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University of New Hampshire, Durham

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FACTORS THAT INFLUENCE THE DECISION REGARDING HORMONE REPLACEMENT THERAPY IN POSTMENOPAUSAL WOMEN

BY

Ruth A. Rogers Reilly
B.S. Florida State University, 1965
M.O.E. University of New Hampshire, 1989

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy

in

Animal and Nutritional Sciences

May, 1998

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Ph.D. DISSERTATION

This dissertation has been reviewed.

Joanne Curran-Celentano
Dissertation Director, Joanne Curran-Celentano, Ph.D.
Associate Professor, Department of Animal & Nutritional Sciences

Kristine M Baber
Kristine M. Baber, Ph.D., Associate Professor
Department of Family Studies

Patricia D Bedker
Patricia D. Bedker, Ph.D., Associate Dean
College of Life Sciences and Agriculture
Associate Professor, Department of Animal & Nutritional Sciences

Thomas L. Foxall
Thomas L. Foxall, Ph.D., Professor
Department of Animal & Nutritional Sciences

Neil B Vroman
Neil B. Vroman, Ph.D., Associate Dean
School of Health and Human Services
Associate Professor, Department of Kinesiology

4-29-98
Date

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DEDICATION

The goal that I have accomplished during the past five years would have not been possible without the love and support of the four most important people in my life:

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My son, Patrick Reilly, for his sense of humor, practical outlook, and continued confidence in my ability.

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My daughter, Shannon Reilly, for teaching me that achievement does not come easily and hard work and dedication are required to reach your goal.

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TABLE OF CONTENTS

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Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF TABLES

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Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

LIST OF FIGURES

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ABSTRACT

FACTORS THAT INFLUENCE THE DECISION REGARDING HORMONE REPLACEMENT THERAPY IN POSTMENOPAUSAL WOMEN

by

Ruth A. Rogers Reilly
University of New Hampshire, May 1998

Cardiovascular disease is the leading cause of death in women in the United States today. Prior to menopause women have lower rates of heart disease than men; however, after menopause a woman's risk for heart disease rises dramatically. This is thought to be due the ovaries' decreased production of estradiol, but the mechanisms for this effect have not been fully elucidated. The need exists to investigate the impact of healthy lifestyles on attenuating the risk of cardiovascular disease in women as they age.

The majority of research on hormone replacement therapy (HRT) has focused on the biomedical model suggesting that menopause reflects an estrogen "deficiency" and is treatable by pharmaceutical intervention. Little information is available that views menopause from a broader perspective which includes psychological and social factors. This research tested the hypothesis that postmenopausal women who practice positive lifestyle interventions, such as health supporting diets and regular exercise, will demonstrate positive biological, social and psychological outcomes independent of HRT.

Seventy healthy postmenopausal women, aged 48-66, who had experienced a surgical or natural menopause were categorized into one of three groups: (1) no hormone

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replacement therapy (n=36); (2) estrogen only (n=11); and (3) estrogen and progestin (n=23). Subjects donated a fasting blood sample and completed a comprehensive lifestyle and medical questionnaire that gathered information regarding health, menopause, decision making, self-esteem, and mood states. Subjects also completed a food frequency and three-day food diary. Anthropometric measurements were performed to determine body mass index and waist hip ratios. Biological assays of collected blood samples included estradiol and progesterone concentrations, lipid profiles, serum beta-carotene and vitamin E in total serum and in LDL, total antioxidants and the resistance of LDL to oxidation. Results indicated that lifestyle factors including quality of dietary intake, duration and intensity of exercise, and body weight may be better predictors of disease risk than use of HRT in postmenopausal women. Results suggest that, regardless of hormone use, positive lifestyle interventions will provide improved quality of life in women as they age.

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CHAPTER I

INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality in women in the industrialized world: one out of every two women will eventually die of heart disease or stroke (1). Prior to menopause women have lower rates of heart disease than men, but at menopause a women's risk of cardiovascular disease rises dramatically. This is thought to be due in part to the ovaries' decreased production of estradiol. The cardioprotective effects of estrogen appear to be modulated through several mechanisms which are currently being investigated. These mechanisms include estrogen's beneficial effects on carbohydrate metabolism, clotting factors, antioxidant ability and effects on the vascular system.

Menopause is defined as the end of menstruation. In industrialized countries this occurs at the average age of 51 (2) and it is diagnosed clinically with the cessation of menses for at least one year (3). Consequences of menopause include vasomotor instability, vaginal dryness, and mood swings and an increased risk for chronic diseases such as coronary heart disease and osteoporosis. The most common medical intervention used to treat both the acute and chronic symptoms of menopause is HRT.

Results of 30 epidemiological studies (4), (5), (6) are now available and have been summarized and compared in three meta-analyses. These studies have shown a significant reduction (relative risk of 0.56-0.65) in the incidence of fatal and nonfatal cardiovascular events in postmenopausal women using HRT. In spite of the fact that the

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health benefits of exogenous hormone use is well documented, compliance among women is poor. In 1994 Hammond estimated that less than 20% of postmenopausal women in the United States have been prescribed HRT. Of the women who use HRT, less than 40% will continue its use after one year, and overall 70% of these women are non-compliant (7). This may be due to a number of factors including fear of cancer, side effects, and unacceptable bleeding patterns. This suggests that women and health care providers need more information and education regarding the risks and benefits of the use of HRT during menopause.

Historically, research on menopause and HRT has been dominated by the medical model, which characterizes menopause as a deficiency disease requiring treatment (8). Less information or research is available that has investigated menopause from a psychological or social perspective. The question of how women make the important decision to use or not to use HRT remains virtually unanswered. There has been limited research into the contributions of lifestyle interventions, such as modifications in diet and exercise, as a alternative or adjuvant to HRT.

There is evidence that the oxidative modification of low-density lipoprotein cholesterol (LDL-C) plays a key role in the development of atherosclerosis. It has been proposed that estrogens alone (9) or with progestins (3) may protect against atherosclerosis in part by inhibiting the oxidation of LDL-C. In addition, the antioxidant nutrients α-tocopherol (vitamin E) and beta-carotene (β-carotene) also inhibit the oxidation of LDL-C (10), (11). LDL carries in it antioxidants, such as β-carotene and vitamin E, which can modify free radicals and prevent the oxidation of LDL. As a result, the cardioprotective effect of estrogen may be conferred in part by its influence on the

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antioxidant content of LDL. One focus of this research is to evaluate the influence of dietary intake of vitamin E and β-carotene, in modulating the risk profile of cardiovascular disease in postmenopausal women.

The contribution of the biological research on menopause and HRT would be strengthened by the inclusion of the social and psychological aspects of menopause. The biopsychosocial perspective attempts to understand health status by recognizing how the biological, psychological, and social elements are related to one another(12). Studies by Engel (13), (14) discussed the limitations of limiting explanations of a dysfunction to any one of its three major components (biological, psychological, social) and emphasized the benefits of their simultaneous inclusion in research models. These three factors have a synergistic effect on one another and their complex interplay should be considered in research protocols (15). Another focus of this research is to apply the biopsychosocial model to a population of women who are making decisions regarding HRT.

Specific Aims of the Study

1. To assess the biological consequences of the choice regarding HRT on cardiovascular risk profiles in postmenopausal women by evaluating the effect of HRT use or disuse on antioxidant (β-carotene and α-tocopherol) concentrations in serum vs. LDL in postmenopausal women.
2. To investigate the effect of positive lifestyle interventions (healthy diet and moderate, frequent exercise) on biological, social and psychological parameters independent of HRT use in postmenopausal women.
3. To investigate the variables influencing a woman's choice of HRT from a biopsychosocial (biological, psychological, and social) perspective.

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Hypotheses

This research seeks to investigate several hypotheses generated by the biopsychosocial model:

Biological

1. Postmenopausal women who use HRT will have higher concentrations of β-carotene and α-tocopherol in both the serum and LDL lipoproteins, and thus less susceptibility to oxidation of LDL than women who do not use HRT.
2. Serum estradiol concentrations would be positively correlated with serum concentrations of β-carotene and ∝-tocopherol, LDL ∝-tocopherol and LDL β-carotene, β-carotene/LDL and ∝-tocopherol/LDL ratios, and lipid profiles in postmenopausal women.
3. The use of HRT will positively affect serum cholesterol and lipoprotein concentrations Lp(a), high density lipoprotein, and low-density lipoprotein in postmenopausal women.
4. Postmenopausal women who practice positive lifestyle interventions (health supporting diets and moderate and frequent exercise) will demonstrate a decreased risk of cardiovascular disease regardless of their use of HRT.
5. Postmenopausal women who use HRT will have lower body mass indexes (BMI) and waist-hip ratios than women who do not use HRT.

Psychological

6. Concerns regarding cancer will be the primary reason reported for not using HRT, or for discontinuing its use in postmenopausal women.

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7. Postmenopausal women who practice positive lifestyle interventions (health supporting diets and moderate and frequent exercise) will report less severe postmenopausal symptoms, as well as more positive self-esteem and moods regardless of their use of HRT.

Social

8. Postmenopausal women will report that their choice to use HRT was strongly affected by outside influences in their lives (medical professionals, media, and significant others).
9. Postmenopausal women will report that acute symptom relief and concern over chronic disease (osteoporosis and/or heart disease) were the most influential factors in making the decision to use HRT.
10. Postmenopausal women who use HRT will consume diets lower in fat and saturated fat than those who do not use HRT.
11. Postmenopausal women who use HRT will be more likely to have used oral contraceptives (OC) in the past when compared to women who do not use HRT.

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CHAPTER II

REVIEW OF THE RELATED LITERATURE

Decision Making

One of the most controversial issues in women's health today is the use of HRT. Women experiencing menopause will be offered advice regarding the risks and benefits of exogenous hormone use. A great deal of the information disseminated to women is contradictory. There is much debate as to whether menopause should be viewed as an endocrine deficiency requiring long-term management with HRT, or as a natural stage in a woman's life requiring little or no medical intervention. These opposing opinions and recommendations communicated to women may compromise their ability to make an informed decision with respect to HRT (16).

There is great variability in the reported prevalence of HRT use in the United States. It is estimated that 50% of women will take HRT at some point in their lifetime (17), and estimates of current HRT use range from 5.3% (18) to 39.3% (19). These estimates vary depending on age, geographic location, study population, and time of the study (20).

The *Commonwealth Foundation National Survey on Women's Health* indicated that either perimenopausal or immediately postmenopausal women were most likely to use HRT, and also use of HRT decreased with advancing age (21). The strongest factors influencing the initiation of hormone use is surgical menopause (20), (7) and

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vasomotor instability (22). Women who have had a surgical menopause are two to three times more likely to use unopposed estrogen after menopause (23).

Historically, the research on HRT has focused on middle-class white women who have the greatest access to health care. Women who are currently using HRT tend to participate in more preventative health care behaviors including nutrient-rich diets and exercise (24). They also tend to be leaner (25),(26), (27), (28), of higher socioeconomic status (25), more highly educated and exercise on a regular basis (27). Hormone users are more likely than nonusers to view menopause as a medical condition and are less likely to consider natural alternatives to treat menopausal symptoms (29).

Several studies have reported that the most important reasons for starting HRT were recommendation of a physician (29), (16), (30), (31), followed by the possible relief of the acute symptoms of menopause (29). It has also been reported that only 19% of physicians allow their patients to stay on HRT for more than three years (32), (33) and that physicians are reluctant to prescribe hormone therapy for women with diabetes, hypertension, or known heart disease (24), (34). In one of the first studies evaluating women's knowledge and attitudes (31) about ERT, 64% of the women who had not used estrogen replacement reported never discussing it with their physician. Asked if their physician recommendation would have an effect on their decision, 75% reported that it would. This study (31) also reported that women who receive care from a gynecologist are much more likely to be prescribed HRT than women who receive care from other medical disciplines and that, in general, women who discontinue HRT use, do so upon the advice of a physician. It is clear that a woman's decision to use HRT is strongly influenced by the medical community.

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A survey conducted by the North American Menopause Society (35) determined that physicians were more likely to discuss the short-term effects of menopause than information on long-term risks such as osteoporosis and heart disease with their patients. When discussing treatment options to women, 84% of the physicians focused on HRT, 3% discussed exercise, and 2% diet. Other lifestyle interventions were mentioned even less frequently. This suggests that physicians tend to focus on the medical treatment of menopause and that women are not receiving information on interventions such as diet and exercise in treating menopausal symptoms and the prevention of chronic disease.

In spite of the fact that the health benefits of long-term estrogen use for the prevention of heart disease and osteoporosis have been well demonstrated, compliance is poor. Hammond (7) estimated that less than 20% of postmenopausal women in the United States have ever been prescribed HRT. Of those women on HRT, less than 40% will continue its use after one year, and 70% of all women on HRT are not compliant and the motivation to continue HRT diminishes over time. Women who have experienced a ovariectomy (20) or who have diagnosis of osteoporosis are most likely to use HRT on a long-term basis. Women with a natural menopause are the most likely to use HRT for a short period of time for the relief of the acute symptoms of menopause (20).

The *Massachusetts Health Study* (20),(29) demonstrated the problems with compliance and continuance of HRT in a longitudinal study of women aged 45-55. In this cohort of 2,425 subjects, 20% stopped treatment within nine months, 10% took the prescription sporadically, and 20-30% never filled the prescription. Women discontinued HRT use because of disappearance of menopausal symptoms, complicated and confusing treatment schedules, side-effects and fear of endometrial and/or breast cancer. Women

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rate unacceptable bleeding patterns(31), (36) and fear of cancer as the main reasons for discontinuing HRT (37).

Discontinuance of HRT is sometimes treated in the medical literature as a compliance problem that could be solved by more education and better designed drug packaging. In discussing compliance among women using HRT, Kaufert (38) refers to a phrase used by Weintraub (39), "Intelligent Noncompliance". This term is defined as a situation "where prescribed medication is purposely not taken and the patient's reason for noncompliance appears valid when analyzed dispassionately". It is suggested that a woman makes the decision to use or not to use HRT by intelligently weighing the risks and benefits of HRT (38). Doren and Schneider (40) suggest that physicians' understanding of compliance involves a unidirectional decision that is synonymous with the patient's acceptance of medical authority.

There are few studies on how women view and utilize the information available to them to make judgments regarding HRT. After controlling for perceived levels of menopausal distress, Logothetis (16) investigated women's beliefs about menopause. She reported that women who were currently using estrogen perceived themselves to be more susceptible to difficulties in menopause and viewed HRT as beneficial in treating these problems. It was found that women make decisions regarding HRT based on their perception of the risk-benefit ratio. The recurrent theme among women in this study was the need for more accessible and reliable information relative to HRT and menopause. A preliminary study, conducted by Rothert et al. (41) collected data for the purpose of designing and evaluating an educational intervention to assist women in becoming more effective decision makers during menopause. The data collected found that women were

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capable of processing information regarding the risk and benefits of HRT, could apply the information given to them consistently, and were influenced by personal and social factors in making their decisions. However, it was also reported that women did not have access to the information needed to enable them to participate in their own health care.

One study (42) investigated attitudes and knowledge of HRT among 600 female graduates of Stanford University with a mean age of 50. Forty-nine percent of these women used HRT. The women in this study perceived their risk of developing heart disease to be low, and breast cancer risk as high. Only 25% of the current users of HRT considered the potential protection against heart disease in their decision to use HRT. When asked what disease they feared most, three times as many women feared breast cancer (48%) when compared to heart disease (16%). This study demonstrates that even though highly educated women know that HRT could possibly reduce their risk of developing heart disease, they do not perceive themselves as vulnerable to heart disease. This group of women reported that they felt use of HRT on a long-term basis would increase their risk of breast cancer. Logothetis (16) compared the characteristics of current estrogen users to nonusers and found that only 10% of the current users reported prevention of chronic disease (heart disease or osteoporosis) as a reason for taking HRT and 75% reported use for less than 2 years.

In a recent study (43), questionnaires were mailed to 126 women, aged 45-55, to determine what factors influenced women's decision making to use HRT. The majority of the women surveyed (81%) knew that the risk for osteoporosis increased after menopause; however, only 53% knew that the risk for heart disease increased as well. Only 48% of the women surveyed realized the inverse relationship between HRT use and

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heart disease risk. This study also found that the most important predictor of HRT use was the recommendation of the health care provider.

To assist women in the decision making process, a mathematical model has recently been developed at New England Medical Center. The purpose of developing this model was to examine the effect of HRT on life expectancy in postmenopausal women with different risk factors for breast cancer, hip fracture, and heart disease. Results indicated that the use of HRT would increase life expectancy in most women. It also concluded that only women with a high risk of breast cancer and a low risk of cardiovascular disease would not benefit from HRT use. These researchers have developed a checklist that can be used by women and their physicians to evaluate their own individual risks and benefits to make an informed decision regarding HRT (44).

One factor in the reluctance to use HRT, as well as the low compliance, may be that the available information is confusing and difficult to obtain. Educational programs need to be developed to inform women about the physiology of menopause and the risk and benefits of HRT (40). Health care professionals also need to be better informed and to take an active role and become partners in the decision process. When provided with accessible and reliable information, women will be more capable of making an informed decision on whether to use or not to use HRT.

Current Status of Cardiovascular Disease In Women

Cardiovascular disease (CVD), which includes heart disease and stroke, is the leading cause of death in American women and accounts for nearly 500,000 deaths annually (1). CVD rarely occurs in premenopausal women, but risk for CVD in women

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increases steadily after menopause. *The Framingham Heart Study* reported that only 6 of every 1600 premenopausal women died of coronary heart disease (45); however CVD represents the leading cause of death in older women and accounts for nearly 53% of all deaths in women over 50 years of age (46). CVD also impacts the quality of life in older women, as heart disease and stroke are the two major causes of morbidity and disability in postmenopausal women (47).

Compared to men, women are most often diagnosed with chronic rather than acute heart disease and develop heart disease on average 10 years later than men (45). In the past, the diagnosis and treatment of heart disease was different for men and women. Women tended to undergo evaluations and treatments for cardiac disease substantially less frequently than men with similar or less severe symptoms, particularly for the evaluation of chest pain (48). A woman who suffered a myocardial infarction was more likely than a man to have a recurrence and to die in the recovery period (49). In fact, 45% of women, compared to 10% of men, were likely to die within a year following a heart attack and 40% of women compared to 13% of men reinfarcted within a year (50). Until the middle 1990's, the rate of early death after a myocardial infarction was higher in women than in men and the mortality rate for women who underwent coronary angioplasty or bypass surgery was higher than that of men. It is not clear if these adverse outcomes were due to older age, smaller body size and more frequent and severe coexisting illnesses sometimes seen in women, or because of substandard or delayed care as compared to men (52). Recently, several coronary care centers have reported similar mortality rates in men and women undergoing angioplasty since 1995. This is thought to be due to gender

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appropriate angioplasty devices designed for women's smaller arteries (53), as well as earlier diagnosis and treatment of heart disease.

Historically, there has been a lack of research on cardiovascular disease in women. Women of childbearing age were excluded from studies presumably due to the perceived confounding factor of the menstrual cycle. Older women were excluded because of co-morbidities. Until recently there had been almost no prospective data on primary prevention strategies for women, and most clinical decisions about the choice and dose of cardiac drugs was based on studies done primarily on middle-aged men (54).

The increased risk of CVD seen in women during the menopausal transition has been suggested to be, at least in part, due to changes in total cholesterol and lipoprotein concentrations. Cross-sectional studies have demonstrated that postmenopausal women have higher levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), lipoprotein (a), and triglycerides (TG) (55), (28), (56), (57). Conversely, high-density lipoprotein cholesterol (HDL-C) shows only a small decrease at the time of menopause (58), (59). Prospective studies have shown that HDL-C decreases slightly years before the last menstrual cycle, whereas the rise in LDL-C coincides with the end of menses (60). It has also been noted that after menopause, women develop more of the smaller, denser LDL-C particles which correlate with an increased triglyceride and decreased HDL-C concentrations. These conditions are considered to be atherogenic (61), (62).

A 1990 longitudinal study (60) investigated the influence of menopause on lipids and lipoproteins in women experiencing the menopausal transition. The 170 women who were not taking HRT were followed at 6 week intervals for two to three years. Serum

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lipids and lipoprotein profiles were examined cross-sectionally dependent on menopausal status: premenopausal, perimenopausal, and postmenopausal. Total cholesterol, LDL-C, and triglyceride serum concentrations increased, and HDL-C decreased as a result of menopause. All these changes occurred within six months of cessation of menses.

Prospective studies suggest that HDL-C may be the best predictor of coronary heart disease (CHD) in women (63), (64), (65). HDL-C was shown to be the strongest predictor CHD in women in the *Framingham Study* (66), *Lipid Research Clinic* (4), and *Donolo-Tel Aviv* studies(67). The *Framingham* and *Lipid Research* studies both demonstrated an association between HDL-C concentration and CVD when it was shown that a 10mg/dL increase in HDL-C serum concentration was correlated with a 40-50% decrease in CVD risk. In the *Tel-Aviv* study, high total cholesterol concentrations were essentially neutralized by high concentrations of HDL-C. In this study, subjects with high concentrations of HDL-C and high levels of total cholesterol (>250mg/dL) had age adjusted rates of heart disease that were not different than the rates in women with lower cholesterol levels. These findings suggest that in postmenopausal women, HDL-C is a critically important factor in determining the risk for cardiovascular disease. It also indicates that interventions such as diet and exercise, which increase HDL-C concentrations, can potentially decrease heart disease risk in women.

There is no direct evidence that decreasing serum cholesterol concentrations in women will decrease coronary morbidity and mortality except at higher concentrations of total cholesterol (68). Studies do concur that women with a total cholesterol concentration of >265mg/dL have three-fold increased risk of CVD when compared to women having the lower cholesterol concentrations (64). However, the evidence is less

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consistent in women with "borderline high" total cholesterol levels (200-240mg/dL). Only when HDL-C is taken into consideration is the relationship between total cholesterol and cardiovascular risk in postmenopausal women consistent (69).

Lipoprotein (a) [Lp(a)] is an atherogenic particle that is structurally similar to LDL-C, but contains a molecule of apolipoprotein (a) attached to apolipoprotein A-100 by a disulfide bond. Elevated serum concentrations of Lp(a) have been shown to be an independent risk factor for cardiovascular disease (70). Lp(a) concentrations are genetically determined and are resistant to alterations in diet and pharmaceutical treatments. Although supplemental niacin does appear to decrease Lp(a) concentrations (71). The *Framingham Offspring Study* reported that Lp(a) serum concentrations were 6% higher in postmenopausal women as compared to premenopausal women (70). Epidemiological studies have reported that Lp(a) concentrations increase with menopause, and that postmenopausal women taking HRT have lower concentrations of Lp(a) than those who do not use HRT (72). Kim et al. (72) showed that a combination HRT (estrogen and progestin) lowered Lp(a) in postmenopausal women. This effect was greatest in subjects with the highest basal levels of Lp(a). Perrone et al. (73) found that Lp(a) was not effected by HRT use in 42 hypercholesterolemic postmenopausal women. Lp(a) has been shown to be a predictor of CHD in both pre- and postmenopausal women (74). The role that Lp(a) plays in the pathogenesis of heart disease in women and the effect of diet or exercise on Lp(a) concentrations requires further clarification.

Clearly there is a strong biological component to cardiovascular risk in women. The risk factors for women include family history of CHD, menopausal status, hypertension, obesity and central adiposity, diabetes mellitus, current cigarette smoking,

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physical inactivity, elevated LDL-C and low HDL-C concentrations (75), (76). When multiple risk factors are taken into consideration, it is evident that this disease is not purely biological. It involves social and psychological factors as well, including low educational level and stress.

Hormone Replacement Therapy

The fact that a woman's risk for heart disease increases after menopause suggests that hormonal factors play a role in the development of heart disease (68). Data suggests that HRT is the treatment of choice for relief of acute symptoms of menopause and the prevention of such chronic diseases as heart disease and osteoporosis. The use of hormone replacement has increased in the last decade. One-fourth of all postmenopausal women in the United States are currently using some form of HRT (77).

The first commercial estrogen preparations became available in 1926, but were not widely used until the 1960's when the link between menopause and cardiovascular disease and osteoporosis began to be widely researched (78). Unopposed estrogen (estrogen without the addition of progestin) was used to prevent these chronic diseases until the 1970's when reports started to appear in the literature linking ERT with an increased risk of endometrial cancer (79). At this time progestin was added to the prescription to eliminate this risk and HRT became the treatment of choice. Currently HRT is the term more frequently used to indicate estrogen alone or in combination with a progestin.

The goal of postmenopausal hormone therapy is to replace lost endogenous estrogen to minimize the health risks associated menopause. For women who do not need endometrial protection (women without a uterus), unopposed estrogen is prescribed.

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Estrogen: Estrogen preparations constitute a wide variety of pharmaceutical options. Exogenous estrogen can be classified by chemical composition (natural or synthetic) or by route of administration (oral or parenteral).

Natural preparations of HRT are six to seven times less potent than the lowest dose of synthetic estrogens (80). The natural estrogens include conjugated equine estrogen (CEE), estradiol (either micronized or as the valerate ester) and estrone sulfate. *Premarin*™ (CEE) is the most commonly prescribed estrogen in the United States. This CEE is made from the urine of pregnant mares and is a mixture of three estrogens. The first component is estrone sulfate, an estrogen synthesized by both humans and horses, composes 50-60% of the preparation. The other two components equilin sulfate (20-30%) and 17 α-dihydroquilin sulfate (15%), are both horse estrogens (81). Other types of oral estrogens include micronized estradiol (*Estrace*™), esterified estrogens (*Estratab*™), and estropipate, or piperazine estrogen sulfate (*Ogen*™). In Europe, estradiol valerate (*Progynin*™) is the most commonly used oral estrogen. The synthetic estrogens ethinyl estradiol (*Estinyl*™), quinesterol (*Estrovis*™), and diethylstibestrol (*DES*), are rarely used today (82).

When taken orally, estrogen is absorbed through the intestinal wall where it begins to be converted to estrone and estradiol. These products are subsequently absorbed via the blood, and progress to the liver. The oral administration of estrogen results in hormone concentrations in hepatic sinusodial blood that are four to five times higher than those in the peripheral blood (83). This is called the "first pass" effect and it promotes the hepatic synthesis and secretion of coagulation factors, renin substrates, sex hormone binding proteins and a variety of lipid apoplipoproteins (84).

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Parenteral routes of estrogen administration include injection, transvaginal (creams, tablets, and silastic rings), transdermal ("patch"), subcutaneous pellets, intranasal and percutaneous (gel) administration (81). The estrogen patch in particular is very popular in Europe and is gaining popularity in the United States. *Estraderm*™, the most commonly used patch, releases 17 β-estradiol, and consists of four layers. Since use of the patch can also result in endometrial hyperplasia, it is commonly used in combination with an oral progestin. The most common side effect with patch use is skin irritation, but because the patch does not adversely affect liver function its use is increasing (85).

Parenteral administration does not result in alterations in renin substrate or clotting factors and the effect on lipoprotein concentrations varies with dose and route of administration. This is thought to be due to the fact that these preparations enter the systemic circulation before reaching the liver and thereby avoid the "first pass" hepatic effect (84).

Progestins: Progestins are added to the estrogen prescription to prevent endometrial hyperplasia in women with a uterus. Numerous types, doses, and schedules are used. Synthetic progestins are the most frequently used because progesterone is poorly absorbed (84). The synthetic progestins are usually two basic types: the 19-nortestosterones (Medrogestone and Norethisterone acetate) and 17-α progestagens (medoxyprogesteroneacetate-MPA-*Provera*™). A natural progesterone in a micronized form is currently available in pill or cream form throughout Europe and in Canada. Micronized progesterone is made by breaking progesterone into small pieces to facilitate better absorption of the drug. In the United States it is available only through alternative

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pharmacies although is expected to be approved by the FDA for use in the United States in the near future (86).

Estrogen and progesterone can be taken *continuously* or *cyclically*. In cyclical therapy, estrogen is typically taken daily and progestin for only the first twelve days of the month, when most women experience breakthrough bleeding. In continuous therapy, both estrogen and progestin are taken daily which results in less breakthrough bleeding. A new formulation, *Prempro*™, combines *Premarin*™ and *Provera*™ in one tablet and is approved for use in the United States (87).

Testosterone: The possible effects of androgen replacement have not been fully examined. Androgens alone or in combination with estrogen or with estrogen and progestin are widely prescribed to postmenopausal women for relief of menopausal symptoms, especially depression and decreased libido. Clinical trials addressing the efficacy of prescribing testosterone to postmenopausal women are few; and the existing data is fraught with cofounders such as differences in study populations (surgical or natural menopause), lack of controls, blinding and randomization. Also, the association between libido and serum testosterone levels is tenuous. Exogenous testosterone appears to only effect women whose blood concentrations of testosterone are low prior to treatment (82). In women who have experienced a surgical menopause, there has been an improvement in menopausal symptoms demonstrated at very high concentrations of testosterone. However, lower doses of testosterone used with women who have experienced a natural menopause have failed to show the same results. *The Nurses' Health Study* (88) found an increased risk of breast cancer in women taking androgens (relative risk 1.64; 95% confidence interval, 0.53 to 5.09). Also, the effect that these preparations have on

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cholesterol levels is not well-defined (89). There is a need for more studies to determine the risk and benefits of androgen replacement therapy use during menopause (90).

Research on HRT is a field of study that is expanding very rapidly as women continue to seek more information regarding the risk/benefit equation of these therapies. Thompson (78) suggests that it is illogical to assume that all women will respond in the same way to exogenous hormones considering the variations in absorption, first-pass metabolism with oral preparations, and plasma binding, all of which can affect the bioavailability of the drug. Therefore, hormone replacement therapy needs to be individualized according to the woman's symptoms, risk factors, and response to the specific dose and type of hormone used.

The Cardioprotective Effect of Estrogen

The first clinical trial investigating estrogen and heart disease was conducted on men and demonstrated an increased risk of myocardial infarction in those subjects receiving supraphysiologic (2.5 mg or 5 mg *Premarin*™) doses of estrogen (91). In 1979, Nachtigall (200) conducted the first prospective randomized clinical trial on estrogen and heart disease on 168 institutionalized women and followed them for ten years. In this study there were eighty-four pairs of randomly assigned postmenopausal women, half receiving no HRT, and the other half receiving a cyclic combination of *Premarin*™ and progestin. Although not statistically significant, the hormone-treated women demonstrated more positive lipid profiles.

Animal studies have also shown a positive correlation between HRT use and decreased risk of cardiovascular disease. Cynomolgus macaque females (*Macaca*

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fascicularis) are excellent models for research on coronary heart disease in both pre-and postmenopausal women. Cynomolgus monkeys are susceptible to atherosclerosis. Females have a twenty eight-day menstrual cycle, sex hormone patterns similar to human females, and natural menopause occurs later in their lifespan (93). Like women, premenopausal females of this species have higher plasma concentrations of HDL-C than postmenopausal females. These monkeys apparently have protection from atherosclerosis premenopausally, but also experience loss of this protection and progressive coronary artery disease with age. Adams et al. (94) used this animal model to investigate the influence of HRT on diet-induced atheroscelerois in ovariectomized adult female monkeys. All the animals were fed an atherogenic diet and menopause was induced by ovariectomy. The animals were then randomly assigned to three treatment groups. Group one received no HRT (n=17). Group two was given continuously administered 17-β estradiol plus cyclical progesterone (n=20), and group three was given continuously administered 17-β estradiol (n=18) only. Physiologic concentrations of plasma estradiol and progesterone were maintained by the administration of the hormones using subcutaneous implants. The experiment lasted for 30 months.

At autopsy, the extent of atherosclerosis was evaluated by measuring the intimal area of carotid arteries and aortas. In the untreated group (no hormone replacement) atherosclerosis was significantly more extensive (50%) than in animals in the two hormone treated groups. There was no significant difference between the two hormone replacement groups. The decreased risk was found to be independent of variations in total plasma cholesterol, LDL-C, apolipoprotein A-1 and B concentrations, as well as HDL-C, and LDL-C particle size.

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Haarbo et al. (50) had similar findings when he studied orally administered 17β-estradiol alone or with progestins in ovariectomized cholesterol-fed rabbits. All hormone treated animals demonstrated reductions in aortic cholesterol accumulation, as well as significant reductions in cholesterol concentrations.

Endogenous estrogen has been shown to confer a positive effect on lipid and lipoprotein concentrations in women prior to menopause. Endogenous estrogen stimulates VLDL-C catabolism by transferring surface components to HDL-C, thus increasing HDL-C levels (95). Total cholesterol and LDL-C increase with age in both genders, but women tend to have lower levels than men. This may be because estradiol stimulates lipoprotein lipase and enhances hepatic uptake of LDL-C, which results in lower LDL-C concentrations (75). In spite of the positive effect endogenous estrogen has on lipids and lipoprotein concentrations, the relationship of physiological levels of estradiol and CHD is based largely on observational studies. Studies on postmenopausal women have demonstrated no positive association between endogenous estrone or estradiol levels and cardiovascular risk factors, especially HDL-C (96). One prospective study followed women through menopause and found a concurrent decrease in estradiol and HDL-C (59).

In 1995 Barrett-Connor and Goodman-Gruen (82) conducted a study in Rancho Bernardo, California to examine the association between serum concentrations of androstenedione, testosterone, and estrone on risk of death from cardiovascular disease in 651 postmenopausal women who were not taking exogenous estrogen. They found no correlation between concentrations of these three sex hormones and risk of heart disease in these subjects. These studies indicate that although endogenous sex hormones seem to

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have a beneficial effect on lipids and lipoproteins, they do not appear to confer protection against heart disease in postmenopausal women.

Observational studies have consistently found a decrease risk of heart disease in postmenopausal women using HRT. In 1991, Stampfer and Colditz (5) conducted a meta-analysis of all studies to date that had investigated the link between estrogen replacement therapy and heart disease. They reported a relative risk of 0.56 (95% confidence interval, 0.50-0.61) for all studies. The same research team conducted a second study in 1996 and examined the relationship between heart disease in postmenopausal women using estrogen and progestin. They reported a relative risk of 0.39 (95% confidence interval, 0.19-0.78) in women using estrogen and progestin, and a relative risk of 0.60 (95% confidence interval, 0.43-0.83) in women taking estrogen only when comparing both to women taking no hormone replacement (97). This data suggested that progestin taken in combination with estrogen, is not as strongly correlated with a decreased cardiovascular risk in postmenopausal women as when estrogen used alone.

Bush et al. (4) followed 2,270 women (aged 40-69) for 8.5 years in the *Lipid Research Clinics Prevalence (LRC) Study*. The age-adjusted relative risk for cardiac disease was 0.34 (95% confidence interval, 0.12-0.81) for estrogen users compared to nonusers. HDL-C was also shown to be inversely correlated with cardiovascular death in this study. The protective effect demonstrated in estrogen users may be due to the increased HDL-C concentrations seen in the subjects who used estrogen.

The *Leisure World Study* (98) followed 8,841 women (aged 40-101) for 5-1/2 years (40,919 person years) using a detailed health survey. The all-cause mortality for

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estrogen users had a relative risk of 0.80 (95% confidence interval, 0.70-0.91) as compared to nonusers, and for fatal MI the relative risk was 0.59 (95% confidence interval, 0.42-0.82). After 7.5 years of follow-up (99), women reporting a history of estrogen use had an age-adjusted all cause mortality risk that was 20% lower than non-estrogen users. Mortality was shown to decrease with increasing duration of estrogen use, and was lower in current users when compared to women who had used estrogen in the past.

In 1996 (100) a cohort of 292 postmenopausal women (aged 55-99) volunteered to participate in a study that was designed to assess the relationship between lipids and lipoprotein concentrations, and unopposed estrogen or combination hormone (estrogen and progestin) replacement use. Estrogen, unopposed or in combination, was negatively correlated to total cholesterol, LDL-C and positively related to HDL-C. This study showed no reduction in the beneficial effect found with unopposed estrogen in the women using combined HRT.

In contrast to the other observational studies, early reports from the *Framingham Heart Study* (101) reported an increased risk for cardiovascular disease in 1,234 postmenopausal women (aged 50-83) who used HRT. The subjects were followed for eight years. Women who reported using estrogen , at one or more examinations, had over a 50% increased risk of cardiovascular morbidity ($p < 0.01$) as women who did not use HRT. However, when the data from this study was reanalyzed (102), excluding an endpoint of angina pectoris, the use of HRT was associated with coronary heart disease in only a small group of women who were 60-69 years old at baseline.

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The *Nurses' Health Study* is the largest ongoing observational study on the relationship of HRT and mortality in women to date (103). The study was initiated in 1976 when questionnaires were mailed to 121,964 female registered nurses aged 30-55. Questionnaires sought information on personal and family medical history, menopause, smoking, height and weight, use of oral contraceptives and/or HRT, cancer, and cardiovascular risk factors. A total of 32,317 postmenopausal women without prior CHD have been followed for over twenty years for a total of 105,786 person years. Information has been updated with biennial questionnaires since 1976 to determine the relationship between postmenopausal hormone use and mortality among participants. Death was documented by medical records. Follow-up studies since 1985 (104), (105), (106), (97) have consistently reported a reduced risk of cardiovascular disease in women using HRT.

The most recent study reported by these researchers investigated the relationship between the use of postmenopausal hormones and mortality among participants of the *Nurses' Health Study* (107). Current hormone users were found to be at decreased risk of death (relative risk 0.63; 95% confidence interval, 0.56-0.70) compared to subjects who had never used hormones. However, this benefit was found to decrease after 10 or more years of HRT use because of the increase in mortality from breast cancer. It was also found that current users with coronary risk factors demonstrated the greatest reduction in mortality (relative risk 0.51; 95% confidence interval, 0.45-0.57), but subjects with low risk demonstrated less benefit (relative risk 0.89; 95% confidence interval, 0.62-1.28).

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The Effect of HRT on Lipids and Lipoproteins

Exogenous estrogen has been demonstrated to exert a cardioprotective effect on postmenopausal women. This is thought to be due in part to alterations in lipids and lipoprotein concentrations. Over the past 30 years, there have been countless studies investigating the effect of postmenopausal HRT on lipid and lipoprotein concentrations. The earlier studies involved unopposed estrogen, and the later studies the addition of progestins. This has offered researchers the opportunity to investigate the effect of estrogen independently and subsequently the modifying role of progestins. A large number of cross-sectional and longitudinal studies have assessed the influence of postmenopausal HRT on plasma lipoprotein concentrations, but the results of these studies reflect differences in study design, the size and characteristics of the subject pool, hormone formulations, treatment regimes, duration of hormone use, and laboratory methods (108).

A 1994 hypothetical decision analysis evaluated the relative risks and benefits of long-term estrogen use in a cohort of 10,000 women at age 50. Health outcomes were extrapolated to age 75 (17). The results suggested that estrogen use for 25 years would decrease fatal CHD events by 48% and deaths from hip fractures by 49%. It is also indicated, however, that HRT use would increase deaths from breast cancer by 21% and endometrial cancer by 207%. In conclusion, the study suggested that the health benefits of 25 years of postmenopausal estrogen therapy exceeded the risks in this theoretical population.

Oral unopposed estrogen has been consistently shown to reduce total cholesterol and LDL-C, while increasing HDL-C and triglyceride concentrations. All forms of ERT (oral, transdermally, percutaneously, or subcutaneously) decrease total cholesterol plasma

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concentrations. Whitehead (109) reported that various studies have demonstrated between 5% and 20% lower values depending on the type of estrogen, the route of administration, and the dose. A review by Bush (110) of 10 randomized crossover studies of normolipidemic women showed an increase in serum triglyceride concentrations of 20-24% over baseline levels and an decrease in LDL-C concentration with oral estrogen use. Walsh (111) showed a dose dependent reduction of LDL-C, 15% with 0.625 mg CEE and 19% with 1.25 mg CEE. The reduction in LDL-C with estrogen use is thought to be due to increased LDL-C catabolism that results in an increased number of LDL receptors and activity (112). Oral estrogens have been shown to increase HDL-C concentrations by 10-15% (110). *The Lipid Research Clinics Study* (113) reported a 10% higher HDL-C concentrations in subjects using CEE when compared to nonusers.

The Addition of Progestins to ERT: During the 1970's reports were appearing in the literature linking ERT with an increased risk of endometrial cancer (79). In order to minimize this risk, progestin was added to ERT. This study appeared to eliminate the risk and HRT became the treatment of choice. There was a concern, however, that the addition of progestins to the prescription might modulate the positive effects of estrogen on the cardiovascular and skeletal systems. A recent controlled clinical trial, *The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial* (63), was designed to investigate the effect of the addition of progestins to ERT on cardiovascular risk factors in postmenopausal women. This study involved 875 healthy postmenopausal women, aged 45-64, who were randomly assigned to placebo, unopposed estrogen, or one of three combined estrogen/progestin regimes. Four aspects related to the risk of CVD were chosen as endpoints: HDL-C plasma concentrations, systolic blood pressure, serum

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insulin, and fibrinogen concentrations. The results of this study indicated that estrogen alone, or in combination with a progestin, improved lipoprotein concentrations and lowered fibrinogen levels without affecting insulin or blood pressure. The *PEPI Trial* provides clear evidence that unopposed estrogen has a more favorable effect on HDL-C than estrogen given with continuous or cyclic progestins. However, all hormone treatments were significantly better than the placebo in increasing HDL-C and lowering LDL-C.

It appears that the metabolic effects of the addition of progestin to ERT are related to dose, the relative androgenic potency of the hormone preparation, and the accompanying dose of estrogen. The addition of progestin is associated with increased triglyceride and VLDL-C plasma concentrations beyond what is seen with unopposed estrogen. Progestins increase hepatic lipase activity and appear to decrease hepatic VLDL-C production. In general, the 19-northestosteron derivatives, which have high androgenic activity, oppose the positive effects of estrogen on lipoprotein metabolism, decreasing HDL-C and increasing LDL-C concentrations. The 17-hydroxyprogesterone derivatives (low androgenic activity) have predominantly neutral effects on lipid and lipoprotein cholesterol levels (62). This was demonstrated in a controlled study conducted by Tremollieres et al. (114) in 1995 which assessed the long-term effect of a low androgenic progestin (*Promegestone*™) on serum lipids, renin substrate, and antithrombin activity. Thirty-five postmenopausal women were randomly assigned to a placebo or progestin group and were followed for 2 years. The results of this study showed no statistically significant differences in the biological parameters between the two groups.

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A study reported by Miller et al. (115) in 1994 investigated the qualitative and quantitative effects of two doses of estrogen before and after the addition of progestin. The 103 postmenopausal women in this study were randomly assigned to one of three groups for 4 months: no estrogen, 0.625 mg/day, or 1.25 mg/day. At four months the estrogen groups were given 10 mg/day medroxyprogesterone acetate (MPA) for 8 months. After 1 year of treatment, the estrogen treated groups showed a decrease in LDL-C, but an increase in HDL-C and triglyceride concentrations when compared to the placebo group.

Nabulsi et al. (116) reported on a cross-sectional study of approximately 5000 postmenopausal women. Subjects were categorized according to current hormone use: unopposed estrogen users, estrogen/progestin users, nonusers who had used HRT in the past, and those who had never used hormone replacement. Subjects using hormone replacement had higher triglyceride, HDL-C, and HDL_2 than nonusers, as well as lower plasma concentrations of LDL-C. These researchers concluded that subjects who used combination HRT appeared to have a lower cardiovascular risk than those using unopposed estrogen.

A Medline search conducted of English-language articles published from 1981-1995 summarized the effect of hormone replacement therapy on lipid and lipoproteins in postmenopausal women (117). The pooled results from 42 studies demonstrated a decrease in total cholesterol of 14.4 mg/dL with continuous and 14.9 mg/dL with sequential HRT; a decrease in LDL-C of 17 mg/dL with continuous and 18.4mg/dL with sequential HRT; as well as an increase in HDL-C of 1.9 mg/dL with continuous and 3.1 mg/dL with sequential HRT.

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The majority of studies conducted in the United States on postmenopausal hormone therapy and heart disease have used estrogen and most often *Premarin*™. There is a need for more randomized, controlled, clinical trials that utilize different forms of estrogen and include the addition of progestin to the formulations. Two new studies, the *Women's Health Initiative* and the *HERS Study*, will address these issues in the near future.

The *Women's Health Initiative* is the largest clinical study ever undertaken by the National Institutes of Health (NIH). Subjects were recruited from 45 communities throughout the United States in the fall of 1993 (118). This study of approximately 57,000 women aged 50-79 year old, will address the risks and benefits of unopposed estrogen and estrogen plus progestin, a low fat diet, and a calcium and vitamin D supplement on cardiovascular disease, cancer, and osteoporosis. Results of the study are expected to be available in 2008 (92).

The *HERS (Heart and Estrogen-Progestin Replacement Study*) will investigate if HRT can prevent a second myocardial infarction (MI) in women who have already had a heart attack, coronary bypass surgery, angioplasty, or have been diagnosed as having less than 50% narrowing of one or more major coronary arteries. Results of this study are expected within the next few years (24).

The use of exogenous hormones has been shown to result in favorable lipoprotein profiles in postmenopausal women. It is important to note that the changes demonstrated with hormone use is dependent on type, potency, combination, and dose of the hormones used. It is still unclear to what extent the alterations in lipoproteins contribute to the decreased incidence of cardiovascular disease demonstrated with HRT.

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Other Mechanisms

It is presently believed that ≈20-25% of the cardioprotective effects of estrogen are mediated by the beneficial changes seen in lipids and lipoproteins (109). The remaining 75-80% is proposed to be mediated by several other mechanisms including estrogen's beneficial effects on carbohydrate metabolism, clotting factors, antioxidant ability and its direct effects on the vascular system.

Disturbances in glucose metabolism have been shown to be predictive of subsequent coronary artery disease (119). This increased risk is accompanied by hyperinsulinemia and insulin resistance. It has been shown that insulin resistance increases progressively in postmenopausal women. It is also evident that ERT increases the pancreatic secretion of insulin and decreases insulin resistance. Androgenic progestins, however, may oppose these beneficial effects (120).

Interest in the effect of HRT on a woman's homeostatic mechanism is growing. It has been demonstrated that the overall balance between coagulation and fibrinolysis results in a reduction in thrombosis in women using HRT (121). The effects of HRT on coagulation and fibrinolytic factors is variable depending on the type and dose of the hormone used (120). A recent study (122) demonstrated the beneficial effects of HRT on fibrinolysis in 30 postmenopausal women (mean age 55 ±5 years). The oral administration of CEE alone or in combination with MPA (medroxyprogesterone acetate) inhibited plasminogen activator inhibitor (PAI-1) by 50%. The *Framingham Offspring Study* (123) reported high concentrations of PAI-1 in postmenopausal women when compared to premenopausal women. This increased concentration of PAI-1 is believed to contribute to the increased risk of atheroslerosis seen in postmenopausal women. In another group of

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women, transdermal estrogen administration, with and without progestin, did not decrease PAI-1 levels. It appears that only oral estrogen has an effect on PAI-1 and this suggests that the hepatic effects of estrogen regulate PAI-1 synthesis, clearance, or both in an important way.

A case-controlled study (124) was conducted as part of the UNHWHS that sought to determine the effect of HRT use on the risk of thrombotic disease in 171 healthy women aged 38-65. The use of HRT was not found to increase the risk of thrombotic disease. The mechanism for protection involves lowing antithrombin III or protein C. The authors suggest that the use of HRT in postmenopausal women could be protective by increasing circulating protein C concentrations.

There is also good evidence that estradiol has a direct and protective effect on the arteries. Animal studies have shown that there are active estrogen receptors present in both the heart and major vessels (125). Losordo et al. (127) showed that the intima and media of most atherosclerotic coronary arteries of both pre- and postmenopausal women lacked estrogen receptors, but receptors were found in the majority of atherosclerosis free vessels. Another study (127) also observed a decreased number of estrogen receptors in the coronary arteries of women with atherosclerosis when compared to normal control subjects, suggesting the receptor levels may be altered in disease states.

There is a growing body of evidence suggesting that a great deal of the cardioprotection of estrogen is mediated by its direct action on the vascular wall (128). Estrogen appears to enhance vasodilation and reduce vascular resistance, which in turn leads to an increased blood flow (129). Estrogen, directly or indirectly, may retard the development of fibrous plaque, favorably affect the vulnerability of existing plaque, or

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reduce the risk of an occlusive thrombus (130), (131). Exogenous estrogen use was found to reduce the incidence of restenosis in 204 postmenopausal women who had undergone a coronary angioplasty or atherectomy. This was thought to be due to estrogen's effect on vasodilation in the coronary arteries, which works to reduce the pathologic vasodilation often seen after angioplasty (132). It is now clear that the beneficial effects of estrogen are mediated by many mechanisms other than its effect on lipid and lipoprotein serum concentrations. It is plausible that the cardioprotective effect of estrogen is mediated by a combination of mechanisms resulting in the overall beneficial effect seen with HRT.

Vitamin E and β-carotene as Antioxidants

It has been suggested that estrogen alone, or in concert with the antioxidant nutrients, may confer protection against the oxidation to LDL-C, thereby preventing the development of heart disease. Epidemiological studies (133), (134) and clinical trials (135), (136) indicate that populations with high intakes of antioxidants have a lower risk of heart disease. It is suggested that vitamin E (α-tocopherol) and beta-carotene (β-carotene) and may prevent the initiation of atherosclerosis and thereby reduce the morbidity and mortality of coronary artery disease.

High serum levels of LDL-C are associated with the development of heart disease. Studies suggest that the atherogenicity of LDL-C is greatly enhanced by oxidative damage (137). In *vitro*, oxidized LDL shows a diminished affinity for the LDL-C receptor and an increased affinity for the macrophage scavenger receptor. This results in foam cell formation. The uptake of oxidized LDL by macrophages is not down-regulated by

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internalized LDL-C, and this results in the lipid loading of the cells. Oxidized LDL-C is cytotoxic towards vascular cells, and chemotactic towards monocytes (138).

Antioxidants may inhibit the oxidation of LDL-C, hence decreasing the risk for the development of atherosclerosis. The possibility that antioxidants may prevent or retard LDL-C oxidation is suggested by the fact that the core of LDL-C contains lipophilic antioxidants, such as α-tocopherol, β-carotene, lycopene, ubiquinol-10, crytoxanthin, and phytofluene (10), as well as estrogenic compounds. The antioxidants present in the LDL-C are believed to prevent the oxidation of polyunsaturated fatty acids within the LDL-C (11). The antioxidant vitamins, β-carotene and α-tocopherol, react with highly reactive free radicals and inhibit LDL-C oxidation in *in vitro* systems and at pharmacological doses *in vivo* (139).

Vitamin E and β-carotene reside in circulating lipoproteins and lipid membranes. These antioxidants are able to terminate chain reactions and quench free radicals that could ultimately result in LDL-C oxidation. Due to its lipid solubility, β-carotene partitions in the lipid domain of biological membranes and lipoprotein particles. Alpha-tocopherol is the most abundant isomer of the vitamin E family and is the principle lipid-soluble chain-breaking antioxidant in tissue and plasma (140). Vitamin E has been shown to inhibit the proliferation of smooth muscle cells *in vitro* (140). When added to plasma, it increases the resistance of LDL-C to oxidation (141). Thus, LDL-C carries within it a number of natural antioxidants that can trap free radicals and interfere with and possibly prevent LDL-C oxidation (142).

Several studies have investigated the correlation between several dietary antioxidants and coronary artery disease with conflicting results. The *Scottish Heart Study*

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(143) investigated the correlation between diet and risk of coronary artery disease in 10,359 men and women (aged 40-59). The men with the highest vitamin E intake demonstrated a significant reduction in risk of coronary heart disease. The trend was not found to be significant in women. *The Optimal Vitamin Study*, a follow-up to *WHO/MONICA Core Study* (134), evaluated 16 European populations regarding age specific CHD mortality. The results demonstrated a strong inverse correlation between lipid standardized α-tocopherol concentrations and CHD mortality rates.

The Nurses' Health Study (144), (145) followed 87,245 female nurses (aged 34-59) for eight years to evaluate their consumption of vitamins through food and dietary supplements. There was an inverse relationship between risk of CHD and consumption of antioxidant vitamins. Women in the highest quintile of vitamin consumption, compared to those in the lowest quintile, demonstrated a relative risk of 0.66 (95% confidence interval, 0.50-0.87) for vitamin E, a relative risk of 0.78 (95% confidence interval, 0.59-1.03) for β-carotene, and a relative risk of 0.70 (95% confidence interval, 0.53-.92) for vitamin A. All the results reported were adjusted for age, smoking, and other CHD factors.

The Physicians Health Study (135), a randomized, double-blind, placebo-controlled 2×2 factorial trial of 22,071 US male physicians (aged 40-84) included a trial of β-carotene and its role in preventing heart disease. Among the physicians who received the β-carotene there was a 50% reduction in the risk of major coronary events, and a 54% reduction in the risk of major vascular events. *The Health Professionals Follow-Up Study* (136) evaluated dietary intake of vitamin E and β-carotene and the risk of cardiovascular disease in a subset of 39,910 healthy male professionals, aged 40-75. After controlling for age and several coronary risk factors, the men with higher intakes of vitamin E had a 41%

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lower risk of coronary artery disease when compared to the men with the lowest intake. The greatest reduction in risk was found in men consuming 100-249 IU of vitamin E a day. β-carotene intake was not associated with a lower risk for coronary artery disease in those men who never smoked, but was inversely correlated with current and former smokers.

The Relationship Between Antioxidants and HRT

There have been several *in vitro* studies that have demonstrated the antioxidant properties of estrogen. When 17β-estradiol was infused directly for 20 minutes into the brachial arteries of postmenopausal women, it delayed the onset and rate of copper-initiated oxidation of isolated LDL when compared to baseline values. Administration of estradiol for three weeks with a transdermal patch resulted in a similar protection from oxidation. This response was elicited at one third of the estradiol plasma concentration when compared to the acute administration method. This study demonstrated an antioxidant effect of physiological levels of estradiol (9).

Recent studies have suggested that estrogen alone or in combination with progestin may exert an antioxidant effect on LDL-C and lipids in the plasma membrane (9). One of the first *in vivo* studies to evaluate the antioxidant activity of three major estrogens found in *Premarin*™, involved eight postmenopausal women in a prospective randomized cross-over study (146). Each subject received a randomized schedule of an oral treatment of estrone sulfate, equilin sulfate, or 17 α-dihydroequilin sulfate for 30 days each. The oxidation resistance of LDL-C was determined by the duration of the lag-phase in copper-ion induced oxidation. The lag time was significantly increased with all three

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estrogens, with estrone sulfate conferring the greatest antioxidant activity. This study may explain in part the cardioprotective effect seen with the use of exogenous hormones in postmenopausal women.

A similar study demonstrated the effect of a combination of estrogen and progestin on LDL-C oxidation in a longitudinal prospective study of 39 healthy postmenopausal women (147). The subjects received a cyclic administration of conjugated estrogen (*Premarin*™) and medrogestone (*Colpro*™). After one year of treatment, this sequential hormone treatment resulted in beneficial effects on lipoprotein concentrations, but the results of LDL oxidizability were inconsistent.

A 1995 study (148) investigated if the cardioprotective effect of estrogen may be conferred in part by its antioxidant influence on LDL-C. Along with dietary assessments, changes in serum lipids, apolipoprotein B, α-tocopherol, β-carotene, retinol, LDL tocopherol, LDL β-carotene, and the susceptibility of LDL to oxidation, were determined. Measurements were taken at five intervals across two menstrual cycles in ten women cycling normally, and ten women taking oral contraceptives. Results showed significant and different changes in serum LDL-C, apolipoprotein B and total cholesterol across cycles in both groups. Serum and LDL β-carotene concentrations, and length of the lag phase varied significantly across cycle phase during the menstrual cycle. Serum retinol concentrations were significantly higher and LDL β-carotene concentrations were significantly lower in the group taking the oral contraceptives. These findings demonstrate a number of hormone related changes that influence the antioxidant protection of LDL-C in conjunction with dietary intakes of these micronutrients.

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Since there is a strong association of lipophilic vitamins with lipoprotein particles, some authors have suggested that this relationship should be expressed as the ratio of α-tocopherol and β-carotene serum levels to LDL content (149), (51). Clemente et al. (150) investigated whether HRT could confer an antioxidant effect on lipids and therefore preserve the vitamin E and β-carotene in the LDL. Fifteenpostmenopausal women were treated with a cyclic administration of transdermal estradiol and oral medroxyprogeserone. After six months of treatment, there was a significant reduction in total cholesterol and LDL-cholesterol in the subjects. Although the serum levels of α-tocopherol and β-carotene did not change, there was a significant increase in α-tocopherol/LDL and β-carotene/LDL ratios. This study suggests that HRT may preserve and enrich the content of α-tocopherol and β-carotene in LDL particles, as well as keep LDL in a reduced antioxidant state.

There appears to be evidence to support the protective effect of α-tocopherol and estrogen in decreasing the susceptibility of LDL to oxidative stress. The evidence for a similar effect due to β-carotene seems less clear. There is a need for more studies to evaluate the effect of an increased dietary intake of vitamin E and/or β-carotene (alone or with supplements) and different regimes of hormone replacement therapy on the oxidizability of LDL.

Lifestyle Interventions

Lifestyle factors have been suggested to influence the development of heart disease. Obesity, smoking, and central adiposity result in increased risk, whereas weight control, regular exercise, stress reduction, and the cessation of smoking result in a

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decreased risk. Much of the research on the prevention and treatment of cardiovascular disease in women has focused on HRT. The benefit of positive lifestyle interventions (health supporting diet and exercise) on the overall health and well-being of older women has not been well documented. Little information is available as to the influence of lifestyle factors in alleviating the acute symptoms of menopause and in preventing the development of chronic disease.

Diet: The role that diet plays in the management of plasma lipid concentrations has been recognized for some time (151). Diets low in total fat, cholesterol, and saturated fat, and high in soluble fiber and mono- and polyunsaturated fat have resulted in positive changes in serum lipid profiles in women. Many epidemiological and dietary studies have shown that increased intake of dietary sources of the antioxidant nutrients (β-carotene, vitamin C, and vitamin E) may also inhibit the development of cardiovascular disease (136), (152).

Epidemiological studies have shown that people who consume high amounts of antioxidant vitamins through diet and supplements have a decreased risk of cardiovascular disease (133). This link between antioxidants and disease is based primarily on evidence involving dietary intake or blood levels that reflect dietary choices, rather than supplements (153). Kushi et al. (154) surveyed 34,486 healthy postmenopausal women using a questionnaire on dietary intake of vitamins from food and supplements. After seven years of follow-up, the women with the highest dietary intake of vitamin E demonstrated the lowest risk of coronary artery disease; women with the lowest intake, the highest risk. No such association was found when intake from supplements and diet was included. This study suggests that women can lower their risk of heart disease without using supplements. No such association was found for β-carotene in this study.

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Many dietary factors affect plasma lipids and lipoproteins. Studies have shown that dietary factors are predictive of blood lipid concentrations in women. High dietary intake of saturated fat is a negative predictor of serum total cholesterol, LDL-C, and HDL-C in premenopausal women (155), (156). In a study on aging and nutrition, Garry et al. (157) assessed dietary intakes and blood lipid concentrations in 92 postmenopausal women over a nine year period. This study found that decreased intakes of total and saturated fat were correlated with beneficial changes in plasma total cholesterol and triglyceride concentrations.

Techniques for Measuring Dietary Intake: Many methods are used to evaluate food intake in research studies. Food records are used to provide quantitatively accurate information on food consumed during the recording period. Food records are considered the "gold standard" against which other dietary assessments are compared. By recording foods as they are consumed, the problem of omission is decreased and foods and portions are described more fully. The food record is a prospective instrument that is used frequently in research and clinical environments (158).

The food frequency questionnaire is a method that is utilized to evaluate the dietary intake of particular foods or food groups on a daily, weekly, or monthly basis. This method determines usual frequency and consumption of foods over the past year and can circumvent recent changes in diet. Unlike the food record, it is retrospective and requires recall on the part of the subject (158). The use of both the food frequency questionnaire and a three day food record in a research study can offer both a prospective and retrospective analysis of dietary intake, and is felt to represent a more comprehensive evaluation of a subject's diet.

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Changes in Body Composition with Aging: Menopause is associated with changes in body composition and fat distribution. During this time there is a decrease in lean body mass and an increase in fat mass, as well as a shift from a gynoid to an android fat distribution (159). Of the 541 women involved in the *Healthy Women Study*, 20% gained 20 or more pounds with an average five pound weight gain during the perimenopausal time (160). The subjects in this study, based on a 24 hour recall and a food frequency questionnaire, reported eating approximately 37% of total calories from fat (13% saturated fat). Few women ate diets with less than 20% total calories from fat.

All levels of overweight are associated with an increased risk of cardiovascular disease in women, although being obese with a body mass index (BMI) of greater than 29 carries the greatest risk (161), (162), (163). Obesity is not an independent risk factor for coronary heart disease, but is associated with other cardiovascular risk factors such as hypertension, dysplipidmia, hyperinsulinemia, insulin resistance, and diabetes mellitus (162). Data from the *Nurses' Health Study* (164) found that the lowest mortality rates were in those women who weighed 15% less than the US average for women of the same age, and among those women who had maintained their weight since age 18. Added to the deleterious effects of body weight on the lipoprotein blood profile, some data suggests that obese subjects (BMI >30) may also have a blunted response to a low fat diet when compared to leaner subjects (165). Denke et al. (166) reported that a higher BMI in both pre- and postmenopausal women was associated with higher triglyceride levels (+35-48mg/dL) and lower HDL-C levels (−5-9mg/dL) than average weight women. Stevenson et al. (167) investigated the influence of age and menopause on serum lipids and lipoproteins in 542 non-obese pre-and postmenopausal women (aged 18-70). This study

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found that BMI was related positively to both total cholesterol and low-density lipoprotein concentrations.

The increase in body fat, particularly upper body (android) fat, that is seen following menopause appears to be an important risk factor in postmenopausal women (168). Although obesity and weight loss affect plasma lipids and lipoproteins, a number of studies have shown that centrally distributed adiposity affects both plasma lipoproteins and the incidence of ischemic heart disease and myocardial infarction (MI) (68). A waist/hip ratio of greater than 0.80 and/or a BMI of greater than 25 is considered high risk in women. In contrast to men, localization of adipose tissue appears to be more important than obesity in the risk of heart disease in women (169). Greater upper body fat and weight gain are positively correlated with high triglycerides, hypertension, and lower HDL-C concentrations (170). In 1,275 women in the *Rancho Bernardo Study* (aged 50-89), fasting triglyceride levels were positively correlated with waist/hip ratio and inversely correlated with HDL (171).

There is a common perception among women that the use of HRT may result in weight gain. Reubinoff et al. (159) reported on the effect of HRT use on body weight and body fat distribution in 63 postmenopausal women (aged 44-54). After one year of use, HRT neither prevented nor increased weight gain and fat accumulation, but did minimize the shift from gynoid to android fat distribution. Changes in body composition (BMI and waist-hip ratios) have been shown to have deleterious effects on lipoprotein profiles in postmenopausal women. It is therefore important to evaluate body composition when determining cardiovascular risk factors in aging women.

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Exercise During and After Menopause: The increased risk of heart disease seen in postmenopausal women is related both to the aging process and the cumulative effects of adverse lifestyle habits, especially diet and exercise. The role that exercise plays in reducing this risk is complex and to date the data is inconclusive and lacking for the amount and type of exercise needed to reduce CVD risk.

Exercise has been shown to beneficially affect the plasma lipoprotein levels in women. Exercise has been shown to stimulate the production of HDL-C providing a cardioprotective effect and active women have higher HDL-C concentrations than their sedentary counterparts (172). Cross-sectional studies report a consistent relationship between exercise and increased plasma HDL-C levels, however few studies have controlled for exercise intensity and duration, age, gender, hormonal status, body weight and composition (67).

Although there is strong evidence of the beneficial effects of exercise in women, studies to date have been difficult to evaluate for several reasons. It was not until the early 1980's that studies on physical activity included women. A 1987 review by Powell (173) of the role of physical activity in the primary prevention of coronary heart disease concluded that physically active individuals are at lower risk for coronary heart disease than less active people. Of the 43 studies included in this review, only five presented data on women separately. Historically, studies on physical fitness have used men, and those who have included women have demonstrated inconsistent results. The flaw in assessing physical activity in women tends to be the instrument used. Most studies have used questionnaires that have been validated and used on men. These questionnaires tended to focus on sports activities and other physical activities in which women typically did not

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participate. Since women have traditionally engaged in less vigorous activities than men, the results tended to demonstrate that there was no decreased risk to women engaging in physical activity. Thus, these studies did not adequately evaluate the activities that women were involved in at the time (child care and homemaking) and therefore miscalculated the exposure variable. When leisure-time activities were included, the differences disappeared (174).

Some research has also suggested that there may be a gender difference in response to exercise. Body composition and hormonal factors may account for the differences in exercise capacity and occurrence of cardiovascular disease. Heart rate is higher and VO_2 max is lower in women than in men at all ages. When Blair (175) used treadmill performance as a measurement of physical fitness in women he reported a relative risk over eight years of 3.92 for women and 1.82 for men in the lowest fitness group as compared to the highest fitness group, suggesting different physiological responses to exercise between women and men. In this study, the lack of an association between inactivity and mortality demonstrated in women was likely due to inadequate measurements of physical activity (176). This suggests that women should not be evaluated in the same way as men in regard to physical fitness and exercise.

Until the 1960's, there was considerable doubt as to whether middle-aged and older people could benefit from exercise, due to the perception that there was a decline in cardiorespiratory function with age. For women over 50, it was thought that the decline in function would mitigate the beneficial effects of exercise, and that the hormonal changes associated with menopause could decrease aerobic fitness. White et al. (177) followed a group of previously sedentary women between the ages of 50-63 through a six

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month program of either aerobic dance or walking. The women were matched with respect to age, weight, past activity, and pretest fitness. Both groups engaged in their respective exercise for 30 minutes a day, four days a week at 70% of their maximal heart rates. At the end of the six months, both groups demonstrated increases in fitness and strength and decreases in resting and recovery heart rates.

Cowen and Gregory (178) investigated the responses of 20 pre- and 18 postmenopausal women to aerobic conditioning throughout their participation in a progressive walking program to determine the ability to improve cardiorespiratory endurance and body composition. The subjects participated in a progressive walking program four days a week for nine weeks at 80% of maximum heart rates. Both premenopausal (mean age 41.3) and the postmenopausal (mean age 55.7) group improved their cardiorespiratory endurance, by 12.1% and 19% respectively, and significantly reduced their percent body fat when compared to controls. Notelovitz et al. (179) also challenged the theory that hormonal changes contribute to cardiorespiratory decline when comparing pre- and postmenopausal women on a bicycle ergometer test. Results showed no significant differences in their estimated aerobic capacity values. These studies demonstrate that women can benefit from physical training programs regardless of menopausal status, and that cardiorespiratory fitness does not decrease with age in physically active women.

In 1991 the American College of Sports Medicine Prevention and Rehabilitative Exercise Committee recommended that healthy women should participate in continuous physical activity a minimum of 20 minutes a day, 3 days a week. The aerobic activity should sustain a heart rate of 70-85% maximal heart rate to avoid a decline in

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cardiorespiratory fitness (180). In 1995, an expert panel (181) comprised of researchers with an expertise in issues related to the health implications of physical activity was established by the Centers of Disease Control and Prevention and the American College of Sports Medicine. The members of this panel reviewed all the pertinent physiological, epidemiological, and clinical evidence from primary and review articles on the health implications of physical fitness. Their recommendation was that every American adult should engage in 30 minutes or more of moderate-intense physical activity most and preferably all days of the week. It was also determined that this activity could take place in short bouts, and that intermittent and moderately intense activity (gardening, housework, walking) confers substantial benefits in decreasing the risk of cardiovascular disease.

Two recent studies have applied the recommendations of the expert panel specifically to women. An hour a day of moderate activity (brisk walk or swimming) was found to reduce the risk of heart attack by 44% and ischemic stroke by 56% in women compared to sedentary controls (182). The second was a case-controlled study (183) that evaluated the benefits of leisure-time activity in postmenopausal women. The case subjects were 268 women who had been hospitalized for a heart attack and the control subjects were 925 healthy matched controls. The women who engaged in modest leisure activity for 30-45 minutes at least three times a week had a 50% decreased risk of having a heart attack when compared to sedentary counterparts.

Although there has been documentation on the benefits of moderate exercise on cardiovascular risk in women, many experts assumed that higher levels of physical activity were needed to impact HDL-C concentrations. Williams (184) evaluated the response of

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HDL-C to exercise and found that women who exercised at levels exceeding the current guidelines had the most substantial increases in their HDL-C plasma concentrations. *The Stanford Weight Control Project II* (185) investigated the effect of diet with or without exercise on HDL-C concentrations in moderately overweight men and women. The 112 women in the study were randomly assigned to one of three groups: control, diet only (low fat and low calorie), or diet and exercise combined. After one year of treatment, both intervention groups lost weight and demonstrated a decrease in LDL-C, but the exercise groups also showed an increase in HDL-C that was not seen in the diet only group. This study demonstrated that physical activity and diet can have interactive effects in improving lipoprotein patterns, thus decreasing risk of coronary heart disease in women.

Since exercise is also known to reduce stress, physical activity may be useful in alleviating some of the secondary symptoms associated with menopause such as fatigue, depression, and tension. Empirical research has clearly established the effects of exercise on physical and psychological well-being. Exercise has been shown to decrease depression and anxiety while positively affecting self-esteem and general mood state (186). A recent study (187) measured mood states and symptoms reported by 93 premonopausal, 32 perimenopausal, and 54 postmenopausal without HRT, and 41 women currently using HRT. Two validated instruments were used to measure moods and symptoms in these women.

The first instrument is the POMS (*Profile of Mood States*) (188) which consists of 65 items and provides subscales on six mood states: depression, tension, anger, fatigue, confusion, and vigor. The second instrument is the WHQ (*Women's Health*

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Questionnaire) (189), a 37-item scale that is designed to assess well-being in middle-aged women. It provides scores on nine factors: depressed mood, memory and concentration problems, somatic symptoms, vasomotor symptoms, anxiety and fears, sleep problems, sexual dysfunction, menstrual symptoms, and perceived physical attractiveness.

The women in the study who exercised regularly (aerobic floor exercises or brisk walking, for a minimum of 2 times a week for 30 minutes a session) were compared to sedentary women using the two instruments. Across all menopausal groups, women who exercised scored significantly higher than sedentary women on seven of nine subscales of the WHQ and on five of the six subscales of the POMS. As a whole, women who exercised and postmenopausal women who were using and not using HRT, and exercised regularly reported fewer symptoms than sedentary women. The same study also investigated the effect of an acute bout of exercise (45-minute aerobic class) on moods and symptoms found significant enhancements in mood and reductions in symptoms immediately following this type of exercise. When evaluated together, these results suggest that women, regardless of menopausal status and use of HRT, exhibit similar and beneficial responses to exercise in regard to mood and symptom relief.

There have been suggestions that exercise might also reduce the incidence and severity of vasomotor instability due to an increase in endorphin levels (190). One study (191) found that physically active women reported fewer moderate to severe hot flashes than women who were sedentary. Women who participated in aerobic exercise reported a increased sense of well-being and were better able to manage stress (68). McCann and Holmes (242) reported that depressed women who did strenuous exercise had significantly

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greater decreases in depression than women who did relaxation exercises or no exercise at all.

The literature clearly demonstrates the beneficial effects of positive lifestyle interventions on the health profiles of postmenopausal women. More research needs to be directed at determining the effect of lifestyle changes as an alternative or adjunct to hormone replacement therapy.

The Psychological and Social Aspects of Menopause

Women's attitudes towards menopause may influence their menopausal experience. Experiences associated with menopause include vasomotor symptoms, such as hot flashes and night sweats; psychosomatic symptoms including palpitations and dizziness; and psychological symptoms, such as fatigue, nervousness, forgetfulness, irritability, and loss of concentration (192). The research does not support the occurrence of these symptoms in the majority of women. Of these symptoms, only vasomotor instability has been unequivocally linked to menopause (193). Although 75% of all women experience hot flashes (22), only 12% report that they have symptoms severe enough to interfere with work or social life (194). Many women start HRT to relieve hot flashes (22) and it has been suggested that some of the symptoms reported during menopause are due to hot flash induced insomnia (195). Most women report that their symptoms are mild and transient and the majority of women regard the menopausal experience as uneventful.

A *Medline* search was conducted for the years 1984-1994 for the purpose of identifying the articles on menopause. The number of articles on menopause from all

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sources was 9,018. The majority of these articles focused on the medical treatment of menopause, the most frequent topic being HRT. Articles having a social or psychological focus totaled 500 (≈ 6%) and the majority of these dealt with depression or moods. A parallel search of the *PsychLit* database cited 227 articles on menopause and only 67 (≈ 30%) were related to psychosocial aspects of menopause; and the most frequent topics were distress and depression (196).

Menopause is a highly complex event with multiple and diverse influences that requires understanding of the psychological, social and cultural influences as well as the biological factors involved (197). To date the majority of information on menopause has focused on the biological aspects and there is a need to include other factors in research on menopause and hormone replacement.

Several authors have suggested that this lack information on the social and psychological aspects of menopause is due to the "medicalization" of menopause (198), (199), (200). Medicalization is defined by Schneider and Conrad (201) as "defining and treating human experiences as medical problems". This also raises the question as to whether menopause is a disease state due to an estrogen deficiency or a natural transition in a woman's life. As Freeman commented in 1989 (202), "menopause remains the one normal female process that is still overtly referred to as a disease in the medical literature".

The impact of these negative perceptions of menopause is confirmed by the literature. In *The Healthy Women Study* (203), (204), an ongoing study of the natural history of menopause, 541 healthy women (aged 42-50) were recruited in Allegheny County, Pennsylvania. These women were not using any type of medication, including HRT upon entry into the study in 1993-1994. At this time, the women's attitudes toward

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menopause and symptoms were evaluated. The women were followed longitudinally at 2, 5, and 8 years and were reevaluated when they had either stopped menstruating or had used HRT for twelve months. At the same time, age-matched premenopausal women were reevaluated so that comparisons could be made cross-sectionally. Women had negative expectations regarding menopause, but were more optimistic about their own experiences than that of other women. At study entry, 80% of the women agreed that women were likely to get depressed during menopause and 55% thought they would get depressed. In actuality, when these women were compared with the premenopausal control group, no differences were found in standardized measures of depression, menopausal symptoms, anxiety, or anger in the women who did not use HRT. The postmenopausal women reported less stress in their lives over time, when compared to the control group. This study concluded that natural menopause did not have negative mental health consequences for the majority of women. Several other studies have reported similar results and concluded that menopausal status is not related to psychological distress (195), (205), (206). Even though women have negative expectations prior to menopause, these are for the majority of women unfounded.

The common belief that menopausal women are anxious and depressed is not confirmed by research studies. Epidemiological studies from the United States (30), (207), Sweden (208) and England (209) have consistently failed to document an increase in depressive symptoms at the time of menopause compared to other times. In fact clinical depression is rarely seen in menopause and is most likely to occur in women who have previously experienced depression (210).

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Depression during menopause has been shown to be more prevalent in perimenopausal (211) rather than postmenopausal women. It is also evident that women who have undergone a surgical menopause tend to score lower on depression scores than women who had experienced a natural menopause (206). Lennon (212) found that the timing of menopause was an important factor in the reported stress associated with menopause. She found that women who reported menopause during mid-life (ages 44-54) did not experience psychological distress. She did however find that women who experienced either an early (<age 44) or late (> age 54) menopause exhibited a significant increase in distress and depression scores.

Attitudes, symptoms, and degree of depression were measured by validated instruments in a random sample of 149 women (aged 35-65) stratified by occupation, age, and race in a study by Wilbur (3). Unlike the majority of studies to date that have involved only middle-class white women, this study included 44% African American and 56% white women. The majority of the women, regardless of ethnicity, education, occupation, or socioeconomic status, had neutral feelings toward menopause, with postmenopausal women reporting the most positive attitudes. A significant correlation was found between positive attitudes toward menopause and an increased number of children, particularly in white women. In contrast to previous reports, this study found that women who had experienced a surgical menopause did not have higher levels of depression, and that physical symptoms were not related menopausal attitudes. A negative attitude toward menopause was associated with depression and low well-being in both white and African-American women independent of age and menopausal status. As

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confirmed by other studies, depressed women have more negative attitudes toward menopause.

It is important to consider the fact that many women in mid-life are often experiencing other changes in their personal lives concurrent with the menopausal experience. Menopausal women are disproportionately at risk for divorce, illness or death of spouse. Also death or disability of a parent, children leaving home, and other major life events (187), (38) are likely to occur at this time. Many of the symptoms reported during menopause may be due to environmental factors or the aging process that are independent of menopause.

The National Health Examination Follow-up Study (195) examined whether menopause was associated with three measures of psychological distress: depression, lack of well-being, and sleep disturbances. A cohort of 3,049 women aged 40-60 were assigned to four groups based on their menopausal status: premenopause, perimenopause, natural menopause, and surgical menopause. In the ten year follow-up interval, no increase in any of the three psychological distress categories was associated with the menopausal transition, the number of years since menopause or the timing of the menopausal transition.

Social, cultural, individual, and environmental factors intersect to influence the menopausal experience in each individual woman (211) and it is important to consider all of these when investigating menopause and the impact of HRT.

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Conclusions

After an extensive review of the related literature, the focus of this dissertation research was to investigate menopause from a comprehensive perspective that involved the physiological, psychological and social factors that influence a woman's choice in regard to hormone replacement therapy and the consequences of that choice.

Physiologically speaking, a woman's risk for certain chronic diseases, including heart disease and osteoporosis, increases following menopause, as do acute symptoms, presumably due to the decreased production of estrogen. Psychologically, a woman's choice may be influenced by moods, depression, or other psychological distress. There are many social influences as well, including expectations and ideals surrounding the aging process. It is important to consider all of these factors when exploring the decision women make regarding HRT.

Physiological studies (4), (213) suggest that between 25-50% of estrogen's positive effects on heart disease risk is due to the changes in HDL-C and LDL-C levels. This implies that ~50-75% of the beneficial effects of estrogen is through other mechanisms. These may include estrogen's beneficial effects on carbohydrate metabolism, clotting factors, antioxidant ability and its effects on the vascular system. LDL-C oxidation is an important occurrence in atherogenesis (214). It has been proposed that estrogen, aside from its effect on lipoprotein metabolism, may protect against atherosclerosis by inhibiting the oxidation of LDL-C, an important component in atherogenesis (214). The antioxidant nutrients, α-tocopherol (vitamin E) and ß-carotene, have been shown to also inhibit oxidation of LDL-C (215).

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Psychological factors may also influence a woman's choice regarding HRT. The idea that women suffer debilitating symptoms and depression during menopause is unfounded. Although 75% of all women report vasomotor instability during this time, the majority do not report that the symptoms are severe enough to result in disruption of normal life. The idea that menopause leads to depression is also unfounded. Only women who are depressed prior to menopause report depression during menopause. The primary reason for the institution of HRT is for the relief to vasomotor instability. Research has demonstrated that for the majority of women, menopause is uneventful and that most women report that their expectations of adverse reactions is unfounded.

Cultural and social factors may influence a woman's choice regarding HRT use also. Attitudes regarding menopause have been created largely by a medical model which portrays menopause from a negative perspective. A 1988 survey mailed to physicians and menopausal women reported that 21% of the physicians when asked to name the major health problem of mid-life women responded with "menopause"; none of the women surveyed offered this response. In regard to attitudes toward menopause and women's health, women in this study were found to be more positive than the physicians 56% of the time (216).

It is also important to take into consideration the limitations of previous studies on when interpreting the HRT-heart disease association. The majority of early studies on HRT and cardiovascular disease involved unopposed and almost exclusively *Premarin*™. The research on HRT has focused on highly educated, middle-class white women who have the greatest access to health care. Socioeconomic status is a powerful predictor and inversely associated with mortality and morbidity, for almost every disease and condition,

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including heart disease and cancer (217). There is also a linear relationship between educational attainment and mortality (218). Thus, subjects in these vanguard studies may enjoy a "healthy cohort effect" which could result, in part, to the decreased risk seen in postmenopausal women using HRT (34). Fifteen years ago physicians were reluctant to prescribe HRT to women with a high risk for heart disease or diabetes, leaving the healthy women to take HRT (34). Women who undergo a surgical menopause are more likely to use HRT and to continue its use on a long-term basis. They are also younger, utilize the health care system more and are more likely to have early diagnoses and treatment of medical problems than women who experience a natural menopause. Many studies include both types of menopause without defining the groups. Although women with a surgical menopause should be included in menopause studies, they should be studied, analyzed, and reported as a separate group. There is also confusion in many studies in regard to duration and type of hormone use, with unclear definitions of "current" vs "ever" vs "past" hormone replacement use. (219).

It is estimated that one fourth of all postmenopausal women in the United States are taking some type of HRT (77). Women who are currently using estrogen tend to participate in more preventative health care regimens and show greater behavior change (17). Hormone replacement therapy is often not available to the women at greatest risk of chronic disease because these women do not typically have access to health care and diagnosis of chronic diseases are often delayed.

Much of the data on the association between CVD and HRT comes from *The Nurses' Health Study*. The results of this study provide an indication of the health risks faced by women who have good access to health care, exercise programs, and healthy

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diets. Kaufert (38) warns that generalizations beyond this social context have to made with extreme caution.

Much research on hormone replacement therapy has been focused on physiological outcomes and treatment of menopausal symptoms. There is a need to also investigate how women view and experience menopause. Understanding menopause merits consideration of the social, cultural, and psychological as well as the biological factors (196).

At the turn of the century , the average life expectancy for an American women was 50, today it is almost 80. Forty million American women are in or past menopause and another 20 million are due to reach menopause in the next decade (220). With increased awareness and emphasis on women's health issues, women are now better able to obtain the knowledge and information necessary to exercise control over their health and well-being.

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CHAPTER III

MATERIALS AND METHODS

Materials

(see Appendix B)

Experimental Design

Subject protocol. A cohort of 70 healthy postmenopausal women, aged 48-66 with a mean age 55.84 (± 4.43 years), who had experienced a surgical or natural menopause and were nonsmokers, were recruited from faculty and staff at the University of New Hampshire in May 1995 and Plymouth State College, Plymouth, New Hampshire in May 1996. Subject were divided into one of three groups dependent on their use of exogenous hormones: no hormone replacement (n = 36); estrogen only (n = 11); and estrogen and progestin (n = 23). Menopause was confirmed by the cessation of menses for at least one year. The women who were taking estrogen and progestin confirmed that they had not had a menstrual cycle for at least one year prior to initiating use of HRT. All women in the hormone replacement groups had been on medication for at least one year. The women in the no hormone group had not used any type of hormone replacement for at least one year.

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Volunteers for the study responded to a letter of interest (Appendix C) mailed to all faculty and staff at the two institutions as part of the *University of New Hampshire Women's Health Study.* Women interested in participating were contacted by phone to confirm that they were non-smokers, postmenopausal, and to determine the dose and type of HRT they were presently using. A brief description of the study and the candidate's participation was explained. Those qualified and interested were scheduled for evaluation and blood sampling within a month's time.

Once accepted into the study, the subjects reported in a fasted state to the study site at the University of New Hampshire or at Plymouth State College. They were then asked to sign a consent form approved by the University of New Hampshire Institutional Review Board (Appendix C). A medical history was taken to verify the type and dose of hormone replacement, as well as prescription and non-prescription drugs and personal and family health risk factors (Appendix C). The subjects also completed a comprehensive lifestyle and medical questionnaire that gathered information regarding health, menopause, decision making, self-esteem, and mood states (*University of New Hampshire Women's Health Study Questionnaire:* Appendix C). Each questionnaire was identified by the number of the subject only to insure confidentiality.

Anthropometric assessments were conducted which included height and weight, body-mass index, and determination of waist-hip ratio. Each participant was weighed in light street clothes without shoes on a balance-beam institutional scale. Height was measured without shoes with subjects facing straight ahead with head against the measuring tool on the balance beam. Body-mass index was calculated using weight in kilograms divided by the square of height in meters. Using a tape measure, waist-to-hip

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ratio was taken by measuring the waist circumference (at the umbilicus) and the hip circumference (at the largest point between waist and buttocks).

The dietary information on the subjects was obtained by using both a food frequency questionnaire and a three-day food record. The three-day food record (Appendix C) included all foods and beverages consumed for two consecutive weekdays and one weekend day. Portion size was demonstrated using food models (Nasco Inc., Fort Atkinson, WI). The data from the food records was then analyzed with a microcomputer using two computerized analysis programs. The food frequency questionnaire utilized the *Health Habits and History Questionnaire: Diet History and Other Risk Factors Dietary Analysis System* (DIETSYS Version 3.0) developed by the National Cancer Institute Information Management Services, Inc. Block Dietary Data Systems (Appendix C). The three-day food diary analysis system was based on data from the United States Department of Agriculture using *Nutritionist IV*™ for Windows Diet Analysis (version 4.1) software (First DataBank, San Bruno, CA).

Collection and Analysis of Blood Samples

A fasting blood sample was drawn from the antecubital vein into four vacutainers: 13 cc serum separation vacutanier tube, 7cc plasma separation vacutainer with EDTA (K3), 7cc plasma separation vacutainer with freeze-dried sodium heparin, and 7cc plasma separation vacutainer with sodium citrate. The blood draws were conducted in the Nursing Laboratory at the University of New Hampshire and Plymouth State College

After the blood was drawn, the tubes were immediately wrapped in aluminum foil to protect the photosensitive pigments from exposure to light. The serum samples

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were then allowed to clot at room temperature for thirty minutes. Both the sera and plasma were then centrifuged at 1400G for 15 minutes at 4°C, and aliquoted into cryovials. Samples were stored at -70°C until assayed.

The analyses of the samples was conducted as follows:

1. *Serum triglyceride, total cholesterol, LDL- and HDL-concentrations.
2. Serum antioxidant concentrations (α-tocopherol and β-carotene) concentrations.
3. Serum progesterone and estradiol concentrations.
4. Serum oxygen radical absorbency capacity (ORAC) for total antioxidants.
5. Plasma →LDL isolation→protein concentration→ LDL antioxidant concentrations.
6. Plasma →LDL isolation→protein concentration→ LDL susceptibility to oxidation.
7. Plasma lipoprotein (a).

* Fresh sera was used in these assays.

Serum Total Cholesterol. Serum total cholesterol concentrations were determined utilizing the *Sigma Diagnostics* #352 Kit. This method measures cholesterol enzymatically and is a modification of the method of Allain et al (221). A cholesterol reagent is used that hydrolyzes the cholesterol esters in the serum to cholesterol. The cholesterol is then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide produced is then coupled with the chromogen to yield a quinoneimine dye which has an absorbence maximum of 500 nm. The intensity of the color produced is directly proportional to the total cholesterol concentration in the sample.

In the assay the lyophilized reagent is reconstituted with 100 mL of deionized water and mixed by inversion. A series of test tubes are set up and 1.0 mL of reagent is

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added to each tube. This is followed by the addition of 10ml of deionized water to the blank, calibrator, control, and sample respectively and each tube is vortexed briefly. All tubes are then incubated for 10 minutes at 30° C and read on a spectrophotometer at 500 nm within 30 minutes after end of the incubation time. Total cholesterol concentration is calculated in mg/dL:

$$\frac{\text{A test-A Blank}}{\text{A Calibrator-A Blank}} \times \text{Calibrator (198 mg/dL)}$$

Serum Triglyceride. Serum triglyceride was quantified using *Sigma Diagnostics* Kit #336. The reagent creates an enzymatic reaction in which triglycerides are first hydrolyzed by lipoprotein lipase to glycerol and free fatty acids. Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to form glycerol-1-phosphate (G-1-P) and adenosine-5-diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK). The G-1-P is oxidized to dihydroxyacetone phosphate (DAP) with the concomitant reduction of nicotinamde adenine dinucleotide (NAD) to NADH in the reaction catalyzed by glycerol-1-phosphate dehydrogenase (G-1-PHD). The NADH is oxidized with the simultaneous reduction of 2-(p-iodophenyl)-3-nitrophenyl-5-phenyltetrazolium chloride (INT) to INTH (formazan in the presence of diaphorase). The resulting formazan is highly colored and has an absorbence at 500nm. The intensity of the color produced is directly proportional to the concentration of triglyceride in the sample.

In the assay the lyophilized reagent is reconstituted with 100 ml of deionized water and mixed by inversion. A series of test tubes are set up and 1.0 mL of reagent is added to each tube. This is followed by the addition of 10µl of deionized water to the blank, calibrator, control, and sample respectively and vortexed briefly. All tubes are then

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incubated for 10 minutes at 30°C and read on a spectrophotometer at 500 nm within 30 minutes after end of the incubation time. The triglyceride concentration is calculated in mg/dL:

$$\frac{\text{A test-A Blank}}{\text{A Calibrator-A Blank}} \quad X \quad \text{Calibrator (250 mg/dL)}$$

Serum HDL Cholesterol. Serum HDL cholesterol was quantified using *Sigma Diagnostics* Kit #352-3. Dextran sulfate and Mg ions precipitate LDL and VLDL, leaving the HDL fraction in solution. The cholesterol in the supernatant is then assayed with the same procedure used for determining total cholesterol concentration, using the 50 ml of HDL reagent and 500 mL of sample. The calculation used to determine HDL concentration in mg/dL:

$$\frac{\text{A test-A Blank}}{\text{A Calibrator-A Blank}} \quad X \quad 50 \quad X \quad 1.1$$

Serum LDL Cholesterol. Serum LDL-C concentration was then calculated using the following formula validated by Friedewald, et al (222).

LDL-C (mg/dL) = Total Cholesterol – HDL Cholesterol – *Triglycerides/5

*Provided Triglycerides are less than 400 mg/dL

Serum α-tocopherol, β-carotene and retinol. The HPLC (high performance liquid chromatography) procedure was used to quantify concentrations of serum α-tocopherol, β-carotene, and retinol. Four hundred ml of serum was precipitated with 200 ml of 100% ethanol, using 200 ml of retinyl acetate and tocopherol acetate in alcohol as an internal standard, and then extracted twice with hexane. The hexane layer was collected in an amber glass vial and evaporated under nitrogen and the sample was then reconstituted in 200 ml of 100% ethanol. The HPLC system (Beckman 338) is a reverse-

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phase HPLC with a C18 Baker Bond wide-pore (5um) column (J.T. Baker, Phillipsburg, NJ), a variable wavelength spectrophotometer (Beckman 406), and a Beckman 167 Scanning Detector Module (Beckman Instruments, Wakefield, MA). The mobile phase uses a gradient, changing from solvent A (methanol and 1% ammonium acetate) to solvent B (methanol: methylene chloride (80:20) and 1% ammonium acetate) over the first four minutes of the run. The wavelengths are set at 292nm for alpha-tocopherol, 325nm for retinol and 452 nm for beta-carotene. The flow rate was set at 1.5ml/min. Twenty µl of sample is injected twice for each sample. The internal standard is used to correct for any volume lost during the preparation.

Isolation of LDL. The LDL was isolated from plasma to determine protein concentration, susceptibility to oxidation, and antioxidant concentrations. The LDL was isolated by a single spin density gradient ultracentrifugation at 65,000 RPM for 1 hour and 20 minutes at 7°C, with a NVT 65.2 rotor, as previously described by Chung et al. (222). Prior to ultrcentrifugation, the density of the plasma was adjusted to about 1.21g/ml by the addition of 0.4898 g KBr (potassium bromide) to 1.5 mL of serum. The sample was then layered under 3.3 ml of saline (0.154 M NaCl) in a Beckman Optiseal tube using a 5cc syringe with a 3" flat bottom 20 ga needle. After centrifugation the LDL band is removed from the Optiseal tube using a 1 mL syringe with a 25G5/8 needle, and then filtered through a acrodisc filter into a test tube.

Quantification of α-tocopherol, β-carotene and retinol in the LDL fraction in plasma. After isolation of the LDL, HPLC was used to determine the plasma concentrations of α-tocopherol, β-carotene, and retinol by method described previously.

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LDL protein. Protein determinations were made on the LDL isolates using a modification of the Lowry method (223). In the procedure, 100 mL of reagent A (2.0% Na_2CO_3, 0.4% NaOH, 0.16% sodium tartrate, and 1.0% SDS, in H_20) was mixed with 1 mL of reagent B (4% $CuSO_4(5H_2O)$ in H_20) to form reagent C, which is the alkaline copper reagent. Folin-Ciocalteau reagent was diluted 1:1 with H_20, for a final concentration of 1N. Bovine serum albumin (BSA) was used as the standard, and the concentrations of the standard curve ranged from 0 to 100 ug/ul. Three mL of reagent C is then added to 50 µl of sample and allowed to incubate at room temperature for 10 minutes. This was followed by the addition of 300 µl of Folin-Ciocalteau reagent while votexing. After a 45-minute incubation at room temperature, the optical density (O.D.) of all standards and samples were read at 660 nm, and measured against a blank. Protein concentrations were then determined from the standard curve.

LDL Resistance to oxidation *ex vivo*. LDL isolated from the plasma and protein was determined as described above. The susceptibility of LDL to oxidation was determined by the measurement of conjugated diene formation as previously described by Esterbauer (224). $CuSO_4$ was used to initiate the formation of a peroxyl radical. The LDL sample (100 µg protein/assay), saline (to increase volume to 710 µl), 250 µl of 20 mM HEPES, and 40 µl of 80 uM $CuSO_4$ were added to a cuvette. After mixing 2 to 3 times by inversion, the absorbency of each sample was recorded at 37°C every 10 minutes for 10 hours on a Varian Cary 1, UV/VIS spectrophotometer (Vaarian Associates Inc., Palo Alto, CA). Ten samples were analyzed simultaneously. Measurements of the lag phase, propagation phase, and total diene formation were determined from the oxidation curve.

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Serum estradiol concentrations. An ELISA (Enzyme-Linked Immunosorbent-Assay) was used for the quantitative analysis of plasma estradiol concentrations. The fundamental principle of this assay involves antigen-antibody reactions and utilizes a 96-well microplate and is used for establishing a standard curve. The *MaxiStop*™ Nunc microplate is used that is precoated with anti-Estradiol rabbit antibody on each well. The assay is specific for 17β-estradiol (100%). Before conducting the ELISA assay, the estradiol must be extracted from the plasma using ethyl ether. One hundred µl of plasma is pipetted into glass tubes to which 1 ml of ethyl ether is added. The tubes are votexed for 30 seconds and the phases are allowed to separate. The organic phase is transferred into a clean tube and the solvent is allowed to evaporate with a stream of N_2. The residue is then dissolved in 500 µl of EIA buffer, votexed and incubated in a 37° water bath for approximately 15 minutes.

A standard curve is established using a stock solution and EIA buffer in increasing concentrations: 0, .02, .04, .10, .20, .40, 1.00, and 2.00 ng/ml. Fifty µl of standards and samples are then added to appropriate wells in the plate in duplicate, followed by the addition of 50µl of diluted enzyme conjugate, called horseradish peroxidase (HRPO), is added to each well and mixed by shaking gently and then allowed to incubate. After incubation the contents of the plate is dumped on a clean lint-free towel. Each well was washed with 200µl of washing buffer three times and 1.5µl of K-Blue Substrate is added to each well, mixed by shaking the plate gently and allowed to stand at room temperature for 30 minutes to 1-1/2 hours. At the end of this time the plate is shaken gently to insure uniform color throughout each well and read on a microplate reader at 650 nm. A standard curve was then established and concentrations were interpolated from this.

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Serum concentration of Progesterone. Serum progesterone concentrations were determined using the *Milenia*™ Progesterone enzyme immunoassay kit. This assay utilizes liquid-phase kinetics and a microplate format. The standard curve is established by pipetting 25 µL (in duplicate) of a stock solution in increasing concentrations of 0, 0.25, 1.0, 5.0, 10, 20, and 40 ng/ml into appropriate wells in the microplate. Twenty-five µl of samples (in duplicate) are then added to the remaining wells that have been coated with anti-rabbit antibody. One hundred µl of Ligand-Labeled progesterone is then added to all wells, followed by 100 µl of Progesterone Antiserum. The plate is then covered, mixed gently, and then rotated on an automatic plate mixer (set at 1300 revolutions/minute and an amplitude of 5) for exactly 60 minutes. After completion of the rotation, 25µl of progesterone enzyme-labeled anti-ligand is added to all wells and the microplate is then rotated again for 30 minutes on the plate reader. After the 30 minute rotation, the plate is decanted and washed four times, each time with 300 µl of wash buffer per well. Two hundred µl of substrate working solution is next added to all wells and the plate is incubated without shaking in the dark for 30 minutes. After the incubation period, 50µl of Stop solution is added to each well and the plate is shaken gently to mix to ensure uniform color throughout each well. The plate is then read on a microplate reader at 450 nm. A standard curve was then established and concentrations were interpolated from this.

Total Antioxidant. Frozen serum samples were transported on dry ice to Dr. Ronald L. Prior at the USDA Human Nutrition Research Center on Aging at Tufts University in Boston Massachusetts. The automated assay or (ORAC) oxygen radical absorbance capacity with the COBAS FARA II method was used to determine total antioxidant concentrations (225).

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ORAC is a sensitive method for quantifying the oxygen radical absorbance capacity of antioxidants in a biological system. Antioxidant systems include enzymes (superoxide dismutase, catalase, glutathione peroxidase), large molecules (ferritin, and albumin), as well as small molecules (∝-tocopherol, β-carotene, ascorbic acid). This assay has been automated utilizing the COBAS FARA II centrifugal analyzer with flourescence. β-phycoerythrin (β-PE) is used as the indicator protein, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as a calibrator for antioxidant activity. This assay is allowed to go to completion and is able to quantify both inhibition time and degree of inhibition (225).

Lipoprotein (a). Lipoprotein (a) "Lp(a)" concentrations were determined using the Macra™ Enzyme Immunoassay Kit. A standard curve was established by the addition of 100 µl (in duplicate) of prediluted standards (0, 5, 10, 40, and 80mg/dL) and two controls to the bottom of test wells of a microtiter plate. Plasma samples were diluted 1:201 by adding 10µl of the sample to 2mL.of sample diluent and vortexed to mix completely. One hundred µl of prediluted samples (in duplicate) were added to the bottom of the remaining wells. The plate was incubated at a controlled room temperature (18°-25°C) for one hour ± 2 minutes on a rotator at 120 rpm. After the incubation period, the plate was aspirated and washed four times with wash buffer. The plate was inverted and forcefully tapped on an absorbent pad to remove any excess wash solution. Using a multi-channel pipet, 100µl of Anti-Lp(a) conjugate was dispensed to the bottom of all wells and the washing procedure was repeated. The plate was then incubated on a rotator again for 20 minutes.

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After this incubation period, 100µl of color substrate was added to each well and plate incubated for an additional 20 minutes and then 50µl of stop reagent was dispensed into each test well. The plate was read on a plate reader at 490 nm. A standard curve was then established and concentrations were interpolated from this. A standard curve was then established and concentrations were interpolated from this.

UNH Women's Health Study (UNHWHS) Questionnaire

This questionnaire (Appendix C) was developed as a collaborative effort by several researchers at the University of New Hampshire. The questionnaire was pilot tested on 120 women in the summer of 1994. A Jury of Experts (Appendix A) reviewed the questionnaire for content and validity and offered comments and suggestions for review. The questionnaire was then revised using the suggestions and comments of the experts. The questionnaire collected information on the following:

Demographics: Age, ethnicity, religion, occupation, education, marital status.

Health History: Primary care provider, present and past health status, access to health care, pregnancy, surgeries.

Drug History: Types, brands, and dose of all prescription and non-prescription drugs and vitamin and mineral supplements.

Exercise Habits: Type, frequency, intensity, and duration of present exercise routine and number of years subject had exercised at the present rate.

Diet History: Information on use of special diets, food allergies, and weight loss history.

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Menstrual History: Changes in menses in last twelve months, past use of oral contraceptives, menopausal status.

HRT History: Present use of HRT (type and dose). Past use of HRT. Factors influencing use of HRT.

The questionnaire was mailed to each participant upon entry into the study. A cover letter (Appendix C) was included that provided instructions on how to complete the questionnaire and that they did not have to answer any question that they chose not to. All questionnaires were returned with only a code number to identify the questionnaire to subject.

A coding system was developed (Appendix C) to compile information from the questionnaires for entry into the computer for analysis.

Evaluation of Dietary Intake

Dietary intake of participants was quantified using the *Dine Score System* (226), (227) from nutrient values obtained from the three-day dietary analysis. In this system, macro- and micronutrients categories are assigned points based upon eating foods that contain nutrients within recommended ranges. The scoring process assigns pluses, zeros, and minuses to the subject's dietary intake. Each category is comprised of one, two or three nutrients with a range of scores from 0-10 points. The *Dine Score* descriptors (10 = perfect; 9-8 = excellent; 7-6 =good; 5-4 = fair; 3-2 = poor; 1-0 = very poor) measure dietary status for eating behavior, determined by the number of nutritional guidelines achieved by the subject. (Appendix C : *Dine Score System*).

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Psychological and Social Assessments

Self-esteem. A 10-item Rosenberg (228) Likert-type scale was used to measure differences in self-esteem among groups. Respondents were asked to rate each of the 10 items on the instrument as: *strongly disagree* (1), *disagree* (2), *agree* (3), or *strongly agree* (4). Items #4 and #5 received negative weights in calculating the total score. The scores for each respondent were then added together and divided by 4 to obtain a total self-esteem score. The range of scores for the self-esteem inventory was 2.5-10 with a higher score indicating a more positive self-esteem. (Appendix C *UNH Women's Health Questionnaire*).

Moods. A 65-item (POMS) Profile of Mood States Inventory (103) was used to measure six identifiable mood or affective states: Tension-Anxiety(T); Depression-Dejection (D); Anger-Hostility (A); Vigor-Activity (V); Fatigue-Inertia (F); and Confusion-Bewilderment (C); as well as a total Mood State, or POMS Score. In this self-administered survey, subjects are asked to respond to the stem, "How have you been feeling during the past week including today"? Examples of the various sub-scales: *tense, on edge, uneasy* (T); *unhappy, sad, worthless* (D); *peeved, spiteful, angry* (A); *energetic, active, lively* (V); *listless, worn out, weary* (F); and *confused, panicky, uncertain* (C). The ranking of scores included: *not at all* (0); *a little* (1), *moderately* (2), *quite a bit* (3), or *extremely* (4). To obtain a score for each mood factor, the sum of the responses was obtained for the adjectives defining the factor. (Refer to Table 11a and 11b for factor loading criteria of each sub-scale). All items defined in each factors were keyed in the same direction except for two items, "*relaxed*" on the Tension-Anxiety scale and "*efficient*" on the Confusion scale. These two items received negative weights in

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calculating the factor scores. A Total Mood Disturbance Score was obtained by summing the scores (with vigor weighted negatively) on the six primary mood factors. The five sub-scale scores were then added together and vigor is subtracted from these scores. The range of scores for the POMS sub-scales was T= 0-36; D= 0-60; A= 0-48; F= 0-28; C= 0-28; and V= 0-32. The range of possible scores for the Total Mood Disturbance Score was 0-168 with higher scores indicating a more negative mood state. (Appendix C. *UNH Women's Health Questionnaire*).

The reliability of the POMS instrument is moderate to high. Internal consistency on all subscales is .90 or above. The test-retest reliability ranges from .65 for vigor to .74 for depression. The correlations for reliability were taken from patients entering treatment at a medical center psychiatric clinic. Product-moment correlations among POMS scores were evaluated at three time points: pretreatment, immediately before the first session, and six weeks post-treatment. The correlation between pretreatment and the first session are lower bound estimates of reliability because of changes in emotional states associated with finding sources of psychiatric treatment. Stability of a fluctuating state like mood is expected, thus rank ordering of individual scores would be expected. Extremely high stability coefficients for mood measures could be taken as evidence of lack of construct validity (103).

Experiences. Symptoms of menopause were evaluated with a 28-item Likert scale that described various symptoms that women experience during menopause. The subject were asked to rank their experiences at present or within the last six months from 0= No to 4= always. The responses to the 28 items from each subject were then added together to obtain a total experience score for each subject. The range of possible scores was 28-112

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with scores weighted toward negative symptoms. (Appendix C: *UNH Women's Health Questionnaire*).

Attitudes Regarding Hormone Replacement Therapy. Subjects responded to several questions regarding their attitudes and use of HRT. The responses involved several major areas of interest: (1) factors that influenced their decision to use HRT; (2) the sources that have provided them information on menopause; (3) other therapies used for treatment of menopausal symptoms; (4) factors that influenced their decision not to use HRT; and (5) factors leading to discontinuance of the use of HRT. Responses to each of the questions were calculated and frequency of the answers was determined. (Appendix C. *UNH Women's Health Questionnaire*).

Statistical Analyses

All analyses were conducted using SPSS® (Statistical Package for the Social Sciences): Graduate Pack™ Advanced Class Version 7.0 for Windows. Chicago, IL. Significance was defined as $p<0.05$.

Data collected in this study was statistically analyzed in two ways: Comparison I investigated relationships between three groups: no hormone replacement therapy (NHRT), estrogen only (EO) and estrogen and progestin (E/P). Comparison II investigated differences and relationships between two groups: no hormone replacement therapy (NHRT) compared to data from both hormone groups (HRT)

A one-way Analysis of variance (ANOVA) was used to detect differences in Comparison I. After significant differences were detected, a least significant difference

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(LSD) post-hoc test was used to determine which groups differed significantly. An independent sample t-test was used to detect differences in Comparison II.

The Pearson product correlation was used to determine associations between variables that were shown to demonstrate trends on the previous analyses. This analysis was also used to compare the average daily intake of selected nutrients based on the three-day records to those based on the food frequency questionnaire as well as other pertinent variables in this research. Correlation statistics are summarized in Table 18.

Frequency distributions were used to analyze data on factors influencing the decision to use HRT, source of information on menopause, alternate therapies use to treat menopausal symptoms, and reasons for choosing not to use HRT.

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CHAPTER IV

RESULTS

Subject Characteristics

The subjects in this study represented a group of healthy free-living postmenopausal women who had been using or not using hormone replacement therapy for at least one year and who responded to a recruitment letter mailed to all faculty and staff at the University of New Hampshire (Durham, New Hampshire) and Plymouth State College (Plymouth, New Hampshire). The age range of the subjects was 48-67 years old, with a mean age of 55.84 ± 4.43 years. Subject characteristics are presented in Tables 1a and 1b.

All subjects donated a blood sample. Sixty-six (94.3%) returned a completed *UNH Women's Health Study Questionnaire* (Appendix C), 55 (78.6%) a completed three-day food diary (Appendix C), and a *Health Habits and History Questionnaire* (Appendix C). Personal and family histories (mortality and morbidity reported in a first degree relative) were collected during a personal interview and are summarized in Table 2. Subjects using HRT were on a variety of exogenous hormone preparations which are summarized in Appendix F.

The majority of the subjects were white (97.0%), and highly educated (42.4% held post-graduate college degrees). Sixty-seven percent were married for a mean of 27.45 ± 7.88 years. Fifteen subjects (21.4%) had experienced a surgical menopause: nine (total-simple hysterectomies), four (total hysterectomies with bilateral salpingo-oophorectomies),

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one (removal of ovaries and retention of uterus), and one subject did not know what type of hysterectomy she had experienced. Six of the hysterectomized women were not taking any hormone replacement therapy. Nine were taking estrogen only.

Of the seventy subjects in this study 82.9% reported having a physical exam, 74.3% a mammogram, and 77.1 % a pap smear within the last 12 months. The primary health care provider identified by subjects (n=66) included gynecologists (7.6%), nurse practitioner (12.1%), internist (36.4%) and family practitioner (43.9%).

There was no significant difference found in number of children, number of pregnancies or years of past oral contraceptive (OC) use among groups. As seen in Table 1b, the women who were presently using HRT (EO and E/P groups) reported 5.44 ± 1.28 years of past OC use, as compared to the NHRT group with 3.94 ± 0.79 years of past use of OC. Although not statistically significant there was a trend toward longer OC use in the HRT group, thus supporting hypothesis #11 that postmenopausal women who use HRT are more likely to have used OC in the past.

The Effect of HRT on Serum Lipids, Estradiol, and Progesterone

The least square means and standard errors of serum concentrations of total cholesterol, LDL-C, HDL-C, triglycerides, Lp(a), estradiol, progesterone and total cholesterol/HDL ratio are presented in Tables 3a and 3b. No statistically significant differences were found in serum estradiol, progesterone, triglycerides, HDL, LDL, Lp(a) concentrations or Total Cholesterol/HDL ratio by HRT use, as seen in Table 3a. However, when the hormone groups were collapsed (Table 3b), a significant difference was found in progesterone concentration between the NHRT and the HRT group

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($p < 0.01$) There was no correlation found between serum estradiol concentrations and serum β-carotene or α-tocopherol, as well as between LDL β-carotene and LDL α-tocopherol or βcarotene/LDL and α-tocopherol/LDL ratios. An ANOVA was used to compare all groups and a t-test to compare the NHRT and HRT group to test hypothesis #3 that the use of HRT would positively affect serum cholesterol, LDL-C, HDL-C, Lp(a) and total cholesterol/HDL ratio was not supported. The Pearson product correlation was used to test hypothesis #3 proposing that HRT would positively affect serum cholesterol and lipoprotein concentrations in postmenopausal women. This hypothesis was also not supported by the data.

The Effect of HRT on Serum Antioxidants (α-tocopherol and β-carotene), LDL α-tocopherol and β-carotene, and α-tocopherol and β-carotene/LDL Ratios.

The least squares means and standard errors of serum antioxidants and LDL antioxidant variables are presented in Tables 4a and 4b. No statistically significant differences were noted in serum α-tocopherol or serum β-carotene concentrations by HRT use when comparing all three groups (Table 4a). However when the groups were collapsed (Table 4b), a trend toward higher concentrations of both antioxidant vitamins in the EO and E/P groups was noted. Serum α-tocopherol concentrations was positively correlated with LDL α-tocopherol ($r = .299$, $p<0.05$) and α-tocopherol/LDL ratio ($r = .777$, $p<0.01$). The α-tocopherol/LDL ratio was also negatively correlated with serum LDL concentration ($r = -.269$, $p<0.05$). Serum β-carotene was positively correlated with and LDL β-carotene ($r = .923$, $p<0.01$) as well as β-carotene/LDL ratio

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($r = .884$, $p<0.01$).

No statistically significant differences were noted in LDL β-carotene or LDL α-tocopherol concentrations by HRT use. The NHRT group had higher concentrations of LDL α-tocopherol (8.556 ± 1.30 mol/mol LDL) than either of the hormone groups; EO (7.070 ± 0.71 mol/mol LDL) and E/P (8.500 ± 0.62 mol/mol LDL), although the difference were not significant. The NHRT group had lower LDL β-carotene than either hormone group (EO and E/P). LDL ∝-tocopherol was positively correlated with ∝-tocopherol/LDL ratio ($r = .260$, $p<0.05$). LDL β-carotene was positively correlated with β-carotene/LDL ratio ($r = .967$, $p<0.01$) and negatively correlated with total cholesterol/HDL ratio ($r = -.288$, $p<0.05$) and serum triglycerides ($r = -.261$, $p<0.05$), as well as LDL ($r = -.241$, $p<0.05$). The β-carotene/LDL ratio was found to be negatively correlated with serum LDL ($r = -.300$, $p<0.05$) and total cholesterol/HDL ratio ($r = -.301$, $p<0.05$). The α-tocopherol/LDL ratio and β-carotene/LDL ratio were not found to be significantly different between groups by HRT use. An ANOVA was used to compare all groups and a t-test to compare the NHRT and HRT group to test hypothesis #1 proposing that postmenopausal women who use HRT will have higher concentrations of serum β-carotene and ∝-tocopherol in both serum and LDL than women who do not use HRT. Although this hypothesis was not supported, a trend was noted towards higher concentrations of both antioxidants in the serum in the HRT groups. The Pearson product correlation was used to test hypothesis #2 that serum estradiol concentrations would be positively correlated with serum concentrations of β-carotene and ∝-tocopherol, LDL

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∝-tocopherol and LDL β-carotene, and β-carotene/LDL and ∝-tocopherol/LDL ratios, and lipid profiles in postmenopausal women. This hypothesis was not supported by the data.

Resistance of LDL to oxidation ex vivo

The values for oxidation of LDL are represented in Tables 4a and 4b. There were no statistically significant differences between groups in length of lag phase or maximum diene formation by HRT use. There was however, a significant difference found in the rate of diene formation between the NHRT and the E/P group ($p<0.001$). The hormone replacement groups (EO and E/P) did demonstrate a trend toward longer lag phases and lower rates of diene formation and maximum diene formation than the NHRT group. A negative correlation was found between rate of diene formation, serum tocopherol ($r = -.366$, $p<0.01$) and serum triglycerides ($r = -.277$, $p=<0.05$): and α-tocopherol/LDL ratio ($r = -.544$, $p<0.01$). There was a negative correlation between serum triglycerides and maximum diene formation ($r = -.303$, $p<0.05$).

Table 4a and 4b report the values for total antioxidants (ORAC: Oxygen Radical Absorbency Capacity) as both μmol Trolox equivalents/l and nmol Tolox/mg protein. No significant differences were found for either calculation between groups by HRT use. There was a trend toward higher ORAC values in the NHRT group as compared to the hormone groups (EO and E/P). ORAC was found to be negatively correlated with LDL α-tocopherol ($r = -.250$, $p<0.05$), ∝-tocopherol/LDL ratio ($r = -.279$, $p<0.05$) and HDL ($r = -.248$, $p<0.05$). A positive correlation was seen between ORAC and rate of diene formation ($r = .314$, $p<0.01$).

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HRT Use and Body Composition: BMI, Waist-Hip Ratio and Body Weight

The least square means and standard errors for body mass index and waist-hip ratio values are presented in Tables 1a and 1b. Height and BMI were the only categories where a significant difference occurred according to HRT use. In Table 1a, a significant difference was found between the NHRT group and the E/P group ($p< 0.05$), and the EO and E/P group ($p< 0.05$) for height; however this was not true when the HRT groups were collapsed (Table 1b). The EO group weighed more than both the NHRT and E/P group, and a significant difference in body mass index was noted between the EO (BMI of 28.42 ± 1.66) and E/P groups (BMI or 24.50 ± 1.04), ($p= <0.05$). This difference also disappeared when the HRT groups were combined. There were no statistically significant difference found in waist-hip ratios between groups. An ANOVA was used to compare all groups and a t-test to compare the NHRT and HRT group to test hypothesis #5. The hypothesis that postmenopausal women who use HRT will have lower BMI's and waist-hip ratios than women who do not use HRT was not supported. The subjects in the EO group tended to be heavier than either the NHRT or E/P groups (164.32, 157.69, and 151.74 pounds respectively) and to have greater waist-hip ratios (07.86, 07.79 and 07.53 respectively). BMI was positively correlated with serum triglycerides ($r = .354$, $p<0.01$); LDL ($r = .265$, $p<0.05$), and total cholesterol/HDL ratios ($r = .391$, $p<0.01$). BMI was negatively correlated with HDL ($r = -.308$, $p<0.05$), serum β-carotene ($r = -.273$, $p<0.05$), LDL β-carotene ($r = -.363$, $p<0.01$), and β-carotene/LDL ratio ($r = -.328$, $p<0.01$). Waist-hip ratio was positively correlated with total cholesterol/HDL ratio ($r = .377$, $p<0.01$) and negatively correlated with serum triglycerides ($r = -.315$, $p<0.01$), HDL ($r = -.342$, $p<0.01$) and LDL β-carotene ($r = -.256$, $p<0.05$). Body weight was found be positively correlated

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with serum triglycerides ($r = .294$, $p<0.05$) and LDL ($r = .262$, $p<0.05$); and negatively correlated with serum β-carotene (r -.327, $p<0.01$), HDL ($r = -.309$, $p<0.05$), LDL β-carotene ($r = -.391$, $p<0.01$), and total cholesterol/HDL ratio ($r = -.373$, $p<0.01$).

Lifestyle Interventions.

The least square means and standard errors for alcohol consumption and the amount and duration of exercise is reported in Tables 5a and 5b. No statistically significant differences were found among the three groups, nor when the groups were collapsed, in any of the categories. However, as shown in Table 5b, the NHRT group did demonstrate a trend toward more frequent episodes of exercise (3.36 ± 0.04 versus 2.58 ± 0.4 times per week) and longer duration (129.69 ± 18.8 versus 94.33 ± 15.06 minutes per week) in exercise regimes than either of the hormone group (EO and E/P). A positive correlation was found between times of exercise per week and LDL β-carotene ($r = .292$, $p<0.05$) and β-carotene/LDL ratio ($r = .291$, $p<0.05$).

The least square means and standard errors of selected nutrients based on the three-day food records was compared to those based on the food frequency questionnaires using the Pearson product correlation. Significant correlations were detected between the two methods for the values for total energy ($r = .424$, $p<0.01$), grams of fat ($r = .414$, $p<0.01$), grams of saturated fat ($r = .454$, $p<0.01$), percent calories from fat ($r = .637$, $p<0.01$), calcium ($r = .579$, $p<0.01$), iron ($r = .327$, $p<0.01$), vitamin E ($r = .588$, $p<0.01$), iron ($r = .414$, $p<0.01$), and fiber ($r = .327$, $p<0.01$). Correlations between methods for vitamin A, cholesterol, or β-carotene were not significant. The least squares means and standard errors for the three-day intake is presented in Tables 6a and

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6b, and for the food frequency questionnaire in Tables 7a and 7b. No significant differences were found for any of the reported nutrients by HRT use.

Forty-one percent of participants reported using some type of dietary supplement. In the NHRT group, 13 subjects used a supplement, as compared to 4 in the EO group and 12 in the E/P group. Of the women who used no hormone replacement therapy (NHRT) 18.6% reported using a supplement as opposed to 22.9% of the women who used HRT.

The least squares means and standard errors for intake of vitamin E from dietary intake, supplements, and total vitamin E intake (diet and supplements) is presented in Tables 8a and 8b. No statistically significant differences were found in any of these parameters by HRT use. The subjects in the E/P group reported greater daily intakes of vitamin E from supplements than either the NHRT or EO groups (224.12 ± 81.1 mg) versus (157.26 ± 48.5 mg) and (54.44 ± 30.7 mg), respectively. When the groups were collapsed the daily total vitamin E intake (Table 8b) was greater in the HRT groups than the NHRT group (179.91 ± 49.1 mg) versus (158.49 ± 44.1 mg) respectively. Although serum α-tocopherol was not correlated with reported vitamin E intake from the three day food diary and the FFQ, it was positively correlated with vitamin E intake from supplements ($r = .270$, $p<0.05$) and total vitamin E intake ($r = .289$, $p<0.05$). LDL α-tocopherol was also positively correlated with dietary vitamin E ($r = .443$, $p<0.05$) and α-tocopherol/LDL ratio was found to be positively correlated with vitamin E from supplements ($r = .443$, $p<0.01$), as well as with total vitamin E (diet and supplements) ($r = .420$, $p<0.01$). Rate of diene formation was negatively correlated with total vitamin E intake ($r = -.264$, $p<0.05$).

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The least squares means and standard errors for intake of β-carotene from dietary intake, supplements, and total β-carotene (diet and supplements) is presented in Tables 9a and 9b. No statistically significant differences were found in any of these parameters by HRT use. The two hormone replacement groups (EO and E/P) reported greater daily intakes of β-carotene from supplements than the NHRT group with EO taking(1000.00 ± 500.00 RE), E/P reporting (909.19 ± 250.63 RE) and NHRT (692.31± 192.31 RE). The NHRT group's dietary intake of β-carotene was greater than either of the EO or E/P groups (3432.88 ± 779.18 RE) versus (1079.39 ± 262.16 RE) and (2778.21 ± 910.94 RE) respectively. When the hormone groups were collapsed (Table 8b) a greater total β-carotene intake was demonstrated in the NHRT group (4088.02 ± 1188.05 RE) when compared to the HRT group (3419.76 ± 1340.36 RE).

There was no statistically significant difference found between serum β-carotene and reported dietary intake of β-carotene in the three day food diary or the FFQ. However, a positive correlation was found between serum β-carotene and β-carotene from supplements ($r = .603$, $p<0.01$). LDL β-carotene was positively correlated with dietary β-carotene ($r = .307$, $p<0.05$), β-carotene from supplements ($r = .408$, $p<0.01$), and total β-carotene ($r = .541$, $p<0.01$). The β-carotene/LDL ratio was found to be positively correlated with dietary intake of β-carotene ($r = .316$, $p<0.05$), β-carotene form supplements ($r = .351$, $p<0.01$) and total β-carotene ($r = .494$, $p<0.01$).

The least square means and standard errors for the *Dine Score* to measure dietary status are presented in Tables 10a and 10b. The DINE scores indicated "good" dietary status for the NHRT (mean score of 6.21 ± 0.31) and EO (mean score of 6.75 ± 0.38)

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groups , and "fair" for the E/P (mean score of 5.62 ±0.35) group. An ANOVA was used to compare all groups and a t-test to compare the NHRT and HRT group to test hypothesis #4 and #10. The subjects in all three groups reported moderate and frequent exercise routines and consuming healthy diets therefore supporting the hypothesis #4 that postmenopausal women who practice positive lifestyle interventions will demonstrate a decreased risk for cardiovascular disease independent of HRT use. However, hypothesis #10 was not supported in that there was no significant differences noted in dietary intake of fat or saturated fat among groups.

Psychological and Social Factors

The least square means and standard errors for the *POMS Mood States* inventory are presented in Tables 11a and 11b. No statistically significant differences were found among the three groups for identified mood states. In noting the range of scores for each mood/affective states in the *POMS* Inventory, mean scores for all sub-scales, as well as Total Mood State scores on the *POMS* Inventory were low for all groups. The total mean scores for the POMS inventory were as follows: NHRT (11.13 ± 5.0), EO (23.22 ± 9.1), and E/P (6.11 ± 6.3). This is indicative of positive mood states reported among subjects in the study.

The least square means and standard errors for the *Self-esteem Score* are presented in Tables 12a and 12b and indicate no statistically significant differences were found by HRT use. The scores of the self-esteem inventory were as follows: NHRT (8.62 ± 0.2), EO (8.07 ± 0.4), and E/P (8.56 ± 0.2). These values are out of a possible 10 points

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indicating positive self-esteem among the participants regardless of hormone replacement use.

The least square means and standard errors for and the *Experiences (symptoms) Inventory* are presented in Tables 13a and 13b. The total score on the Experiences inventory is weighted toward negative symptoms. The scores were as follows: NHRT (50.38 ± 2.8); EO(52.20 ± 4.8); and E/P(48.13 ± 2.0). These scores were out of a possible 112 points indicating moderate menopausal symptoms among subjects.

An ANOVA was used to compare all groups and a t-test to compare the NHRT and HRT group to test hypothesis #7. This hypothesis is supported in that postmenopausal women who practice positive lifestyle interventions (health supporting diets and moderate and frequent exercise) will report less severe postmenopausal symptoms, as well as positive self-esteem and moods regardless of their use of HRT.

Factors Influencing Hormone Replacement Therapy Use.

Percentages of subject's responses regarding the *Factors Influencing the Decision to Use HRT* is presented in Table14 and Figure 1. These responses were only from the subjects who were using HRT (EO and E/P groups). For the purposes of comparison, the subject's responses were divided into three categories (1) Health concerns (hysterectomy, relief of symptoms, concern about heart disease, and concern about osteoporosis); (2) Advice of others (advice of MD or health professional; advice of friend, co-worker, or family; and advice of partner); and (3) Psychological and Social Factors (mood changes, concern about aging, and sexual problems). Responses of "moderately or extremely influential" were reported for hysterectomy (13.6 %), relief of menopausal symptoms

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(72.4%) , concern about heart disease (77.7%), and concern about osteoporosis (77.4 %) in the *Health Concerns* Category. Responses of "moderately or extremely influential" were reported for advice of MD or health professional (82.8 %), advice of friend, co-worker, or family (8.3 %), and advice of partner (4.2 %) in the *Advice of Others* category. Responses of "moderately or extremely influential" were reported for mood changes (26.9 %), concern about aging (34.6 %), and sexual problems (4.2 %) in the *Psychological-Social* category.

Table 15 and Figure 2 summarize the responses of subjects for *Sources of Information on Menopause*. An MD or health care professional was reported by 70.5 % of the subjects as "moderately or extremely informative" sources of information on menopause. Other influences, though not as strong, were mother (4.2 %), media:radio and TV (33.4 %), workshops and conferences (40.0 %), books and pamphlets (70.5%), partner (0%) and friend or relative (27.1 %). Frequency distributions were used to test hypotheses #8 and #9. Hypothesis #8 was supported in that outside influences were important in the choice to use HRT in postmenopausal women in this study, with the health care professional having the strongest influence in this decision. Hypothesis #9 was also supported as postmenopausal women reported that acute symptom relief and concern over chronic disease (heart disease and osteoporosis) were the most influential factors in making the decision to use HRT.

Alternate Therapies Used to Treat Menopausal Symptoms were reported by 27.3% (n=18) of the subjects and results are presented in Table 16. Vitamins and minerals (9.4 %), exercise (4.7 %), and meditation (6.3%) were the most frequently used alternate therapies reported by subjects. One subject identified each of the following (diet, massage

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therapy, natural hormone replacement therapy, relaxation techniques, and vaginal creams) as alternate therapies used to treat menopausal symptoms.

Percentages of subjects' *Reported Reasons for Not Using HRT* are presented in Table 17 and Figure 3. The responses presented here are only from those subjects who were not using HRT (NHRT group). The primary reason reported for not using HRT (45.9 %) was concern about cancer, personal history of breast cancer (3.8 %), family history of breast cancer (11.5 %), family history of other types of cancer (4.0 %), fear of breast cancer (3.8 %), concern over uterine or ovarian cancer (7.4 %), and concern over cancer in general (15.4 %). Other responses included fibrocystic breast disease (4.0%), not liking to take medication (16.0%), no risk factors/no need to use HRT (20.0%), MD or health professional discouraged use of HRT (8.0%), not wishing to start menstruating again (4.0%), cost of medication (4.0%), and not remembering to take medication (2.9%). Figure 3 categorizes the reported reasons for not using hormone replacement therapy as: cancer concerns (49.9%), medication concerns (31.7%), MD advice (8.0%), and no need to take HRT or no perceived risk factors (20.0%). Frequency distributions were used to test hypothesis #6. The hypothesis was supported in that fear of cancer was the primary reason reported for not using HRT, or discontinuing its use in this group of subjects.

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CHAPTER V

DISCUSSION

Women entering mid-life today will have many decisions to make concerning their health and the quality of their lives as they age. One of the most difficult and controversial decisions will be whether or not to use HRT, and if so, for how long. Menopause is a life stage that encompasses many facets of a woman's life. To confine an investigation of menopause to only the biological, the psychological, or the social aspects would be limiting. By integrating these three aspects of menopause, the results will offer an expanded and much more comprehensive view of the complex interplay that effect women's perceptions and choices during this time. The present study, to the best of my knowledge, is the first investigation that has utilized the biopsychosocial approach to determine the factors that influenced the decision to use hormone replacement therapy in seventy postmenopausal women.

This study was an outgrowth of a larger study, *The University of New Hampshire Women's Health Study*, as well as part of the NE-172 regional project, *Assessment of Nutritional Risk in the Elderly*. The objectives for both of these studies were integrated into the study described in this dissertation. In 1995 the UNHWHS collected information on women's health from 303 questionnaires mailed to women from ages 35-70 in New Hampshire. This research is from a cohort of 70 postmenopausal women recruited from

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the larger study population. The subjects in this study were very similar in age, weight, and height (Table 1a and 1b). They all reported good access to health care and reported regular exercise and consumption of healthy diets.

The impact on cardiovascular disease risk shown by previous research appears to be attenuated when study design and subject selection are taken into consideration. Subjects who participated in previous studies appeared to enjoy a "healthy cohort effect" (34). The majority of these women were healthy, middle-class, and well educated. They were also relatively lean (25), exercised on a routine basis (27), (24), and had good access to health care. The earlier studies used ambiguous terms (219), such as "current user", "past user", "never user", when describing HRT status and estrogen alone (primarily *Premarin*™) was the hormone treatment. The majority of studies were retrospective observational studies using mailed questionnaires to collect data and the focus was primarily on biological outcomes, with the most common theme being the effect of HRT on lipoprotein concentrations. Very few studies included any social or psychological information on HRT use. Therefore, the subjects used in these vanguard studies may have been healthy entering the study which may have resulted in a decreased risk of CVD, independent of HRT use.

Taking all these factors into consideration, the present study recruited a similar group of free-living postmenopausal women some of whom were not taking HRT and others who were, on varied doses and formulations of hormone replacement therapy (Appendix F) for a least one year. As shown in Table 2 three women reported that they had heart disease, five were presently taking medication to treat hyperlipidemia, twelve were hypertensive and one had been treated for breast cancer. This similar subject selection allowed for comparison with past studies which demonstrated a 50% reduction in

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cardiovascular risk with HRT use (63), (103),(104), (106). Similar to previous studies, the subjects in this study were well-educated, healthy, and reported positive lifestyle interventions (health supporting diets and frequent exercise). As seen in previous studies, the women in the present study appeared to enjoy the "healthy cohort effect" also. It is difficult to recruit women at greatest risk for cardiovascular disease. Until recently women at greatest risk were often not prescribed HRT. Many of these women did not have good access to health care or information on menopause and HRT and therefore would be less likely to participate in research protocols. Since the present study was the first study to use the biopsychosocial perceptive to investigate menopause and HRT, it was thought that the similarity in subject populations would allow comparisons between previous studies and the one described in this dissertation. With all this in mind, this research sought to determine if postmenopausal women who practice positive lifestyle interventions would demonstrate more positive biological, social, and psychological outcomes independent of hormone replacement therapy.

The Biological Outcomes Associated with HRT Use

Since the early 1970's, HRT has been used to treat acute symptoms of menopause (22), (20) and to prevent the onset of chronic disease, such as osteoporosis and cardiovascular disease. Research has consistently shown that HRT use can decrease the risk of cardiovascular disease in postmenopausal women by as much as 50% (63), (103), #110], (104), (106). The primary mechanism identified has been the beneficial effect of HRT on lipids and lipoproteins (109).

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The subjects in this study had already made their choice regarding HRT. This research investigated the biological outcomes of this choice on cardiovascular risk, with specific emphasis placed on the role of the antioxidant nutrients of dietary origin (∝-tocopherol and β-carotene), and estradiol on cardiovascular risk factors in postmenopausal women.

Naturally occurring changes in lipoproteins are seen at the time of menopause. Total cholesterol, LDL-C (55), (28), (56), (57), and Lp(a) (70) concentrations increase, while HDL-C is only moderately affected (58), (59). HRT has been shown to attenuate this lipid profile by decreasing total cholesterol, LDL-C (104), (109) and Lp(a) (72) while increasing HDL-C (117), although wide variation in these changes are seen dependent on type of estrogen, the addition of progestin, the route of administration, and dose (109). Various studies have indicted that only ~25-50% of the cardioprotective effects of estrogen is thought to be mediated by the beneficial changes in lipids and lipoproteins (109). *The Nurses' Health Study* (104), the *Healthy Women's Study* (229), and the *Lipid Research Follow-Up Study* (4), (69) have all demonstrated beneficial effects of exogenous hormone use. The subjects in the present study did not demonstrate any significant differences in triglycerides, HDL-C, LDL-C, total cholesterol or Lp(a) concentrations (Table 3a and 3b).

When evaluating the effect of lipid and lipoprotein concentrations on cardiovascular risk in women, it is important to keep several issues in mind. *The National Cholesterol Education Program's Expert Panel* has identified a HDL-C of <35 mg/dL, total cholesterol of >240, and a LDL-C of >160 mg/dL all as cardiovascular risk factors in women (68). HDL-C is the best predictor of cardiovascular risk in women (63), (64), (67) and positive lifestyle changes in diet and exercise can increase HDL-C concentrations (67).

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As depicted in Table 3a, all three groups of subjects in the present study had HDL-C concentrations > 35mg/dL NHRT (52.76 mg/dL ± 2.26), EO (55.91 mg/dL ± 3.75) and E/P (51.94mg/dL ±2.62). This was also true for both total cholesterol and LDL-C. The three groups had total cholesterol concentrations of (224.59mg/dL ±6.22), (235.32mg/dL ±11.56), (212.48 mg/dL ±8.81) respectively; and LDL-C concentrations of (148.06mg/dL ±5.26), (147.67mg/dL ± 9.09), and (138.66mg/dL ±7.72) respectively. One might expect the women who are taking HRT to have a more positive lipid profile than the women who were not using HRT. The similarity in values could be explained by the fact that the women had been using HRT for a least a year. The women who chose HRT may have done so because of negative lipid profile. HRT use should have resulted in more positive concentrations of the lipoprotein concentrations.

HDL-C has been identified as the most important lipoprotein in women. The women in this study reported frequent exercise patterns and consumption of healthy diets which could have resulted in the positive HDL-C concentrations. The fact that the subjects in this study were healthy and practiced positive lifestyle behaviors, including health supporting diets, control of body composition, and routine exercise, may have contributed to positive blood lipid profiles independent of HRT use.

Estrogen, both endogenous and exogenous, has been studied widely to determine the mechanism involved in its ability to offer protection to heart and bone, while conferring deleterious effects on the breast and endometrium. Several studies have demonstrated no association between serum estradiol concentrations and the risk of cardiovascular disease in women (96), (59), (82). The present study found no significant differences in serum estradiol concentrations between the women who used HRT and those who did not. As

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shown in Table 3a and 3b there was little difference in estradiol concentrations between the three groups or when the HRT groups were collapsed. The similarity in estradiol concentrations could be explained by the fact that the ELISA used to measure estradiol in the present study was very specific for 17 β-estradiol and was not able to quantify the other estrogens present. There was a wide variability in the types and route of administration of HRT regimes among subjects (Appendix F). Most HRT formulations contain a combination or estrogens. Oral estrogens are also subject to the first-pass hepatic effect, where 60-90% of the drug will be metabolized by the liver. Administration of estrogen by transdermal patch does not cause this hepatic effect and serum levels of estradiol remains much more constant (230).

It is also important to note that the women in the present study had their blood samples drawn after a 12 hour fast and many had not taken their HRT medication in over 24 hours. This would have modulated the first-pass effect seen with oral estradiol administration where serum levels rise acutely for 6-10 hours and then return to baseline within 48 hours (231). All subjects in this study had been on HRT for at least one year, and it is possible that the women in the NHRT group may not have been suffering from acute menopausal symptoms because they had higher concentrations of endogenous estrogen to begin with. There is also great intra- and inter variability in serum estradiol among women who use HRT, and the limited number of subjects in this study may not have been sufficient to see significant differences in estrogen concentrations. In future studies the measurements of estrone, the predominant circulating estrogen in postmenopausal women, might be a better indicator of endogenous hormone concentrations. This study did not measure the total estrogenic activity in these subjects.

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At menopause the production of progesterone is limited to a small amount produced by the adrenal gland which is used as a precursor for the synthesis of other steroids. Although unexpected, there was a significant difference ($p < 0.01$) found in progesterone concentrations when comparing the NHRT group to the HRT group (Table 3b). The NHRT group had higher concentrations of progesterone (0.4778 ± 0.08) when compared to the HRT group (0.3245 ± 0.02) which at first seemed contradictory. As with the estradiol assay, the ELISA used in this study was specific for progesterone and therefore did not measure the progestins in the HRT formulations, but rather the endogenous progesterone *in vivo*. Progestins are not well absorbed and are broken down by the liver. The stimulation of the liver by the progestins may have stimulated the steroidogenic pathway resulting in more endogenous progesterone being utilized as a precursor resulting in the lower concentrations of progesterone seen in the HRT group seen in this study.

Studies have indicted that only ~25-50% of the cardioprotective effects of estrogen is thought to be mediated by the beneficial changes in lipids and lipoproteins (109). Several other mechanisms are currently being investigated and the present study sought to determine the impact of HRT and lifestyle factors on the oxidative modification of LDL-C. Previous studies have supported the belief that the oxidative modification of LDL is an important step in the development and progression of atherosclerosis (232). Estrogen alone or acting synergistically with antioxidants confers protection from oxidation to the LDL-C (10), (11), (139). Estrogen affects the synthesis and secretion of lipoproteins by the liver and because the lipoproteins are the carriers of the antioxidant nutrients in the

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peripheral circulation, this study investigated the potential effect of estrogen (endogenous and exogenous) on the antioxidant content of LDL-C; as well as the oxidizability of LDL-C. Human LDL-C is also protected from oxidative modification by lipid soluble antioxidants present in the same particle.

The concentration of the two antioxidants of dietary origin were measured in both the serum and the isolated LDL. The basis of this research was from a study reported by Clemente et al (150) which suggested that the effect of HRT may be its ability to conserve and enrich the antioxidants in the LDL-C. Clemente suggests that the ratio of total serum antioxidants to LDL antioxidants is a more appropriate way to indicate estrogen's ability to protect LDL-C from oxidative damage. Therefore, a higher ratio would indicate more enrichment of the LDL-C, despite of the amount found in the serum. The subjects in the present study did not demonstrate significant differences in serum or in LDL α-tocopherol and β-carotene concentrations (Table 4a). In comparing the NHRT group to the HRT group (Table 4b) a trend was noted toward higher α-tocopherol/LDL (NHRT group 34.80 μmol/l $\pm$ 1.77 verses HRT group 38.52 μmol $\pm$ 2.7) and β-carotene/LDL (NHRT group (0.17 $\pm$ 0.02 verses HRT group 0.259 $\pm$ 0.06) ratios in the HRT group. These results suggest that hormone replacement therapy may exert some effect on the ability to preserve the antioxidant nutrients in LDL, although further studies would be required to determine this effect.

Two *in vitro* oxidation assays were performed in this study. The *in vitro* oxidation of isolated LDL-C with copper is a more traditional method used to indicate resistance of the LDL-C to oxidation. In this method lag phase, rate and maximum diene formation are quantified. Longer lag times, the time prior to oxidation when antioxidants are being

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depleted, indicates more protection to the LDL-C. Higher rate of diene formation and maximum diene formation values are indicative of more oxidation occuring and therefore less protection to the LDL. No significant differences were found in lag phase or maximum diene formation among subjects in this study. However, there was a trend toward longer lag phase and higher maximum diene formation (Table 4b) in the HRT group. There was a significant difference in rate of diene formation between the NHRT (12.94 ±0.33) and HRT (11.41 ± 0.30) group at $p < 0.01$.

ORAC is a method for quantifying the total antioxidants present in a biological system. The subjects using HRT had a lower ORAC value (3769.65 μmol Trolox equiv/l ±138.73) than the NHRT group (4300.70 μmol Trolox equiv/l ± 201.700) at $p < 0.05$ (Table 4b). This seems contradictory to what might be expected, however ORAC is a measure of not only the fat soluble antioxdants (∝-tocopherol and β-carotene); but also water soluble vitamin C, and enzymes (superoxide dismutase, catalase, glutathione peroxidase), as well as macromolecules such as ferritin, and albumin. It is possible that the women who were not using HRT had more total antioxidants regardless of HRT use. This data suggests that HRT may have conferred some protection from oxidation to the LDL, although further studies are needed to confirm this.

The Effect of Lifestyle Interventions on Cardiovascular Risk Profile

Positive lifestyle interventions, health supporting diets and routine exercise, result in decreased risk for cardiovascular disease. Studies have consistently shown the diets low in total fat, saturated fat, and high in monounsaturated fat and fiber result in a decreased cardiovascular risk (155), (156). Consumption of high amounts of the antioxidant

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nutrients (∝-tocopherol and β-carotene) from diet and supplements results in a decreased risk of CVD (143), (134), (145), (135), (144). Two validated instruments (three day food diary and a food frequency questionnaire) were used in this study to assess the quality of dietary intake and to relate dietary intake to the serum measurements. Collecting retrospective data from a food frequency questionnaire and prospective data from a three-day intake allowed for a comprehensive assessment of the subject's dietary patterns. Information gathered from the three-day food diary (Tables 6a and 6b) indicated that all groups were consuming diets that were low in fat, saturated fat, as well as containing adequate intakes of vitamin E, calcium, iron, and dietary fiber. Data from the food frequency questionnaire (Tables 7a and 7b) indicated similar dietary intakes with the exception of percent calories from fat, which was > 30% in all three groups. The overall assessment of the subjects' dietary intake from the *DINE* Score (Tables 10a and 10b)was rated as "good" for the NHRT and EO groups and "fair" for the E/P group. The reported dietary intakes from the subjects in the present study indicate the consumption of diets that were low in total fat, cholesterol, and saturated fat. The fact that lipid and lipoprotein concentrations among subjects met the NCEP guidelines appears to support the hypothesis that women who consume health supporting diets will demonstrate a decreased risk of cardiovascular disease independent of their use of hormone replacement therapy.

The intakes of the antioxidant nutrients, β-carotene and α-tocopherol, from diet and supplements is represented in Tables 8a, 8b, 9a, and 9b. The NHRT group consumed more vitamin E from dietary sources than either of the hormone replacement groups (15 mg/day ± 2.2), (13.97 mg/day ± 3.5), and (11.86 mg/day ± 1.3) respectively. However, when vitamin E supplements were taken into consideration, the E/P group consumed more

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total vitamin E (234.80 mg/day ± 68.5), than the NHRT group (158.49 mg/day ± 44.1) or the EO group (64.57 mg/day ± 28.0) respectively . The NHRT group also consumed more β-carotene from dietary sources (3432.88 RE/day ± 779.18) than the two EO (1079.39 RE/day ± 262.16) or the E/P group (2778.21 RE/day ± 910.94). However, as seen with vitamin E, both HRT groups reported higher intakes from supplements: EO (1000 RE/day ± 500), E/P (909.09 RE/day ± 250.63) verses NHRT (692.31 RE/day ± 192.31). These results suggest that women who use HRT are more likely to use dietary supplements and may in general feel more comfortable with the use of "pills" to treat or prevent disease. This was also seen with past use of oral contraceptives with the women using HRT reporting (5.44 years ± 1.28) of past use as compared to NHRT group reporting (3.94 years ± 1.28). Results from all the dietary instruments indicate that all three study groups were consuming adequate and health-supporting diets regardless of HRT use.

Changes in body composition with age have been shown to be associated with an increased risk of cardiovascular disease. Although obesity is not an independent risk factor in cardiovascular disease, it is secondary to hypertension, diabetes mellitus, and dyslipidemia (162). With age there is a decrease in lean body mass, an increase in fat mass, and a shift from a gynoid to an android fat distribution (159). Body mass index is associated with an increase in triglyceride and LDL-C (167) and a decrease in HDL-C concentrations (166), (170). In the present study the EO group was heavier, had higher body mass indexes than either the NHRT group or the E/P group. In fact a significant in BMI was found between the EO group (28.42 ± 1.66) and the E/P group (24.50 ± 1.04) at ($p<0.01$). The NHRT and the E/P group were both within desirable ranges for BMI (26.71

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± 0.86) and (24.50 ± 1.04) respectively. However the EO group was slightly above the desirable limits with a body mass index of (28.42 ± 1.66). In the present study body mass index was positively correlated with serum triglycerides ($r = .354$, $p<0.01$), LDL ($r = .265$, $p<0.05$), and total cholesterol/HDL ratio ($r = .391$, $p<0.01$); and negatively correlated with HDL ($r = -.308$, $p<0.05$) which is consistent with previous research (167), (166).

Although the use of HRT has not been shown to increase or decrease weight in women who use it, it has been suggested that its use will prevent the shift to the android fat pattern (159) and therefore decrease the risk of cardiovascular disease. All three groups in the present study had waist-hip ratios that within desirable limits (<0.8). Waist-hip ratio was positively correlated with serum triglycerides ($r = .354$, $p<0.01$) and total cholesterol/HDL ratio ($r = .377$, $p<0.01$) and negatively correlated with LDL β-carotene ($r = -.256$, $p<0.05$) and serum HDL ($r = -.342$, $p<0.01$). Although we are unable to determine if HRT affected body composition in this population, the acceptable body mass indexes and waist-hip ratios found in the NHRT and E/P groups may have contributed to the benefits to serum HDL-C and triglyceride concentrations found in these subjects, which in turn would result in lower cardiovascular risk.

It has been shown that postmenopausal women can benefit greatly from engaging in routine exercise (177), (178), (179). Exercise has been shown to increase HDL-C, the most important lipoprotein parameter in women (172), (67). Exercise also reduces stress (68), reduces the severity of vasomotor instability (190) and depression (2). *The American College of Sports Medicine Prevention and Rehabilitative Exercise Committee* now recommends 30 minutes of moderate exercise most, if not all, days of the week to decrease cardiovascular risk. It is also suggested that this exercise does not have to be

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continuous, but in fact short bouts of exercise can confer the same benefits (182). In the present study, all three groups reported participating in the recommended exercise routine. As demonstrated in Tables 5a and 5b, women had been participating in a routine exercise program for an average of 6.62 years ± 1.17. Subjects in this study engaged in moderate and frequent exercise, report less severe postmenopausal symptoms, more positive self-esteem and moods regardless of HRT use. All of these factors may have contributed to the low cardiovascular risk demonstrated in these subjects.

Factors Affecting the Choice of HRT in Postmenopausal Women

Women entering menopause today will be given various opinions from the experts regarding the risks and benefits of HRT. Reported prevalence rates on the use of HRT range from 5.3% (18) to 39.3% (19) throughout the United States. Although women are being prescribed HRT at a much greater rate than ten years ago, they continue to take it for short-term symptom relief (22) and not for the treatment and prevention of chronic disease (20). Women who have had a hysterectomy are also the most likely to use HRT (20), (7). There is a great deal of evidence in the literature that women are not compliant (20), (29), (40) in following their HRT regimes and tend to discontinue use after one year (7). It is important to acknowledge that some of this noncompliance is due to side effects from the progestin component of the prescription, such as undesirable bleeding patterns and not to women's indecision regarding the efficacy of HRT. Weintraub (39) describes this as "intelligent noncompliance" indicating that women are not using HRT after carefully weighing the risks and benefits and making an intelligent and informed choice based on this information.

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Table 14 and Figure 1 represent the factors that influenced the decision to use HRT. Subjects reported health concerns as the major factor influencing their choice to use HRT. Previous research has reported relief of symptoms as the strongest factors in choosing HRT (22). Subjects in the current study also reported concern about osteoporosis, and heart disease equally as important as relief of acute symptoms. This may indicate that women today have more information on HRT's role in the prevention of such chronic diseases as heart disease and osteoporosis. Although hysterectomy was reported as a concern by 13.6% of the subjects, the limited number of women in the hysterectomy group may altered interpretation of the results.

Past research has consistently shown that health care professionals strongly influence the decision to use HRT (29), (16). As seen in Table 14 and Figure 1, subjects in this study also reported that advice of the physician or other health care providers strongly influenced their decision to use HRT, with advice of friends, co-workers, family members and partners as having little influence.

Women in the present study who chose to use HRT do not tend to use alternative therapies to treat menopausal symptoms (31). As represented in Table 16, subjects in the present study did not report any significant use of alternative therapies such as meditation, yoga, exercise. This could be explained by the fact that this information was gathered after women had been using HRT for at least one year and they may have not perceived these activities as alternatives or treatment for symptoms that were no longer present. As seen in Tables 5a and 5b subjects had been exercising at their present duration and intensity for 3.35-9.70 years. This indicates that many of the subjects were exercising premenopausally and did not see this activity as a method to treat menopausal symptoms.

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Women also reported that their primary source of information on menopause came from health care professionals (Table 15). They also reported that psychological or social factors (mood changes, concern about aging, or sexual problems) had little influence on their choice. Although past research has demonstrated health benefits with the use of HRT, women remain reluctant to use HRT and that health care providers have reservations about prescribing HRT to women for long term use.

Many factors influence a women's decision to use hormone replacement therapy during menopause. The influence that the health care provider has on a women's decision regarding HRT is well documented in the literature (29), (16), (30), (31). The present study demonstrated the same trend as 82.8% of subjects reported that the advice of the physician or health care professional was "moderately influential" or "extremely influential" in making the decision to use HRT (Figure 1) and 70.5% reported MD or health care professional as the "moderately informative" or "extremely informative" in providing them with information on menopause (Figure 2). In view of the fact that health care providers have such a profound influence on women's choice of HRT, one might question where health care providers are obtaining their information on hormone replacement therapy and the efficiency of prescribing it to their patients? The review of the literature attests to a wide body of information related to menopause and HRT in professional journals, magazines and books; however, physicians also receive much of their information directly from the pharmaceutical industry. Recommendations and information from the drug representatives or literature mailed to them by the pharmaceutical company also influence the information that health care providers disseminate to their patients. In the past, physicians have been reluctant to prescribe HRT to women with chronic health problems,

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such as hypertension, diabetes, or heart disease (24). When discussing HRT with their patients, physicians are more likely to talk about short-term symptom relief rather than treatment of chronic disease or the value of lifestyle changes (35). The message and information being conveyed to health care professionals may emphasize symptom relief instead of the positive effects that HRT has on heart and bone. In the present study the subjects were concerned with relief of symptoms, but more concerned with heart disease and osteoporosis. It is therefore important that health care providers convey accurate and current information not only on the risks and benefits, but also discussion on alternatives to HRT. The role of the health care professional is vital as they serve as the conduit between the pharmaceutical industry and their patients.

In spite of the fact that a women's lifetime risk of developing heart disease is greater than that of breast cancer, studies have reported that women have a greater fear breast cancer than heart disease (42) and that they do not perceive the risk of heart disease as great (16). One out of two postmenopausal women will develop coronary artery disease, as compared to a one in eight lifetime chance of breast cancer (77). In the present study 45.9% of respondents reported cancer concerns as a reason for not using HRT (Table 17, Figure 3). Skafar, et al in a recent article noted that "cardiovascular disease is the leading cause of mortality in women, a fact that is underappreciated by women and their physicians" (233). Women and their physicians may not perceive heart disease as important a risk as breast cancer because cardiovascular disease usually occurs later in life after the decision regarding HRT has already been made. The majority of women contemplate HRT use when symptoms first appear, which is usually during the perimenopausal years when cardiovascular symptoms are not as likely to be present.

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However, this is the time when the threat of breast cancer increases, making women very aware of the potential of developing breast cancer. Although both heart disease and breast cancer take years to develop, when the diagnosis of breast cancer is made, treatment usually begins immediately. On the other hand, cardiovascular disease manifests itself in many ways, to different degrees, and with varied symptoms. A women can have heart disease without severe or identifiable symptoms. Diagnosis is often not made until there is a major cardiovascular event. The increased risk of breast cancer associated with HRT use appears to be related to the duration of estrogen use. Women who use estrogen for ≤ 5 years appear to have no increased risk, but for women who use estrogen 8-15 years the relative risk is 1.25-1.3 respectively (6). The most recent evidence from the *Nurses' Health Study* (107) has also shown an increased risk of breast cancer with 10 or more years of HRT use. This fact may be one of the reasons that physicians tend to prescribe HRT to women for short periods of time, agreeing with their patients that long term use could increase the risk of breast cancer.

When evaluating family medical history from all the subjects (Table 2), the NHRT had a combined family history of heart disease (heart disease and deaths from heart disease) of 66%, and the HRT group a 64% family risk. In contrast the NHRT had a combined family history of breast cancer (breast cancer and deaths from breast cancer) of 16.6%, and the HRT group a 8.8% family risk. Although the women in the current study reported a higher family risk for cardiovascular disease than breast cancer, cancer concerns were the primary reason for not using HRT among subjects. The results of the current study supports the past research that women are more aware of the risks of breast cancer than of heart disease related to the use of hormone replacement therapy (42). Table 17 and Figure

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3 present the reported reasons that the women in the present study chose not to use HRT. The data supports the original hypothesis as 43.9% of the women surveyed reported cancer as a major concern.

In the present study all but one subject in the EO group had experienced a hysterectomy. Historically, hysterectomized women have been excluded in HRT studies even though they represent a large percentage of postmenopausal women and are the most likely group to be using HRT (20), (7). One third of women in the United States will experience a hysterectomy by age 65 (234). It is estimated that 570,000 women a year in the United States undergo a hysterectomy and for approximately 50% of these women surgery includes a bilateral oophorectomy (234). Unlike women without hysterectomies, women who have experienced a hysterectomy have a high adherence to HRT over time (235). One of the factors that may contribute to the higher adherence and long-term use by hysterectomized women may be that they are able to take unopposed estrogen, without progestin, and therefore do not suffer from withdrawal bleeding. They also do not have the concern of developing endometrial cancer, which may increase adherence rates. It was determined that women who had experiences a hysterectomy should be included in the present research, however, the lack of sufficient numbers made it difficult to interpret the results from this group.

Impact of HRT on Psychological and Social Factors

The perception that menopause is a negative experienced is not supported by the literature (194), (203), (204), (208). In fact most women go through menopause with a minimum of stress and women are not more depressed during this time when compared to

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premenopausal counterparts (236), (204). Much of the negative perception of menopause has been created by the medical model which characterizes menopause as a "deficiency disease" treatable by HRT, as opposed to a natural transition in a woman's life. The impact of media and pharmaceutical public relations literature may be partly responsible for this perception. *The Healthy Women's Study* reported that women have more negative expectations prior to menopause than they actually experience (203), (204). Women who are depressed prior to menopause tend to be depressed during menopause also (210). It is important to take into consideration that women during mid-life are also likely to be experiencing other changes in addition to menopause (children leaving home, aging parents and partners, health concerns, career changes) which could also contribute to their psychological well-being during this time. It is difficult to separate these factors when evaluating women's reactions to and perceptions of menopause.

The data from the present study demonstrated that women were not depressed or overwhelmed with menopausal symptoms. The POMS Inventory (Tables 11a and 11b) measuring mood states demonstrated that use of HRT did not have an impact on any of the sub-scales or total mood score. The women in all three groups demonstrated positive mood states using this instrument. The same trend was found in self-esteem scores (Tables 12a and 12b) with the use of HRT having little effect on women's self-esteem as all three groups reporting high levels of self-esteem. Subjects in this study did not report negative symptoms due to menopause, as demonstrated in tables 13a and 13b. All of this data supports the past literature that has shown that for the majority of women, menopause is not a negative experience. The results also suggest that women who practice positive lifestyle interventions (exercise and health-supporting diets) demonstrate less severe

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menopausal symptoms, higher self-esteem, and more positive mood states regardless of HRT use.

The perception that menopause is associated with a decline in quality of life is not supported by the literature or by the present study.

Limitations of the Study.

1. It is important to acknowledge that the present study is observational and retrospective. The information collected was from postmenopausal women one year after they had made their choice regarding HRT. The data related to prior health and psychological factors is therefore subjective in nature and therefore difficult to evaluate which of these elements can be directly attributed to the choice of HRT.
2. Only one blood sample was evaluated in this study. Repeated measures would have provided more data and better evaluation of biological variables.
3. The trends seen in several variables under study might have been statistically significant with a larger subject pool.

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CHAPTER VI

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Heart disease remains the leading cause of death in women and accounts for one-third of all hospitalizations in women over the age of 55 (237). An impressive body of evidence supports the possibility that HRT offers protection against cardiovascular disease in women, although the mechanism/s have not been fully elucidated. It is important to remember that epidemiological studies, while suggesting a relationship, do not prove cause and effect. Closely controlled clinical trials and observational research protocols are needed before it can be determined if HRT decreases cardiovascular risk for the majority of postmenopausal women. Until that time, women and their health care providers need to collaborate to evaluate the risk-benefit equation for each women individually. Women with a strong personal or family history of heart disease and a low risk for breast cancer, may chose to use HRT, whereas a woman with a high breast cancer risk may not make the same choice. There also needs to be continued research investigating alternatives to HRT for women who cannot or chose not to use HRT.

A recent study from a cohort of the *Nurses' Health Study* (238) investigated the effect of folate intake from diet and supplements on the risk of CHD in 80,000 women with no cardiovascular risk factors. The women were followed for 14 years and a FFQ was used to determine dietary intake. Women who consumed 696μg of folate/day as compared to 158 μg/day had a RR of 0.67 (95% confidence interval (CI), 0.55-0.87]. Folate is a nutrient that is easily obtained from a variety of foods, is water soluble and therefore

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unlikely to be toxic, and has demonstrated a 67% reduction in cardiovascular disease in this study. Results of this study indicate that there may be alternatives to HRT that could provide decrease risk from chronic disease without the risks and expense.

The present study involving a free-living population of postmenopausal women offered a unique look at the menopausal experience. The women who participated in this study were very similar to the subjects in the previous observational studies and therefore appropriate for comparison. The use of the biopsychosocial model in this research allowed a broader perceptive from which to observe menopause, hormone replacement therapy, and the factors that determine a women's use of HRT.

The results suggests that women who practice positive lifestyle interventions will demonstrate low risk for CVD regardless of HRT use. There were no significant differences in either physiological outcomes nor in self-esteem, moods, and symptoms reporting in relation to HRT. The 70 women participating in the study practiced preventative health care, ate health-supporting diets, controlled their body composition, and exercised on a routine basis.

Several prospective, randomized placebo-controlled studies are presently in progress which may offer more answers to the questions that women and physicians have regarding hormone replacement therapy. The *Women's Health Initiative* will hopefully offer some insight within the next 5-10 years. Until this and other clinical trials offer more information, women will need to make the decision regarding HRT use on an individual basis, after weighing the risk and benefits of the options. In the future many women will have spend a large portion of their adult years on some type of exogenous hormones. The efficacy of this choice will be evaluated as each generation reaches menopause. In the

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meantime Susan Love suggests that "any women taking hormones has to realize that she's part of a large experiment" (87).

Future Research

The research presented in this dissertation investigated the biological, social, and psychological outcomes of the choice to use or not to use hormone replacement therapy in postmenopausal women who have already made their choice regarding HRT. A prospective study would allow evaluation of the biological, social, and psychological factors before and after making the choice to use or not to use HRT.

An ongoing study is presently underway that is following the subjects described in this dissertation. Subjects have donated a blood sample and biological assays are being repeated. Information from a second questionnaire will collect information on present health status, current HRT use, and reasons for any change in HRT status one to two years after the initial study.

A longitudinal study is also planned to follow the 301 women who completed the *UNWHS Questionnaire* through each phase of the menopausal transition.

More research is needed to determine different dose and methods of administration of exogenous hormones so the formulations can be more individualized and safer for women. More research is needed on alternatives to hormone replacement therapy, including the use of phytoestrogens, natural HRT, and lifestyle interventions.

The findings presented here also indicate a need to examine the mechanisms for the effects of the steroidal hormones of hormone replacement therapy on lipids and lipoproteins, clotting factors, antioxidants, carbohydrate metabolism, effects on the

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vascular system and the vascular wall. Additional studies are needed to evaluate the effect of both endogenous and exogenous hormones on the oxidizability of LDL and the role of that antioxidants play in this mechanism.

This research indicates that women need more information on aging, menopause, and the risks and benefits of hormone replacement therapy. Health care providers need to be educated and updated on these issues to enable them to work as collaborators with their patients to offer accurate and timely information.

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Table 1a. Subject Characteristics by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen & Progestin (E/P).

	NHRT (n=36)	EO (n=11)	E/P (n=23)
Age (years)	56.63 (± 0.79)	56.00 (± 1.41)	54.57 (± 0.79)
Height (inches)	64.51 (± 0.35)[a]	63.70 (± 0.62)[a]	66.00 (± 0.52)
Weight (pounds)	157.69 (± 5.27)	164.32 (± 9.83)	151.74 (± 7.13)
Body Mass Index	26.71 (± 0.86)	28.42 (± 1.66)[a]	24.50 (± 1.04)
Waist/Hip Ratio	0.779 (± 0.01)	0.786 (± 0.03)	0.753 (± 0.01)
# children	2.19 (± 0.23)	2.09 (± 0.37)	2.43 (±0.30)
# pregnancies	2.39 (± 0.25)	3.36 (± 0.62)	2.67 (± 0.40)
Past OC use (years)	3.94 (± 0.79)	4.00 (± 1.73)	5.86 (± 1.59)

Values represent least square mean ± SEM.
[a]Group significantly different from E/P group ($p<0.05$).

Table 1b. Subject Characteristics by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P).

	NHRT (n=36)	HRT (n=34)
Age (years)	56.63 (± 0.79)	55.03 (± 0.70)
Height (inches)	64.51 (± 0.35)	65.26(± 0.44)
Weight (pounds)	157.69 (± 5.27)	155.32 (± 5.78)
Body Mass Index	26.71 (± 0.86)	25.77 (± 0.93)
Waist/Hip Ratio	0.779 (± 0.01)	0.764 (± 0.01)
# children	2.19 (± 0.23)	2.31 (± 0.23)
# pregnancies	2.39 (± 0.25)	2.31 (± 0.34)
Past OC use (years)	3.94 (± 0.79)	5.44 (± 1.28)

Values represent least square mean ± SEM. There were no significant differences among groups.

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Table 2. Summary of reported personal and family health history

	NHRT (n= 36) (% of group)	EO (n=11) (% of group)	E/P (n=23) (% of group)
Personal History			
Breast Cancer	1 (2.8%)	0 (0%)	0 (0%)
Diabetes Mellitus (Type II)	0 (0%)	0 (0%)	1 (4.3%)
Fibrocystic Breast Disease	3 (8.3%)	2 (18.2%)	2 (8.7%)
Heart Disease	1 (2.8%)	1 (9.1%)	1 (4.3%)
Hyperlipidemia being treated with medication	4 (11.1%)	0 (0%)	1 (4.3%)
Hypertension	9 (25.0%)	2 (18.2%)	1 (4.3%)
Osteoporosis	1 (2.8%)	0 (0%)	2 (8.7%)
Other Cancers	1 (2.8%)	0 (0%)	0 (0%)
Family History			
Breast Cancer	3 (8.3%)	0 (0%)	1 (4.3%)
Breast Cancer/Death	3 (8.3%)	2 (18.2%)	0 (0%)
Heart Disease	18 (50%)	7 (63.6%)	6 (26.1%)
Heart Disease/Death	6 (16.7%)	5 (45.5%)	4 (17.4%)
Other Cancers	1 (2.8%)	0 (0%)	0 (0%)
Osteoporosis	3 (8.3%)	1 (9.1%)	2 (8.7%)

Table 3a. Serum and Plasma Values of Serum Lipids, Serum Estradiol and Progesterone, by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen and Progestin (E/P).

	NHRT (n=36)	EO (n=11)	E/P (n=23)
Serum triglyceride (mg/dL)	118.75 (± 15.07)	165.61 (± 34.55)	127.50 (± 12.40)
Serum HDL-cholesterol (mg/dL)	52.76 (± 2.26)	55.91 (± 3.75)	51.94 (± 2.62)
Serum LDL-cholesterol (mg/dL)	148.06 (± 5.26)	147.67 (± 9.09)	138.66 (± 7.72)
Serum total cholesterol (mg/dL)	224.59 (± 6.22)	235.32 (± 11.56)	212.48 (± 8.81)
Serum Lp (a) (mg/dl)	20.55 (± 2.86)	20.27 (± 5.43)	14.64 (± 3.50)
Total Cholesterol/HDL ratio	4.56 (± 0.25)	4.27 (± 0.27)	4.44 (± 0.32)
Estradiol (pg/ml)	16.93 (± 2.08)	21.85 (± 9.46)	17.87 (± 2.05)
Progesterone (ng/ml)	0.4778 (± 0.08)	0.3545 (± 0.05)	0.3152 (± 0.03)

Values represent least square mean ± SEM. There were no significant differences among the groups.

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Table 3b. Serum and Plasma Values of Serum Lipids, Serum Estradiol and Progesterone, by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P).

	NHRT (n=36)	HRT (n= 34)
Serum triglyceride (mg/dL)	118.75 (± 15.07)	139.83 (± 14.00)
Serum HDL-cholesterol (mg/dL)	52.76 (± 2.26)	53.18 (± 2.14)
Serum LDL-cholesterol (mg/dL)	148.06 (± 5.26)	141.48 (± 5.98)
Serum total cholesterol (mg/dL)	224.59 (± 6.22)	219.87 (± 7.18)
Serum Lp (a) (mg/dl)	20.55 (± 2.86)	16.46 (± 2.94)
Total Cholesterol/HDL ratio	4.56 (± 0.25)	4.39 (± 0.23)
Estradiol (pg/ml)	16.93 (± 2.08)	19.16 (± 3.28)
Progesterone (ng/ml)	0.4778 (± 0.08)[a]	0.3245 (± 0.02)

Values represent least square mean ± SEM.

[a] Groups significantly different ($p<0.01$).

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Table 4a. Serum Antioxidants, LDL Antioxidants, ORAC and LDL Oxidation by HRT Use. Comparison of groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen and Progestin (E/P).

	NHRT (n=36)	EO (n=11)	E/P (n=23)
Serum α-tocopherol (μmol/l)	34.80 (± 1.77)	38.19 (± 5.11)	38.68 (± 3.16)
Serum β-carotene (μmol/l)	0.631 (± 0.07)	0.945 (± 0.43)	0.777 (± 0.16)
Serum retinol (μmol/l)	2.085 (± 0.09)	2.471 (± 0.22)	2.362 (± 0.10)
Serum lutein (μmol/l)	0.3008 (± 0.03)	0.3172 (± 0.05)	0.2837 (± 0.02)
LDL α-tocopherol (mol/mol LDL)	8.556 (± 1.30)	7.070 (± 0.71)	8.500 (± 0.62)
LDL β-carotene (mol/mol LDL)	0.315 (± 0.04)	0.378 (± 0.14)	0.411 (± 0.10)
α-tocopherol/LDL ratio	9.24 (± 0.45)	9.74 (± 1.25)	11.05 (± 0.85)
β-carotene/LDL ratio	0.17 (±0.02)	0.24 (± 0.10)	0.27 (± 0.08)
ORAC (μmol Trolox equiv/l)	4300.70 (± 201.70)	3792.79 (± 181.63)	3758.59 (± 188.40)
ORAC (nmol Trolox equiv/mg protein)	45.94 (± 2.29)	39.01 (± 5.86)	41.44 (± 10.93)
LDL oxidation			
lag phase(min)	38.39 (± 2.97)	39.11 (± 8.67)	41.83 (± 4.50)
rate of diene formation (ng/min/mg LDL protein)	12.94 (± 0.33)[a]	12.32 (± 0.64)	11.05 (± 0.31)
maximum diene formation (ng/mg LDL protein)	431.22 (± 5.70)	409.11 (± 12.30)	419.09 (± 8.32)

Values represent least square mean ± SEM.

[a] Group significantly different from E/P group ($p<0.001$)

Table 4b. Serum Antioxidants, LDL Antioxidants, ORAC, and LDL Oxidation by HRT Use. Serum and Plasma Values of Serum Lipids, Serum Estradiol and Progesterone, by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P).

	NHRT (n=36)	HRT (n= 34)
Serum α-tocopherol (μmol/l)	34.80 (± 1.77)	38.52 (± 2.7)
Serum β-carotene (μmol/l)	0.631 (± 0.07)	0.831 (± 0.27)
Serum retinol (μmol/l)	2.085 (± 0.09)[a]	2.398 (± 0.10)
Serum lutein (μmol/l)	0.3008 (± 0.03)	0.2938 (± 0.18)
LDL α-tocopherol (mol/mol LDL)	8.556 (± 1.30)	8.037 (± 0.49)
LDL β-carotene (mol/mol LDL)	0.315 (± 0.04)	0.400 (± 0.81)
α-tocopherol/LDL ratio	9.24 (± 0.45)	10.64 (± 0.70)
β-carotene/LDL ratio	0.17 (±0.02)	0.259 (0.06)
ORAC (μmol Trolox equiv/l)	4300.70 (± 201.70)[a]	3769.65 (±138.73)
ORAC (nmol Trolox equiv/mg protein)	45.94 (± 2.29)	40.66 (± 1.64)
LDL oxidation		
lag phase(min)	38.39 (± 2.97)	41.06 (± 3.98)
rate of diene formation (ng/min/mg LDL protein)	12.94 (± 0.33)[b]	11.41 (± 0.30)
maximum diene formation (ng/mg LDL protein)	431.22 (± 5.70)	416.28 (± 6.85)

Values represent least square mean ± SEM.

[a] Groups significantly different ($p<0.05$).

[b] Groups significantly different ($p<0.01$).

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Table 5a. Lifestyle Factors by HRT Use. Comparison of All Groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen & Progestin (E/P).

	NHRT (n=33)	EO (n= 11)	E/P (n= 22)
Alcohol (drinks/mo)	12.27 (± 2.77)	7.64 (± 3.70)	9.64 (± 2.05)
Exercise (years)	6.80 (± 1.8)	9.70 (± 4.0)	3.35 (± 1.0)
Exercise (min/wk)	129.69 (± 18.8)	103.33 (± 29.2)	90.48 (± 17.9)
Exercise (times/wk)	3.36 (± 0.4)	3.18 (± 0.8)	2.27 (± 0.4)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 5b. Lifestyle Factors by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n=33)	HRT (n= 33)
Alcohol (drinks/mo)	12.27 (± 2.77)	8.97 (± 2.1)
Exercise (years)	6.80 (± 1.8)	5.47 (± 1.12)
Exercise (min/wk)	129.69 (± 18.8)	94.33 (± 15.06)
Exercise (times/wk)	3.36 (± 0.4)	2.58 (± 0.4)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 6a. Dietary Intake of Selected Nutrients from Three-day Diet Record by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen and Progestin (E/P).

	NHRT (n= 28)	EO (n= 9)	E/P (n= 17)
Calories (kcals)	1790.46 (± 59.46)	1712.56 (± 136.4)	1765.65 (± 71.8)
Fat (g)	51.69 (± 4.3)	48.68 (± 6.8)	52.76 (± 4.2)
Fat (% of kcals)	24.79 (± 1.6)	24.03 (± 2.2)	25.59 (± 1.5)
Cholesterol (mg)	190.40 (± 19.2)	150.80 (± 29.8)	179.70 (± 28.4)
Saturated Fat (g)	16.50 (± 1.8)	15.11 (± 2.5)	15.36 (± 1.7)
Vitamin E (mg)	15.00 (± 2.2)	13.97 (± 3.5)	11.86 (± 1.3)
α-tocopherol (mg)	7.96 (± 1.5)	8.63 (± 3.0)	6.83 (± 0.9)
Vitamin A (RE)	1513.72 (± 201.5)	1521.58 (± 206.1)	1443.63 (± 320.2)
β-carotene (RE)	3432.88 (± 779.2)	1215.90 (± 267.7)	2778.21 (± 910.9)
Calcium (mg)	869.39 (± 67.7)	924.87 (± 65.1)	803.1 (± 87.0)
Iron (mg)	16.73 (± 2.6)	13.52 (± 1.2)	12.77 (± 0.9)
Dietary fiber (g)	19.44 (± 1.4)	18.30 (± 2.6)	22.96 (± 3.1)

Values represent least square mean ± SEM. There were no significant differences among groups.

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Table 6b. Dietary Intake of Selected Nutrients from Three-day Diet Record by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n= 28)	HRT (n=26)
Calories (kcals)	1790.46 (± 59.46)	1747.27 (± 65.2)
Fat (g)	51.69 (± 4.3)	51.35 (± 3.6)
Fat (% of kcals)	24.79 (± 1.6)	25.05 (± 1.2)
Cholesterol (mg)	190.40 (± 19.2)	169.69 (± 21.1)
Saturated Fat (g)	16.50 (± 1.8)	15.27 (± 1.4)
Vitamin E (mg)	15.00 (± 2.2)	12.59 (± 1.5)
α-tocopherol (mg)	7.96 (± 1.5)	7.46 (± 1.2)
Vitamin A (RE)	1513.72 (± 201.5)	1470.61 (± 218.3)
β-carotene (RE)	3432.88 (± 779.2)	2237.41 (± 614.2)
Calcium (mg)	869.39 (± 67.7)	845.24 (± 61.4)
Iron (mg)	16.73 (± 2.6)	13.03 (± 0.7)
Dietary fiber (g)	19.44 (± 1.4)	21.34 (± 2.2)

Values represent least square mean ± SEM. There were no significant differences among groups.

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Table 7a. Dietary Intake of Selected Nutrients from Food Frequency Questionnaire by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen and Progestin (E/P).

	NHRT (n= 30)	EO (n= 10)	E/P (n=17)
Calories (kcals)	1327.75 (± 81.2)	1403.20 (± 170.7)	1449.06 (± 123.1)
Fat (g)	45.86 (± 3.8)	48.88 (± 9.6)	51.86 (± 5.6)
Fat (% of kcals)	33.02 (± 1.6)	30.50 (± 2.4)	32.61 (± 2.3)
Cholesterol (mg)	166.89 (± 14.2)	177.89 (± 33.3)	205.70 (± 43.3)
Saturated Fat (g)	14.77 (± 1.4)	15.71 (± 3.6)	17.29 (± 2.1)
Vitamin E (∝-tocopherol equivalents)	9.93 (± 1.7)	9.78 (± 2.5)	8.21 (± 0.9)
Vitamin A (RE)	1191.81 (± 110.84)	1297.57 (± 166.99)	1270.59 (± 189.53)
Retinol (MCG)	602.51 (± 59.5)	713.99 (± 100.9)	623.01 (± 77.9)
β-carotene (MCG)	3111.35 (± 385.9)	3025.96 (± 773.3)	3491.32 (± 834.3)
Lutein (MCG)	3494.35 (± 636.8)	2973.09 (± 1031.3)	2611.14 (± 540.2)
Calcium (mg)	826.5267 (± 86.6)	971.79 (± 112.0)	860.24 (± 105.2)
Iron (mg)	11.45 (± 1.1)	12.29 (± 1.7)	12.32 (± 1.1)
Dietary fiber (g)	12.32 (± 1.1)	12.18 (± 1.2)	13.02 (± 1.4)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 7b. Dietary Intake of Selected Nutrients from Food Frequency Questionnaire by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n= 30)	HRT (n= 27)
Calories (kcals)	1327.75 (± 81.2)	1432.07 (± 98.1)
Fat (g)	45.86 (± 3.8)	50.76 (± 5.0)
Fat (% of kcals)	33.02 (± 1.6)	32.16 (± 1.7)
Cholesterol (mg)	166.89 (± 14.2)	195.40 (± 29.6)
Saturated Fat (g)	14.77 (± 1.4)	16.70 (± 1.9)
Vitamin E (∝-tocopherol equivalents)	9.93 (± 1.7)	8.79 (± 1.1)
Vitamin A (RE)	1191.81 (± 110.84)	1280.59 (± 132.29)
Retinol (MCG)	602.51 (± 59.5)	656.71 (± 61.1)
β-carotene (MCG)	3111.35 (± 385.9)	3318.97 (± 590.2)
Lutein (MCG)	3494.35 (± 636.8)	2745.19 (± 500.6)
Calcium (mg)	826.5267 (± 86.6)	901.56 (± 77.5)
Iron (mg)	11.45 (± 1.1)	12.31 (± 0.9)
Dietary fiber (g)	12.32 (± 1.1)	12.71 (± 1.0)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 8a. Vitamin E Intake with Diet and Supplements by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen and Progestin (E/P).

	NHRT (n=31)	EO (n= 10)	E/P (n= 21)
Vitamin E from Diet (mg)	15.00 (± 2.2)	13.97 (± 3.5)	11.86 (± 1.3)
Vitamin E:Supplements (mg)	157.26 (± 48.5)	54.44 (± 30.7)	224.12 (± 81.1)
Total Vitamin E (mg)	158.49 (± 44.1)	64.57 (± 28.0)	234.80 (± 68.5)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 8b. Vitamin E Intake with Diet and Supplements by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n=31)	HRT (n= 31)
Vitamin E from Diet (mg)	15.00 (± 2.2)	12.59 (± 1.5)
Vitamin E from Supplements (mg)	157.26 (± 48.5)	165.38 (± 55.9)
Total Vitamin E (mg)	158.49 (± 44.1)	179.91 (± 49.1)

Values represent least square mean ± SEM. There were no significant differences among groups.

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Table 9a. β-Carotene Intake with Diet and Supplements by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen and Progestin (E/P).

	NHRT (n=28)	EO (n=8)	E/P (n=17)
Dietary β-Carotene Diet (RE)	3432.88 (± 779.18)	1079.39 (± 262.16)	2778.21 (± 910.94)
β-Carotene:Supplements(RE)	692.31 (± 192.31)	1000.00 (± 500.00)	909.09 (± 250.63)
Total β-Carotene (RE)	4088.02 (± 1188.05)	2009.90 (± 492.90)	3822.57 (± 1716.55)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 9b. β-Carotene Intake with Diet and Supplements by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n=28)	HRT (n=25)
β-Carotene from Diet (RE)	3432.88 (± 779.18)	2237.41 (± 614.23)
β-Carotene from Supplements (RE)	692.31 (± 192.31)	928.57 (± 215.20)
Total β-Carotene (RE)	4088.02 (± 1188.05)	3419.76 (±1340.36)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 10a. Dine Score of Three-Day Food Intake by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen and Progestin (E/P).

	NHRT (n=28)	EO (n= 9)	E/P (n= 17)
Dine Score	6.21 (± 0.31)	6.75 (± 0.38)	5.62 (± 0.35)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 10b. Dine Score of Three-Day Food Intake by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n=28)	HRT (n= 26)
Dine Score	6.21 (± 0.31)	6.01 (± 0.28)

Values represent least square mean ± SEM. There were no significant differences among groups.

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Table 11a. Mean Scores of POMS Inventory Sub-scales by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen & Progestin

	Range of Scores	NHRT (n= 31)	EO (n= 10)	E/P (n=21)
Tension-Anxiety (T)	0-36	6.97 (± 0.8)	8.90 (± 1.6)	6.96 (± 1.0)
Depression-Dejection (D)	0-60	5.20 (± 1.8)	9.20 (± 2.8)	4.16 (± 1.1)
Anger-Hostility (A)	0-48	4.81 (± 1.0)	5.81 (± 1.7)	4.65 (± 1.2)
Fatigue (F)	0-28	7.63 (± 0.9)	7.30 (± 2.7)	5.24 (± 1.2)
Confusion-Bewilderment (C)	0-28	4.66 (± 0.7)	6.18 (± 1.6)	4.25 (± 0.8)
Vigor (V)	0-32	18.23 (± 1.1)	17.10 (± 1.7)	18.00 (± 1.7)
Total Mood Score (T+D+A+F+C)- (V)	0-168	11.13 (± 5.0)	23.22 (± 9.1)	6.11 (± 6.3)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 11b. Mean Scores of POMS Inventory Sub-scales by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n= 31)	HRT (n=31)
Tension-Anxiety (T)	6.97 (± 0.8)	7.45 (± 4.7)
Depression-Dejection (D)	5.20 (± 1.8)	5.90 (± 1.3)
Anger-Hostility (A)	4.81 (± 1.0)	5.06 (± 1.0)
Fatigue (F)	7.63 (± 0.9)	5.90 (± 1.2)
Confusion-Bewilderment (C)	4.66 (± 0.7)	4.94 (± 0.7)
Vigor (V)	18.23 (± 1.1)	17.69 (± 1.3)
Total Mood Score (T+D+A+F+C)- (V)	11.13 (± 5.0)	11.81 (± 5.3)

Values represent least square mean ± SEM. There were no significant among groups.

Table 12a. Mean Scores for Self-esteem Scale by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT),Estrogen Only (EO), and Estrogen & Progestin (E/P).

	NHRT (n= 33)	EO (n= 11)	E/P (n= 21)
Self-esteem	8.62 (± 0.2)	8.07 (± 0.4)	8.56 (± 0.2)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 12b. Mean Scores for Self-esteem Scale by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT: Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n= 33)	HRT (n= 32)
Self-esteem	8.62 (± 0.2)	8.39 (± 0.2)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 13a. Mean Scores for Experiences (Symptoms) Scale by HRT Use. Comparison of all groups: No Hormone Replacement (NHRT), Estrogen Only (EO), and Estrogen & Progestin (E/P).

	NHRT (n= 16)	EO (n= 5)	E/P (n= 16)
Experiences	50.38 (± 2.8)	52.20 (± 4.8)	48.13 (± 2.0)

Values represent least square mean ± SEM. There were no significant differences among groups.

Table 13b. Mean Scores for Experiences (Symptoms) Scale by HRT Use. Comparison of No Hormone Replacement Therapy (NHRT) Group to Hormone Replacement Therapy Group (HRT): Estrogen Only (EO) and Estrogen and Progestin (E/P) Groups.

	NHRT (n= 16)	HRT (n= 21)
Experiences	50.38 (± 2.8)	49.01 (1.8)

Values represent least square mean ± SEM. There were no significant differences among groups.

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Table 14. Factors Influencing Decision to Use HRT.

	Not influential at all (%)	*Somewhat influential (%)*	*Moderately influential (%)*	*Extremely influential (%)*
Health Concerns (M: 2.5)				
Hysterectomy (n=22)	72.7	13.6	0	13.6
Relief of Symptoms (n=29)	20.7	6.9	31.0	41.4
Concern about Heart Disease (n=27)	22.2	0	44.4	33.3
Concern about Osteoporosis (n= 31)	6.5	16.1	25.8	51.6
Advice of Others (M:1.8)				
Advice of MD/Health Professional (n=29)	10.3	6.9	34.5	48.3
Advice friend/co-worker/family (n=24)	83.3	8.3	8.3	0
Advice of partner (n=24)	95.8	0	4.2	0
Psychological-Social Factors (M:1.6)				
Mood changes (n=26)	69.2	3.8	7.7	19.2
Concern about aging (n=26)	42.3	23.1	23.1	11.5
Sexual problems (n=24)	79.2	16.7	4.2	0

Table 15. Sources of Information on Menopause

	Least informative (%)	*Somewhat informative (%)*	*Moderately informative (%)*	*Extremely informative (%)*
MD/Health care professional (n=61)	9.8	19.7	45.9	24.6
Mother (n=48)	77.1	18.8	4.2	0
Radio/television (n=51)	33.3	33.3	21.6	11.8
Workshops or Conferences (n=50)	50.0	10.0	20.0	20.0
Books/Pamphlets (n=61)	13.1	16.4	27.9	42.6
Partner (n=27)	95.3	4.7	0	0
Friend/relative (n=48)	41.7	31.3	20.8	6.3

Table 16. Alternate Therapies Used to Treat Menopausal Symptoms.

	Percentage Reporting Use (n=66)
Diet (n=1)	1.6
Exercise (n=3)	4.7
Massage (n=1)	1.6
Meditation (n=4)	6.3
Natural HRT (n=1)	1.6
Relaxation techniques (n=1)	1.6
Vaginal Creams (n=1)	1.6
Vitamins/Minerals (n=6)	9.4

Table 17. Reported Reasons For Not Using HRT.

	%
Fibrocystic breast disease (n= 25)	4.0
Do not like to take medication (n= 25)	16.0
No risk factors/ no need to use HRT (n= 25)	20.0
MD or health professional discourage use of HRT (n =25)	8.0
Do not want to have menstrual cycle (n= 25)	4.0
Too costly (n= 25)	4.0
Cannot remember to take medications (n= 26)	7.7
Breast cancer (n= 26)	3.8
Family history of breast cancer (n= 25)	11.5
Family history of cancer other than breast cancer (n= 25)	4.0
Fear of breast cancer (n= 26)	3.8
Concern about uterine or ovarian cancer (n= 27)	7.4
Concern over cancer in general (n=26)	15.4
Total of cancer related concerns	*45.9*

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Table 18. Correlations between variables in study

		r
Serum Lipoproteins		
Serum HDL	number alcoholic drinks/month	.267*-
Serum HDL	Body Mass Index (BMI)	-.308*
Serum HDL	Waist-Hip Ratio	-.342**
Serum HDL	ORAC	-.248*
Serum HDL	Serum Lutein	.248*
Serum HDL	Body Weight	-.309*
Serum LDL	α-tocopherol/LDL ratio	-.269*
Serum LDL	LDL β-carotene (mol/mol) LDL	-.241*
Serum LDL	Body Mass Index (BMI)	.265*
Serum LDL	Body Weight	.262*
Serum LDL	Serum retinol	.362**
Serum triglycerides	Body Mass Index (BMI)	.354**
Serum triglycerides	Serum retinol	.325*
Serum triglycerides	Waist-Hip Ratio	-.315**
Serum triglycerides	Maximum diene formation	-.303*
Serum triglycerides	Rate diene formation	-.277*
Serum triglycerides	LDL β-carotene (mol/mol) LDL	-.261*
Serum triglycerides	Body Weight	.294*
Antioxidants		
Serum α-tocopherol	Vitamin E supplements	.270*
Serum α-tocopherol	Total vitamin E intake (diet & supplements)	.289*
Serum α-tocopherol	Rate of diene formation	-.366**
Serum α-tocopherol	α-tocopherol/LDL ratio	.777**
Serum α-tocopherol	LDL α-tocopherol (mol/mol LDL)	.299*
Serum α-tocopherol	Serum triglycerides	.381**
Serum α-tocopherol	Total Cholesterol/HDL Ratio	.288*
Serum β-carotene	LDL β-carotene (mol/mol) LDL	.923**
Serum β-carotene	β-carotene/LDL ratio	.884**
Serum β-carotene	β-carotene from supplements	.603**
Serum β-carotene	α-tocopherol/LDL ratio	.358**
Serum β-carotene	Body weight	-.327**
Serum β-carotene	Body Mass Index	-.273*
LDL α-tocopherol(mol/mol) LDL	ORAC	-.250*
LDL α-tocopherol(mol/mol) LDL	α-tocopherol/LDL ratio	.260*
LDL α-tocopherol(mol/mol) LDL	Dietary vitamin E	.443*
LDL β-carotene (mol/mol) LDL	Total cholesterol/HDL Ratio	-.288*
LDL β-carotene (mol/mol) LDL	Total β-carotene (diet + supplements)	.541**
LDL β-carotene (mol/mol) LDL	β-carotene from supplements	.408**
LDL β-carotene (mol/mol) LDL	Body Weight	-.391**
LDL β-carotene (mol/mol) LDL	Body Mass Index (BMI)	-.363**
LDL β-carotene (mol/mol) LDL	Waist-Hip Ratio	-.256*

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LDL β-carotene (mol/mol)LDL	β-carotene/LDL ratio	.967**
LDL β-carotene (mol/mol LDL)	Dietary β-carotene	.307*
LDL β-carotene (mol/mol) LDL	Times exercise/week	.292*
α-tocopherol/LDL ratio	Rate diene formation	-.544**
α-tocopherol/LDL ratio	ORAC	-.279*
α-tocopherol/LDL ratio	Vitamin E from supplements	.443**
α-tocopherol/LDL ratio	Total vitamin E (diet & supplements)	.420**
β-carotene/LDL ratio	Times exercise per week	.291*
β-carotene/LDL ratio	Dietary β-carotene	.316*
β-carotene/LDL ratio	Total β-carotene (diet & supplements)	.494**
β-carotene/LDL ratio	β-carotene intake from supplements	.351**
β-carotene/LDL ratio	Body Weight	.363**
β-carotene/LDL ratio	Serum LDL	-.300*
β-carotene/LDL ratio	Total cholesterol/HDL Ratio	-.301*
β-carotene/LDL ratio	Times exercise per week	.291*
Body Mass Index (BMI)	Total Cholesterol/HDL Ratio	.391**
Body Mass Index (BMI)	β-carotene/LDL Ratio	-.328**
Waist -Hip Ratio	Total cholesterol/HDL Ratio	.377**
Rate diene formation	ORAC	.314**
Rate diene formation	Total vitamin E intake (diet +supplements)	-.264*
Rate diene formation	Maximum diene formation	.593**
Lifestyle Factors		
Number drinks per month	Total cholesterol/HDL ratio	-.264*
Number drinks per month	minutes exercise/week	.270*
Number drinks per month	Lp(a)	-.255*
Body Weight	Total cholesterol/HDL ratio	.373**
Food Frequency/Food Diary		
Food Diary: Total Calories	FFQ: Total Calories	.424**
Food Diary: Fat (grams)	FFQ: Fat (grams)	.414**
Food Diary: Saturated Fat	FFQ: Saturated Fat (grams)	.454**
Food Diary: % calories from fat	FFQ: % calories from fat	.637**
Food Diary: Calcium (mg)	FFQ: Calcium (mg)	.579**
Food Diary: Iron (mg)	FFQ: Iron (mg)	.414**
Food Diary: Fiber (grams)	FFQ: Fiber (grams)	.327**
Food Diary: Vitamin E	FFQ: Vitamin E	.588**

* $p = < 0.05$ (2-tailed)
** $p = < 0.01$ (2-tailed)

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Figure 1. Factors influencing decision to use HRT

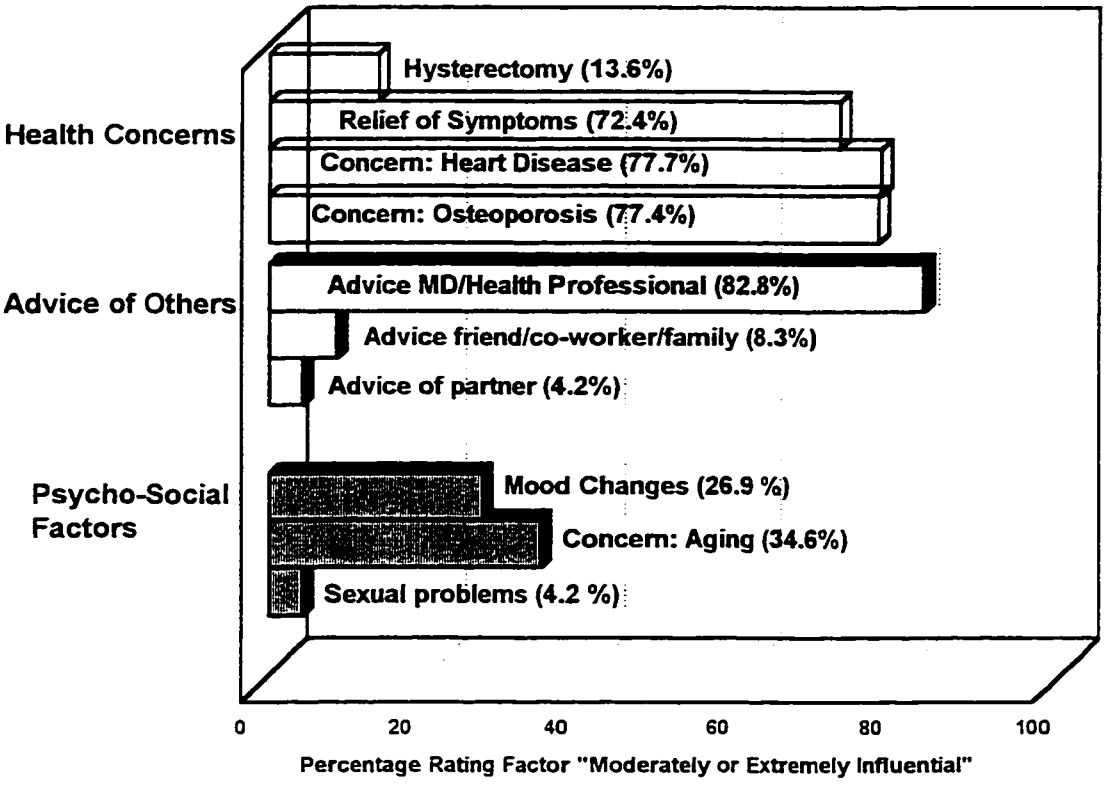

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Figure 2. Sources of information on menopause

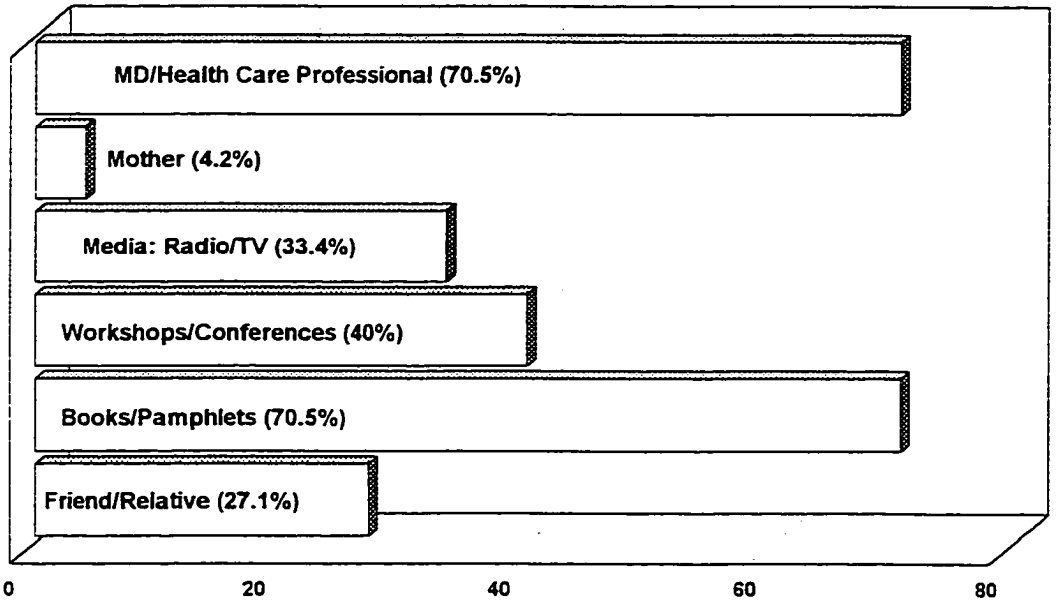

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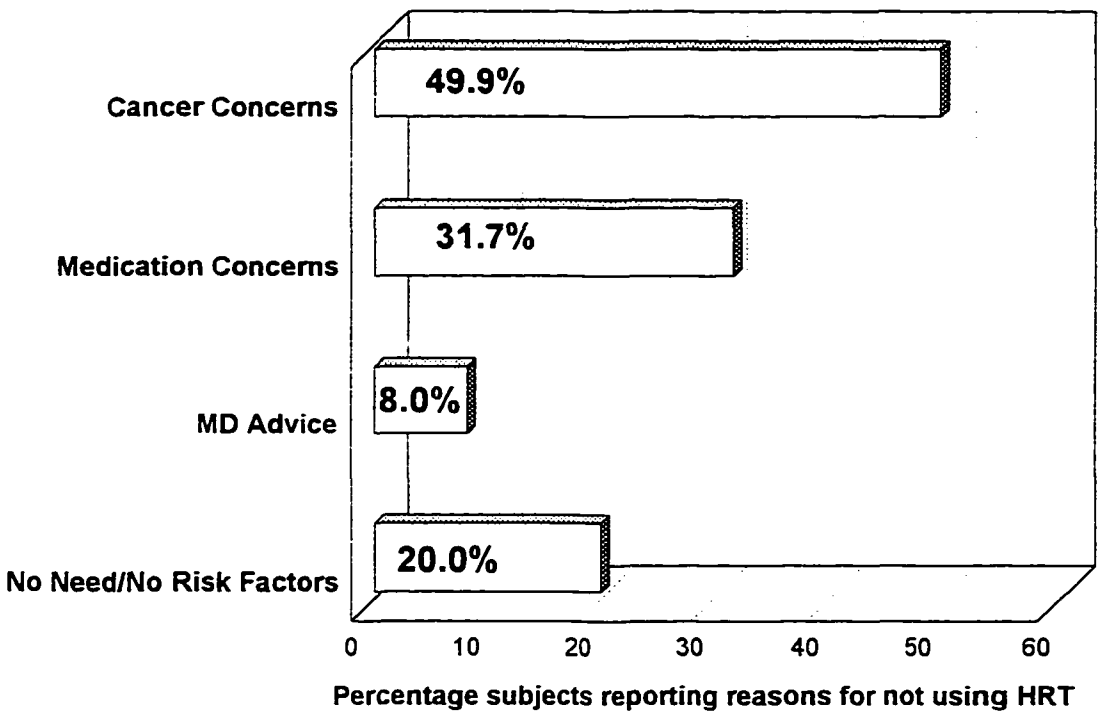
Figure 3. Reported reasons for not using HRT



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APPENDIX A

JURY OF EXPERTS

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JURY OF EXPERTS

Nancy Brown
The Clinic
P.O. Box 797
Dover, New Hampshire 03820

Ellen S. Cohn, Ph.D.
Professor of Psychology
University of New Hampshire
Durham, New Hampshire 03824

Susan Frankel
215 Pond Hill Road
Barrington, New Hampshire 03867

Valerie A. Long, M.O.E., R.D.
Extension Specialist
University of New Hampshire Cooperative Extension
Durham, New Hampshire 03824

Pamela M. Quinlan, M.S.
Department of Animal and Nutritional Sciences
University of New Hampshire
Durham, New Hampshire 03824

Elizabeth C. Smith, Ph.D.
Department of Animal and Nutritional Sciences
University of New Hampshire
Durham, New Hampshire 03824

Mary W. Temke, Ph.D.
Extension Educator and Extension Specialist
University of New Hampshire
Durham, New Hampshire 03824

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APPENDIX B

MATERIALS

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MATERIALS

Items:	Purchased from:
food models	Nasco, Fort Atkinson, Wisconsin
Nutritionist IV™ Diet Analysis for Windows (version 4.1) software	First Data Bank, San Bruno, CA.
SPSS® (Statistical Package for the Social Sciences): Graduate Pack™ (Advanced Class Version 7.0 for Windows) software	SPSS, Inc. 444 North Michigan Avenue Chicago, Illinois 60611
serum separation	Fisher Scientific, Pittsburgh, PA.
Plasma separation vacutainer tubes	Fisher Scientific, Pittsburgh, PA.
Nalgene cryovials	Fisher Scientific, Pittsburgh, PA.
breakfast for subjects	Bagelry, Durham, NH Market Basket, Lee, NH
Optiseal tubes (#362185)	Beckman Instruments, Inc., Fullerton, CA.
Acrodisc filters	Fisher Scientific, Pittsburgh, PA.
Folin-Ciocalteau reagent	Sigma Chemical Company, St. Louis, MO[a]
HPLC-grade hexane	VWR Scientific, Boston, MA.
HPLC- grade methonol	VWR Scientific, Boston, MA.
Methylene chloride	VWR Scientific, Boston, MA.
HPLC- grade ammonium acetate	Fisher Scientific, Pittsburgh, PA.
Borosilicate amber glass vials	Scientific Specialties Service Inc., Randallstown, MD.
ultrapure gas, N_2	Northeast Airgas, Manchester, NH
pipetman adustable pipettes	Rainin Instrument Co., Woburn. MA.
pipet tips	Fisher Scientific, Pittsburgh, PA.
petroleum ether	Fisher Scientific, Pittsburgh, PA.
Macra™ Lp(a) Enzyme Immunoassay Kit	Strategic Diagnostics, Newark, Delaware
Milenia™ Progesterone EIA Kit	Diagnostic Products Corporation, Los Angeles, CA.
Estradiol ELISA Test Kit	Neogen Corporation, ELISA Technologies®Division, Lexington, KY.
Triglyceride (INT) Enzymatic Kit (Catalog # 336-10)	Sigma Diagnostics, St. Louis, MO.
Cholesterol Enzymatic Kit (Catalog # 352-20)	Sigma Diagnostics, St. Louis, MO.
HDL Enzymatic Kit (Catalog # 352-3)	Sigma Diagnostics, St. Louis, MO.

[a] All other necessary supplies and equipment for the LDL isolation, and the LDL protein and oxidation measurements, were made available in the laboratory of Dr. Robert Nicolosi at the University of Massachusetts, Lowell, MA.

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APPENDIX C

INSTRUMENT

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UNIVERSITY OF NEW HAMPSHIRE

Department of Animal and Nutritional Sciences
College of Life Sciences and Agriculture
Kendall Hall
Durham, New Hampshire 03824-3590

UNH WOMEN'S HEALTH STUDY

Women's health issues have taken the spotlight in the 1990's. More research is being done in the areas of heart disease, osteoporosis, and cancer in order to improve the quality of life for women. Last summer 367 women participated in the *UNH Women's Health Study* here at the University of New Hampshire. We would like to expand this study population by inviting the Plymouth State College community to join us in our efforts.

You, or someone you know, are invited to participate in our ongoing research study on women's health issues. The study covers areas such as physical, emotional, and social issues for women in society today. This current study will consist of three parts.

The *first* part of the study is a comprehensive questionnaire, for women age 35 and over, covering many aspects of women's health and will take approximately 20-30 minutes to complete. The individual responses to the questionnaire will remain confidential.

The *second* part of the study will involve an observational study investigating the effects of hormones on the risk factors associated with heart disease in postmenopausal women. The study will involve a diet history questionnaire and a blood test to analyze serum lipids, antioxidant nutrients, and coagulation factors as they relate to physiological and nutritional well being. Women who are nonsmokers, between the ages of 50-65, and interested in learning more about this part of the study, may indicate so by checking the appropriate box on the attached sheet and they will be contacted by phone to determine eligibility. All information gathered will remain confidential and participants will receive results of their blood cholesterol, lipid profile, blood type and dietary analysis for their participation.

The *third* part of the study involves an interview on relationship and sexual issues. Women 35 and over who are interested in participating, may indicate so by checking the appropriate box on the attached sheet and they will be contacted with further information.

If interested in any or all of the components of this phase of the Women's Health Study, please check the appropriate box(s) on the attached sheet. If you are ineligible because of age or gender please pass this invitation on to someone else who may be interested in participating in the study. Feel free to address you questions to any of the undersigned.

Sincerely yours,

Kristine M. Baber, Ph.D. (862-2151)

Karol Lacroix, Ph.D. (862-3007)

Kathryn Cataneo, M.B.A. (862-2001)

Ruth A. Reilly, M.O.E., R.D. (862-2573)

Joanne Curran-Celentano, Ph.D. (862-2573)

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UNH WOMEN'S HEALTH STUDY

Please indicate by checking the appropriate box/s your interest in participating in any or all parts of the study and return in the enclosed envelope.

I (sign your name) _____ am interested in participating in the following parts of the study:

- ☐ **Part I: HEALTH QUESTIONNIARE (Age 35 and over)**
- ☐ **Part II: MENOPAUSE STUDY (Age 50-65)**
- ☐ **Part III: RELATIONSHIP & SEXUALITY INTERVIEW (Age 35 and over)**

Name _____

Home Address _____

_____

Home Phone _____

Work Address _____

_____

Work Phone _____

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UNIVERSITY OF NEW HAMPSHIRE

Department of Animal and Nutritional Sciences
College of Life Sciences and Agriculture
Kendall Hall
Durham, New Hampshire 03824-3590

UNH WOMEN'S HEALTH STUDY

Dear Participant,

Thank you for agreeing to participate in the Women's Health Study at the University of New Hampshire. The study covers physical, emotional, and social issues for women in society today.

Please do not include your name or any other personal identifiers on the questionnaire to help ensure the confidentiality of your responses. The code number on the questionnaire will be used only if you participate in other parts of the study. If there are any questions you feel uncomfortable responding to, simply skip those items and continue on with the remainder of the questionnaire.

After you have completed filling out the questionnaire, please place it in the large self-addressed envelope provided and mail. If you would like to receive a summary of the results of the study when available, please complete the enclosed self-addressed postcard and mail (please do not mail the postcard with the questionnaire).

Please feel free to address your questions to any of the undersigned.

Sincerely yours,

Kristine M Baber
Kristine Baber, Ph.D. (862-2151)

Kathryn Cataneo
Kathryn Cataneo, M.B.A. (862-2001)

Joanne Curran-Celentano
Joanne Curran-Celentano, Ph.D. (862-2573)

Karol Lacroix
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Ruth A Reilly
Ruth A. Reilly, R.D. (862-2573)

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Physiological Effects of Menopause

Objectives:

The objectives of this study is to determine if there is a relationship between hormone status, diet, and exercise and the risk of heart disease and stroke. We will evaluate this by measuring the levels of lipids, clotting proteins, hormones and vitamins in the blood of women who chose to use hormone replacement therapy and those who do not.

Protocol:

1. You will be notified if qualified for this phase of the study.
2. An appointment will be scheduled for you to have a fasting blood sample drawn. A trained phlebotomist will perform the blood draw from your arm (anticubetal vein) and approximately 20 ml (2/3 oz.) will be taken.
3. You will be given juice and a snack and asked to remain seated for about 15 minutes to assure that you are comfortable following the blood draw.
4. Your height, weight and waist/hip ratio will be recorded.
5. You will be asked to complete a three day food diary and a health habits-history questionnaire.

Risks Associated with the Study:

There are very few risks associated with the blood draw procedure when taken by a trained phlebotomist. Occasionally, a hematoma (black and blue mark) will result if the phelbotomist has difficulty getting the specimen or if pressure is not properly applied following the process. If the subject is anxious on rare occassion she may feel light headed or faint. In the event that this happens, please notify the plebotomist, place your head between your knees and smelling salt will be administered.

INFORMED CONSENT

PLEASE READ THE FOLLOWING STATEMENTS AND RESPONDE AS TO WHETHER YOU ARE WILLING TO PARTICIPATE:

1. I understand that the use of human subjects in this project has been approved by the UNH Institutional Review Board for the Protection of Human Subjects in Research.

2. I understand the scope, aims and purpose of this research project and the procedures to be followed and the expected duration of my participation.

3. I have received a description of any reasonable foreseeable risks or discomforts associated with my being a subject in this research, had them explained an understand them.

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4. I have received a description of any potential benefit that may be accrued from this research and understand how they may affect me and others.

5. I understand that the confidentiality of all data and records associated with my participation in this research , including my identity will be fully maintained.

6. I understand that my consent to participate in this research is entirely voluntary and that my refusal to participate will involve no prejudice, penalty or loss of benefit to which I would otherwise be entitled.

7. I further understand that if I consent to participate, I may discontinue my participation at any time without prejudice or penalty.

8. I confirm that no coercion of any kind was used in seeking my participation.

9. I understand that if I am injured or require medical treatment, I may seek treatment at the University Health Services Center and will be charged for services rendered, unless covered by paid student-health fee.

10. I understand that if I have any questions pertaining to the research, I am encouraged to call Joanne Curran-Celentano at 862-2573 to discuss concerns in confidence.

11. I understand that I will not be provided financial incentive for my participation by the University of New Hampshire.

12. I understand that I will be given a summary of my results and will be invited to hear about the outcome of the research at the conclusion of the study.

I,_____ consent/agree to participate in this research project.

_____Date_____
signature of subject

_____Date_____
witness

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Health History Inventory

Subject # _____ Date_____
Previous Study _____

Name _____ DOB _____ Age _____

Height _____ Weight _____ BMI _____
Waist _____ Hip _____ W/H Ratio _____

Menopause Information:
How long have you been post? _____
Do you use any type of HRT? _____ How long have you taken it? _____

What type _____ Dose _____
Time of day _____

Have you previously taken HRT? _____ If yes when/how long? _____
Hysterectomy? Y N When? _____ Type? _____

Personal Health History:

_____ Diabetes _____ Hypertension _____ Heart Disease
_____ Thyroid _____ Osteoporosis _____ Breast Cancer

When is the last time you had your blood lipids measured? _____
Do you know the results? _____ TC _____ HDL _____ LDL

Family Health History:

_____ Diabetes _____ Hypertension _____ Heart Disease
_____ Thyroid _____ Osteoporosis _____ Breast Cancer
_____ High Blood Cholesterol

Medications: Do you presently take either over the counter or prescribed medications (other than HRT?)

Name of Drug	Reason for Taking	Dose	Frequency	How long

Supplements:

Name of Drug	Reason for Taking	Dose	Frequency	How long

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Code #: _____
Group #: _____

Remember, if there are any questions you feel uncomfortable responding to, please feel free to skip those items and continue on with the remainder of the questionnaire.

DEMOGRAPHIC AND HEALTH HISTORY INVENTORY

Today's Date: _____

Date of Birth: _____
Present Height: _____ Present Weight: _____

Ethnicity: White: _____ African-American: _____ Asian/American _____
Hispanic: _____ Native American: _____ Other: _____

Religious Affiliation: _____

Current Relationship Status: *(check all that apply)*

Never married: _____		
Cohabitating: _____	# Years:	_____
Married: _____	# Years:	_____
Divorced: _____	# Years:	_____
Remarried: _____	# Years:	_____
Separated: _____	# Years:	_____
Widowed: _____	# Years:	_____

Highest Education Level: *Please circle the highest grade in school you have completed or check the highest degree you hold:* 1 2 3 4 5 6 7 8 9 10 11

High School/GED: _____
Associate's Degree: _____
Bachelor's Degree: _____
Master's Degree: _____
Doctorate Degree or other professional degree _____

Present Occupation: _____

Health History

1. Do you presently have medical insurance? Yes_____ No____
2. Your primary health care provider is *(check one)*:
   - A gynecologist_____
   - A nurse practitioner_____
   - An internist_____
   - A family practitioner_____
   - Other (i.e. Naturopathic physician)_____
3. In the last five years have you been told that you have *(check all that apply)*:

_____ Diabetes	_____ Heart disease	_____ Ulcers
_____ Gastrointestinal disorder	_____ Lung disease	_____ Cancer
_____ High blood pressure	_____ Kidney disease	_____ Thyroid Disease
_____ Hardening of arteries	_____ Liver disease	_____ Sexually transmitted
_____ Blood clotting problems	_____ Stroke	diseases
_____ Gall bladder disease	_____	Other

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4. Number of pregnancies: _____
5. Number of children and ages: _____
_____

6. Your age when first child was born: _____ Your age when last child was born: _____

7. Please check when you had your last:

	within 0-12 months	*1-2 years ago*	*3-5 years ago*	*more than 5 years ago*
Physical Exam	_____	_____	_____	_____
Mammogram	_____	_____	_____	_____
Pap Smear	_____	_____	_____	_____

8. Briefly describe any problems with pregnancies, labor, delivery: _____
_____
_____

9. Age at first mammogram: _____
10. List past surgical operations (with approximate dates): _____
_____
_____

Drug History

If you check "yes" to any of the following, please indicate the type of drug, your reason for taking it, and how often you take it

1. Do you use any prescribed medications? Yes_____ No_____

Name of drug	Reason for taking/how often
_____	_____
_____	_____
_____	_____
_____	_____

2. Do you use over-the-counter medication (such as aspirin)? Yes_____ No_____

Name of drug	Reason for taking/how often
_____	_____
_____	_____
_____	_____
_____	_____

3. Do you take vitamins/mineral supplements, herbs, or other health remedies? Yes_____ No_____

Name &/or nutrient	Reason for taking/how often
_____	_____
_____	_____
_____	_____
_____	_____

4. Do you currently smoke cigarettes? Yes___ No___

If "yes", the number of cigarettes, on the average, you smoke each day. _____

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5. Do you drink alcohol? Yes ____ No____

If "yes" please indicate, on the average, the number of servings (serving = 1½ oz. liquor or liquer; 12 oz. beer; or 4 oz. wine) you consume:
Per month_____ Per week_____ Per day_____

Exercise Habits

(Please check which best represents your exercise routine.)

1. No regular exercise _____
Exercise 2 times a week for(greater than or equal to)
___20 min. ___30 min. ___40 min. ___50 min. ___60 min.
Exercise 3 times a week for (greater than or equal to)
___20 min. ___30 min. ___40 min. ___50 min. ___60 min.
Exercise 4 times a week for (greater than or equal to)
___20 min. ___30 min. ___40 min. ___50 min. ___60 min.
Exercise 5 times a week for (greater than or equal to)
___20 min. ___30 min. ___40 min. ___50 min. ___60 min.

2. Number of years exercised at above rate: _____

3. Briefly describe your exercise routine: _____
_____

4. If you no longer exercise, why did you stop? _____
_____

Diet History

1. Is your present weight your "usual weight"? _____

2. Do you believe that your present weight is a healthy weight for you? Yes____ No___
If you answered "no" what would you consider a healthy weight for you? _____

3. Are you on a special diet? Yes____ No____
If "yes" what kind of diet?_____

4. Are there any other facts about your lifestyle that you think might influence your nutritional habits?
Yes____ No____
If "yes", explain: _____
_____
_____

5. Do you know of any foods to which you are allergic? Yes____ No____
If "yes", explain: _____
_____

6. Do you consistently limit or eliminate any foods or food groups from your diet? Yes_____ No____
If "yes", please explain: _____
_____
_____

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Menstrual History

1. To the best of your recollection, how old were you when you first started your monthly period? _____

2. Within the last 12 months have you noticed any changes in your monthly period? Yes_____ No_____
If "yes" please read the following statements and for each circle the response that corresponds to your experience at the present time.

	No	*Sometimes*	*Often*	*Always*
My monthly period is less frequent than it used to be.	1	2	3	4
My monthly period is more frequent than it used to be.	1	2	3	4
My monthly period has decreased in number of days per month.	1	2	3	4
My monthly period has increased in number of days per month.	1	2	3	4
My monthly period blood flow is lighter than usual for me.	1	2	3	4
My monthly period blood flow is heavier than usual for me.	1	2	3	4
In my monthly period, more clots are expelled in the blood.	1	2	3	4
In my monthly period, fewer clots are expelled in the blood.	1	2	3	4

3. At present are you using an oral contraceptive? Yes _____ No _____
If "yes", Brand/type: _____ dose: _____

4. If you have used oral contraceptives in the past, how many years in total did you do so? _____

5. Since you started menstruating, has your cycle stopped for more than three months (other than during pregnancy or breast-feeding)? Yes_____ No_____
If "yes", please explain: _____
_____
_____

6. According to the following definitions, which represents your present status?
_____ Premenopausal (Regular menstrual periods)
_____ Perimenopausal (In the last 12 months you have skipped your period or you have noted changes in regularity or flow)
_____ Menopausal (No regular menstrual cycle for 6-12 months)
_____ Postmenopausal (No menstrual period for 12 months or more)
_____ Hysterectomy: At what age? _____

Hormone Replacement Therapy (HRT) History

1. Do you presently take any of the following?: Yes_____ No_____

	Brand	Dose	When did you begin?
Estrogen	_____	_____	_____
Estrogen and Progesterone	_____	_____	_____
Progesterone	_____	_____	_____
Testosterone (i.e. *Estratest*)	_____	_____	_____

If "yes" please answer questions #2, 3 and 4.
If "no" please answer questions #3, 4, 5 and 6.

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2. Please indicate by circling 1, 2, 3, or 4 to what extent each of the following factors influenced your decision to take hormone therapy.

	Not influential at all	*Somewhat influential*	*Moderately influential*	*Extremely influential*
Hysterectomy	1	2	3	4
Relief of physical menopausal symptoms (hot flashes, urinary tract problems, vaginal dryness, etc.)	1	2	3	4
Concern about heart disease	1	2	3	4
Concern about osteoporosis	1	2	3	4
Advice of M.D. or other health professional	1	2	3	4
Advice of friend/coworker/family member	1	2	3	4
Advice of partner	1	2	3	4
Mood changes	1	2	3	4
Concern about aging	1	2	3	4
Sexual problems	1	2	3	4
Other: _____	1	2	3	4

3. Please indicate by circling the responses 1, 2, 3, or 4 to what extent the following sources have provided you with information on menopause:

	Least informative	*Somewhat informative*	*Moderately informative*	*Extremely informative*
MD or other health professional	1	2	3	4
Mother	1	2	3	4
Radio/ television	1	2	3	4
Workshops or conferences	1	2	3	4
Books, pamphlets	1	2	3	4
Partner	1	2	3	4
Friend or relative	1	2	3	4
Other (please explain)_____	1	2	3	4

_____

_____

4. Are there any other therapies you use for treatment of menopausal symptoms (such as massage therapy, supplements, meditation etc)? Yes___ No___
If "yes", please explain: _____

_____

_____

_____

5. Explain why you have chosen not to use hormone therapy: _____

_____

_____

_____

6. Have you taken HRT in the past? Yes _____ No_____
If "yes", how long did you take it and why did you stop taking it? _____

_____

_____

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Code #_____
Group #_____

RELATIONSHIP

1.	Are you currently in an intimate relationship with someone you would define as a primary or exclusive emotional and/or sexual partner? (If you answered "No" skip to question 11)	Yes	No
2.	What is the sex of this partner?	Male	Female
3.	How long have you been in this relationship?		

FOR THE FOLLOWING QUESTIONS, PLEASE CIRCLE THE RESPONSE THAT INDICATES BEST HOW YOU SEE YOUR RELATIONSHIP.

4.	In general, how close do you feel emotionally to your partner?	Not close at all	Somewhat distant	Neutral	Somewhat close	Extremely close
5.	How much conflict is there in your relationship?	None	Very little	Average amount	Very much	An extreme amount
6.	How committed are you to staying in this relationship?	Very uncommitted	Somewhat uncommitted	Neutral	Fairly Committed	Highly Committed
7.	In general, how emotionally satisfied are you with your relationship?	Very dissatisfied	Somewhat dissatisfied	Neutral	Somewhat satisfied	Very satisfied
8.	In general, how sexually satisfied are you with your relationship?	Very dissatisfied	Somewhat dissatisfied	Neutral	Somewhat satisfied	Very satisfied
9.	How well does your partner meet your emotional needs?	Not at all	Meets few of my needs	Meets most of my needs	Meets all of my needs	
10.	How well does your partner meet your sexual needs?	Not at all	Meets few of my needs	Meets most of my needs	Meets all of my needs	

FOR THE FOLLOWING QUESTIONS, PLEASE CIRCLE THE RESPONSE THAT INDICATES HOW FREQUENTLY YOU PARTICIPATE IN THE FOLLOWING SEXUAL ACTIVITIES.

11.	Fantasy	Not at all	Less than once/week	1-3 times a week	Daily
12.	Kissing	Not at all	Less than once/week	1-3 times a week	Daily
13.	Masturbation	Not at all	Less than once/week	1-3 times a week	Daily
14.	Touching/holding partner	Not at all	Less than once/week	1-3 times a week	Daily
15.	Oral sex	Not at all	Less than once/week	1-3 times a week	Daily
16.	Vaginal intercourse	Not at all	Less than once/week	1-3 times a week	Daily
17.	Anal intercourse	Not at all	Less than once/week	1-3 times a week	Daily

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SELF

Code #_____
Group #_____

DIRECTIONS: PLEASE READ EACH OF THE STATEMENTS LISTED BELOW AND INDICATE HOW MUCH YOU PERSONALLY AGREE WITH EACH STATEMENT BY CIRCLING THE APPROPRIATE RESPONSE.

HOW DO YOU FEEL ABOUT YOURSELF AS A PERSON?___	Strongly Disagree	Disagree	Agree	Strongly Agree
1. I FEEL THAT I AM A PERSON OF WORTH, AT LEAST ONE ON EQUAL BASIS WITH OTHERS.	1	2	3	4
2. I FEEL THAT I HAVE A NUMBER OF GOOD QUALITIES.	1	2	3	4
3. ALL IN ALL, I AM INCLINED TO FEEL THAT I AM A FAILURE.	1	2	3	4
4. I AM ABLE TO DO THINGS AS WELL AS OTHER PEOPLE.	1	2	3	4
5. I FEEL I DO NOT HAVE MUCH TO BE PROUD OF.	1	2	3	4
6. I TAKE A POSITIVE ATTITUDE TOWARD MYSELF.	1	2	3	4
7. ON THE WHOLE, I AM SATISFIED WITH MYSELF.	1	2	3	4
8. I WISH I COULD HAVE MORE RESPECT FOR MYSELF.	1	2	3	4
9. I CERTAINLY FEEL USELESS AT TIMES.	1	2	3	4
10. AT TIMES I THINK I AM NO GOOD.	1	2	3	4

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Code #_____
Group #_____

EXPERIENCES

DIRECTIONS: PLEASE READ THE FOLLOWING STATEMENTS AND CIRCLE THE NUMBER YOU FEEL CORRESPONDS TO YOUR EXPERIENCES AT THE PRESENT TIME OR WITHIN THE LAST SIX MONTHS.

I AM EXPERIENCING OR HAVE RECENTLY EXPERIENCED...	No	Sometimes	Often	Always
1. CHANGES IN MY BODY TEMPERATURE THAT MAKE ME FEEL COLD, OR HOT AND FLUSHED IN THE FACE.	1	2	3	4
2. SWEATING DURING THE NIGHT.	1	2	3	4
3. VAGINAL DRYNESS CAUSING PAIN, SORENESS, OR DISCOMFORT DURING SEXUAL ACTIVITY.	1	2	3	4
4. LACK OF FIRMNESS IN MY VAGINAL MUSCLES.	1	2	3	4
5. VAGINAL INFECTIONS INCLUDING YEAST INFECTIONS.	1	2	3	4
6. FEELINGS OF LIGHT HEADEDNESS.	1	2	3	4
7. SENSATIONS OF SICKNESS TO MY STOMACH OR NAUSEA.	1	2	3	4
8. TENSION AND PAIN IN MY HEAD (HEADACHES).	1	2	3	4
9. NOTABLE CHANGES IN MY HEART'S BEATING PATTERN.	1	2	3	4
10. CHANGES IN SLEEP PATTERNS (INSOMNIA).	1	2	3	4
11. CHANGES IN MY BODY LIMBS SUCH AS TINGLING, NUMBNESS.	1	2	3	4
12. MORE FREQUENT PASSING OF WATER (URINATION).	1	2	3	4
13. MORE PAINFUL PASSING OF WATER (URINATION).	1	2	3	4
14. ACCIDENTS IN PASSING WATER (URINARY INCONTINENCE).	1	2	3	4
15. FORGETFULNESS AND A LACK OF REMEMBERING.	1	2	3	4
16. LOW AROUSAL OR EXCITEMENT DURING SEXUAL ACTIVITY.	1	2	3	4
17. HIGH LEVELS OF AROUSAL OR EXCITEMENT DURING SEXUAL ACTIVITY.	1	2	3	4
18. A NEED FOR LONGER PERIOD OF STIMULATION TO ACHIEVE ORGASM.	1	2	3	4
19. LESS FREQUENT ORGASMS THAN IN THE PAST.	1	2	3	4
20. MORE FREQUENT ORGASMS THAN IN THE PAST.	1	2	3	4
21. LESS INTENSE ORGASMS THAN IN THE PAST.	1	2	3	4
22. MORE INTENSE ORGASMS THAN IN THE PAST.	1	2	3	4
23. PAINFUL ORGASMS.	1	2	3	4
24. BLEEDING DURING OR AFTER INTERCOURSE.	1	2	3	4
25. PAIN OR BURNING DURING INTERCOURSE.	1	2	3	4
26. LESS INTEREST IN SEXUAL ACTIVITY.	1	2	3	4
27. MORE INTEREST IN SEXUAL ACTIVITY.	1	2	3	4
28. EXTREME CHANGES IN MY MOOD.	1	2	3	4

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MOODS

Code #_____
Group #_____

DIRECTIONS: BELOW IS A LIST OF WORDS THAT DESCRIBE FEELINGS PEOPLE HAVE. PLEASE READ EACH ONE CAREFULLY AND CIRCLE THE NUMBER THAT BEST DESCRIBES YOUR ANSWER.

HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY....	Not at All	A Little	Moderately	Quite a Bit	Extremely
1. FRIENDLY	0	1	2	3	4
2. TENSE	0	1	2	3	4
3. ANGRY	0	1	2	3	4
4. WORN OUT	0	1	2	3	4
5. UNHAPPY	0	1	2	3	4
6. CLEAR-HEADED	0	1	2	3	4
7. LIVELY	0	1	2	3	4
8. CONFUSED	0	1	2	3	4
9. SORRY FOR THINGS DONE	0	1	2	3	4
10. SHAKY	0	1	2	3	4
11. LISTLESS	0	1	2	3	4
12. PEEVED	0	1	2	3	4
13. CONSIDERATE	0	1	2	3	4
14. SAD	0	1	2	3	4
15. ACTIVE	0	1	2	3	4
16. ON EDGE	0	1	2	3	4
17. GROUCHY	0	1	2	3	4
18. BLUE	0	1	2	3	4
19. ENERGETIC	0	1	2	3	4
20. PANICKY	0	1	2	3	4
21. HOPELESS	0	1	2	3	4
22. RELAXED	0	1	2	3	4
23. UNWORTHY	0	1	2	3	4
24. SPITEFUL	0	1	2	3	4
25. SYMPATHETIC	0	1	2	3	4
26. UNEASY	0	1	2	3	4
27. RESTLESS	0	1	2	3	4
28. UNABLE TO CONCENTRATE	0	1	2	3	4
29. FATIGUED	0	1	2	3	4
30. HELPFUL	0	1	2	3	4
31. ANNOYED	0	1	2	3	4
32. DISCOURAGED	0	1	2	3	4
33. RESENTFUL	0	1	2	3	4

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MOODS

Code #_____
Group #_____

	Not at All	A Little	Moderately	Quite A Bit	Extremely
34 NERVOUS	0	1	2	3	4
35 LONELY	0	1	2	3	4
36 MISERABLE	0	1	2	3	4
37 MUDDLED	0	1	2	3	4
38 CHEERFUL	0	1	2	3	4
39 BITTER	0	1	2	3	4
40 EXHAUSTED	0	1	2	3	4
41 ANXIOUS	0	1	2	3	4
42 READY TO FIGHT	0	1	2	3	4
43 GOOD-NATURED	0	1	2	3	4
44 GLOOMY	0	1	2	3	4
45 DESPERATE	0	1	2	3	4
46 SLUGGISH	0	1	2	3	4
47 REBELLIOUS	0	1	2	3	4
48 HELPLESS	0	1	2	3	4
49 WEARY	0	1	2	3	4
50 BEWILDERED	0	1	2	3	4
51 ALERT	0	1	2	3	4
52 DECEIVED	0	1	2	3	4
53 FURIOUS	0	1	2	3	4
54 EFFICIENT	0	1	2	3	4
55 TRUSTING	0	1	2	3	4
56 FULL OF PEP	0	1	2	3	4
57 BAD-TEMPERED	0	1	2	3	4
58 WORTHLESS	0	1	2	3	4
59 FORGETFUL	0	1	2	3	4
60 CAREFREE	0	1	2	3	4
61 TERRIFIED	0	1	2	3	4
62 GUILTY	0	1	2	3	4
63 VIGOROUS	0	1	2	3	4
64 UNCERTAIN ABOUT THINGS	0	1	2	3	4
65 BUSHED	0	1	2	3	4

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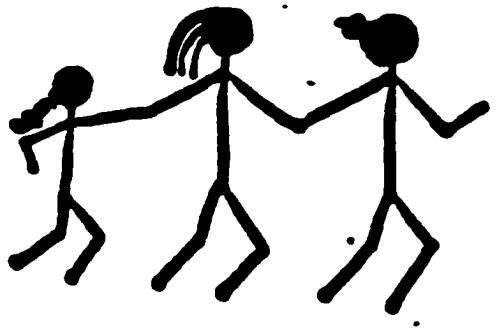
FOOD DIARY

UNH WOMEN'S HEALTH STUDY



1. Record everything you eat or drink. Use a new diet intake sheet for each day. Indicate the name of the MEAL or the TIME of the snack, WHERE the food is provided or prepared (cafeteria, at home, vending machine, etc.), the FOOD ITEM, the AMOUNT eaten, and briefly describe how it was prepared (fried, boiled, broiled, etc.). If the item was a brand name product, please include the name.

2. Try to eat what you normally eat and record everything. This dietary survey will only be useful if you give an accurate account of what you eat.

3. List amounts in common household units that you are familiar with (i.e., teaspoon, cup, pat, ounce, inch, etc.).

4. MILK: Indicate whether milk is whole, low fat (1 or 2%), or skim. Include flavoring if one is used.

5. VEGETABLES and FRUITS: One average serving of cooked or canned fruits and vegetables is about a half cup. Fresh whole fruits and vegetables should be listed as small, medium or large. Be sure to indicate if sugar or syrup is added to fruit and list if any margarine, butter, cheese sauce or cream sauce are added to vegetables. When recording salad, list items comprising salad and be sure to include salad dressing used.

6. EGGS: Indicate method of preparation (scrambled, fried, poached, etc.) and the number eaten.

7. MEAT - POULTRY - FISH: Indicate approximate size (i.e., 2" x 2" x 1") or weight in ounces of the serving. Be sure to include any gravy, sauce, or breading added.

8. CHEESE: Indicate kind, number of ounces, cubic inches, or slices and whether it is made from whole milk, part skim or is low calorie.

9. CEREAL: Specify kind, whether cooked or dry, and measured or estimated in terms of cups or ounces.

10. BREAD and ROLLS: Specify kind (whole wheat, enriched white, rye, etc.), number and thickness of slices, or size in inches. Remember to include in your description any butter or margarine used on bread.

11. BEVERAGES: Include every item you drink including water. Be sure to record cream and sugar used in tea and coffee, whether juices are sweetened or unsweetened and whether soft drinks are diet or regular.

12. FATS: Remember to record all the butter, margarine, oil and any other fats used in cooking or on food.

13. VITAMIN and/or MINERAL SUPPLEMENTS: Indicate type and quantity consumed and amount of nutrients provided.

14. MEDICATIONS: Indicate name and prescribed dosage.

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TIME	M/S	LOCATION	FOOD ITEM	AMT.	DESCRIPTION	CODE

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HEALTH HABITS AND DIET QUESTIONNAIRE

This form asks you a variety of questions about your background, environment, and habits, which may affect or be related to your health. The information you provide will help scientists to understand more about the causes of disease. This questionnaire will take about 12-15 minutes to complete. Please fill in the information requested, or place a check in the appropriate space. If you are not sure about an answer, please estimate.

THIS SPACE FOR OFFICE USE

TODAY'S DATE: Month ___ - Day ___ - Year ___ (11–16)

Please PRINT YOUR NAME (name of study participant)

17 LAST 31 FIRST 40 MIDDLE 48

A 79 80

1-10*

ADDRESS: 11 STREET 34

35 CITY 49 STATE 52 ZIP 61

62 ___ Area Code

TELEPHONE: (___) ___ - ___ (64–73)

B 79 80

1. When were you born? ___/___/___ Month Day Year — 11

2. How old are you? ___ years — 18

3. Sex: 1 ___ Male 2 ___ Female — 20

4. Race or ethnic background:
   1 ___ White, not of Hispanic origin
   2 ___ Black, not of Hispanic origin
   3 ___ Hispanic
   4 ___ American Indian/Alaskan native
   5 ___ Asian
   6 ___ Pacific Islander — 21

5. Please circle the highest grade in school you have completed:
   1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17+ — 22

6. How tall are you? ___ feet ___ inches 7. How much do you weigh? ___ pounds — 24

8. Do you smoke cigarettes now? 1 ___ No 2 ___ Yes — 30
   IF YES: On the average, about how many cigarettes a day do you smoke now? ___ cigarettes — 31

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9. During the past year, have you taken any vitamins or minerals?
1 ___ No 2 ___ Yes, fairly regularly 3 ___ Yes, but not regularly If Yes, → 23 —

What do you take fairly regularly?	# of PILLS per DAY, WEEK, etc.		
Multiple Vitamins			
One-a-day type	____ pills per ____		24 ____
Stress-tabs type	____ pills per ____		27 ____
Therapeutic, Theragran type	____ pills per ____	How many milligrams or IUs per pill?	30 ____
Other Vitamins			
Vitamin A	____ pills per ____	____ IU per pill	33 ____
Vitamin C	____ pills per ____	____ mg per pill	37 ____
Vitamin E	____ pills per ____	____ IU per pill	41 ____
Calcium or dolomite	____ pills per ____	____ mg per pill	45 ____

Other (What?) 1 ___ Yeast 2 ___ Selenium 3 ___ Zinc 4 ___ Iron 5 ___ Beta-carotene
6 ___ Cod liver oil 7 ___ Other _____ 49 —

Please list the brand of multiple vitamin/mineral you usually take: _____

C 79 80

10. This section is about your *usual* eating habits. Thinking back over the past year, how often do you usually eat the foods listed on the next page?

First, check (✓) whether your usual serving size is small, medium or large. (A small portion is about one-half the medium serving size shown, or less; a large portion is about one-and-a-half times as much, or more.)

Then, put a *NUMBER* in the most appropriate column to indicate *HOW OFTEN*, on the average, you eat the food. You may eat bananas *twice a week* (put a *2 in the "week"* column). If you never eat the food, check "Rarely/Never." Please DO NOT SKIP foods. And please BE CAREFUL which column you put your answer in. It will make a big difference if you say "Hamburger once a day" when you mean "Hamburger once a week"!

One item says "in season." Indicate how often you eat this just in the 2-3 month time when that food is in season. (Be careful about overestimating here.)
Please look at the *example* below. This person
1) eats a medium serving of cantaloupe once a week, in season.
2) has ½ grapefruit about twice a month.
3) has a small serving of sweet potatoes about 3 times a year.
4) has a large hamburger or cheeseburger or meat loaf about four times a week.
5) never eats liver.

EXAMPLE:

	Medium Serving	Your Serving Size S	M	L	How often? Day	Week	Month	Year	Rarely/ Never
Cantaloupe (in season)	¼ medium		✓			1			
Grapefruit	(½)		✓				2		
Sweet potatoes, yams	½ cup	✓						3	
Hamburger, cheeseburger, meat loaf	1 medium			✓		4			
Liver	4 oz.								✓

FOR OFFICE USE
Q 9, mg or IU: 1=50-100 2=200-250 3=400-500 4=1000 5=5000 6=10,000 7=20,000-25,000 8=50,000 9=Unk.

On the following two pages, code the four characters for each food as follows:

___ S-1 M-2 L-3 NS-9

___ ___ No. Times NS-99

___ Da-1 Wk-2 Mo-3 Yr-4 Nev-5 NS-9

If respondent places a checkmark in the "How often" columns, do not impute "01", once. Instead, code "99", Not Stated. If respondent does not check a portion size, do not impute medium, but code "9".

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	Medium Serving	Your Serving Size			How often?					OFFICE USE
					Day	Week	Month	Year	Rarely/ Never	
FRUITS & VEGETABLES		S	M	L						
EXAMPLE – Apples, applesauce, pears	(1) or ½ cup		✓			4				
Apples, applesauce, pears	(1) or ½ cup									11 ____
Cantaloupe (in season)	¼ medium									15 ____
Oranges	1 medium									19 ____
Orange juice or grapefruit juice	6 oz. glass									23 ____
Grapefruit	(½)									27 ____
Other fruit juices, fortified fruit drinks	6 oz. glass									31 ____
Beans such as baked beans, pinto, kidney, limas, or in chili	¼ cup									35 ____
Tomatoes, tomato juice	(1) or 6 oz.									39 ____
Broccoli	½ cup									43 ____
Spinach	½ cup									47 ____
Mustard greens, turnip greens, collards	½ cup									51 ____
Cole slaw, cabbage, sauerkraut	½ cup									55 ____
Carrots, or mixed vegetables containing carrots	½ cup									59 ____
Green salad	1 med. bowl									63 ____
Salad dressing, mayonnaise (including on sandwiches)	2 Tblsp.									67 ____
French fries and fried potatoes	¼ cup									71 ____
Sweet potatoes, yams	½ cup									75 ____ D 79 80
Other potatoes, incl. boiled, baked, potato salad, mashed	(1) or ½ cup									11 ____
Rice	¼ cup									15 ____
MEAT, MIXED DISHES, LUNCH ITEMS		S	M	L	Da	Wk	Mo	Yr	Nv	
Hamburgers, cheeseburgers, meat loaf	1 medium									19 ____
Beef—steaks, roasts	4 oz.									23 ____
Beef stew or pot pie with carrots, other vegetables	1 cup									27 ____
Liver, including chicken livers	4 oz.									31 ____
Pork, including chops, roasts	2 chops or 4 oz.									35 ____
Fried chicken	2 sm. or 1 lg. piece									39 ____
Chicken or turkey, roasted, stewed or broiled	2 sm. or 1 lg. piece									43 ____
Fried fish or fish sandwich	4 oz. or 1 sand.									47 ____
Other fish, broiled, baked	4 oz.									51 ____
Spaghetti, lasagna, other pasta with tomato sauce	1 cup									55 ____
Hot dogs	2 dogs									59 ____
Ham, lunch meats	2 slices									63 ____
Vegetable soup, vegetable beef, minestrone, tomato soup	1 med. bowl									67 ____
BREADS / SALTY SNACKS / SPREADS		S	M	L	Da	Wk	Mo	Yr	Nv	
White bread (including sandwiches), bagels, etc., crackers	2 slices, 3 cracks									71 ____
Dark bread, including whole wheat, rye, pumpernickel	2 slices									75 ____ E 79 80
Corn bread, corn muffins, corn tortillas	1 med. piece									11 ____
Salty snacks (such as chips, popcorn)	2 handfuls									15 ____
Peanuts, peanut butter	2 Tblsp.									19 ____
Margarine on bread or rolls	2 pats									23 ____
Butter on bread or rolls	2 pats									27 ____
BREAKFAST FOODS		S	M	L	Da	Wk	Mo	Yr	Nv	
High fiber, bran or granola cereals, shredded wheat	1 med. bowl									31 ____
Highly fortified cereals, such as Product 19, Total, or Most	1 med. bowl									35 ____
Other cold cereals, such as Corn Flakes, Rice Krispies	1 med. bowl									39 ____
Cooked cereals	1 med. bowl									43 ____
Eggs (1 egg = small, 2 eggs = medium)										47 ____
Bacon	2 slices									51 ____
Sausage	2 patties or links									55 ____

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SWEETS	Medium Serving	Your Serving Size S	M	L	How often? Day	Week	Month	Year	Rarely/ Never	OFFICE USE
Ice cream	1 scoop									59 ____
Doughnuts, cookies, cakes, pastry	1 pc. or 3 cookies									63 ____
Pies	1 med. slice									67 ____
Chocolate candy	small bar, 1 oz.									71 ____
DAIRY PRODUCTS, BEVERAGES		S	M	L	Da	Wk	Mo	Yr	Nv	
Cheeses and cheese spreads, not including cottage	2 slices or 2 oz.									75 ____ F 79 80
Whole milk and bevs. with whole milk (not incl. on cereal)	8 oz. glass									11 ____
2% milk and bevs. with 2% milk (not incl. on cereal)	8 oz. glass									15 ____
Skim milk, 1% milk or buttermilk (not incl. on cereal)	8 oz. glass									19 ____
Regular soft drinks (not diet)	12 oz. can or bottle									23 ____
Beer	12 oz. can or bottle									27 ____
Wine	1 med. glass									31 ____
Liquor	1 shot									35 ____
Milk or cream in coffee or tea	1 Tblsp.									39 ____
Sugar in coffee or tea, or on cereal	2 teaspn.									43 ____

	1 Seldom/Never	2 Sometimes	3 Often/Always	
11. How often do you eat the skin on chicken?	_____	_____	_____	47 —
How often do you eat the fat on meat?	_____	_____	_____	48 —
How often do you add salt to your food?	_____	_____	_____	49 —
How often do you add pepper to your food?	_____	_____	_____	50 —

12. Not counting salad or potatoes, about how many servings of vegetables do you eat per day or per week? _____ vegetables per _____ day, week — 51 ____

13. Not counting juices, how many servings of fruits do you usually eat per day or per week? _____ fruits per _____ day, week — 54 ____

G 79 80

THANK YOU VERY MUCH for taking the time to fill out this information.

Reviewed by _____

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Coding Sheet (5-29-96)

Coder _____

Date _____

LINE 1

Column #	Variable	
1-5	Subject #	____
6	Group #	___
7-8	Age	___
9-11	Weight	___
12	Ethnicity	___
13	SPACE	
14	Religion	___
15	Relationship Status (never married)	___
16-17	Relationship Status (Cohabitating)	___
18-19	Relationship Status (Married)	___
LINE 1 20-21	Relationship Status (Divorced)	___
22	SPACE	
23-24	Relationship Status (Remarried)	___
25-26	Relationship Status (Separated)	___
27-28	Relationship Status (Widow)	___
29	Highest Educational Level	___
30	Present Occupation	___
31	Present Occupation (health related)	___
32	Medical Insurance	___
33	Primary Healthcare Provider	___
34	SPACE	

If no answer given code "0's"

35	Diabetes	___
36	Gastrointestinal Disorder	___
37	High Blood Pressure	___
38	Hardening of Arteries	___
39	Blood Clot Problems	___
40	Gall Bladder disease	___
41	Heart disease	___
42	Lung disease	___
43	Kidney disease	___
LINE 1 44	Liver disease	___
45	Stroke	___
46	Ulcers	___
47	Cancer	___
48	Thyroid disease	___
49	Sexually transmitted diseases	___
50	Elevated cholesterol	___
51	Arthritis	___
52	Asthma/Allergies	___
53	Hyperlipidemia	___
54	Osteoporosis	___
55	Other	___
56	SPACE	
57-58	Number of Pregnancies	___
Line 1 59-60	Number of Children	___
61	Number of Children (Young child)	___
62	Number of Children (Teens)	___
63-64	Number of Adult Children	___

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DINE Scoring Sheet for 3-Day Diet Analysis

Date:_____
Subject No:_____
Researcher:_____

Selected Nutrients	*Criterion*	*Possible Score*	*Criterion*	*Possible Score*	*Subject's Score*
Total Calories	Within 10% of ICL for individual	+1.0	Less than 1,000kcal or >50% of ICL	-1.0	
Protein	10-15% of total kcals	+1.0	<6% ICL or >30% ICL	-1.0	
Total Fat			>45% ICL	-1.0	
Saturated Fat	<10% of total calories	+1.0			
Monounsaturated Fat	<10% of total calories	+0.5			
Polyunsaturated Fat	<10% of total calories	+0.5			
Complex Carbohydrates	45-80% of total kcal	+0.5	<25% ICL or >80% ICL	-0.5	
Dietary Fiber	20-35 grams	+0.5	<12 grams	-0.5	
Added Sugar	<10% of total kcals	+1.0	>20% ICL	-1.0	
Cholesterol	300 mg or less	+1.0			
Sodium	500-2400 mg	+0.5			
Potassium	2000-5625 mg	+0.5			
Vitamin A	800 RE or more	+0.5			
Vitamin C	60 mg or more	+0.5			
Iron	15 mg or more	+0.5			
Calcium	800 mg or more	+0.25			
Phosphorus	800 mg or more	+0.25			
Alcohol			>95 kcal/day	-1.0	
	TOTAL	10.0		-6.0	

ICL = Ideal Caloric Level
* Any value that does not fall within either of the given ranges is scored as 0

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APPENDIX D

GLOSSARY OF TERMS

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Glossary of Terms

Estrogen Replacement Therapy: Use of estrogen alone during menopause; typically not recommended for women with a uterus because of increased risk of endometrial cancer.

Hormone Replacement Therapy: Term usually used to denote the use of estrogen in combination with progestin during menopause. Also used as a generic term to denote any use of postmenopausal hormone therapy, including estrogen alone or testosterone alone or estrogen combination with progestin and/or testosterone.

Premenopause: the time when a woman is having regular menstrual cycles.

Perimenopause (Menopausal Transition): the time (usually a few years before the last menstrual period) when cycles are irregular and that is accompanied by wide hormonal fluctuations (239) which ends with menopause.

Menopause: defined as the last menstrual period and is usually diagnosed retrospectively after 12 months of amenorrhea (240).

Postmenopause: the time after menopause

Total (simple) hysterectomy: removal of the uterus alone(241).

Total (simple) hysterectomy: hysterectomy with bilateral salpingo-oophorectomy: removal of the uterus, both fallopian tubes, and both ovaries (241).

Salipingo-oophorectomy: removal of one fallopian tube and one ovary (241).

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APPENDIX E

GLOSSARY OF ABBREVIATIONS

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Glossary of Abbreviations

α-tocopherol: alpha tocopherol

β-carotene: beta-carotene

BMI: body mass index

CEE: conjugated equine estrogen

CHD: coronary heart disease

CVD: cardiovascular disease

EO: estrogen only group

E/P: estrogen and progestin group

ELISA: enzyme linked immunosorbent assay

FFQ: food frequency questionnaire

HDL-C: high-density lipoprotein cholesterol

HHHQ: Health Habits and History Questionnaire

HRT: hormone replacement therapy

LDL-C: low-density lipoprotein

Lp(a): lipoprotein (a)

MI: myocardial infarction (heart attack)

MPA: medroxyprogesterone acetate.

NCEP: National Cholesterol Education Panel

NHRT: no hormone replacement therapy group

OC: oral contraceptive

ORAC: oxygen radical absorbency capacity

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POMS: Profile of Mood States Inventory

TC: total cholesterol

TG: (triacylglycerol) triglyceride

VLDL-C: very-low density lipoprotein

WHR: waist-hip ratio

UNHWHS: *University of New Hampshire Women's Health Study*

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APPENDIX F

HORMONE REPLACEMENT FORMULATIONS

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Hormone Replacement Formulations

SUBJECT	ESTROGEN	DOSE/day	PROGESTIN	DOSE	REGIME
3	Premarin®	.3 mg			
4	Premarin®	.625	Provera®	.5 mg	continuous
9	Premarin®	.3 mg			every other day
10	Premarin®	.625 mg	Provera®	2.5 mg	continuous
15	Estraderm® Patch	.005 mg	Provera® or Cycrin®	10 mg.	cyclic
18	Premarin®	.625 mg	Provera®		cyclic
*20	Prempro®	.625 mg	Prempro®	2.5 mg	1 tab. 2x a day
21	Estrace®	1 mg	Provera®	2.5 mg	continuous
23	Premarin®	.625 mg			
27	Premarin®	.625 mg			
33	Premarin®		Provera®		continuous
36	Premarin®	.625 mg			
51	Premarin®	.625 mg	Provera® or Cycrin®	5 mg	cyclic (Premarin® 1-21d, Provera®/Cycrin® 16-21d)
53	Premarin®	.625 mg	Provera®		cyclic (Premarin® 1-15d, Provera ®10-26d)
55	Estrace® Patch	1 mg	Cycrin®	2.5 mg	cyclic
**56	Estratest®		Provera®	2.5 mg	continuous
57	Premarin®	.3 mg			
59	Premarin®		Provera®		continuous
**76	Estratest®	.62/1.2 mg	Aygestin®	5 mg	continuous
79	Estrace®	1 mg	Provera®	10 mg	cyclic (Premarin® 25d, Provera®10d)
80	Estraderm® Patch	.05 mg	IC-Provera®/ Cycrin®	2.5 mg	continuous
82	Premarin®		Cycrin®		continuous
89	Premarin®	.9 mg	Provera®	2.5 mg	continuous
95	Estrace®	.5 mg			
288	Premarin®	.625 mg	Provera®	2.5 mg	continuous
295	Premarin®	.625 mg	Provera®	2.5 mg	continuous
306	Estrace®	1 mg	Cycrin®	10 mg	cyclic (Estrace® 25d, Cycrin® 10d)
334	Premarin®	.625 mg			
336	Premarin®	.625 mg			
337	Estrace® Patch	1 mg	Provera®	2.5 mg	continuous
344	Premarin®	.625 mg	Provera®	5 mg	cyclic (Premarin® 1-25d, Provera® 16-25d)
*358	Prempro®	.625 mg	Prempro®	2.5 mg	
368	Estrace® Patch	1 mg			
375	Ogen®	.625 mg			

*Prempro® is one pill that contains both estrogen and progestin. **Estratest® contains testosterone and estrogen.

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CHAPTER VII

LIST OF REFERENCES

1. Heart and Stroke Statistical Update. Dallas, TX.: American Heart Association, 1997.

2. Hargarten KM. Menopause, how exercise mitigates symptoms. **The Physician and Sportsmedicine** 1994; 22:49-56.

3. Wilbur J, Miller A, Montgomery A. The influence of demographic characteristics, menopausal status, and symptoms on women's attitudes toward menopause. **Women & Health** 1995; 23:19-39.

4. Bush TL, Barrett-Connor E, Cowan LD, et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Follow-up Study. **Circulation** 1987; 75:1102-1109.

5. Stampfer MJ, Colditz GA. Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence. **Preventive Medicine** 1991; 20:47-63.

6. Grady D, Rubin SM, Petitti DB, et al. Hormone therapy to prevent disease and prolong life in postmenopausal women. **Annals of Internal Medicine** 1992; 117:1038-1041.

7. Hammond CB. Women's concerns with hormone replacement therapy-compliance issues. **Fertility and Sterility** 1994; 62 (Suppl 2):157S-160S.

8. Wagner PJ, Kuhn S, Petry LJ, Talbert FS. Age differences in attitudes toward menopause and estrogen replacement therapy. **Women & Health** 1995; 23:1-15.

9. Sack MN, Rader DJ, Cannon RO. Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women. **Lancet** 1994; 343:269-270.

10. Esterbauer H, Puhl H, Dieber-Rotheneder M, Waeg M, Rabl H. Effect of antioxidants on oxidative modification of LDL. **Annals of Medicine** 1991; 23:573-581.

11. Kwiterovich PO. The effect of dietary fat, antioxidants, and pro-oxidants on blood lipids, lipoproteins, and atherosclerosis. **Journal of the American Dietetic Association** 1997; 97:S31-S41.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

12. Julian TW, Gavazzi SM. Toward a biopsychosocial model of domestic violence. **Journal of Marriage and the Family** 1995; 57:307-320.

13. Engel GL. The need for a new medical model: a challenge for biomedicine. **Science** 1977; 196:129-136.

14. Engel GL. The biopsychosocial model and medical education. **The New England Journal of Medicine** 1982; 306:802-805.

15. McDaniel SH, Hepworth J, Doherty WJ. Medical Family Therapy: A biopsychosocial approach to families with health problems. New York: Basic Books, 1992.

16. Logothetis ML. Women's decisions about estrogen replacement therapy. **Western Journal of Nursing** 1991; 13:458-474.

17. Gorsky RD, Koplan JP, Peterson HB, Thacker SB. Relative risks and benefits of long-term estrogen replacement therapy: A decision analysis. **Obstetrics and Gynecology** 1994; 83:161-166.

18. Derby CA, Hume AL, Barbour MM, J.B. M, Lasater TM, Carleton RA. Correlates of postmenopausal estrogen use and trends through the 1980s in two southeastern New England communities. **American Journal of Epidemiology** 1993; 137:1125-1135.

19. Criqui MH, Suarez L, Barrett-Connor E. Postmenopausal estrogen use and mortality. Results from a prospective study in a defined, homogeneous community. **American Journal of Epidemiology** 1988; 128:606-614.

20. Johannes CB, Crawford SL, Posner JG, McKinlay SM. Longitudinal patterns and correlates of hormone replacement therapy use in middle-aged women. **American Journal of Epidemiology** 1994; 140:439-452.

21. Woods N. Midlife women: health care patterns and choices. In: Falik M, Collins K, eds. **Women's Health: The Commonwealth Fund Survey.** Baltimore: Johns Hopkins Press, 1996:145-174.

22. Ross ME. Approaching menopause. **Melpomene Journal** 1992;11:23-25.

23. Scalley EK, Henrich JB. An overview of estrogen replacement therapy in postmenopausal women. **Journal of Women's Health** 1993; 2:289-294.

24. Barrett-Connor E. Postmenopausal estrogen and heart disease. **Atherosclerosis** 1995; 118:S7-S10.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

25. Matthews KA, Kuller LH, Wing RR, Meilahan, Plantinga P. Health prior to hormone use: Matthers et al. Reply to Grodstein. **American Journal of Epidemiology** 1996; 143:893-894.

26. Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prior to use of estrogen replacement therapy, are users healthier than nonusers? **American Journal of Epidemiology** 1996; 143:971-978.

27. Cauley JA, Cummings SR, Black DM, Mascioli SR, Seeley DG. Prevalence and determinants of estrogen replacement therapy in elderly women. **American Journal of Obstetrics and Gynocology** 1990; 163:1438-1444.

28. Manolio TA, Furberg CD, Shemanski L, et al. Associations of postmenopausal estrogen use with cardiovascular disease and its risk factors in older women. **Circulation** 1993; 88:2163-2171.

29. Ravnikar VA. Compliance with hormone therapy. **American Journal of Obstetrics and Gynecology** 1987; 156:1332-1334.

30. McKinlay SM, Brambilla DJ, Pasner JG. The normal menopausal transition. **Maturitas** 1992; 14:103-115.

31. Ferguson KJ, Heogh C, Johnson S. Estrogen replacement therapy: A survey of women's knowledge and attitudes. **Archives of Internal Medicine** 1989; 149:133-136.

32. Pasley BH, Standfast SJ, Katz SH. Prescribing estrogen during menopause: physician survey of practices in 1974 and 1981. **Public Health Report** 1984; 99:424-429.

33. Kennedy DL, Baum C, Forbes MB. Noncontraceptive estrogens and progestins: use patterns over time. **Obstetrics and Gynecology** 1985; 65:441-446.

34. Posthuma WFM, Westendorp RGJ, Vandenbroucke JP. Cardioprotective effect of hormone replacement therapy in postmenopausal women: is the evidence biased? **British Medical Journal** 1994; 308:1268-1269.

35. Utian W, Schiff I. Survey on women's knowledge, information sources and attitudes to menopause and hormone replacement therapy. **Menopause: The Journal of the North American Menopause Society** 1994;1:39-48.

36. Corson SL. A practical guide to prescribing estrogen replacement therapy. **International Journal of Fertility** 1995; 40:229-227.

37. Samsioe G. Hormone replacement therapy; aspects of bleeding problems and compliance. **International Journal of Fertility** 1996; 41:11-15.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

38. Kaufert PA. A health and social profile of the menopausal woman. **Experimental Gerontology** 1994; 29:343-350.

39. Weintraub M. Intelligent noncompliance and conscious compliance. In: Lasagna L, ed. **Patient Compliance**. New York: Futura Publishing Company, 1976:40.

40. Doren M, Schneider HPG. The impact of the different HRT regimens on compliance. **International Journal of Fertility** 1996; 41:29-39.

41. Rothert M, Padonu G, Holmes-Rovner M, et al. Menopausal women as decision makers in health care. **Experimental Gerontology** 1994; 29:463-468.

42. Pilote L, Hlatky MA. Attitudes of women toward hormone therapy and prevention of heart disease. **American Heart Journal.** 1995; 129:1237-1238.

43. Walsh JME, Brown JS, Kagawa M, Grady D. Postmenopausal hormone therapy: factors influencing women's decision making. **The Journal of the North American Menopause Society** 1997; 4:39-45.

44. Col NF, Eckman MH, Karas RH, et al. Patient-specific decisions about hormone replacement therapy in postmenopausal women. **Journal of the American Medical Association** 1997; 277:1140-1147.

45. Castelli WP. Cardiovascular disease in women. **American Journal of Obstetrics and Gynecology** 1988; 158:1553-1560.

46. Statistics National Center for Health. Vital Statistics of the United States 1986. : Public Heath Service, U.S. Government Printing Office, Washington, D.C.

47. Moss AJ, Parsons VL. Current estimates for the National Health Interview Survey, United States 1995. : Department of Health Statistics, Washington D.C. U.S. Printing Office, Washington, D.C.

48. Gorodeski G. Impact of the menopause on the epidemiology and risk factors of coronary artery disease in women. **Experimental Gerontology** 1994; 9:357-375.

49. American Heart Association . *Heart and Stroke Facts: 1995 Statistical Supplement.* Dallas, Texas, 1995.

50. Haarbo J, Leth-Espensen L, Stender S, al. e. Estrogen monotherapy and combined estrogen-progestogen replacement therapy attenuate aortic accumulation of cholesterol in ovariectomized cholesterol-fed rabbits. **Journal of Clinical Investigation** 1991; 87:1174-1279.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

51. Dithurnham, Davies JA, Crump BJ, Situnayake RD, Davis M. The use of different lipids to express serum tocopherol: lipid ratios for the measurement of vitamin E status. **Annals of Clinical Biochemistry** 1986; 23:514-520.

52. Wenger NK, Speroff L, Packard B. Cardiovascular health and disease in women. **New England Journal of Medicine** 1993; 329:247-256.

53. Bell MR, Grill DE, Garratt KN, Gersh BJ, Holmes DR. Long-term outcome of women compared with men after successful coronary angioplasy. **Circulation** 1995; 91:2876-2881.

54. Hartz RS. Gender differences in cardiovascular outcomes. **International Journal of Fertility** 1996; 41:101-108.

55. Brown SA, Hutchinson R, Morrisett J, et al. Plasma lipid, lipoprotein cholesterol, and apoprotein distribution in selected US communities. **Arteriosclerosis and Thrombosis** 1993;13:1139-1158.

56. Campos H, McNamara JR, Wilson PWF, Ordovas J, Schaefer EJ. Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women. **Journal of Clinical Endocrinology and Metabolism** 1988; 67:30-35.

57. Ettinger WH, Wahl PW, Kuller LH. Lipoprotein lipids in older people: Results from Cardiovascular Health Study. **Circulation** 1992; 86:858.

58. Cauley JA, Gutai JP, Kuller LH, Powell JG. The relation of endogenous sex steroid hormone concentrations to serum lipid and lipoprotein levels in postmenopausal women. **American Journal of Epidemiology** 1990; 132:884-894.

59. Kuller LH, Gutai JP, Meilahn E, Matthews KA, Plantinga P. Relationship of endogenous sex steroid hormones to lipids and apoproteins in postmenopausal women. **Arteriosclerosis** 1990; 10:1058-1066.

60. Jensen J, Nilas L, Christiansen C. Influence of menopause on serum lipids and lipoproteins. **Maturitas** 1990; 12:321-331.

61. Miller VT. Dyslipoproteinemia in women. Special considerations. **Endocrinology Metabolic Clinics of North America** 1990; 322:173-177.

62. Kafonek SD. Postmenopausal hormone replacement therapy and cardiovascular risk reduction A review. **Drugs** 1994; 47:16-24.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

63. The Writing Group of the PEPI Trial. Effects of estrogen or estrogen/progestin regimes on heart disease risk factors in postmenopausal women: The postmenopausal estrogen/progestin interventions (PEPI) trial. **Journal of the American Medical Association** 1995; 273:199-208.

64. Bush TL, Fried LP, Barrett-Connor E. Cholesterol, lipoproteins, and coronary heart disease in women. **Clinical Chemistry** 1988; 34:B60-70.

65. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoproteins cholesterol and cardiovascular disease. Four prospective American studies. **Circulation** 1989; 79:8-15.

66. Lerner DJ, W.B. K. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26 year follow-up of the Framingham population. **American Heart Journal** 1986; 11:383-390.

67. Brunner D, Weisbort J, Meshulam N, et al. Relation of serum total cholesterol and high-density lipoprotein cholesterol percentage to the incidence of definite coronary events: Twenty-year follow up of the Donolo-Tel Aviv prospective artery disease study. **American Journal of Cardiology** 1987; 59:1271-1276.

68. Kris-Etherton PM, Krummel D. Role of nutrition in the prevention and treatment of coronary heart disease in women. **Journal of the American Dietetic Association** 1993; 93:987-993.

69. Bush T. The epidemiology of cardiovascular disease in postmenopausal women. In: Flint M, Kroneneberg F, Utian W, eds. **Multidisciplinary Perspectives on Menopause**. New York: Annals of the New York Academy of Sciences, 1990:263-271.

70. Jenner JL, Ordovas JM, Lamon-Fava S, et al. Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels: the Framingham offspring study. **Circulation** 1993; 87:1135-1141.

71. Conrad J, Basdevant A, J. T, et al. Cardiovascular risk factors and combined estrogen-progestin replacement therapy: a placebo-controlled study with nomegestrol acetate and estradiol. **Fertility and Sterility** 1995; 64:957-962.

72. Kim CJ, Jang HC, Cho DH, Min YK. Effects of hormone replacement therapy on lipoprotein (a) and lipids in postmenopausal women. **Arteriosclerosis and Thrombosis** 1994;14:275-281.

73. Perrone G, Stefanutti C, Galoppi P, et al. Effect of oral and transdermal hormone replacement therapy on lipid profile and Lp(a) level in menopausal women with hypercholesterolemia. **International Journal of Fertility** 1996; 41:509-515.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

74. Orth-Gomer K, Mittleman MA, Schenck-Gustafsson K, et al. Lipoprotein(a) as a determinant of coronary heart disease in young women. **Circulation** 1997; 95:329-334.

75. Krummel DA. Cardiovascular Disease. In: Debra A. Krummel PMK-E, ed. **Nutrition in Women's Health**. Gaithersburg, Maryland: Aspen Publishers, Inc., 1996:383-417.

76. Hanson MJS. Modifiable risk factors for coronary heart disease in women. **American Journal of Critical Care** 1994; 3:177-186.

77. Hormone replacement therapy: weighing the hazards and rewards. **Clinician Reviews** 1997; 7:53-72.

78. Thompson W. Estrogen replacement therapy in practice: trends and issues. **American Journal of Obstetrics and Gynecology** 1995; 173:990-993.

79. Hubert HB, Eaker ED, Garrison RJ, Castelli WP. Life-style correlates of risk factor change in young adults: an eight-year study of coronary heart disease risk factors in the Framingham offspring study. **American Journal of Epidemiology** 1987; 125:812-831.

80. Lobo RA. Estrogen and cardiovascular disease. In: Flint M, Kroneneberg F, Utian W, eds. **Multidisciplinary Perspectives on Menopause**. New York: Annals of the New York Academy of Sciences, 1990:286-294.

81. Levrant SG, Barnes RB. Pharmacology of estrogens. In: Lobo RA, ed. **Treatment of the Postmenopausal Woman: Basic and Clinical Aspects**. 1 ed. New York: Raven Press, 1994:57-68.

82. Barrett-Connor E, Goodman-Gruen D. Prospective study of endogenous sex hormones and fatal cardiovascular disease in postmenopausal women. **British Medical Journal** 1995;311:1193-1196.

83. Kuhl H. Pharmacokinetics of oestrogens and progestogens. **Maturitas** 1990; 12:171-97.

84. Arafat ES, Hargrove JT, Maxson WS, Desiderio DM, Wentz AC, Andersen RN. Sedative and hypnotic effects of oral administrations of micronized progesterone may be mediated through its metabolites. **American Journal of Obstetrics and Gynecology** 1988;159:1203-1209.

85. Jewelewicz R. New developments in topical estrogen therapy. **Fertility and Sterility** 1997;67:1-12.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

86. Cutler WR, Garcia C. Hormone replacement therapy I. In: Cutler WG, C., ed. **Menopause: A Guide for Women and Those Who Love Them**. New York-London: W.W. Norton & Company, 1992:120-148.

87. Love SM. Hormones: The menu of options. Dr. Susan Love's Hormone Book. First ed. New York: Random House, 1997:269-270.

88. Colditz GA, Hankinson SE, Hunter DJ, et al. The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. **The New England Journal of Medicine** 1995; 332:1589-1593.

89. Hickok LR, Toomey C, Speroff L. A comparison of esterified estorgens with and without methyltestosterone: effects on endometrial histology and serum lipoproteins in postmenopausal women. **Obstetrics and Gynecology** 1993; 82:919-924.

90. Casson PR, Carson SA. Androgen replacement therapy in women: myths and realities. **International Journal of Fertility** 1996; 41:412-422.

91. The Coronary Drug Projedt Research Group. The coronary drug project: Initial findings leading to modifications of its research protocol. **Journal of the American Medical Association** 1970; 214:1303-131312.

92. Cotton P. Women's health initiative leads the way as research begins to fill gender gaps. **Journal of the American Medical Association** 1992;267:469-470.

93. Adams MR, Kaplan JR, Koritnik DR. Psychosocial influences on ovarian endocrine and ovulatory function cynomolgus monkeys (*Macaca fascicularis)*. **Physiology Behavior** 1985;35:935-940.

94. Adams MR, Kaplan JR, Manuck SB, et al. Inhibition of coronary artery athreoscelerosis by 17-beta estradiol in ovariectiomized monkeys. Lack of effect of added progesterone. **Circulation** 1990;10:1051-1057.

95. Knopp RH. Cardiovascular effects of endogenous and exogenous sex hormones over a woman's lifetime. **American Journal of Obstetrics and Gynecology** 1988;158:1630-1643.

96. Barrett-Connor E. Endogenous and exogenous sex hormones and heart disease in men and women. In: Zipes DP, Rowlands DJ, eds. **Progress in Cardiology**. Philadelphia: Lea & Febiger, 1991:115-126.

97. Grodstein F, Stampfer MJ, Manson JE, et al. Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. **The New England Journal of Medicine** 1996;335:453-461.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

98. Henderson BE, Paganini-Hill A, Ross RK. Estrogen replacement therapy and protection from acute myocardial infarction. **American Journal of Obstetrics and Gynecology** 1988;159:312-317.

99. Henderson BE, Paganini-Hill A, Ross RK. Decreased mortality in users of estrogen replacement therapy. **Arch of Internal Medicine** 1991;151:75-76.

100. Paganini-Hill A, Dworshy R, Krauss RM. Hormone replacement therapy, hormone levels, and lipoprotein cholesterol concentrations in elderly women. **American Journal of Obstetrics and Gynecology** 1996;174:897-902.

101. Wilson PWF, Garrison RJ, Castelli WP. Postmenopausal estrogen use, cigarette smoking, and cardiovascular morbidity in women over 50: The Framingham Study. **New England Journal of Medicine** 1985;313:1038-1043.

102. Eaker ED, Castelli WP. Coronary heart disease and its risk factors among women in the Framingham Study. In: Eaker E, Packard B., Wagner, N.K., Clarkson, T.B., Tyroler, H.A., ed. **Coronary heart disease in women**. New York: Haymarket Doyma, 1987:122-132.

103. Hennekens CH, Speizer FE, Rosner B, C.J. B, Belanger C, Peto R. Use of permanent hair dyes and cancer among registered nurses. **Lancet** 1979:1390-1403.

104. Stampfer MJ, Willett WC, Colditz GA, Rosner B, Speizer FE, Hennekens CH. A prospective study of postmenopausal estrogen therapy and coronary heart disease. **New England Journal of Medicine** 1985;313:1044-1049.

105. Stampfer MJ, Colditz GA, Willett WC, et al. Postmenopausal estrogen therapy and cardiovascular disease: ten-year follow-up from the Nurses' Health Study. **New England Journal of Medicine** 1991;325:756-762.

106. Colditz GA, Willett WC, Stampfer MJ, Rosner B, Speizer FE, Hennekens CH. Menopause and the risk of coronary heart disease in women. **The New England Journal of Medicine** 1987;316:1105-1110.

107. Grodstein F, Stampfer MJ, Colditz GA, et al. Postmenopausal hormone therapy and mortality. **The New England Journal of Medicine** 1997;336:1769-1775.

108. Sacks FM, Walsh BW. The effects of reproductive hormones on serum lipoproteins: Unresolved issues in biology and clinical practice. In: Flint M, Kroneneberg F, Utian W, eds. **Multidisciplinary Perspectives on Menopause**. New York: Annals of the New York Academy of Sciences, 1990:272-285.

109. Whitehead M. Progestins and androgens. **Fertility and Sterility** 1994;62:161S-167S.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

110. Bush TL, Miller VT. Effects of pharmacologic agents used during menopause: impact on lipids and lipoproteins. In: Mishell DR, ed. **Menopause: Physiology and Pharmacology**. Chicago: Year Book Medical Publishers, 1987:187-208.

111. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. **New England Journal of Medicine** 1991;325:1196-1204.

112. LaRosa JC. Metabolic effects of estrogens and progestins. **Fertility and Sterility** 1994;62:140S-146S.

113. Wahl P, Walden C, Knopp R, et al. Effect of estrogen/progestin potency on lipid/lipoprotein cholesterol. **New England Journal of Medicine** 1983;308:862-867.

114. Tremollieres FA, Pouilles J, Ribot CA. A prospective two-year study of progestin given alone in postmenopausal women: Effect on lipid and metabolic parameters. **American Journal of Obstetrics and Gynecology** 1995;173:85-89.

115. Miller VT, Muesing RA, LaRosa JC, Stoy DB, Fowler SE, Stillman RJ. Quantitative and qualitative changes in lipids, lipoproteins, apolipoprotein A-I, and sex hormone-binding globulin due to two doses of conjugated equine estrogen with and without progestin. **Obstetrics and Gynecology** 1994;83:173-179.

116. Nabulsi AA, Folsom AR, White A, et al. Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women. **New England Journal of Medicine** 1993;328:1069-1075.

117. Udoff L, Langenberg P, Adashi EY. Combined continuous hormone replacement therapy: a critical review. **Obstetrics and Gynecology** 1995;86:306-316.

118. Marshall E. Women's health initiative draws flak. **Science** 1993;262:838.

119. Fuller JH, Shipley MJ, Rose G, Jarrett RJ, Keen H. Mortality from coronary heart disease and stroke in relation to degree of glycaemia: the Whitehall Study. **British Medical Journal** 1983;287:867-870.

120. Stevenson JC, Crook IF, Collins P, Whitehead MI. Hormone replacement therapy and the cardiovascular system nonlipid effects. **Drugs** 1994;47:35-41.

121. Paganini-Hill A, Ross RK, Henderson BE. Postmenopausal oestrogen treatment and stroke. a prospective study. **British Medical Journal** 1988;297:519-522.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

122. Koh KK, Mincemoyer R, Bui MN, et al. Effects of hormone replacement therapy on fibrinolysis in postmenopausal women. **The New England Journal of Medicine** 1997;336:683-690.

123. Gebara OCE, Mittleman MA, Sutherland P. Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. **Circulation** 1995;91:1952-1958.

124. LaCroix KA, Bean C, Reilly R, Curran-Celentano J. The effects of hormone replacement therapy on antithrombin III and protein C levels in menopausal women. **Clinical Laboratory Science** 1997;10:145-148.

125. Penotti M, Farina M, Sironi L, et al. Long-term effects of postmenopausal hormone replacement therapy on pulsatility index of internal carotid and middle cerebral arteries. **Menopause** 1997;4:101-104.

126. Legato MJ. Coronary artery disease in women. **International Journal of Fertility** 1996;41:94-100.

127. Losordo DW, Kearney M, Kim BA, Jekanowski J, Isner JM. Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries in premenopausal women. **Circulation** 1994;89:1501-1510.

128. Samsoie G. Introduction to steroids in the menopause. **American Journal of Obstetrics and Gynecology** 1992;166:1980-1985.

129. McKeon VA. Hormone replacement therapy: evaluating the risks and benefits. **Journal of Obstetrics and Gynecology and Neonatal Nursing** 1993;23:647-657.

130. Okumura K, Yasue H, Matsuyama K, et al. Effect of acetylcholine on the highly stenotic coronary artery: difference between the constrictor response of the infarct-related coronary artery and that of the noninfarct-related artery. **Journal of the American College of Cardiology** 1992;19:752-758.

131. Bogaty P, Hackett D, Davies G, Maseri A. Vasoreactivity of the culprit lesion in unstable angina. **Circulation** 1994;90:5-11.

132. OBrien JE, Peterson ED, Keeler GP. Relation between estrogen replacement therapy and restenosis after percutaneous coronary interventions. **Journal of the American College of Cardiology** 1996;28:1111-1118.

133. Hennekens CH, Gaziano JM, Manson JE, Buring JE. Antioxidant vitamin-cardiovascular disease hypothesis is still promising, but still unproven: the need for randomized trials. **American Journal of Clinical Nutrition** 1995;62:1377S-1380S.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

134. Gey KF, Puska P, Jordan P, Moser UK. Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. **American Journal of Clinical Nutrition** 1991;53:326S-334S.

135. Gaziano JM, Hennekens CH. Vitamin antioxidants and cardiovascular disease. **Current Opinion in Lipidology** 1992;3:291-294.

136. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. **The New England Journal of Medicine** 1993;328:1450-1456.

137. Belcher JD, Balla J, Jacobs DR, Gross M, Jacob HS, Vercellotti GM. Vitamin E, LDL, and endothelium: Brief oral vitamin supplementation prevents oxidized LDL-mediated vascular injury in vitro. **Arteriosclerosis and Thrombosis** 1993;13:1779-1789.

138. Esterbauer H, Striegl G, Puhl H, Rotheneder M. Continuous monitoring of *in vitro* oxidation in human low density lipoprotein. **Free Radical Research Comms.** 1989;6:67-75.

139. Riemersma R. Antioxidant vitamins, atherosclerosis, and coronary heart disease. In: Weber PC, Leaf A, eds. **Atherosclerosis Reviews**. New York: Raven Press, Ltd., 1993:287-292.

140. Boscoboinik D, Szewczyk A, Hensey C, Azzi A. Inhibition of cell proliferation by α-tocopherol: role of protein kinase C. **Journal of Biological Chemistry** 1991:6188-6194.

141. Esterbauer H, Dieber-Rotheneder M, Striegl M, Waeg G. Role of vitamin E in preventing the oxidation of low-density lipoprotein. **American Journal of Clinical Nutrition** 1991;53:314S-321S.

142. Gaziano JM, Manson JE, Buring JE, Hennekens CH. Dietary antioxidants and cardiovascular disease. In: Sauberlich HE, Machlin LJ, eds. **Beyond Deficiency: New Views on the Function and Health Effects of Vitamins**. New York: The New York Academy of Sciences, 1992:249-259.

143. Bolton-Smith C, Woodward M, Tunstall-Pedroe H. The Scottish Heart Study. Dietary intake by food frequency questionnaire and odds ratio for coronary heart disease risk II. The antioxidant vitamins and fiber. **European Journal of Clinical Nutrition** 1992;46:85-93.

144. Stampfer MJ, Manson JE, Colditz GA, Rosner B, Willerr WC. Vitamin E consumption and the risk of coronary disease in women. **New England Journal of Medicine** 1993;328:1444-1449.

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145. Manson JE, Stampfer MJ, Willett WC, et al. A prospective study of antioxidant vitamins and incidence of coronary heart disease in women. **Circulation** 1991;84:546.

146. Wilcox JG, Hwang J, Hodis HN, Sevanian A, Stanczyk FZ, Lobo RA. Cardioprotective effects of individual conjugated equine estrogens through their possible modulation of insulin resistance and oxidation of low-density lipoprotein. **Fertility and Sterility** 1997;67:57-62.

147. van der Mooren MJ, Demacker PNM, Blom HJ, de Rijke YB, Rolland R. The effect of sequential three-monthly hormone replacement therapy on several cardiovascular risk estimators in postmenopausal women. **Fertility and Sterility** 1997;67:67-73.

148 Quinlan PM. Variation in serum and low-density lipoprotein antioxidants during the normal menstrual cycle: comparison with oral contraceptive use. : University of New Hampshire, 1995.

149. Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. **Free Radical Biology & Medicine** 1992;13:341-390.

150. Clemente C, Caruso MG, Berloco P, Giannandrea B, DiLeo A. α–Tocopherol and β–carotene serum levels in post-menopausal women treated with transdermal estradiol and oral medroxyprogesterone acetate. **Hormone Metabolism Research** 1996;28:558-561.

151. Kris-Etherton PM, Krummel D, Russell M, et al. The effect of diet on plasma lipids, lipoproteins, and coronary heart disease. **Journal of the American Dietetic Association** 1988;88:1373-1400.

152. Stampfer MJ, Rimm EB. Epidemiologic evidence for vitamin E in prevention of cardiovascular disease. **American Journal of Clinical Nutrition** 1995;62:1365S-1369S.

153. Rock CL, Jacob RA, Bowen PE. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. **Journal of the American Dietetic Association** 1996;96:693-702.

154. Kushi LH, A.R. F, Prineas RJ, Mink PJ, Wu Y, Bostick RM. Dietary antioxidant vitamins and health from coronary heart disease in postmenopausal women. **New England Journal of Medicine** 1996;334:1156-1162.

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155. Van Horn L, Balleew C, Liu K. Diet, body size, and plasma lipids/lipoproteins in young adults: differences by race and sex. **American Journal of Epidemiology** 1991;133:9-23.

156. Krummel DA, Mashaly MM, Kris-Etherton PM. Prediction of plasma lipids in a cross-sectional sample of young women. **Journal of the American Dietetic Association** 1992;92:942-948.

157. Garry PJ, Hunt WC, Koehler KM. Longitudinal study of dietary intake and plasma lipids in healthy elderly men and women. **American Journal of Clinical Nutrition** 1992;55:682-688.

158. Thompson FE, Byers T. Dietary Assessment Resource Manual. **Journal of Nutrition** 1994;124:2245S-2317S.

159. Reubinoff BE, Wurtman J, Rojansky N, et al. Effects of hormone replacement therapy on weight, body composition, fat distribution, and food intake in early postmenopausal women: a prospective study. **Fertility and Sterility** 1995;64:963-968.

160. Kuller LH, Meilahn EN, Cauley JA, Gutai JP, Matthews KA. Epidemiologic studies of menopause: changes in risk factors and disease. **Experimental Gerontology** 1994;29:495-509.

161. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. **Circulation** 1983;67:968-977.

162. Manson JE, Colditz GA, Stampfer ME. A prospective study of obesity and major weight gain and risk of coronary heart disease in women. **New England Journal of Medicine** 1990;322:882-889.

163. Blackburn GL, Kanders BS. Medical evaluation and treatment of obese patients with cardiovascular disease. **American Journal of Cardiology** 1987;60:55G-58G.

164. Manson JE, Willett WC, Stampfer MJ, et al. Body weight and mortality among women. **The New England Journal of Medicine** 1995;333:677-685.

165. Cole TG, Bowen PE, Schmeisser D. Differential reduction in plasma cholesterol by the American Heart Association phase 3 diet in moderately hyercholesterlemic, premenopausal women with different body mass indexes. **American Journal of Clinical Nutrition** 1992;55:385-394.

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166. Denke MA, Sempos CT, Grundy SM. Excess body weight. An under-recognized contributor to dyslipidemia in white American women. **Archives of Internal Medicine** 1994;154:401-410.

167. Stevenson JC, Crook D, Godsland IF. Influence of age and menopause on serum lipids and lipoproteins in healthy women. **Atherosclerosis** 1993;98:83-90.

168. Ley CJ, Lees R, Stevenson JC. Gender and menopause-associated changes in body fat distribution. **American Journal of Clinical Nutrition** 1992;55:950.

169. Bengtsson C, Bjorkelund C, Lapidus L, Lissner L. Associations of serum lipid concentrations and obesity with mortality in women: 20 year follow up of participants in prospective study in Gothenburg, Sweden. **British Medical Journal** 1993;307:1385-1388.

170. Wing RR, Matthews KA, Kuller LH, Meilahn EN, Plantinga P. Weight gain at the time of menopause. **Archeives of Internal Medicine** 1991;151:97-102.

171. Barrett-Connor E. Heart disease in women. **Fertility and Sterility** 1994;62 (Suppl.2):127S-132S.

172. Cauley JA, LaPorte RE, Sandler RB, Orchard TJ, Siemenda CW. The relationship of physical activity on high density lipoprotein cholesterol in postmenopausal women. **Journal of Chronic Disease** 1986;39:687-697.

173. Powell KE, Thompson PD, Caspersen CJ, Kendrick JS. Physical activity and the incidence of coronary heart disease. **Annual Review of Public Health** 1987;8:253-287.

174. King AC, Blair SN, Bild DE, et al. Determinants of physical activity and interventions in adults. **Medicine and Science in Sports and Exercise** 1992;24:S221-S236.

175. Blair SN, Kannel WB, Kohl HW, Goodyear N, Wilson PWF. Surrogate measures of physical activity and physical fitness. **American Journal of Epidemiology** 1989;129:1145-1155.

176. Blair SN, Kohl HW, Barlow CE. Physical activity, physical fitness, and all-cause mortality in women: do women need to be active? **Journal of the American College of Nutrition** 1993;12:368-371.

177. White MK, Yeater RA, Martin RB. Effects of aerobic dancing and walking on cardiovascular function and muscular strength in postmenopausal women. **Journal of Sports Medicine and Physical Fitness** 1984;24:159-166.

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178. Cowan MM, Gregory LW. Responses of pre- and post-menopausal females to aerobic conditioning. **Medicine and Science in Sports and Exercise** 1985;17:138-143.

179. Notelovitz M, Fields C, Caramelli K, Dougherty M, Schwartz AL. Cardiorespiratory fitness evaluation in climacteric women: Comparison of two methods. **American Journal of Obstetrics and Gynecology** 1986:1009-1013.

180. Committee American College of Sports Medicine, Preventative and Rehabilitative Exercise. Guidelines for Exercise Testing and Prescription. . 4 ed. Philadelphia: Lea & Febiger, 1991:100.

181. Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA. Physical activity and public health. A recommendation from the centers of disease control and prevention and the American college of sports medicine. **Journal of the American Medical Association** 1995;273:402-407.

182. Manson JE, Stampfer MJ, Willett WC, Colditz GA, Speizer FE, Hennekens CH. Physical activity and incidence of coronary heart disease and stroke in women. **Circulation** 1995;91:927.

183. Lemaitre RN, Heckbert SR, Psat;y BM, Siscovick DS. Leisure-time activity and the risk of non-fatal myocardial infarction in post-menopausal women. **Circulation** 1995;91:927.

184. Williams PT. High-density lipoprotein cholesterol and other risk factors for coronary heart disease in female runners. **The New England Journal of Medicine** 1996;334:1298-1303.

185. Wood PD, Stefanick ML, Williams PT, Haskell WL. The effects on plasma lipoproteins of a prudent weight-reducing diet, with and without exercise, in overweight men and women. **New England Journal of Medicine** 1991;325:461-466.

186. Blumenthal JA, Emery CF, Madden DJ, et al. Cardiovascular and behavioral effects of aerobic exercise training on healthy older men and women. **Journal of Gerontology: Medical Sciences** 1989;44:147-157.

187. Slaven L, Lee C. Mood and symptom reporting among middle-aged women: the relationship between menopausal status, hormone replacement therapy, and exercise participation. **Health Psychology** 1997;16:203-208.

188. McNair DM, Loor M, Droppleman LF. Profile of mood states. San Diego, CA: Educational and Industrial Testing Service, 1971.

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189. Hunter M. The Women's Health Questionnaire: a measure of mid-aged women's perceptions of their emotional and physical health. **Psychology and Health** 1992;7:45-54.

190. Lutter JM, Bertrand M, Strom SM, Grumstump K. Menopause and physical activity: What is the relationship? **Melpomene Journal** 1993;12:14-22.

191. Hammer M, Berg G, Lindgren R. Does physical exercise influence the frequency of postmenopausal hot flushes? **Acta Obstetrica et Gynecologica Scandinavica** 1990;69:409-412.

192. Dennerstein L, Smith A, Morse C, et al. Menopausal symptoms in Australian women. **Medical Journal of Australia** 1993;159:232-236.

193. Organization WH. Research on the menopause. Geneva, Switzerland., 1981.

194. McKeon VA. Estrogen replacement therapy . **Journal of Gerontological Nursing** 1990;16:6-11.

195. Busch CM, Zonderman AB, Costa PT. Menopausal transition and psychological distress in a nationally representative sample: Is menopause associated with psychological distress? **Journal of Aging and Health** 1994;6:209-228.

196. Rostosky SS, Travis CB. Menopause research and the dominance of the biomedical model 1984-1994. **Psychology of Women Quarterly** 1996;20:285-312.

197. Gannon L, Ekstrom B. Attitudes toward menopause: The influence of sociocultural paradigms. **Psychology of Women Quarterly** 1993;17:275-288.

198. Bell S. Changing ideas: The medicalization of menopause. **Social Science and Medicine** 1987;24:535-542.

199. Bell S. Sociological perspectives on the medicalization of menopause. In: Flint M, Kroneneberg F, Utian W, eds. **Multidisciplinary Perspectives on Menopause**. New York: Annals of the New York Academy of Sciences, 1990:173-177.

200. Nachtigall LE. The medicalization of menopause. In: Flint M, Kroneneberg F, Utian W, eds. **Multidisciplinary Perspectives on Menopause**. New York: Annals of the New York Academy of Sciences, 1990:179-180.

201. Schneider JW, Conrad P. The medical control of deviant contests and consequences. In: Roth J, ed. **Research in the Sociology of Health Care**. Greenwich, CT.: JAI Press, 1980:3.

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202. Freeman J. **Women: a feminist perspective**. Fourth ed. Mountain View, California: Mayfield Publishing Company, 1989.

203. Matthews KA, Kuller LH, Wing RR, Meilahn EN. Biobehavioral aspects of menopause: lessons from the healthy women study. **Experimental Gerontology** 1994;29:337-342.

204. Matthews KA, Wing RR, Kuller LH, Meilahn EN, Costello EJ, Caggiula AW. Influence of natural menopause on psychological characteristics and symptoms of middle-aged health women. **Journal of Consulting and Clinical Psychology** 1990;58:345-363.

205. Bromberger JT, Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prospective study of the determinants of age at menopause. **American Journal of Epidemiology** 1997;145:124-133.

206. McKinlay JB, McKinlay SM, Brambilla D. The relative contributions of endocrine changes and social circumstances to depression in mid-aged women. **Journal of Health and Social Behavior** 1987;28:345-363.

207. Neugarten BL, Kraines RJ. Menopausal symptoms in women of various ages. **Psychosomatic Medicine** 1965;27:266-273.

208. Hallstrom T, Samuelsson S. Mental health in the climacteric: the longitudinal study of women in Gothenburg. **Archeives of Sexual Behavior** 1985;130:13-18.

209. McKinlay SM, Jeffreys M. The menopausal syndrome. **British Journal of Prev. Soc.Med.** 1974;28:108-115.

210. Mendell D. A study of menopausal women. In: Offermna-Zuckerberg J, ed. **Analytic and Critical Psycho-physical passages in the life of a woman: A Psychodynamic Approach**. New York: Plenum Medical Book, 1988:183-197.

211. Sherwin BB. Sex hormones and psychological functioning in postmenopausal women. **Experimental Gerontology** 1994;29:423-430.

212. Lennon MC. The psychological consequences of menopause: the importance of timing of a life stage event. **Journal of Health and Social Behavior** 1982;23:353-366.

213. Gruchow HW, Anderson AJ, Barboriak JJ. Postmenopausal use of estrogen and occlusion of coronary arteries. **American Heart Journal** 1988;115:954-963.

214. Steinberg D, Participants W. Antioxidants in the prevention of human atherosclerosis. **Circulation** 1992;85:2338-2344.

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215. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. **Journal of the American Medical Association** 1991;265:1861-1867.

216. Delorey C. Women at midlife: women's perceptions, physicians' perceptions. **Journal of Women and Aging** 1989;1:57-69.

217. Gilligan DM, Quyyumi AA, Cannon RO. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. **Circulation** 1994;89:2545-2551.

218. Cauley JA, Gutai JP, Kuller LH, Dai WS. Usefulness of sex steroid hormone levels in predicting coronary artery disease in men. **American Journal of Cardiology** 1987;60:771-777.

219. McKinlay SM. Issues in design, measurement, and analysis for menopause research. **Experimental Gerontology** 1994;29:479-493.

220. Brody JE. Liberated at last from the myths about menopause. **New York Times** 1995.

221. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzyamatic determination of total serum cholesterol. **Clinical Chemistry** 1974;20:470-475.

222. Chung BH, Sagrest JP, Ray MJ, et al. Single vertical spin density gradient ultracentrifugation. **Methods in Enzymology** 1986;128:181-209.

223. Markwell MK, Hass SM, Bieber LL, Tolbert NE. A modification of the Lowry Procedure to simplify protein determination in membrane and lipoprotein samples. **Analytical Biochemistry** 1978;87:206-210.

224. Esterbauer H, Rotheneder M, Striegl G, et al. Vitamin E and other lipophilic antioxidants protect LDL against oxidation. **Fat Science Technology** 1989;91:316-324.

225. Cao G, Verdon CP, Wu AHB, Wang H, Prior RL. Automated assay of oxygen radical absorbance capacity with the COBAS FARA II. **Clinical Chemistry** 1995;41:1738-1744.

226. Dennerstein L, Smith AMA, Morse C. Psychological well-being, mid-life and the menopause. **Maturitas** 1994;20:1-11.

227. Sullivan JM. Coronary arteriography in estrogen-treated postmenopausal women. **Progress in Cardiovascular Disease** 1995;308:211-222.

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228. Rosenberg M. **Society and adolescent self-image**. Princeton, N.J.: Princeton University Press, 1965.

229. Egeland G, Kuller L, Matthews K, Kelsey S, Cauley J, Guzick D. Hormone replacement therapy and lipoprotein changes during early menopause. **Obstetrics and Gynecology** 1990;76:776.

230. Lievertz RW. Pharmacology and pharmacokinetics of estrogens. **American Journal of Obstetrics and Gynecology** 1987;156:1289-1293.

231. Englund DE, Johansson EDB. Plasma levels of oestrone, oestradiol and gonadotrophins in postmenopausal women after oral and vaginal administration of conjugated equine oestrogens(Premarin). **British Journal of Obstetrics and Gynecology** 1978;85:957-964.

232. Witztum JL. The oxidation hypothesis of atherosclerosis. **The Lancet** 1994;344:793-794.

233. Skafar DF, Xu R, Morales J, Ram J, Sowers JR. Clinical Review 91: Female sex hormones and cardiovascular disease in women. **Journal of Clinical Endocrinology and Metabolism** 1997;82:3913-3918.

234. Wilcox LS, Koonin LM, Pokras R, Strauss LT, Xia Z, Peterson HB. Hysterectomy in the United States, 1988-1990. **Obstetrics and Gynecology** 1994;83:549-555.

235. Langenberg P, Kjerulff KH, Stolley PD. Hormone replacement therapy and menopausal symptoms following hysterectomy. **American Journal of Epidemiology** 1997;146:870-880.

236. Carolan MT. Beyond deficiency: Broadening the view of menopause. **The Journal of Applied Gerontology** 1994;13:193-205.

237. Carlson KJ, Eisenstat SA, Ziporyn T. Hysterectomy. **The Harvard Guide to Women's Health**. Cambridge: Harvard University Press, 1996:308.

238. Rimm E, Willett WC, Hu FB, et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. **Journal of the American Medical Association** 1998;279:359-364.

239. Lobo RA. The postmenopausal state and estrogen deficiency. In: Robert Lindsay DWD, and V. Craig Jordan, ed. **Estrogens and Antiestrogens**. Philadelphia: Lippincott-Raven Publishers, 1997:63-71.

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240. Longcope C. The endocrinology of menopause. In: Lobo RA, ed. **Treatment of the Postmenopausal Woman: Basic and Clinical Aspects**. 1 ed. New York: Raven Press, 1994:47-53.

241. Petitti DB. Coronary heart disease and estrogen replacement therapy can compliance bias explain the results of observational studies? **Annals of Epidemiology** 1994;4:115-118.

242. McCann IL, Holmes DS. Influence of aerobic exercise on depression. **Journal of Personality and Social Psychology.** 1994; 46:1142-1147.

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IMAGE EVALUATION
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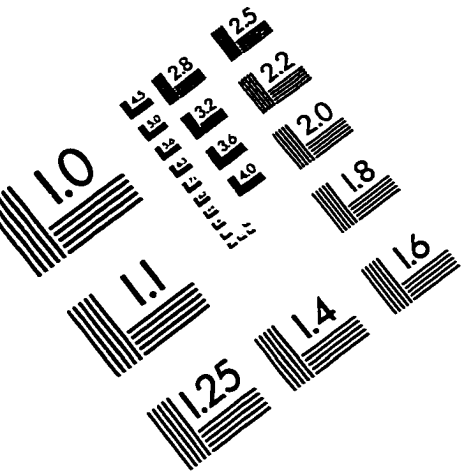

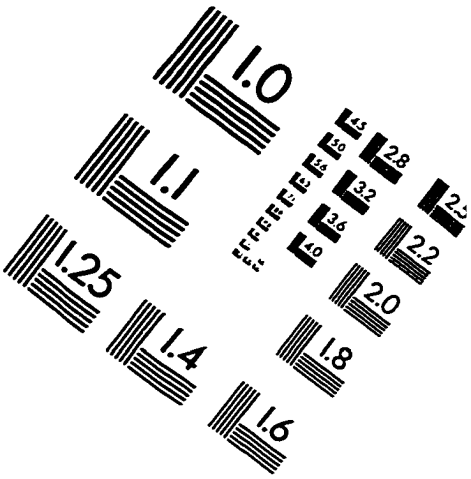

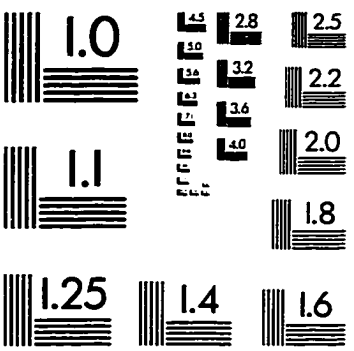

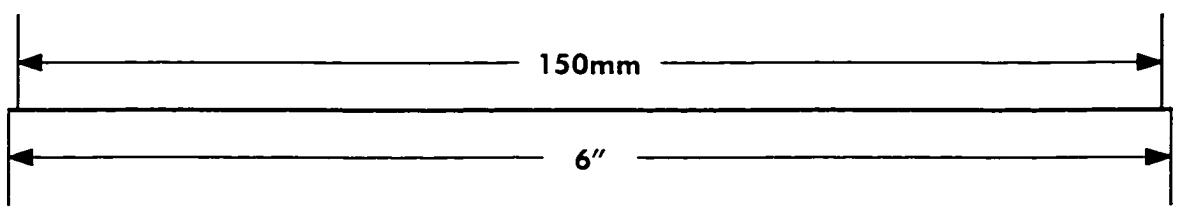

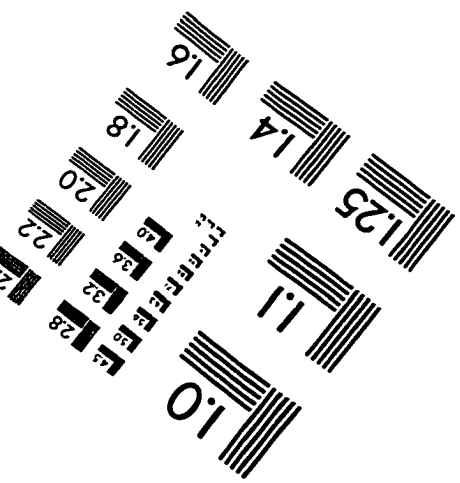

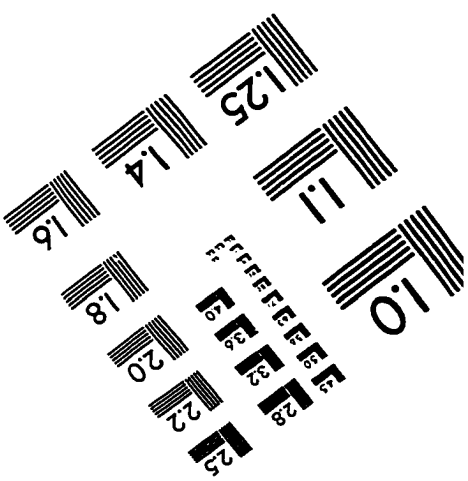

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1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989

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