818-823.
Julius S. 1995. The defense reaction: A common denominator of coronary risk and blood pressure in neurogenic hypertension? Clin Exp Hypertens 17:375-386.
Kaplan JR. 1977. Patterns of fight interference in free-ranging rhesus monkeys. Am J Phys Anthropol 47:279-288.
Kaplan JR. 1987. Dominance and affiliation in the Cercopithecini and Papionini: A comparative examination. Monogr Primatol 10:127-150.
Kaplan JR, Adams MR, Anthony MS, Morgan TM, Manuck SB, Clarkson TB. 1995. Dominant social status and contraceptive hormone treatment inhibit atherogenesis in premenopausal monkeys. Arterioscler Thromb Vasc Biol 15:2094-2100.
Kaplan JR, Adams MR, Clarkson TB, Koritnik DR. 1984. Psychosocial influences on female "protection" among cynomolgus macaques. Atherosclerosis 53:283-295.
Kaplan JR, Adams MR, Clarkson TB, Manuck SB, Shively CA, Williams JK. 1996. Psychosocial factors, sex differences, and atherosclerosis: Lessons from animal models. Psychosom Med 58:598-611.
Kaplan JR, Adams MR, Koritnik DR, Rose JC, Manuck SB. 1986. Adrenal responsiveness and social status in intact and ovariectomized *Macaca fascicularis*. Am J Primatol 11:181-193.
Kaplan JR, Manuck SB. 1999. Status, stress, and atherosclerosis: The role of environment and individual behavior. Ann N Y Acad Sci 896:145-161.
Kaplan JR, Manuck SB, Anthony MS, Clarkson TB. 2002a. Premenopausal social status and hormone exposure predict postmenopausal atherosclerosis in female monkeys. Obstet Gynecol 99:381-388.
Kaplan JR, Manuck SB, Clarkson TB, Prichard RW. 1985. Animal models of behavioral influences on atherogenesis. Adv Behav Med 1:115-163.
Kaplan JR, Manuck SB, Fontenot MB, Mann JJ. 2002b. Central nervous system monoamine correlates of social dominance in cynomolgus monkeys (*Macaca fascicularis*). Neuropsychopharmacology 26:431-443.
Karas RH, Clarkson TB. 2003. Considerations in interpreting the cardiovascular effects of hormone replacement therapy observed in the WHI: Timing is everything. Menopaus Med 10:8-12.
Kaufman BA, Warren MP, Dominguez JE, Wang J, Heymsfield SB, Pierson RN. 2002. Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels. J Clin Endocrinol Metab 87:2777-2783.
Kelley K, Daniels GE, Poe J, Easser R, Monroe R. 1954. Psychological correlations with secondary amenorrhea. Psychosom Med 16:129-147.
Keys A, Brožek J, Henschel A, Mickelsen O, Taylor HL, eds. 1950. Sexual function. In: The Biology of Human Starvation. Minneapolis: The University of Minnesota Press. p 749-763.
Khosla S, Bilezikian JP. 2003. The role of estrogens in men and androgens in women. Endocrinol Metab Clin N Am 32:195-218.
Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, Ellis SG, Lincoff AM, Topol EJ. 2003. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 290:898-904.
Klibanski A, Biller BMK, Schoenfeld DA, Herzog DB, Saxe VC. 1995. The effects of estrogen administration on trabecular bone loss in young women with anorexia nervosa. J Clin Endocrnol Metab 80:898-904.
Klinefelter Jr HF, Albright F, Griswold GC. 1943. Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis. J Clin Endocrinol 3:529-544.
Knobil E. 1988. The neuroendocrine control of ovulation. Hum Reprod 3:469-472.
Knox SS. 2001. Psychosocial stress and the physiology of atherosclerosis. Adv Psychosom Med 22:139-151.
Kroger WS, Freed SC, eds. 1956. Psychosomatic Gynecology: Including Problems of Obstetrical Care. Glencoe IL: The Free Press.
Laatikainen TJ. 1991. Corticotropin-releasing hormone and opioid peptides in reproduction and stress. Ann Med 23:489-496.
Lane MA, Black A, Handy AM, Shapses SA, Tilmont EM, Kiefer TL, Ingram DK, Roth GS. 2001. Energy restriction does not alter bone mineral metabolism or reproductive cycling and hormones in female rhesus monkeys. J Nutr 131:820-827.
Laughlin GA, Dominguez CE, Yen SSC. 1998. Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 83:25-32.
La Vecchia C, Decarli A, Franceschi S, Gentile A, Negri E, Parazzini F. 1987. Menstrual and reproductive factors and the risk of myocardial infarction in women under fifty-five years of age. Am J Obstet Gynecol 157:1108-1112.
Laven JS, Imani B, Eijkemans MJ, Fauser BC. 2002. New approach to polycystic ovary syndrome and other forms of anovulatory infertility. Obstet Gynecol Surv 57:755-767.
Lee RB, ed. 1984. The Dobe !Kung. Toronto: International Thomson Publishing.
LeMaire GS. 1987. The luteinized unruptured follicle syndrome: Anovulation in disguise. J Obstet Gynecol Neonat Nurs 16:116-120.
Libby P. 2002. Inflammation in atherosclerosis. Nature 420:868-874.
Liu JH. 1990. Hypothalamic amenorrhea: Clinical perspectives, pathophysiology, and management. Am J Obstet Gynecol 163:1732-1736.
Loucks AB, Laughlin GA, Mortola JF, Girton L, Nelson JC, Yen SSC. 1992. Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. J Clin Endocrinol Metab 75:514-518.
Loucks AB, Mortola JF, Girton L, Yen SSC. 1989. Alterations in the hypothalamic-pituitary-ovarian and the hypothalamic-pituitary-adrenal axes in athletic women. J Clin Endocrinol Metab 68:402-411.
Loucks TL, Talbott EO, McHugh KP, Keelan M, Berga SL, Guzick DS. 2000. Do polycystic-appearing ovaries affect the risk of cardiovascular disease among women with poycystic ovary syndrome? Fertil Steril 74:547-552.
Loucks AB, Thuma JR. 2003. Luteinizing hormone pulsatility is disrupted at threshold of energy availability in regularly menstruating women. J Clin Endocrinol Metab 88:297-311.
Manson JE. 1994. Postmenopausal hormone therapy and atherosclerotic disease. Am Heart J 128:1337-1343.
Marcus MD, Loucks TL, Berga SL. 2001. Psychological correlates of functional hypothalamic amenorrhea. Fertil Steril 76:310-316.
Marmot M. 1999. Epidemiology of socioeconomic status and health: Are determinants within countries the same as between countries? Ann NY Acad Sci 896:16-29.
Matthews KA, Kuller LH, Sutton-Tyrrell K, Chang YF. 2001. Changes in cardiovascular risk factors during the perimenopause and postmenopause and carotid artery atherosclerosis in healthy women. Stroke 32: 1104-1111.
Mayr E, ed. 1982. The Growth of Biological Thought. Diversity, Evolution and Inheritance. Cambridge MA: The Belknap Press of Harvard University Press.
McEwen BS. 2001. Invited review: Estrogens effects on the brain: Multiple sites and molecular mechanisms. J Appl Physiol 91: 2785-2801.
McGill HC, Stern NP. 1979. Sex and atherosclerosis. Atheroscler Rev 4:157-242.
McNeey MJ, Soules MR. 1988. The diagnosis of luteal phase deficiency: A critical review. Fertil Steril 50:1-l5.
Meczekalski B, Tonetti A, Monteleone P, Bernardi F, Luisi S, Stomati M, Luisi M, Petraglia F, Genazzani AR. 2000. Hypothalamic amenorrhea with normal body weight: ACTH, allopregnanolone and cortisol responses to corticotropin-releasing hormone test. Eur J Endocrinol 142: 280-285.
Melton LJ 3rd, Kan SH, Frye MA, Wahner HW, O'Fallon WM, Riggs BL. 1989. Epidemiology of vertebral fractures in women. Am J Epidemiol 129:1000-1011.

Mendelsohn ME, Karas RH. 1999. The protective effects of estrogen on the cardiovascular system. N Engl J Med 340:1801-1811.
Michelson D, Stratakis C, Hill L, Reynolds J, Galliven E, Chrousos G, Gold P. 1996. Bone mineral density in women with depression. N Engl J Med 335:1176-1181.
Miller KK, Parulekar MS, Schoenfeld E, Anderson E, Hubbard J, Klibanski A, Grinspoon SK. 1998. Decreased leptin levels in normal weight women with hypothalamic amenorrhea: The effects of body composition and nutritional intake. J Clin Endocrinol Metab 83:2309-2312.
Moses EL, Drevets WC, Smith G, Mathis CA, Kalro BN, Butters MA, Leondires MP, Greer PJ, Lopresti B, Loucks TL, Berga SL. 2000. Effects of estradiol and progesterone administration on human serotonin 2A receptor binding: A PET study. Biol Psychiatry 48:854-860.
Münster K, Helm P, Schmidt L. 1992. Secondary amenorrhoea: Prevalence and medical contact—A cross-sectional study from a Danish county. Br J Obstet Gynaecol 99:430-433.
Nordstrom CK, Dwyer KM, Bairey Merz CN, Shircore A, Dwyer JH. 2003. Leisure time physical activity and early atherosclerosis: The Los Angeles Atherosclerosis Study. Am J Med 115:19-25.
Novak E. 1931. Menstruation and Its Disorders. New York: D. Appleton and Company.
O'Connor KA, Holman DJ, Wood JW. 1998. Declining fecundity and ovarian ageing in natural fertility populations. Maturitas 30:127-136.
O'Connor KA, Holman DJ, Wood JW. 2001. Menstrual cycle variability and the perimenopause. Am J Hum Biol 13:465-478.
Ott SM. 1990. Attainment of peak bone mass. J Clin Endocrinol Metab 71:1082A-1082C.
Perkins RB, Hall JE, Martin KA. 2001. Aetiology, previous menstrual function and patterns of neuro-endocrine disturbance as prognostic indicators in hypothalamic amenorrhoea. Hum Reprod 16:2198-2205.
Pirke KM, Schweiger U, Lemmel W, Krieg JC, Berger M. 1985. The influence of dieting on the menstrual cycle of healthy young women. J Clin Endocrinol Metab 60:1174-1179.
Pope NS, Gordon TP, Wilson ME. 1986. Age, social rank and lactational status influence ovulatory patterns in seasonally breeding rhesus monkeys. Biol Reprod 35:353-359.
Prior JC, Vigna YM, Schechter MT, Burgess AE. 1990. Spinal bone loss and ovulatory disturbances. N Engl J Med 323:1221-1227.
Promislow DEL, Harvey PH. 1990. Living fast and dying young: A comparative analysis of life-history variation amount mammals. J Zool Lond 220: 417-437.
Punnonen R, Jokela H, Aine R, Teisala K, Salomäki A, Uppa H. 1997. Impaired ovarian function and risk factors for atherosclerosis in premenopausal women. Maturitas 27:231-238.
Recker R, Lappe J, Davies K, Heaney R. 2000. Characterization of perimenopausal bone loss: A prospective study. J Bone Miner Res 15: 1965-1973.
Reid I. 1997. Glucocorticoid osteoporosis—Mechanisms and management. Eur J Endocrinol 137:209-217.
Reifenstein Jr EC. 1946. Psychogenic or "hypothalamic" amenorrhea. Med Clin N Am 30:1103-1114.
Reindollar RH, Novak M, Tho SP, McDonough PG. 1986. Adult-onset amenorrhea: A study of 262 patients. Am J Obstet Gynecol 155:531-543.
Rhine RJ, Norton GW, Wasser SK. 2000. Lifetime reproductive success, longevity, and reproductive life history of female yellow baboons (*Papio cynocephalus*) of Mikumi National Park, Tanzania. Am J Primatol 51:229-241.
Ridker PM. 2002. On evolutionary biology, inflammation, infection, and the causes of atherosclerosis. Circulation 105:2-4.
Riggs BL, Khosla S, Melton LJ 3rd. 2002. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 23:279-302.
Ripley HS, Papinicolaou GN. 1942. The menstrual cycle with vaginal smear studies in schizophrenia, depression, and elation. Am J Psychiatry 98:567-574.
Robel P, Baulieu EE. 1995. Neurosteroids: Biosynthesis and function. Crit Rev Neurobiol 9:383-394.
Roberts SB, Pi-Sunyer X, Kuller L, Lane MA, Ellison P, Prior JC, Shapses S. 2001. Physiologic effects of lowering caloric intake in nonhuman primates and nonobese humans. J Gerontol A Biol Sci Med Sci 56A(Spec Iss):66-75.
Rosenberg L, Hennekens CH, Rosner B, Belanger C, Rothman KJ, Speizer FE. 1981. Early menopause and the risk of myocardial infarction. Am J Obstet Gynecol 139:47-51.
Rothenbacher D, Hoffmeister A, Brenner H, Koenig W. 2003. Physical activity, coronary heart disease, and inflammatory response. Arch Intern Med 163:1200-1205.
Rowell TE. 1970. Baboon menstrual cycles affected by social environment. J Reprod Fert 21:133-141.
Rowell TE. 1971. Organization of caged groups of cercopithecus monkeys. Anim Behav 19:625-645.
Sade DS. 1973. An ethogram for rhesus monkeys. I. Antithetical contrasts in posture and movement. Am J Phys Anthropol 38:537-542.
Sade DS, Cushing K, Cushing P, Dunaif J, Figueroa A, Kaplan JR, Lauer C, Rhodes D, Schneider J. 1976. Population dynamics in relation to social structure on Cayo Santiago. Yearbook Phys Anthropol 20:253-262.
Samuels MH, Luther M, Henry P, Ridgway EC. 1994. Effects of hydrocortisone on pulsatile pituitary glycoprotein secretion. J Clin Endocrinol Metab 78:211-215.
Shanan J, Brzezinski A, Sulman F, Sharon M. 1965. Active coping behavior, anxiety, and cortical steroid excretion in the prediction of transient amenorrhea. Behav Sci 10:461-465.
Shively CA, Clarkson TB. 1994. Social status and coronary artery atherosclerosis in female monkeys. Arterioscler Thromb 14:721-726.
Shively C, Kaplan J. 1984. Effects of social factors on adrenal weight and related physiology of *Macaca fascicularis*. Physiol Behav 33:777-782.
Shively CA, Laber-Laird K, Anton RF. 1997. Behavior and physiology of social stress and depression in female cynomolgus monkeys. Biol Psychiatry 41:871-882.
Silk JB. 2002. Practice random acts of aggression and senseless acts of intimidation: The logic of status contests in social groups. Evol Anthropol 11:221-225.
Solomon CG, Hu FB, Dunaif A, Rich-Edwards JE, Stampfer MJ, Willett WC, Speizer FE, Manson JE. 2002. Menstrual cycle irregularity and risk for future cardiovascular disease. J Clin Endocrinol Metab 87: 2013-2017.
Soules MR, Clifton DK, Cohen NL, Bremner WJ, Steiner RA. 1989a. Luteal phase deficiency: Abnormal gonadotropin and progesterone secretion patterns. J Clin Endocrinol Metab 69:813-820.
Soules MR, McLachlan RI, Ek M, Dahl KD, Cohen NL, Bremner WJ. 1989b. Luteal phase deficiency: Characterization of reproductive hormones over the menstrual cycle. J Clin Endocrinol Metab 69:804-812.
Sowers M, Crutchfield M, Bandekar R, Randolph JF, Shapiro B, Schork MA, Jannausch M. 1998a. Bone mineral density and its change in pre- and perimenopausal white women: The Michigan Bone Health Study. J Bone Miner Res 13:1134-1140.
Sowers M, Randolph JF, Crutchfield M, Jannausch ML, Shapiro B, Zhang B, LaPietra M. 1998b. Urinary ovarian and gonadotropin hormone levels in premenopausal women with low bone mass. J Bone Miner Res 13:1191-1202.
Stampfer MJ, Colditz GA, Willett WC. 1990. Menopause and heart disease: A review. Ann N Y Acad Sci 592:193-203.
Strassmann BI. 1996. The evolution of endometrial cycles and menstruation. Q Rev Biol 71:181-220.
Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman WP 3rd, Herderick EE, Cornhill JF. 1999. Prevalence and extent of atherosclerosis in adolescents and young adults: Implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA 281:727-735.
Strong JP, Restrepo C, Guzman M. 1978. Coronary and aortic atherosclerosis in New Orleans. II. Comparison of lesions by age, sex, and race. Lab Invest 39:364-369.
Suh BY, Liu JH, Berga SL, Quigley ME, Laughlin GA, Yen SS. 1988. Hypercortisolism in patients with functional hypothalamic-amenorrhea. J Clin Endocrinol Metab 66:733-739.

Sutton-Tyrrell K, Lassila HC, Meilahn E, Bunker C, Matthews KA, Kuller LH. 1998. Carotid atherosclerosis in premenopausal and postmenopausal women and its association with risk factors measured after menopause. Stroke 29:1116-1121.

Troxler RG, Sprague EA, Albanese RA, Fuchs R, Thompson AJ. 1997. The association of elevated plasma cortisol and early atherosclerosis as demonstrated by coronary angiography. Atherosclerosis 26:151-162.

Tsigos C, Chrousos GP. 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res 53:865-871.

Tuzcu EM, Kapadia SR, Tutar E, Ziada KM, Hobbs RE, McCarthy PM, Young JB, Nissen SE. 2001. High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: Evidence from intravascular ultrasound. Circulation 103:2705-2710.

Vaitukaitis JL. 1997. Anovulation and amenorrhea. In: Seibel MM, ed. Infertility—A Comprehensive Text. 2nd ed. Stamford CT: Appleton and Lange. p 111-119.

Vitzthum VJ. 2001. Why not so great is still good enough. Flexible responsiveness in human reproductive functioning. In: Ellison PT, ed. Reproductive Ecology and Human Evolution (Evolutionary Foundations of Human Behavior). New York: Aldine de Gruyter. p 179-202.

Vitzthum VJ, Bentley GR, Spielvogel H, Caceres E, Thornburg J, Jones L, Shore S, Hodges KR, Chatterton RT. 2002. Salivary progesterone levels and rate of ovulation are significantly lower in poorer than in better-off urban-dwelling Bolivian women. Hum Reprod 17:1906-1913.

Wagner JD, Kaplan JR, Burkman RT. 2002. Reproductive hormones and cardiovascular disease mechanism of action and clinical implications. Obstet Gynecol Clin N Am 29:475-493.

Walker ML, Gordon TP, Wilson ME. 1983. Menstrual cycle characteristics of seasonally breeding rhesus monkeys. Biol Reprod 29:841-848.

Waller K, Reim J, Fenster L, Swan SH, Brumback B, Windham GC, Lasley B, Ettinger B, Marcus R. 1996. Bone mass and subtle abnormalities in ovulatory function in healthy women. J Clin Endocrinol Metab 81:663-668.

Warren MP. 1996. Clinical Review 77: Evaluation of secondary amenorrhea. J Clin Endocrinol Metab 81:437-442.

Warren MP, Brooks-Gunn J, Fox RP, Holderness CC, Hyle EP, Hamilton WG. 2002. Osteopenia in exercise-associated amenorrhea using ballet dancers as a model: A longitudinal study. J Clin Endocrinol Metab 87:3162-3168.

Warren MP, Brooks-Gunn J, Fox RP, Lancelot C, Newman D, Hamilton WG. 1991. Lack of bone accretion and amenorrhea: Evidence for a relative osteopenia in weight-bearing bones. J Clin Endocrinol Metab 72:847-853.

Warren MP, Brooks-Gunn J, Hamilton LH, Warren LF, Hamilton WG. 1986. Scoliosis and fractures in young ballet dancers: Relation to delayed menarche and secondary amenorrhea. N Engl J Med 314:1348-1353.

Warren MP, Fried JL. 2001. Hypothalamic amenorrhea: The effects of environmental stresses on the reproductive system: A central effect of the central nervous system. Neuroendocrinology 30:611-629.

Warren MP, Voussoughian F, Geer EB, Hyle EP, Adberg CL, Ramos RH. 1999. Functional hypothalamic amenorrhea: Hypoleptinemia and disordered eating. J Clin Endocrinol Metab 84:873-877.

Wasser SK. 1999. Stress and reproductive failure: An evolutionary approach with applications to premature labor. Am J Obstet Gynecol 180:S272-S274.

Wasser SK, Barash DP. 1983. Reproductive suppression among female mammals: Implications for biomedicine and sexual selection theory. Q Rev Biol 58:513-538.

Wasser SK, Place NJ. 2001. Reproductive filtering and the social environment. In: Ellison PT, ed. Reproductive Ecology and Human Evolution (Evolutionary Foundations of Human Behavior). New York: Aldine de Gruyter. p 137-157.

Wells G, Tugwell P, Shea B, Guyatt G, Peterson J, Zytaruk N, Robinson V, Henry D, O'Connell D, Cranney A. 2002. Meta-analyses of therapies for postmenopausal osteoporosis. V. Meta-analysis of the efficacy of hormone replacement therapy in treating and preventing osteoporosis in postmenopausal women. Endocr Rev 23:529-539.

Whitacre FE, Barrera B. 1944. War amenorrhea: A clinical and laboratory study. JAMA 24:399-403.

Wilks JW, Hodgen GD, Ross GT. 1976. Luteal phase defects in the rhesus monkey: The significance of serum FSH:LH ratios. J Clin Endocrinol Metab 43:1261-1267.

Wilks JW, Hodgen GD, Ross GT. 1977. Anovulatory menstrual cycles in the rhesus monkey: The significance of serum follicule-stimulating hormone/luteinizing hormone ratios. Fertil Steril 28:1094-1100.

Williams JK, Shively CA, Clarkson TB. 1994. Determinants of coronary artery reactivity in premenopausal female cynomolgus monkeys with diet-induced atherosclerosis. Circulation 90:983-987.

Williams NI, Caston-Balderrama AL, Helmreich DL, Parfitt DB, Nosbisch C, Cameron JL. 2001a. Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: Abrupt transition to exercise-induced amenorrhea. Endocrinology 142:2381-2389.

Williams NI, Helmreich DL, Parfitt DB, Caston-Balderrama A, Cameron JL. 2001b. Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. J Clin Endocrinol Metab 86:5184-5193.

Williams RF, Hodgen GD. 1982. The reproductive cycle in female macaques. Am J Primatol Suppl 1:181-192.

Wood CE, Cline JM, Anthony MS, Kaplan JR. 2003. Adrenal effects of soy isoflavones and conjugated equine estrogens in monkeys: Implications for postmenopausal androgen deficiency. Submitted: 14th Annual Meeting of NAMS.

Writing Group for the Women's Health Initiative Investigators. 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women. Principal results from the Women's Health Initiative randomized controlled trial. JAMA 288:321-333.

Xiao E, Xia-Zhang L, Ferin M. 2002. Inadequate luteal function is the initial clinical cyclic defect in a 12-day stress model that includes a psychogenic component in the rhesus monkey. J Clin Endocrinol Metab 87:2232-2237.

Yazici KM, Akinci A, Sütçü A, Özçakar L. 2003. Bone mineral density in premenopausal women with major depressive disorder. Psychiatry Res 117:271-275.

Yen SSC. 1991. The human menstrual cycle: Neuroendocrine regulation. In: Yen SSC, Jaffe RB, eds. Reproductive Endocrinology Physiology, Pathophysiology and Clinical Management. 3rd ed. Philadelphia: WB Saunders Company. p 273-308.

Young EA, Midgley AR, Carlson NE, Brown MB. 2000. Alteration in the hypothalamic-pituitary-ovarian axis in depressed women. Arch Gen Psychiatry 57:1157-1162.

Zuckerman S. 1930. The menstrual cycle of the primates. I. General nature and homology. Proc Zool Soc Lond p 691-754.

Zuckerman S, Parks MA. 1932. The menstrual cycle of primates. V. The cycle of the baboon. Proc Zool Soc Lond p 138-191.