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Rosemary C. Wander

The present cross-sectional study evaluated the relationship of self-reported physical activity behavior and hormone replacement therapy (HRT) with serum apolipoprotein B (apo B) and plasma apolipoprotein A1 (apo A1) concentrations in a subgroup of healthy postmenopausal women. The 78 women were distributed into the following six groups: no HRT/low physical activity (n=18), no HRT/high physical activity (n=17), oral unopposed estrogen/low physical activity (n=12), oral unopposed estrogen/high physical activity (n=7), oral estrogen + progestin/low physical activity (n=10) and oral estrogen + progestin/high physical activity (n = 14). Both the Stanford 7-day physical activity recall and the Ainsworth compendium of physical activities were used to quantify physical activity. Dietary intake was assessed using the Diet Habit Survey and baseline characteristics of the subjects were determined. Fasting blood samples were analyzed for apo B and apo A1 concentrations. Higher amounts of self-

reported physical activity (11.4 – 12.6 kcal/kg/day) were significantly ($P<0.05$) associated with lower body weights and lower body mass indexes. There was no association of HRT with apo B concentrations. However, there was suggestive evidence ($P=0.0507$) indicating that higher amounts of self-reported physical activity was associated with lower apo B concentrations. There was suggestive evidence ($P=0.056$) indicating an interaction between self-reported physical activity and HRT on plasma apo A1 concentrations. Women reporting higher amounts of physical activity and using oral unopposed estrogen possessed greater mean apo A1 levels than the women who reported similar physical activity without HRT. This trend was attenuated in the estrogen + progestin group, indicating that this form of HRT lowers the beneficial relationship of higher reported physical activity on apo A1 concentrations. Conversely, in the women characterized by low physical activity, the concentration of apo A1 was similar in both HRT groups. Although self-reported physical activity and plasma apo A1 were not significantly related, there was convincing evidence ($P=0.0001$) that HRT was associated with higher apo A1 concentrations. These data suggest that women reporting higher amounts of physical activity may have lower apo B concentrations, whereas women using HRT are likely to have higher apo A1 concentrations, indicating a complementary relationship between higher amounts of self-reported physical activity and HRT.

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Relationship of Self-Reported Physical Activity Behavior and Hormone Replacement Therapy with Apolipoprotein B and Apolipoprotein A1 in Postmenopausal Women

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Aaron D. Curtis, Author

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CONTRIBUTION OF AUTHORS

Dr. Rosemary Wander and Dr. Dan Williams were involved in the design of the study and the interpretation of the data. The blood samples were collected by Dr. Dan Williams. The assays were conducted in the laboratory of Dr. Rosemary Wander.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF APPENDICES

Relationship of Self-Reported Physical Activity Behavior and Hormone Replacement Therapy with Apolipoprotein B and Apolipoprotein A1 in Postmenopausal Women

Introduction

It is well established that the incidence of cardiovascular disease (CVD) increases in women after the onset of menopause.[1] Prior to menopause, women are four times less likely to suffer a CVD event than men. Within ten years following the onset of menopause, women are just as likely as their age-matched male counterparts to suffer a CVD event.[6,7] Despite the elevated risk for CVD events in postmenopausal women, specific interventions aimed at CVD risk factor reduction in women have not been established.

Plasma apolipoprotein B (apo B) concentrations reflect the number of atherogenic lipoprotein particles, since one molecule of apo B is present in each of the atherogenic lipoprotein particles (chylomicron, very-low-density lipoprotein, and LDL).[11,12] As a result, apo B concentration has been reported to be a stronger risk factor in case-control studies in patients with CVD than that of other plasma lipids and lipoproteins.[13-15] Apolipoprotein A1 (apo A1), the most abundant apolipoprotein in HDL, has been shown to be an important risk factor that is inversely related to CVD.[20-23]

The studies that have evaluated the influence of endurance exercise on the concentrations of apo B and apo A1 have yielded variable results in women.[33-36] The differences in the apolipoprotein responses to endurance exercise training may be due to

factors such as time of the menstrual cycle; menopausal status; use of exogenous hormones; body composition changes; and frequency, intensity, duration and overall caloric expenditure of the exercise bout.[37-40]

In spite of the overwhelming evidence from observational studies that postmenopausal women who take hormone replacement therapy (HRT), whether unopposed estrogen or a combination of estrogen and progestin, have a lower risk of CVD compared to those who do not,[41-43] the use of HRT failed to reduce the overall rate of CVD events in postmenopausal women with established coronary disease in the only large randomized clinical trial to date.[44] However, in this 4.1 year study, Hulley et al. reported a net 11% decrease in LDL-C and a 10% increase in HDL-C in the HRT group compared with the placebo group. These findings are in agreement with others where estrogen replacement therapy has been demonstrated to decrease LDL-C and to increase both HDL-C and triglycerides in postmenopausal women,[29,30,43,45-47] whereas the inclusion of progesterone tends to lower, but not cancel, the effect on HDL-C.[45] Furthermore, numerous studies have shown that HRT lowers apo B concentrations[48-53] and raises apo A1 concentrations[46,48,51-54] in postmenopausal women. Although there is substantial evidence indicating that HRT alters plasma lipids and apolipoproteins in a favorable manner, additional trials need to be completed in order to establish the relationship between HRT and CVD risk.

To date, only three intervention studies[29-31] and one cross-sectional study[32] have examined the combined effects of endurance exercise and HRT on plasma lipids in postmenopausal women. Lindheim and coworkers[29] demonstrated no added improvement in lipid measures when six months of conjugated equine estrogen and

exercise were combined compared to the effect of estrogen or exercise alone in healthy postmenopausal women. In contrast, Binder et al.[30] reported that 11 months of exercise in combination with conjugated estrogens + medroxyprogesterone significantly decreased total cholesterol and LDL-C concentrations while increasing HDL-C concentrations in healthy postmenopausal women. Further, the exercise training prevented the HRT-related increase in triglyceride concentrations suggesting a significant exercise and HRT interaction. These results suggest that CVD risk would be lowered if both exercise and HRT were combined as a treatment in this population group. Klebanoff et al.[31] reported that 12 weeks of aerobic activity did not produce any changes in blood lipid profiles in healthy postmenopausal women taking conjugated equine estrogen or not taking the hormone therapy. In a recent cross-sectional study, Haddock et al.[32] reported that higher levels of cardiorespiratory fitness (determined by maximal treadmill testing) were associated with lower total cholesterol concentrations, total cholesterol-to-HDL-C ratio and triglycerides and higher HDL-C concentrations in healthy postmenopausal women.

Previously, Williams and coworkers (Med Sci Sports Exerc 31:S289, 1999) assessed the CVD risk for high fasting insulin, low HDL-C and high fasting triglyceride levels in postmenopausal women reporting lower levels of physical activity behavior. The sample included 137 healthy postmenopausal women with three levels of HRT: no HRT, oral unopposed estrogen, and oral estrogen + progestin. The study compared the lowest tertile of physical activity versus the upper two tertiles of physical activity (combined). A Stanford 7-day physical activity recall[85] and the Ainsworth compendium of physical activities[87] were used to quantify physical activity. They observed that women reporting lower physical activity, regardless of HRT use, had approximately a

four-fold greater risk for high fasting insulin and low HDL-C as compared to women reporting higher physical activity levels. The purpose of this study was to extend their work by assessing the relationship of self-reported physical activity and three levels of HRT on apo B and apo A1 concentrations in a subgroup (n=78) of postmenopausal women who fell into the lowest and highest levels of reported physical activity.

Review of Literature

Cardiovascular Disease in Postmenopausal Women

It is well established that the incidence of cardiovascular disease (CVD) increases in women after the onset of menopause.[1] Menopause is associated with a progressive rise in low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglycerides, and generally lower levels of high-density lipoprotein cholesterol (HDL-C).[2-5] Prior to menopause, women are at a fourfold lower risk for CVD than men. Within ten years following the onset of menopause, the risk of CVD in women climbs to the level observed in men.[6,7] Despite the elevated risk for CVD events in postmenopausal women, specific interventions aimed at CVD risk factor reduction in women have not been established.

For the most part, guidelines for the prevention and management of CVD have centered around LDL-C. Increased levels of LDL-C are associated with a higher risk of CVD.[8] However, it has been reported that LDL-C is not a superior lipid risk factor in women.[9] Bass et al. reported that HDL-C and triglyceride levels were stronger lipid predictors of CVD death in women than were LDL-C and total cholesterol.[9] In support of these findings, Livshits and coworkers[10] demonstrated that HDL-C had the highest predictive value for risk of future CVD in women and men when compared to total cholesterol, blood pressure, body mass index and smoking. Neither of these studies measured apolipoprotein B (apo B) or apolipoprotein A1 (apo A1) concentrations as potential lipid risk factors for CVD.

One molecule of apo B is present in each of the atherogenic lipoprotein particles (chylomicron, very-low-density lipoprotein, and LDL) and therefore plasma apo B concentrations reflect the number of atherogenic lipoprotein particles.[11,12] As a result, apo B concentration has been reported to be a stronger risk factor in case-control studies in patients with CVD than that of other plasma lipids and lipoproteins.[13-15] In a recent cross-sectional study, Westerveld et al.[16] reported that apo B concentration was superior in predicting the presence or absence of CVD in women from the general population who were referred for angiography, compared to cholesterol, LDL-C, HDL-C, and triglyceride concentrations. Many prospective studies also support apo B as a major CVD risk factor, although the extent to which it was the strongest predictor has varied.[17-19]

The argument for apo B concentrations as the major risk factor for CVD is not without opposition. Stampfer and associates[17] determined that HDL-C concentration was a stronger predictor for risk of myocardial infarction than apo B concentration in a case-control study of men with new myocardial infarction. In another case-control study, Coleman et al.[18] reported that total cholesterol concentration was a better predictor for risk of myocardial infarction than apo B concentration in a group of middle-aged women. Lastly, Sigurdsson and coworkers reported[19] that apo A1 and apolipoprotein(a) concentrations were stronger predictors of CVD than apo B among Icelandic men.

Apo A1 is the most abundant apolipoprotein in HDL. Since many studies have reported HDL-C as a major CVD risk factor,[8-10] it would therefore seem plausible that apo A1 would mimic the same relationship. Most studies have shown an inverse relationship between apo A1 and CVD, although apo A1 was never found to be a stronger predictor of

CVD than HDL-C.[20-23] In contrast, Westerveld and coworkers[16] reported that apo A1 was not associated as a CVD predictor in women undergoing their first coronary angiography.

The Effect of Endurance Exercise on Blood Lipids, Lipoproteins and Apolipoproteins

Physical inactivity is a major CVD risk factor. Low cardiorespiratory fitness and a sedentary lifestyle are each associated with a twofold increase in CVD risk.[24] Most research examining the relationship of endurance exercise and CVD lipid risk factors have focused on men. Hence, the potential benefits of endurance exercise on lipids in older women merit further study. It is generally accepted that endurance exercise increases HDL-C concentration and decreases triglycerides while not affecting total cholesterol concentration,[25,26] although these findings are not universal. For example, two intervention studies reported that endurance exercise did not significantly change lipids in older women.[27,28] In contrast, Lindheim et al.[29] showed that six months of aerobic exercise significantly lowered total cholesterol, LDL-C and triglyceride concentrations. In support of these findings, Binder and coworkers[30] reported a significant reduction in total cholesterol and LDL-C concentrations with 11 months of endurance exercise training in healthy postmenopausal women. Lastly, in a recent cross-sectional study, Haddock et al.[32] reported that higher levels of cardiorespiratory fitness (determined by maximal treadmill testing) were associated with significantly lower total cholesterol and triglyceride concentrations and higher HDL-C concentration after controlling for hormone replacement therapy (unopposed estrogen or a combination of estrogen and progestin), age, year of testing, and blood glucose level.

There have been less data reported on the influence of endurance exercise on apo B and apo A1 in women. Despres et al.[33] reported lower apo B concentrations and higher apo A1 levels after 14 months of aerobic exercise training in obese premenopausal women. Blumenthal and coworkers[34] demonstrated that 12 weeks of aerobic exercise in premenopausal and postmenopausal women resulted in no change in apo B levels, while apo A1 levels significantly increased. In contrast, Grandjean et al.[35] reported a significant increase in apo B concentrations and no change in apo A1 levels after 12 weeks of aerobic exercise in premenopausal and postmenopausal women, whereas Stefanick and coworkers[36] observed no significant changes in apo B and apo A1 in men and postmenopausal women, with low HDL-C and high LDL-C, after one year of aerobic exercise alone. The differences in blood lipid, lipoprotein and apolipoprotein responses to endurance exercise training may be due to factors such as time of the menstrual cycle, menopausal status, use of exogenous hormones, body composition changes, and frequency, intensity, duration and overall caloric expenditure of the exercise bout.[37-40]

The Effect of HRT on Blood Lipids, Lipoproteins and Apolipoproteins

In spite of the overwhelming evidence from observational studies that postmenopausal women who take hormone replacement therapy (HRT), whether unopposed estrogen or a combination of estrogen and progestin, have a lower risk of CVD compared to those who do not,[41-43] the use of HRT failed to reduce the overall rate of CVD events in postmenopausal women with established coronary disease in the only large randomized clinical trial to date.[44] However, in this 4.1 year study, Hulley et al.[44]

did report a net 11% decrease in LDL-C and a 10% increase in HDL-C in the HRT group compared with the placebo group. These findings are in agreement with others where estrogen replacement therapy has been demonstrated to decrease LDL-C concentration and to increase both HDL-C and triglyceride concentrations in normocholesterolemic and hypercholesterolemic postmenopausal women,[29,30,43,45-47] whereas the inclusion of progesterone tends to lower, but not cancel, the effect on HDL-C.[45] Although there is substantial evidence indicating that HRT alters plasma lipids and lipoproteins in a favorable manner, additional trials need to be completed in order to establish the relationship between HRT and CVD risk.

HRT has also been reported to have a positive influence on apo B and apo A1 concentration. Many studies have shown a reduction in apo B levels as a result of HRT use in postmenopausal women.[48-53] In support of this finding, Haines and associates[49] demonstrated that 2 mg per day of oral estradiol and 5 mg medroxyprogesterone acetate for 12 days per month decreased mean apo B concentrations from 116.1 to 111.6 mg/dl in an open longitudinal study. Numerous studies have also demonstrated that HRT significantly increases apo A1 levels in postmenopausal women.[30,46,48,51-54] In support of this response, Folsom et al.[52] reported that starting estrogen plus progestin therapy raised the mean concentration of apo A1 to a similar degree as did starting estrogen alone (13.5 mg/dL and 11.1 mg/dL, respectively) over a three-year interval in postmenopausal women.

The Effect of the Combination of Endurance Exercise and HRT on Blood Lipids, Lipoproteins and Apolipoproteins

Previous studies have examined the individual effects of exercise or HRT on plasma lipids. Only three intervention studies[29-31] and one recent cross-sectional study[32] have examined the combined effects of both factors in healthy postmenopausal women. Binder et al.[30] reported that 11 months of endurance exercise training (two months of flexibility exercises followed by nine months of walking, jogging, and/or stair climbing three or more days per week for 45 minutes per day at 65-85% of maximal heart rate) significantly reduced total cholesterol and LDL-C concentrations, while no change was observed in HDL-C or triglyceride concentrations in postmenopausal women. Further, HRT (0.625 mg/day of conjugated estrogens and 5 mg/day of medroxyprogesterone acetate for 13 days every third month) significantly lowered LDL-C and raised HDL-C and triglyceride concentrations, but had no significant effect on total cholesterol concentration. Exercise in combination with HRT significantly decreased total cholesterol and LDL-C while increasing HDL-C. Further, the exercise training prevented the HRT-related increase in triglyceride concentrations suggesting a significant exercise and HRT interaction. In comparison, Lindheim et al.,[29] who studied the combined effects of six months of aerobic exercise (walking on a treadmill and pedaling a stationary bicycle three times per week for 30 minutes at 70% of maximal heart rate) and estrogen replacement therapy (0.625 mg of conjugated equine estrogen on days 1 - 25 of each month) on lipids in postmenopausal women, reported that exercise alone resulted in a significant decrease in total cholesterol, triglyceride and LDL-C concentrations.

Moreover, a reduction in LDL-C concentration and an increase in HDL-C and apo A1 concentrations were observed in the group using HRT alone. Furthermore, it was reported that although apolipoprotein A1 significantly increased from baseline at three and six months in the exercise group, the estrogen alone, and the estrogen with exercise, apolipoprotein B did not significantly change from baseline or as compared to the control group. Lastly, the study demonstrated no added improvement in lipid measures when conjugated equine estrogen and exercise were combined compared to the effect of estrogen or exercise alone. In contrast, Klebanoff et al.[31] reported that 12 weeks of aerobic activity failed to significantly affect total cholesterol, triglyceride, HDL-C and LDL-C concentrations in healthy postmenopausal women receiving or not receiving conjugated equine estrogen (0.625 mg per day), although body weight appeared to be a modulating factor.

In the first cross-sectional study that assessed the association of cardiorespiratory fitness on blood lipid and fibrinogen concentrations while controlling for HRT, Haddock and associates[32] reported that the postmenopausal women using no HRT had a significantly lower HDL concentration and a higher total cholesterol-to-HDL-C ratio (TC/HDL) than those taking HRT (unopposed estrogen or a combination of estrogen + progestin). Further, they showed a significant two-way interaction between HRT and the upper and lower cardiorespiratory fitness levels (divided into quintiles based on total treadmill time to exhaustion) for TC/HDL. Higher cardiorespiratory fitness was associated with significantly higher TC/HDL in the no HRT group and the unopposed estrogen group, but not in the estrogen + progestin group.

While all four of these studies provided varying results, it is important to note that three of the four failed to perform statistical analyses for dietary intakes of the subjects. Binder et al. reported that the subjects completed 7-day food records at baseline and upon completion of the study, but nowhere do they mention the results.[29] Lindheim et al.[30] and Haddock et al.[32] failed to give any reference to a diet evaluation on their subjects. Klebanoff et. al.[31] was the only group that conducted a 72-hour dietary record four times throughout the study and analyzed the data.

The Influence of Diet on Blood Lipids and Lipoproteins

It is well known that high dietary intake of saturated fatty acids increases serum total cholesterol concentration.[55-58] Saturated fatty acids raise total cholesterol by primarily elevating LDL-C concentrations relative to the effects of polyunsaturated fatty acids, *cis* monounsaturated fatty acids and carbohydrates.[55] Although saturated fatty acids generally increase LDL-C concentrations, different saturated fatty acids vary in their influence on LDL-C. For example, palmitic acid (16:0), the primary saturated fatty acid in the U.S. diet, has been shown to significantly increase LDL-C concentrations.[55] Two other saturated fatty acids, myristic (14:0)[58] and lauric (12:0),[56,59] also have been reported to significantly increase LDL-C concentrations. In contrast, stearic acid (18:0) has been shown not to raise LDL-C concentrations relative to unsaturated fatty acids.[60,61] One explanation for this finding is that stearic acid is converted readily into oleic acid (18:1), a *cis* monounsaturated acid, once it enters the body.[62] Generally, dietary *cis* monounsaturated fatty acids and polyunsaturated fatty acids have been shown not to

increase LDL-C concentration when added to a low-fat diet, although an increase in HDL-C concentration is observed.[63,64] However, dietary *trans* fatty acids, produced by hydrogenation of vegetable oils, have been reported to increase LDL-C concentration.[56,65,66] *Trans* fatty acids account for only about 6% of the dietary fatty acids in the typical American diet.[67]

Controlled feeding studies have shown that a lower intake of total dietary fat and saturated fatty acids decreases total cholesterol and LDL-C concentrations.[68,69] For example, a Step I diet (30% total kcal from fat, with 8% - 10% from saturated fatty acids and less than 300 mg cholesterol) has been shown to lower total cholesterol and LDL-C concentrations by approximately 7% - 9% compared with the average American diet.[68,69] Furthermore, a Step II diet (30% total kcal from fat, with less than 7% from saturated fatty acids and less than 200 mg cholesterol) has been reported to decrease total cholesterol and LDL-C concentrations by 10% - 20%.[68,69] Lower-fat diets seem to lower LDL-C concentration only when accompanied by a reduction in saturated fatty acids.[70] Barr et al.[70] reported that a reduction of dietary fat intake from 37% to 30% failed to lower total cholesterol and LDL-C concentrations in men unless the reduction in total fat was achieved by decreasing saturated fatty acids from 14% to 9%. Controlled feeding studies have also demonstrated that low-fat (less than 25% of total kcal) diets raise triglycerides and lower HDL-C concentrations when body weight is maintained.[69,71] In contrast, many studies have reported that low-fat diets, when accompanied with weight loss and often exercise, result in a reduction of total cholesterol, LDL-C and triglyceride concentrations and no change in HDL-C concentration.[72-75]

Measurement of Apo B and Apo A1 Concentrations

The analysis of apo B and apo A1 is performed by immunochemical techniques. When these apolipoproteins were first measured, electroimmunoassay (EIA), radial immunodiffusion (RID) and radioimmunoassay (RIA) were used, although all of these methods have drawbacks. Both EIA and RID require large amounts of antisera and have matrix difficulties. In addition, they are difficult to automate for measuring large sample sizes.[76] In contrast, RIA requires smaller amounts of antisera and avoids the matrix problems seen with EIA and RID. Furthermore, RIA has better precision and greater sensitivity than the other two methods, although it is relatively difficult to automate.[77]

Advances in the production of monospecific and monoclonal antibodies and the development of enzymic labels led to the establishment of enzyme-linked immunosorbent assays (ELISA) and more accurate nephelometric and turbimetric assays.[78] ELISA is moderately precise and is highly sensitive, although requires the use of large dilutions. Most routine clinical laboratories find it difficult to automate this particular method, so immunonephelometric or immunoturbidimetric assays are most often chosen. Both of these methods possess the ability to measure large numbers of samples and are much easier to automate than ELISA. In addition, the assays are generally accurate and can be calibrated against internationally acceptable reference materials for both apo B and apo A1.[79,80] However, both methods require large amounts of antisera and are likely to encounter matrix effects.[76] Furthermore, grossly lipemic samples can cause interference with both assays.[81]

The most significant issue encompassing the measurement of apo B and apo A1 has been lack of standardization, which has led to analytical variation among labs.[82] This matter was finally addressed with the adoption of international reference materials endorsed by the World Health Organization in 1994. There are several problems with attempting to standardize these particular apolipoproteins. Since the lipid moiety and apolipoproteins are closely associated with each other, there is a concern that this commonality may affect the immunoreactivity of the apolipoproteins. Furthermore, antibodies raised against whole lipoproteins sometimes fail to react against delipidated apolipoproteins. In addition, antibodies to synthetic apolipoprotein peptides do not consistently react with apolipoproteins associated with lipids in lipoproteins. The apo B assay is at a distinct advantage since each molecule of apo B is contained in a separate particle. In contrast, more than one molecule of apo A1 can be associated with a single HDL particle. Preanalytical and storage factors can initiate a release of apo A1 molecules from HDL particles, which have multiple molecules of apo A1, associated with them. If an apo A1 assay is sensitive to particle number, these additional molecules will reduce the accuracy of the method.[76]

Both apolipoproteins pose their unique difficulties in the development of primary standards and reference methods. Apo B tends to produce aggregates; hence, developing a stable lyophilized reference material has been a problem.[83] To overcome this difficulty, a primary standard based on ultracentrifugally separated LDL (1.030 - 1.050 kg/L) was produced. Then, a secondary standard consisting of a liquid reference material was created based on the first standard. This procedure was successful in the production of a standard material, although there is significant variation between assay methods. It has

been found that immunonephelometric and immunoturbidimetric assays produce higher apo B values than RIA and ELISA. Conversely, the purification of Apo A1 to create a stable lyophilized standard has proven much less difficult. The greatest challenge is obtaining an acceptable antisera for apo A1 measurements.[84] Many of the protein's immunoreactive sites cannot be found, due to the close association of apo A1 and lipids.[76]

Although numerous assays have been developed for measuring apolipoproteins, the immunoturbidimetric method was chosen for the present study for several reasons. The method is standardized, affordable, generally accurate and is widely used among research laboratories. Since a consensus regarding a standard method for quantitating apolipoproteins has not been attained, further research needs to be completed in order to resolve this issue.

Materials and Methods

Overview

The present study was a cross-sectional study assessing the relationship of self-reported physical activity behavior and hormone replacement therapy (HRT) on apolipoprotein B (apo B) and apolipoprotein A1 (apo A1) concentrations in older women. The present sample included 78 healthy postmenopausal women with no individual history of cardiovascular disease or diabetes and who reported the lowest and highest levels of reported physical activity, as previously defined (Williams et al. Med Sci Sports Exerc 31:S289, 1999). Furthermore, 43 of the subjects used HRT (oral unopposed estrogen [n = 19] or oral estrogen + progestin [n = 24]), while the remainder (n = 35) did not.

Subjects

The present sample of subjects is a subsample from a larger parent study examining the hormonal and behavioral determinants of the insulin resistant metabolic syndrome. The parent study was conducted by Dr. Dan P. Williams from the Department of Exercise and Sport Science at Oregon State University (Corvallis, OR). In the parent study, postmenopausal women were screened for inclusion and exclusion criteria from interested telephone respondents to print media articles describing the study. In the parent study, a total of 140 subjects were measured, and 283 subjects were ineligible after screening. The exclusion criteria used in the subject sample selection included: a prior

history of a cardiovascular disease event (stroke or myocardial infarction), diagnosis of cardiovascular/peripheral vascular disease or unstable angina, percutaneous transluminal angiography or a coronary artery bypass graft, diagnosis of insulin-dependent diabetes mellitus, early surgical menopause (<40 years of age), use of non-oral HRT, heavy smoking (>10 cigarettes per day), excessive alcohol consumption (> four drinks per day), corticosteroid medications, thyroid dysfunction/thyroid medication, and uric acid metabolic dysfunction/gout medication. To be included in the study, women had to be postmenopausal and 50 - 78 years of age. In the parent study, blood was collected from 135 out of the original 140 women. For the present study, the 78 subjects were chosen who had blood drawn, consumed diets that contained 20 - 30% of total kilocalories from fat (determined by the Diet Habit Survey[88]) and who fell into the lowest (1.7 – 3.0 kcal/kg/day) and highest (11.4 – 12.6 kcal/kg/day) tertiles of reported physical activity, as previously defined (Williams et al. Med Sci Sports Exerc 31:S289, 1999). The lower fat diets (20 - 30% fat range) were chosen from the survey to help eliminate possible confounding factors relating to the two extreme diets (37% and 10% fat). The two extreme physical activity tertiles were chosen to maximize the behavior differences in physical activity, which in turn, improves the ability to determine whether significant interactions between physical activity and HRT exist for apo B and apo A1. Out of the 78 samples, 43 of the women used HRT, while 35 did not. The HRT group was further divided into two subgroups: one with oral unopposed estrogen therapy (n = 19), and the other with oral estrogen + progestin therapy (n = 24). To determine the main and interactive effects of self-reported physical activity and HRT on blood apolipoproteins, the subjects were distributed into the following six groups: no HRT/low physical activity

(n = 18), no HRT/high physical activity (n = 17), oral unopposed estrogen/low physical activity (n = 12), oral unopposed estrogen/high physical activity (n = 7), oral estrogen + progestin/low physical activity (n = 10) and oral estrogen + progestin/high physical activity (n = 14).

Physical Activity

Physical activity was assessed by the interviewer-administered, Stanford 7-day physical activity recall.[85] A copy of the form is given in **Appendix A**. This assessment was chosen because it focuses on quantifying the amount, frequency, and duration of those activities which are of a moderate or high level of intensity. The Postmenopausal Estrogens/Progestins Intervention (PEPI) Study reported that only those activities that were of moderate or high intensities were associated with biological CVD risk factors in postmenopausal women.[86] In the parent study, caloric expenditure was calculated by estimating the energy expenditure for each specific activity using the Ainsworth Compendium,[87] rather than assuming a constant MET level for each of the three intensity categories as per the original recall protocol.[85] The Ainsworth Compendium is a coding scheme for classifying physical activity rate by energy expenditure.[87]

Diet

Dietary information was assessed using The Diet Habit Survey, developed by the Lipid-Atherosclerosis Nutrition Staff at Oregon Health Sciences University.[88] A copy of the survey is given in **Appendix A**. Nutritional supplements and medications were

recorded on the day that the blood was drawn. The subjects also reported their use of supplements and medications for the three days prior to the draw.

Sample Collection

The blood samples were collected in plasma EDTA (1 g/L) Vacutainer® tubes after the subjects had fasted overnight for 12 hours. Plasma tubes were then centrifuged at 1500 x g for 15 minutes to separate the plasma from the blood cells. After separation, the plasma was subdivided and placed in specific, labeled vials and stored at -70^0 C for 20 months prior to analysis. The blood samples were also collected in serum Vacutainer® tubes containing a gel clot activator. The tubes were allowed to sit for 30 minutes for coagulation to occur and then centrifuged for 15 minutes. The serum was subdivided and placed in specific, labeled vials and stored at -70^0 C for 19 months prior to analysis.

Laboratory Procedures

All of the assays were performed in the Lipid Laboratory at Oregon State University. Serum apo B concentrations were determined using an immunoturbidimetric procedure in a commercial diagnostic kit (Sigma Diagnostics, St. Louis, MO - No. 357). Briefly, 0.5 ml of antibody reagent was added to 5 µl of each calibrator, commercial controls (I and II, Sigma Diagnostics) and sample (in duplicate) and allowed to incubate at room temperature for six minutes. Apo B formed an insoluble complex resulting in turbidity of the mixture, proportional to the apo B concentration. The turbidity was

measured using a Shimadzu UV160U Spectrophotometer (Columbia, MD) at 340 nm. The concentration of apo B was then determined from a calibration curve (**Appendix B**) obtained from the five apo B calibrators available from Sigma. A detailed description of this method is given in **Appendix B**. Plasma apolipoprotein A1 (apo A1) concentrations were determined using an International Federation of Clinical Chemistry standardized turbidimetric immunoassay (Wako Diagnostics, Richmond, VA - No. 991-27201). In the procedure, 750 µl of buffer was added to 9 µl of each sample (in duplicate), a single level calibrator (Wako Diagnostics - No. 992-27591) and a control (in duplicate) and incubated for five minutes at 37°C. Then 78 µl of antibody reagent was added to each cuvette to yield an insoluble aggregate that caused increased turbidity. The turbidity was measured optically using a Shimadzu UV160U Spectrophotometer (Columbia, MD) at 700 nm. The concentration of apo A1 was calculated using simple proportion analysis against the assigned calibrator value. A detailed account of the method is given in **Appendix B**.

Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA) with physical activity and HRT as the two factors. Specifically, HRT included the following three levels: no HRT, unopposed estrogen, and estrogen + progestin; while physical activity included the following two levels: high and low. This analysis enabled us to examine the independent effects of HRT and physical activity, as well as the interactive effect of HRT and physical activity on apo B and apo A1. SAS 6.11 (SAS Institute Inc., Cary, NC.

1985) was used to perform the analysis. Data were expressed as mean ± standard error and $P < 0.05$ was considered significant.

Results

Subjects

The baseline characteristics of the subjects are presented in **Table 1.** The women in the low physical activity groups reported physical activity expenditures of 1.7 – 3.0 kcal/kg/day, whereas the women in the high physical activity groups reported physical activity expenditures of 11.4 – 12.6 kcal/kg/day. There were no significant interactions between self-reported physical activity and HRT among the groups with respect to all of the subject characteristics. Furthermore, HRT was not significantly associated with any of the subjects' characteristics. Higher amounts of self-reported physical activity were significantly ($P<0.05$) associated on average with lower body weights and lower body mass indexes.

Dietary characteristics of the women are presented in **Table 2.** All of the subjects had lower fat diets that contained 20 - 30 % of total kilocalories from fat. In addition, 53 of the women (68%) reported present consumption of alcohol, and 21 subjects (27%) had past smoking behavior. Ten women (13%) were taking anti-hypertensive medications, while five women (6%) were taking lipid medications. Out of the 78 subjects, 45 of them (58%) had a family history of myocardial infarction.

Table 1. Baseline Characteristics of the 78 Healthy Postmenopausal Women[1]

Physical Activity	Low			High			P-value		
Variable	No HRT (n=18)	E[2] (n=12)	E + P[3] (n=10)	No HRT (n=17)	E (n=7)	E + P (n=14)	PA	HRT	INT[4]
Age (y)	60.3 ± 1.5	62.4 ± 2.2	62.7 ± 2.2	63.9 ± 2.0	65.3 ± 2.7	61.1 ± 2.0	0.3502	0.6490	0.3808
Menopause age (y)	49.2 ± 0.8	44.2 ± 1.7	51.1 ± 1.3	46.7 ± 1.9	47.3 ± 2.3	48.8 ± 1.5	0.6864	0.0677	0.2055
Height (cm)	164.9 ± 1.5	161.4 ± 2.0	164.0 ± 1.2	160.7 ± 1.3	163.3 ± 2.5	159.0 ± 1.8	0.1004	0.7198	0.1618
Weight (kg)	72.4 ± 3.8	72.4 ± 4.5	70.2 ± 4.0	60.9 ± 2.5	63.1 ± 4.6	59.1 ± 2.8	0.0012	0.7374	0.9580
BMI[5] (kg/m^2)	26.8 ± 1.5	27.6 ± 1.3	26.1 ± 1.4	23.5 ± 0.8	23.5 ± 1.2	23.4 ± 1.2	0.0040	0.8600	0.8841
PAE[6] (kcal/kg/day)	3.0 ± 0.8	1.7 ± 1.3	2.7 ± 0.8	12.6 ± 5.6	11.4 ± 3.9	11.7 ± 4.8	0.000	0.474	0.925

[1]Values are means ± SEM, except for PAE (means ± SD)
[2]Oral unopposed estrogen group
[3]Oral estrogen + progestin group
[4]Interaction between physical activity (PA) and hormone replacement therapy (HRT)
[5]Body mass index
[6]Physical activity expenditure as previously defined by Williams et al. (Med Sci Sports Exerc 31:S289, 1999)

Table 2. Dietary Characteristics[1] of the 78 Healthy Postmenopausal Women[2]

Physical Activity	Low			High		
Nutrient Category[3]	No HRT (n=18)	E[4] (n=12)	E + P[5] (n=10)	No HRT (n=17)	E (n=7)	E + P (n=14)
Fat (% calories)						
30	50%	75%	70%	53%	29%	36%
25	39%	25%	30%	41%	71%	50%
20	11%	0%	0%	6%	0%	14%

[1]Data from the Diet Habit Survey
[2]Values, from total scores, are presented in percentage of total subjects/group who fell into each nutrient category
[3]The nutrient categories correspond with each other. Fat (30, 25, 20 % calories) corresponds to Cholesterol (<300, <200, <100 mg/day); Saturated fat (10, 8, 5 % calories); Cholesterol-Saturated Fat Index (37, 28, 16); Carbohydrate (55, 60, 65 % calories), and Protein (15, 15, 15 % calories)
[4]Oral unopposed estrogen group
[5]Oral estrogen + progestin group

Serum Apolipoprotein B

Table 3 lists the relationship of self-reported physical activity and/or HRT with apo B and apo A1 concentrations. There was not a significant interaction between physical activity and HRT on serum apo B concentrations. Therefore, significant differences of main effects were evaluated. There was no evidence to support that HRT was associated with mean apo B values. However, there was suggestive evidence (P = 0.0507) indicating that higher amounts of self-reported physical activity were associated with lower apo B concentrations. The women in the high physical activity groups had mean apo B values 11% lower than their female counterparts in the low physical activity groups (58 ± 4.5 and 66 ± 4.4, respectively).

Plasma Apolipoprotein A1

The relationship of self-reported physical activity and/or HRT with apo A1 values is listed on **Table 3**. There was suggestive evidence (P = 0.056) indicating an interaction between self-reported physical activity and HRT on plasma apo A1 levels (**Figure 1**). Higher amounts of self-reported physical activity and oral unopposed estrogen seemed to interact in a beneficial manner, since women in this group possessed greater mean apo A1 levels than the women of similar reported physical activity without HRT. This trend attenuated in the estrogen + progestin group, indicating that this form of HRT lowers the beneficial relationship of higher reported physical activity on apo A1 concentrations. In contrast, in the women characterized by low physical activity, the concentration of apo A1 was similar in both HRT groups. Although self-reported physical activity and plasma apo A1 were not significantly related, there was convincing evidence (P = 0.0001) that HRT was associated with higher apo A1 concentrations. The HRT groups (oral unopposed estrogen only and oral estrogen + progestin) were estimated to have had 17% higher mean plasma apo A1 values compared to the no HRT groups.

Table 3. Relationship of Self-Reported Physical Activity and/or HRT with Apo B and Apo A1 Concentrations[1]

Physical Activity	Low			High			P-value		
Variable	No HRT (n=18)	E[3] (n=12)	E + P[4] (n=10)	No HRT (n=17)	E (n=7)	E + P (n=14)	PA	HRT	INT[2]
Apo B (mg/dL)	62 ± 4.1	69 ± 4.5	66 ± 4.7	60 ± 4.1	59 ± 5.3	56 ± 4.2	0.0507	0.7571	0.6023
Apo A1 (mg/dL)	152 ± 3.6	180 ± 3.5	184 ± 6.2	162 ± 4.4	191 ± 6.3	175 ± 3.9	0.3330	0.0001	0.0558

[1]Values are means ± SEM
[2]Interaction between physical activity (PA) and hormone replacement therapy (HRT)
[3]Oral unopposed estrogen group
[4]Oral estrogen + progestin group

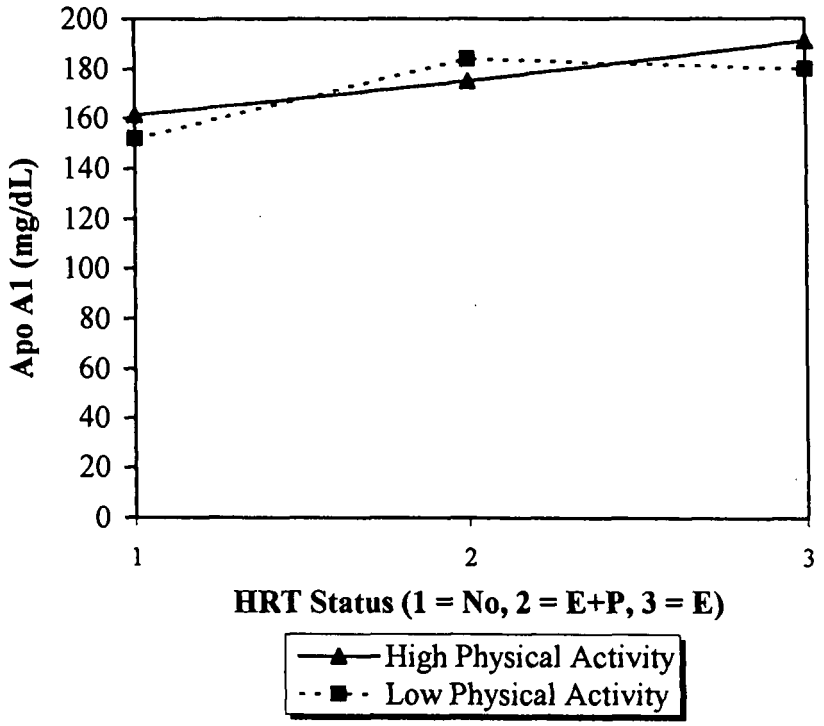

Figure 1. Relationship of Self-Reported Physical Activity and HRT with Apo A1 Concentrations

Discussion

Since it has been shown that diet can have a profound effect on blood lipids and lipoproteins,[57,63,64,69] it is important that studies examining the relationship of physical activity and HRT on CVD control for dietary intake. It is our recommendation that dietary intake be used as a covariate in the statistical analyses to help determine if part of the differences observed are due to varying dietary patterns of the subjects. Although there are many methods for collecting and evaluating dietary data, it is generally recommended to use a multiple-day food record if feasible. The multiple-day food record (usually 3 to 9 days) has been shown to be one of the most valid tools to assess dietary intake.[89,90] Subjects can be instructed on how to keep food records, which minimizes recording errors and reduces recall errors because foods are recorded soon after consumption. There are potential disadvantages associated with using food records, including required literacy skill of individuals to record data, necessary time to educate the individuals in keeping accurate records, potential confounding variable of recording information, and substantial cost and time to analyze the data.

However, in the present study, a brief dietary questionnaire (the Diet Habit Survey) was used to assess individual dietary behaviors.[88] The Diet Habit Survey is a 32-item eating behavior test that was developed with a white, middle-class population. The questions are grouped into six summary scores: a cholesterol-saturated fat score, a carbohydrate score, a beverage score, a salt score, a restaurant and recipe score, a seafood score and a total score. The summary and total scores are categorized into the present

U.S. (37% fat) and four fat diets (30% fat, 25% fat, 20% fat, and 10% fat). Although the survey has demonstrated strong test-retest reliability, its measures are not quantitatively meaningful and it lacks the ability to assess the entire diet of an individual.[91] Thus, the present study excluded the data from the Diet Habit Survey in the statistical analyses, although originally we attempted to control the diet by excluding subjects who fell outside the 20 - 30% fat range.

To date, only three intervention studies have evaluated the combined effects of chronic exercise and HRT on lipids and lipoproteins in postmenopausal women.[29-31] In addition, only one recent cross-sectional study has examined the relationship of cardiorespiratory fitness (determined by maximal treadmill testing) on blood lipid and fibrinogen concentrations while controlling for HRT (unopposed estrogen and estrogen + progestin) in postmenopausal women.[32] The present cross-sectional study is the first to evaluate the relationship of self-reported physical activity behavior and HRT (unopposed and estrogen + progestin) on two independent apolipoprotein CVD risk factors (apo B and apo A1) in healthy postmenopausal women.

Previous studies that have examined the influence of chronic exercise on apo B and apo A1 concentrations in postmenopausal women have yielded variable results.[29,34-36] The results of the present study indicated that higher self-reported physical activity was suggestively ($P = 0.0507$) associated with lower apo B concentrations in healthy postmenopausal women. In support of this finding, Despres et al.[33] reported lower apo B concentrations after 14 months of aerobic exercise training in obese premenopausal women, although postmenopausal women were not included in the study. In contrast to this finding, several studies have demonstrated no significant changes in apo B with

exercise training in postmenopausal women.[29,34,36] Grandjean and coworkers[35] reported a significant increase in apo B concentrations after 12 weeks of exercise training.

In the present study, self-reported physical activity was not associated with mean apo A1 values. This finding was in accordance with Grandjean et al.[35] and Stefanick and associates[36] who reported that apo A1 concentrations were not significantly changed after aerobic exercise training (12 weeks and 1 year, respectively) in postmenopausal women. In contrast to this finding, Blumenthal et al.[34] reported that 12 weeks of aerobic training significantly increased apo A1 concentrations in postmenopausal women. Reasons for the variable apolipoprotein responses to endurance exercise training may be due to factors such as time of the menstrual cycle, menopausal status, use of exogenous hormones, body composition changes, and frequency, intensity, duration and overall caloric expenditure of the exercise bout.[37-40]

It has been well established that HRT, whether unopposed estrogen or estrogen + progestin, significantly lowers apo B concentrations[48-53] and raises apo A1 concentrations[46,48,51-54] in postmenopausal women. The present study demonstrated that there was a strong association ($P = 0.0001$) between HRT (both unopposed estrogen and estrogen + progestin) use and higher apo A1 concentrations. In contrast, one of the most surprising findings in this study was the lack of an association with HRT and apo B, although Lindheim et al.[29] reported that HRT (0.625 mg conjugated equine estrogen) did not significantly change apo B in a six month intervention study. It was hypothesized that HRT would be beneficially associated with both apolipoproteins and that the combination of higher reported physical activity and HRT would have an additive positive relationship.

The only evidence indicating an interaction between self-reported physical activity and HRT in the present study, although suggestive (P = 0.056), was on plasma apo A1 concentrations. Higher amounts of reported physical activity seemed to interact with unopposed estrogen therapy in a beneficial manner, since women in this group possessed greater mean apo A1 concentrations than the women of similar reported physical activity without HRT. This trend was attenuated in the estrogen + progestin group, indicating that the addition of progestin to estrogen therapy lowered the beneficial relationship of higher reported physical activity on mean apo A1 values. Conversely, the subjects characterized by lower physical activity had similar mean apo A1 values in both HRT groups. In contrast to this relationship, Lindheim et al.[29] reported no such interaction between exercise training and HRT (conjugated equine estrogen) on apo A1 in their group of healthy postmenopausal women.

Williams et al. (Med Sci Sports Exerc 31:S289,1999) previously measured total cholesterol, LDL-C, HDL-C and triglyceride concentrations in the parent study of the present study. A two-way ANOVA was conducted on the subgroup of the parent study contained in the present study. **Appendix C** lists the relationship of self-reported physical activity and/or HRT with plasma total cholesterol, LDL-C, HDL-C and triglyceride concentrations. We had hypothesized that apo B and apo A1 concentrations would be related to reported physical activity and HRT in a manner similar to LDL-C and HDL-C concentrations, respectively. Physical activity was significantly associated with HDL-C and triglyceride concentrations. The present study failed to observe a significant association of reported physical activity with apo A1 concentrations, although a suggestive interaction between reported chronic physical and HRT was shown. Since

most studies have shown that apo A1 was never found to be a stronger predictor of CVD than HDL-C,[20-23] a possible explanation for this discrepancy is that HDL-C concentration was more highly related to physical activity. HRT was significantly associated with only HDL-C concentrations. This finding supports the present study since HRT was significantly related to apo A1. There was no evidence to support an interaction between reported physical activity and HRT on these lipid and lipoprotein variables. The most intriguing finding was that reported physical activity was not associated with LDL-C, whereas the present study demonstrated a suggestive association with apo B. A plausible explanation for the observed differences is that apo B concentration is a more direct measure of LDL particle number compared to the measurement of LDL-C concentration, since apo B concentrations reflect the number of atherogenic lipoprotein particles.[11,12]

Conclusions

In summary, the present study showed that higher amounts of self-reported physical activity behavior (11.4 – 12.6 kcal/kg/day) and HRT are associated with complementary benefits on apo B and apo A1 concentrations in healthy postmenopausal women. These data suggest that women reporting higher physical activity behaviors may have lower apo B concentrations, whereas women using HRT are likely to have higher apo A1 concentrations. The results in the present study also suggest a beneficial interaction between higher reported physical activity behaviors and oral unopposed estrogen with apo A1 concentrations. Since both of these apolipoproteins are important CVD risk factors, these results suggest that a recommendation of higher physical activity and HRT may aid in the prevention of CVD. It is recommended that further studies, such as a randomized clinical trial with known dietary intakes, address this association of physical activity and HRT on CVD risk factors in this healthy population group.

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APPENDICES

APPENDIX A

EVALUATION FORMS

Endocrine and Metabolism Laboratory
Oregon State University
Department of Health and Human Performance

7-Day Physical Activity Recall

Acrostic ☐☐☐☐☐☐-☐☐
Name _____
Birthdate _____
Weight _____
Date _____

Day of the week form completed: _____

1. Were you employed in the last 7 days (work & volunteering)? ☐ Yes ☐ No
2. How many total days of the last 7 did you work outside the home? ☐ no. of days (round to nearest day)
3. How many total hours did you work in the last seven days? ☐☐ at work ☐☐ at home ☐☐ total hours
4. What days of the week do you consider to be your weekend or non-work days? For most people this would be Saturday and and Sunday but it may be different for you.

   ☐ Sunday ☐ Monday ☐ Tuesday ☐ Wednesday ☐ Thursday ☐ Friday ☐ Saturday
5. If you did not work your usual week, why did you work less than usual?

   _____

   _____
6. For the past seven days, and thinking only about activities that are at least of **moderate** intensity how many days did you do activity or exercise that added up to at least 30 minutes each day? ☐ number of days (0 to 7)

7-day recall — explain segments of day
am - wake-lunch
pm - lunch-dinner
eve - dinner-sleep

go to PAR work →

Acrostic ☐☐☐☐☐☐-☐☐

One Week Ago

Yesterday

	Sleep							
Morning	Moderate							
	Hard							
	Very Hard							

1) work pd & volunt

2) sleep week
sleep weekend
naps

3) what did you do
where did you go
work
household
leisure

morn
pm
eve

4) lastly,
any other activities

Calculated Energy Expenditure ____.__ kcal/kg/day

Acrostic ☐☐☐☐☐☐☐☐

		One Week Ago						Yesterday
	Sleep							
Afternoon	Moderate							
	Hard							
	Very Hard							

1) work pd & volunt

2) sleep week
sleep weekend
naps

3) what did you do
where did you go
work
household
leisure

morn
pm
eve

4) lastly,
any other activities

Calculated Energy Expenditure ____.__ kcal/kg/day

Acrostic ☐☐☐☐☐☐-☐☐

One Week Ago

Yesterday

Evening								
	Sleep							
	Moderate							
	Hard							
	Very Hard							

1) work pd & volunt

2) sleep week
sleep weekend
naps

3) what did you do
where did you go
work
household
leisure

morn
pm
eve

4) lastly,
any other activities

Calculated Energy Expenditure ____.__ kcal/kg/day

Acrostic ☐☐☐☐☐☐-☐☐

12 Months Physical Activity Recall

1. Thinking about the things you usually did **at work** during the last 12 months, how would you describe the kind of physical activity you performed?

   _____ Inactive _____ Light _____ Moderate _____ Heavy _____ Not applicable

2. Thinking about the things you usually did **in your home** during the last 12 months, how would you describe the kind of physical activity you performed?

   _____ Inactive _____ Light _____ Moderate _____ Heavy

3. Thinking about the things you usually did **in your leisure time** during the last 12 months, how would you describe the kind of physical activity you performed?

   _____ Inactive _____ Light _____ Moderate _____ Heavy

Inactive	Light	Moderate	Heavy
sitting	teaching	mail carrier	regular vigorous activity
standing quietly	light gardening	construction	
	light regular household		carrying heavy boxes
deskwork		brisk walking	strenuous farm work
	walking >10min	recreational tennis	strenuous gardening
reading	leisure bicycle ride	swimming	
watching TV	fishing	moderate housework	jogging
quiet pursuits	bowling	moderate gardening	singles tennis
	golf		high-intensity aerobics

Acrostic ☐☐☐☐☐☐☐☐

7. Was this a typical week in terms of your usual pattern of activity or exercise?

   ☐ Yes

   ☐ No Were you more or less active in the past week than you usually are? ☐ More ☐ Less

3-Month Physical Activity Recall

8. During your **work week**, on average how many hours per day do you spend sitting quietly? ☐☐ average hours per day
   (e.g., watching TV, working at a desk or computer, eating, or reading) **(consider all waking time - before work and after)**

   During your **weekend**, on average how many hours per day do you spend sitting quietly? ☐☐ average hours per day
   (e.g., watching TV, working at a desk or computer, eating, or reading) **(consider all waking time - before work and after)**

9. How many flights of stairs do you climb up each day? (1 flight = 10 steps) ☐☐ number of flights

10. If you had to add together the **total minutes you spend walking** during the day, how many minutes would that be? Remember, add up your **actual walking time** and don't add in the time spent just standing. Include your to and from walking and any fitness walking. Don't try to remember every step, just give a general idea of the time spent walking. ☐☐☐ total minutes per day

11. What is your usual pace of walking? Mark **one** only.

    ☐ Casual or strolling (less than 2 miles per hour)
    ☐ Fairly brisk (3 to 4 miles per hour)
    ☐ Average or normal (2 to 3 miles per hour)
    ☐ Brisk or striding (4 miles per hour or faster)

12. Do you regularly do strength and flexibility exercises like sit-ups, push-ups, yoga, or stretching?

    ☐ Yes How many days per week do you do these exercises? ☐ number of days (0-7) ☐☐☐ total minutes ea session

    ☐ No

Acrostic ☐☐☐☐☐☐-☐☐

Interviewer Evaluation

1. Were there any problems with this survey?

Yes No Explain

2. Do you think this was a valid interview?

Yes No Explain

3. List any activities reported by participant which you don't know how to classify

THE DIET HABIT SURVEY

(A Quiz to Determine Your Diet Composition)

This questionnaire will help evaluate your current eating habits and compare them with the goals of a lower fat, higher carbohydrate eating style. The results will allow us to identify eating habit changes that can help you move closer to the goals. Slow, steady change is the path to permanent change.

_____

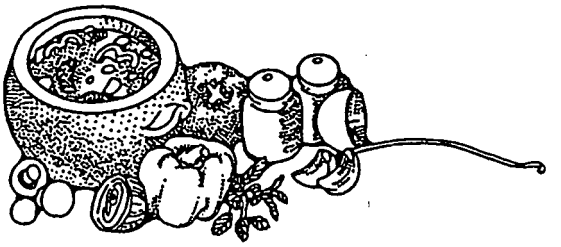
Patient's Name

_____

Date

DIRECTIONS

- For each question, indicate the choices that best describe your eating habits **during the last month.**
- *YOU MAY SELECT MORE THAN ONE CHOICE FOR A QUESTION.* **Do not score the questionnaire.**
- Check with the person who shops and cooks -- it will help with the accuracy.
- Bring the completed quiz to your appointment. A dietitian will:
  1) Score the quiz and compute your diet composition
  2) Estimate how much you can lower your blood cholesterol level by diet
  3) Provide you with low-fat product information and recipes



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MEAT, FISH AND POULTRY

Consider your eating habits during the last month. For each question, circle as many numbers as apply.

1. **Which type of ground meat do you usually eat?** office use only
   1 Regular hamburger (30% fat)
   2 Lean ground beef (25% fat)
   3 Extra lean/ground chuck (20% fat)
   4 Super lean/ground round (15% fat)
   5 Ground sirloin (10% fat), ground turkey breast, ground chicken breast
   6 **Eat no ground meat** Score_____

2. **Which best describes your typical lunch? "Lunch meat" means ham, bologna, salami, pastrami, etc.**
   1 Cheeseburger, pizza, typical cheeses, egg dishes (egg salad, quiche, frittata, etc)
   2 Sandwich (lunch meat, hamburger, etc), meat or chicken entree (plain or fried)
   3 Sandwich (tuna, peanut butter, chicken or turkey lunch meat/light mayo, fish), hot dog (reg or turkey), vegetarian dishes
   4 Tuna sandwich/mayo 1 gm fat or less/tbsp, Gardenburger, entree (fish [not fried], small bits of chicken or meat)
   5 Salad (low-cal dressing), low-fat vegetarian dishes, low-fat yogurt, hot dog (0-2 gm fat), sandwich/mayo 1 gm fat or less/tbsp (thinly sliced deli meats, fat free lunch meat)
   6 Fat free vegetarian dishes, salad (fat free dressing), Gardendog, Garden Vegan (fat free burger), nonfat yogurt Score_____

3. **Circle all of the choices that reflect the entree at your main meal.**
   1 Cheese (Cheddar, Jack, etc), eggs, organ meats (liver, etc), pizza, vegetarian dishes once a week or more
   2 Beef, lamb, pork or ham once a week or more
   3 Very lean red meat (top round or flank steak), veal, venison or elk once a week or more
   4 Chicken, turkey, rabbit, crab, lobster or shrimp twice a week or more
   5 Fish, scallops, oysters, clams, low-fat vegetarian dishes twice a week or more
   6 Fat free vegetarian dishes, fat free seafood dishes every day Score_____

4. **Estimate the number of ounces of meat, cheese, fish *and* poultry you eat in a typical day. *Include all meals and snacks.***
   To guide you in your estimate (a piece the size of a deck of cards = 3 oz):

1 wiener	*= 1 1/2 oz*		
4 strips bacon	*= 1 oz*	*1 chicken thigh*	*= 2-3 oz*
1 small burger patty	*= 3-4 oz*	*1/2 chicken breast*	*= 3 oz*
meat in most sandwiches	*= 2-3 oz*	*1 average T-bone steak*	*= 8 oz*
1 slice cheese	*= 1 oz*	*1-inch cube cheese*	*= 1 oz*

   1 Eleven or more ounces a day
   2 Nine to 10 ounces a day
   3 Six to 8 ounces a day
   4 Four to 5 ounces a day
   5 Up to 1 ounce cheese *or* 3 oz lean meat, poultry, shrimp, crab, lobster *or* 6 oz fish, clams, oysters, scallops a day
   6 Up to 3 ounces shrimp, crab, lobster *or* 6 ounces fish, clams, oysters, scallops a day *or* **none** Score_____

5. **Which of these have you eaten in the past month?**
   1 Bacon, sausage
   2 Canadian bacon, turkey or chicken sausage
   4 Vegetarian sausage (soy)
   5 Garden Sausage
   6 **None** Score_____

TOTAL SCORE (MEAT, FISH AND POULTRY)_____

DAIRY PRODUCTS AND EGGS

Consider your eating habits during the last month. For each question, circle as many numbers as apply.

6. Which do you usually use for drinking (don't forget lattes) or cooking? office use only

1 Whole milk
2 Two percent milk
4 One percent milk, buttermilk, nondairy beverages (Edensoy, Rice Dream, etc)
5 Skim (nonfat) milk, light nondairy beverages (Edensoy light, Rice Dream light, etc) or **none**

Score_____

7. Which toppings do you use?

1 Sour cream (real or imitation including IMO), whipped cream
2 Light sour cream, Cool Whip
3 Light Cool Whip, regular cottage cheese, whole milk yogurt
4 Low-fat yogurt , Dream Whip, low-fat cottage cheese
5 1% fat cottage cheese
6 Nonfat yogurt, nonfat sour cream, nonfat cottage cheese or **none**

Score_____

8. Which frozen desserts are you most likely to eat at least once a month?

1 Ice cream
3 Ice milk, most soft ice cream, frozen yogurt (cream added)
4 Sherbet, low-fat frozen yogurt
5 Nonfat frozen yogurt, sorbets, ices, Popsicles or **none**

Score_____

9. Which kind of cheese do you use for snacks or sandwiches?

1 Cheddar, Swiss, Jack, Havarti, Brie, Feta, Montrachet, cream cheese, cheese slices, cheese spreads
2 Part-skim mozzarella, light cream cheese/Neufchatel, Lappi, string cheese, Weight Watchers, light Cheddar, light Jack (Kraft Light Naturals, Alpine Lace-Lo, Velveeta Light or other part-skim cheeses)
4 Jarlsberg Lite, low-cholesterol "filled" cheese (Hickory Farms Lyte)
5 Light part-skim mozzarella, low-fat ricotta, Light Laughing Cow, Lite-Line
6 Fat free cheeses (Cheddar, Jack, ricotta, cream, Healthy Choice, Alpine Lace, etc) or **none**

Score_____

10. Which kind of cheese do you use in cooking (casseroles, vegetables, etc)?

1 Cheddar, Swiss, Jack, Brie, Feta, Montrachet, cream cheese, processed cheese (Velveeta or American)
3 Part-skim mozzarella, light cream cheese, Lappi, Weight Watchers, light Cheddar, light Jack, (Kraft Light Naturals, Alpine Lace-Lo, Velveeta Light or other part-skim cheeses)
4 Jarlsberg Lite, low-cholesterol "filled" cheese (Hickory Farms Lyte)
5 Light part-skim mozzarella, low-fat ricotta, Lite-Line
6 Fat free cheeses (Cheddar, Jack, ricotta, cream, Healthy Choice, Alpine Lace, etc) or **none**

Score_____

11. Check the type and number of "visible" eggs you eat (scrambled, fried, etc).

1 Six or more whole eggs a week
2 Three to five whole eggs a week
3 One to two whole eggs a week
4 One whole egg a month
5 Egg white, egg substitute (Nulaid, Egg Beaters, Scramblers, Second Nature, etc) or **none**

Score_____

12. Check the type of eggs usually used in food prepared at home or bought in grocery stores.

1 Whole eggs or mixes containing whole eggs (complete pancake mix, slice-and-bake cookies, etc)
3 Combination of egg white, egg substitute and whole egg
5 Egg white, egg substitute or **none**

Score_____

TOTAL SCORE (DAIRY PRODUCTS AND EGGS)_____

FATS AND OILS

Consider your eating habits during the last month. For each question, circle as many numbers as apply.

13. Which kinds of fats are used most often to cook your food (vegetables, meats, etc)? office use only

1 Butter, shortening (with animal fat), lard, bacon grease, chicken fat
2 Shortening (with vegetable fat)
3 Tub or stick margarine (all except canola), vegetable oil (soybean, cottonseed)
4 Vegetable oil (safflower, corn, olive), tub or stick margarine (canola)
5 Vegetable oil (canola)
6 **None** or use nonstick cooking spray

Score_____

14. How much of these "added" fats do you eat in the typical day: peanut butter, margarine, mayonnaise, or salad dressing (including those made with olive oil)?

Examples of amounts people often use:

on toast: 2 tsp margarine
on sandwiches: 6 tsp mayonnaise
6 tsp peanut butter
2 tsp margarine
on salads: 12 tsp salad dressing
on potatoes: 3 tsp margarine
on vegetables: 3 tsp margarine
on pasta, rice: 3 tsp margarine or oil
on pasta, etc: 6 tsp pesto

1 Ten teaspoons or more
2 Eight to 9 teaspoons
3 Six to 7 teaspoons
4 Four to 5 teaspoons
5 Three teaspoons
6 **None**

Do not count fat free products

Score_____

15. How often do you eat potato chips, corn or tortilla chips, fried chicken, fish sticks, French fries, doughnuts, other fried foods, croissants or Danish pastries?

1 Two or more times a day
2 Once a day
3 Two to 4 times a week
4 Once a week
5 Less than twice a month
6 **Never**

Do not count fat free products

Score_____

16. Which best describes the amount of margarine, butter, peanut butter, mayonnaise or cream cheese that you put on breads, muffins, bagels, etc?

1 Average (1 teaspoon or more per serving)
2 Lightly spread (can see through it)
4 "Scrape" (can barely see it)
5 **None**

Do not count fat free products

Score_____

17. Which kind of salad dressings do you use?

1 Real mayonnaise
2 Miracle Whip, French, Roquefort and blue cheese dressings
3 Ranch, vinegar and oil, Russian, Thousand Island and Italian dressings
4 Light mayo, Miracle Whip Light, Ranch Dressing (buttermilk and light mayo or Miracle Whip Light)
5 Best Food's Low-Fat Mayo (1 gm fat/tbsp), low-cal salad dressing, Ranch Dressing (buttermilk and low-fat yogurt)
6 Fat free mayonnaise, fat free Miracle Whip, fat free salad dressings, Ranch Dressing (buttermilk and nonfat yogurt), vinegar, lemon juice or **use no salad dressing**

Score_____

TOTAL SCORE (FATS AND OILS)_____

SWEETS AND SNACKS

Consider your eating habits during the last month. For each question, circle as many numbers as apply.

18. How often do you eat desserts or baked goods (sweet rolls, doughnuts, muffins, scones, cookies, cakes)? office use only

1 Once a day
2 Five to 6 times a week
3 Three to 4 times a week *Do not count fat free versions*
4 Two times a week
5 One time a week
6 **Never**

Score_____

19. Which of the following baked goods have you eaten as a dessert or snack in the last month?

1 Croissants, cheesecake, typical cakes including carrot cake with cream cheese frosting
2 Pies, cookies, cupcakes, muffins, scones
4 Low-fat muffins, desserts made using low-fat recipes, low-fat cookies (fig bars, ginger snaps, Snackwell's)
5 Fat free desserts including angel food cake, fat free cookies (Snackwell's)
6 Fruit or **never eat baked goods listed above**

Score_____

20. Which of the following snacks have you eaten in the last month?

1 Chocolate, frosted doughnut, commercial popcorn, typical homemade popcorn
2 Nuts, plain doughnut, potato chips, tortilla chips, Cheetos, party/snack crackers, microwave popcorn, French fries, peanut butter
4 Light microwave popcorn, lightly buttered popcorn (1 tsp margarine for 3 cups popcorn), low-fat crackers (soda, graham)
5 Baked tortilla chips, baked potato chips, pretzels, fat free soda crackers and other fat free crackers
6 Fruit, vegetables or **do not eat snacks**

Score_____

TOTAL SCORE (SWEETS AND SNACKS)_____

GRAINS, BEANS, FRUITS AND VEGETABLES

Consider your eating habits during the last month. For this part of the quiz, list the number of servings of the following foods you eat each day or week, as specified for the question.

office use only

21. How many pieces of fruit or cups of fruit juice do you consume a dey ? (not "fruit-flavored" drinks)

_____ cups or pieces Score (cups x 5)_____

22. How many cups of vegetables do you eat e day (tossed salad, cooked vegetables, soups, casseroles, etc)? (A typical serving elze for tossed salad is 1 to 1 1/2 cups)

_____ cups Score (cups x 5)_____

23. How many cups of legumes do you eat e week (refried beans, split peas, white beans, black beens, blackeye peas, lentils, chili, etc)?

_____ cups Score (cups x 5)_____

24. List the number of servings of the following you ate last week. (A typical cereal bowl holde 1 1/2 to 2 cups; people typically eat 9 to 12 cups of popcorn).

	Amount eaten LAST WEEK
cooked cereal	_____ bowls/week
ready-to-eat cereal	_____ bowls/week
English muffin	_____ #/week
hamburger bun	_____ #/week
bagel	_____ #/week
Pita or pocket bread	_____ #/week
eight-inch tortilla	_____ #/week
plain popcorn (4 cups/serving)	_____ servings/week
fat free or low-fat muffin	_____ muffins/week
cornbread	_____ pieces/week

Total_____ Score (svgs x 1.2)_____

	Amount eaten LAST WEEK
bread or toast	_____ slices/week
dinner or hard roll	_____ rolls/week
French bread	_____ slices/week
four-inch pancake	_____ pancakes/week
low-fat crackers such as soda, etc (8/serving)	_____ servings/week
rice cakes (3/serving)	_____ servings/week
pretzels (1 cup or 1 large soft)	_____ cups or #/week

Total_____ Score (svgs x 0.7)_____

25. How many servings of grains end potatoes did you eat last week? Be sure to count these foods when they ere in e mixed dish (casserole, burrito, etc). This includes breakfast, lunch and dinner.

	Number of servings eaten LAST WEEK
macaroni, spaghetti and other pastas	_____ cups/week
mashed potato	_____ cups/week
baked potato	_____ large potato/week
rice, corn, bulgur, barley, other grains	_____ cups/week

Score_____

Score: (cups macaroni, etc x 1.5) + (cups mashed potato x 1.5) + (number baked potatoes x 2) + (cups rice, corn, etc x 2)

TOTAL SCORE (GRAINS, BEANS, FRUITS AND VEGETABLES)_____

BEVERAGES

Consider your eating habits during the last month. For each question, circle as many numbers as apply.

26. Which of the following reflects your habits regarding alcoholic beverages? **office use only**

1 drink = *12 ounces beer*
1 1/2 ounces whiskey, gin, rum, etc
4 ounces wine
1 ounce liqueur

1 One or more drinks a day
2 Four to 6 drinks a week
3 Three drinks a week
4 One to 2 drinks a week
5 One to 3 drinks a month
6 **Do not drink alcoholic beverages**

Score_____

27. Which of the following reflects your habits regarding soda pop, sweetened seltzers, sports drinks, fruit punch, etc?

12 ounce can = 1 1/2 cups
16 ounce bottle = 2 cups
32 ounce bottle = 4 cups

1 One or more cups a day or 7 cups a week ***Do not count sugar free (diet) drinks***
2 Four to 6 cups a week
3 Three cups a week
4 One to 2 cups a week
5 **None** or less than one cup a week

Score_____

28. How much coffee do you drink? This includes espressos, lattes, etc.

1 Six or more cups a day
3 Four to 5 cups a day
4 One to 3 cups a day
5 **None** or less than 1 cup a day

Score_____

TOTAL SCORE (BEVERAGES)_____

8

SALT

Consider your eating habits during the last month. For each question, circle as many numbers as apply.

29. Which type of "salt" do you normally use? office use only

1 Regular salt, sea salt, flavoring salts (seasoned salt, garlic salt, onion salt, celery salt, lemon pepper, etc), regular soy sauce
3 Combination of regular and Lite Salt
4 Lite Salt, lower-sodium soy sauce, reduced-sodium flavoring salts
5 None or salt substitute (100% potassium chloride)

Score_____

30. How often do you add salt to your food at the table?

1 Always
2 Frequently
4 Occasionally
5 Never

Score_____

31. Which type of salt and how much do you use in cooking potatoes, rice, pasta, vegetables, meat, casseroles and soups?

1 Regular salt (typical amount) or eat in restaurants 4 or more times a week
2 Regular salt (1/2 typical amount) or Lite Salt (typical amount)
4 Lite Salt (1/2 typical amount)
5 None, salt-free products (Mrs. Dash, etc) or salt substitute

Score_____

32. Which type of cereals do you use?

1 Typical dry cereals (sweetened or unsweetened) or cereals cooked with regular salt (typical amount)
3 Combination of typical dry cereals and salt-free dry cereals (Shredded Wheat, Puffed Wheat, Puffed Rice) or cereals cooked with regular salt (1/2 typical amount) or Lite Salt (typical amount)
5 Eat salt-free dry cereals (Shredded Wheat, Puffed Wheat, Puffed Rice, etc) or cereals cooked without salt *or* do not eat cereal

Score_____

33. How often do you use typical canned, bottled or packaged foods: salad dressings, salsa, picante sauce, ketchup, cured meats (lunch meat, ham, etc), vegetables, soups (remember chicken broth), chili, entrees, beans and sauces?

1 More than 15 times a week or eat in restaurant 4 or more times a week
2 Ten to 14 times a week
3 Six to 9 times a week
5 Five times a week or less

Score_____

TOTAL SCORE (SALT)_____

RESTAURANTS AND RECIPES

Consider your eating habits during the last month. For each question, circle as many numbers or check the choices that apply.

34. How often do you eat breakfast at a restaurant or cafeteria? office use only

1 More than twice a week
2 Once or twice a week
3 Once a week if you eat low-fat (unbuttered toast or English muffin, oatmeal)
5 Less than once a month
6 **Never**

Score_____

35. How often do you eat lunch at a restaurant or cafeteria or eat "take out"?

1 Daily
2 Five days a week
3 Two to four days a week
4 One day a week
5 Less than once a month
6 **Never**

Score_____

36. How often do you eat dinner at a restaurant or cafeteria or eat "take out"?

1 More than 3 times a week
2 Two to 3 times a week
3 Once a week
4 Once or twice a month
5 Less than once a month
6 **Never**

Score_____

37. Check the choices you make when eating in restaurants or cafeterias.

___Select restaurants that offer low-fat choices and order those choices
___Order toast, muffins, cereal, pancakes, waffles for breakfast
___Order soup (not cream), salad or other meatless, cheeseless entrees for lunch
___Order vegetarian pizzas with half the cheese
___Avoid cheese, eggs, bacon on salads and avoid potato and macaroni salads
___Put garbanzo or kidney beans on salad at the salad bar
___Use a very small amount of salad dressing
___Order a fish, shellfish, chicken or lean red meat entree (but not fried)
___Use no more than 1 pat of margarine at any meal
___Order fruit, sorbet, sherbet, frozen yogurt or skip dessert

SCORE: (0-1 checks = 1; 2-3 checks = 2; 4-5 checks = 3; 6-7 checks = 4;
8-10 checks, or eat out less than once a month = 5)

Score_____

38. How often do you eat foods made using low-fat recipes or cook low-fat without recipes?

1 Once a month or less
2 One to 2 times a week
3 Three to 4 times a week
4 Five to 6 times a week
5 Everyday

Score_____

TOTAL SCORE (RESTAURANTS AND RECIPES)_____

SEAFOOD

Consider your eating habits during the last month. For each question, circle all items that apply.

39. How often do you eat fish? (tuna, snapper, perch, sole, halibut, cod, salmon, shrimp [prawns], crab, lobster, scallops, clams, oysters, sardines, etc). office use only

1 Do not eat fish or eat fish less than once a month
2 One to three times a month
3 Once a week
4 Two times a week
5 Three or more times a week *or* **eat vegetarian with no added fat**

Score_____

40. Which fish (fresh, frozen or canned) have you eaten in the last month?

1 Ate no fish in the last month
2 Tuna, clams, scallops, lobster, mussels
3 White fish (snapper, perch, cod, sole, halibut, catfish, etc), shrimp (prawns), crab, snowcrab (surimi), oysters, squid
4 Salmon (pink, silver or coho), trout, steelhead
5 Salmon (Chinook, king or red), sardines, herring, mackerel *or* **eat vegetarian with no added fat**

Score_____

TOTAL SCORE (FISH)_____

SCORING THE DIET HABIT SURVEY FOR 2000 CALORIES

WOMEN AND CHILDREN

Place the score for each category in the appropriate blank space.

Circle the scores for each category. Identify the categories that are closer to the goals and those that are further from the goals.

The TOTAL SCORE will give you an idea of the person's overall eating style.

The nutrients listed below the total scores provide a good estimate of the patient's diet composition.

Finally, there is space for you or your patient to list at least three ways he/she can change eating habits towards the goals.

	Current U.S. Diet 37% fat	Lower fat diet 30% fat	25% fat	20% fat	10% fat	Patient's Score
Meat, Fish and Poultry	<13	13-15	16-21	22-29	30	
Dairy Products and Eggs	<22	22-27	28-32	33-37	38	
Fats and Oils	<15	15-18	19-22	23-28	29	
Sweets and Snacks	<11	11	12-13	14-16	17-18	
Grains, Beans, Fruits, and Vegetables	<45	45-65	66-83	84-104	105-136	
Beverages	<9	9-11	12	13-16	13-16	
Salt	<14	14-17	18-21	22-25	22-25	
Restaurants and Recipes	<13	13-16	17-19	20-25	26-28	
Seafood	<5	5	6-7	8-10	8-10	
TOTAL	<147	147-190	191-235	236-287	288-330	

These total scores correspond to a diet with the following nutrient composition:

Cholesterol, mg/day	400	<300	<200	<100	<75
Saturated fat, % calories	13	10	8	5	2
Cholesterol-Saturated Fat Index/day	49	37	28	16	8
Fat, % calories	37	30	25	20	10
Carbohydrate, % calories	48	55	60	65	75
Protein, % calories	15	15	15	15	15
Sodium, mg/day	>2875	2875	2300	1725	1725
Potassium, mg/day	<2535	2535	3900	3900	3900

< means "less than"
> means "more than"

Suggestions for changing eating habits toward your goals:

SCORING OF THE DIET HABIT SURVEY FOR 2800 CALORIES

MEN AND TEENS

Place the score for each category in the appropriate blank space.

Circle the scores for each category. Identify the categories that are closer to the goals and those that are further from the goals.

The TOTAL SCORE will give you an idea of the person's overall eating style.

The nutrients listed below the total scores provide a good estimate of the patient's diet composition.

Finally, there is space for you or your patient to list at least three ways he/she can change eating habits towards the goals.

	Current U.S. Diet 37% fat	Lower Fat Diet 30% fat	25% fat	20% fat	10% fat	Patient's Score
Meat, Fish and Poultry	<12	12-14	15-20	21-29	30	_____
Dairy Products and Eggs	<22	22-28	29-32	33-37	38	_____
Fats and Oils	<14	14-17	18-21	22-28	29	_____
Sweets and Snacks	<11	11	12-13	14-16	17-18	_____
Grains, Beans, Fruits, and Vegetables	<70	70-96	97-127	128-166	167-195	_____
Beverages	<9	9-11	12	13-16	13-16	_____
Salt	<14	14-17	18-21	22-25	22-25	_____
Restaurants and Recipes	<13	13-16	17-19	20-25	26-28	_____
Seafood	<5	5	6-7	8-10	8-10	_____
TOTAL	<170	170-220	221-277	278-349	350-389	

These total scores correspond to a diet with the following nutrient composition:

Cholesterol, mg/day	500	<350	<220	<140	<100	_____
Saturated fat, % calories	13	10	8	5	2	_____
Cholesterol-Saturated Fat Index/day	67	49	36	23	10	_____
Fat, % calories	37	30	25	20	10	_____
Carbohydrate, % calories	48	55	60	65	75	_____
Protein, % calories	5	15	15	15	15	_____
Sodium, mg/day	>4025	4025	3220	2415	2415	_____
Potassium, mg/day	<3549	3549	5460	5460	5460	_____

< means "less than"
> means "more than"

Suggestions for changing eating habits toward your goals:

THE DIET HABIT SURVEY

GOAL SCORES FOR INDIVIDUAL QUESTIONS FOR 2000 CALORIES
WOMEN/CHILDREN

Question Number	Current U.S. Diet 37% fat	Lower Fat, Higher Carbohydrate Diet			
		30% fat	25% fat	20% fat	10% fat
1	<3*	3	4	5	6
2	<2	2	3	4-5	6
3	<3	3	3-4	4-5	6
4	<3	3	4	5	6
5	<2	2	3	5	6
6	<3	3	4	5	5
7	<4	4	5	5-6	6
8	<3	3	4	4-5	5
9	<2	2	4-5	4-6	6
10	<3	3	3-4	4-6	6
11	<4	4	5	5	5
12	<3	3-5	5	5	5
13	<4	4	4	5-6	6
14	<3	3	4	5	6
15	<3	3	4	5	6
16	<2	2	4	4-5	5
17	<3	3	4	5-6	6
18	<4	4	5	6	6
19	<3	3	4	4-6	5-6
20	<4	4	4	4-5	5-6
21	<10	10-11	12-13	14-16	14-16
22	<5	5-8	9-12	13-18	19-25
23	<3	3-7	8-10	11-15	16-23
24	<24	24-29	30-33	34-37	38-45
25	<3	3-7	8-12	13-18	19-27
26	<3	3	4	4-6	4-6
27	<3	3	4	5	5
28	<3	3	4	4-5	4-5
29	<3	3	4	4-5	4-5
30	<4	4	5	5	5
31	<2	2	4	4-5	4-5
32	<3	3	3	5	5
33	<2	2	3	5	5
34	<2	2	2	3	6
35	<3	3	4	4-5	6
36	<2	2-3	3	4-5	6
37	<3	3	4	5	5
38	<3	3	4	5	5
39	<3	3	4	4-5	4-5
40	<2	2	3	3-5	3-5

*< means less than

THE DIET HABIT SURVEY

GOAL SCORES FOR INDIVIDUAL QUESTIONS FOR 2800 CALORIES
MEN/TEENS

Question Number	Current U.S. Diet 37% fat	Lower Fat, Higher Carbohydrate Diet			
		30% fat	25% fat	20% fat	10% fat
1	<3*	3	4	5	6
2	<2	2	3	4-5	6
3	<3	3	3-4	4-5	6
4	<2	2	3	4	6
5	<2	2	3	5	6
6	<3	3	4	5	5
7	<4	4	5	5-6	6
8	<3	3	4	4-5	5
9	<2	2	4-5	4-6	6
10	<3	3	3-4	4-5	6
11	<4	4-5	5	5	5
12	<3	3-5	5	5	5
13	<4	4	4	5-6	6
14	<2	2	3	4	6
15	<3	3	4	5	6
16	<2	2	4	4-5	5
17	<3	3	4	5-6	6
18	<4	4	5	6	6
19	<3	3	4	4-6	5-6
20	<4	4	4	4-5	5-6
21	<15	15-18	19-21	22-25	22-25
22	<8	8-12	13-17	18-25	26-35
23	<10	10-15	16-19	20-27	28-35
24	<32	32-39	40-48	49-55	56-60
25	<5	5-9	10-19	20-34	35-40
26	<3	3	4	4-6	4-6
27	<3	3	4	5	5
28	<3	3	4	4-5	4-5
29	<3	3	4	4-5	4-5
30	<4	4	5	5	5
31	<2	2	4	4-5	4-5
32	<3	3	3	5	5
33	<2	2	3	5	5
34	<2	2	2	3	6
35	<3	3	4	4-5	6
36	<2	2-3	3	4-5	6
37	<3	3	4	5	5
38	<3	3	4	5	5
39	<3	3	4	4-5	4-5
40	<2	2	3	3-5	3-5

*< means less than

SCORES FOR 2000 CALORIES (WOMEN/CHILDREN)

Score	Present U.S. Diet	Lower-Fat Diets 30% fat	25% fat	20% fat	10% fat
Cholesterol-Saturated Fat	<61.0	61.0-71.5	71.6-88.0	88.1-110.0	110.1-115.0
Carbohydrate	<45.0	45.0-64.5	64.6-82.5	82.6-105.0	105.1-136.0
Beverages	<9.0	9.0-11.5	11.6-12.5	12.6-16.0	12.6-16.0
Salt	<14.0	14.0-16.5	16.6-21.0	21.1-25.0	21.1-25.0
Restaurants and Recipes	<13.0	13.0-15.5	15.6-19.0	19.1-25.5	25.6-28.0
Seafood	≤5.0	5.0-6.0	6.1-7.5	7.6-10.0	7.6-10.0
TOTAL	<147.0	147.0 - 185.8	185.9-230.8	230.9-282.0	282.1-330.0
These total scores above correspond to a diet with the following nutrient composition:					
Cholesterol, mg/day	400	<300	<200	<100	<75
Saturated fat, % calories	13	10	8	5	2
CSI*/day	49	37	28	16	8
Fat, % calories	37	30	25	20	10
Carbohydrate, % calories	48	55	60	65	75
Protein, % calories	15	15	15	15	15
Sodium, mg/day	>2875	2875	2300	1725	1725
Potassium, mg/day	<2535	2535	3900	3900	3900

SCORES FOR 2800 CALORIES (MEN/TEENS)

Score	Present U.S. Diet	Lower-Fat Diets 30% fat	25% fat	20% fat	10% fat
Cholesterol-Saturated Fat	<59.0	59.0-70.0	70.1-86.0	86.1-108.5	108.6-115.0
Carbohydrate	<70.0	70.0-95.5	95.6-126.5	126.6-166.5	166.6-195.0
Beverages	<9.0	9.0-11.5	11.6-12.5	12.6-16.0	12.6-16.0
Salt	<14.0	14.0-16.5	16.6-21.0	21.1-25.0	21.1-25.0
Restaurants and Recipes	<13.0	13.0-15.5	15.6-19.0	19.1-25.5	25.6-28.0
Seafood	≤5.0	5.0-6.0	6.1-7.5	7.6-10.0	7.6-10.0
TOTAL	<170.0	170.0-215.3	215.4-272.8	272.9-342.0	342.1-389.0
These total scores above correspond to a diet with the following nutrient composition:					
Cholesterol, mg/day	500	<350	<220	<140	<100
Saturated fat, % calories	13	10	8	5	2
CSI*/day	67	49	36	23	10
Fat, % calories	37	30	25	20	10
Carbohydrate, % calories	48	55	60	65	75
Protein, % calories	15	15	15	15	15
Sodium, mg/day	>4025	4025	3220	2415	2415
Potassium, mg/day	<3549	3549	5460	5460	5460

< means "less than", > means "more than"

* CSI = Cholesterol-Saturated Fat Index (JADA 1989; 89:807-816)

SCORING THE DIET HABIT SURVEY FOR RESEARCH STUDIES

Scoring the questions:

- The score for questions 1-20, 26-36, 38, 39 and 40 is the number corresponding to the option selected. If more than one option is selected, the score is the average of the options selected.

  For example, with respect to question 5, if a patient circled 1 bacon, sausage and also circled 5 Garden Sausage, the score is: 1 + 5 = 6 divided by 2 = 3.0.

- The score for questions 21-23 is 5 points per serving per day.

- To make it easier for people to answer question 24, we have them estimate for a week and we have divided the foods into two groupings. For the top group, the score is number of servings x 8.5 divided by 7 (number of servings x 1.2). For the bottom group, the score is the number of servings x 5 divided by 7 (number of servings x 0.7).

- The score for question 25 is 10 per cup of mashed potato, macaroni, spaghetti and other pastas divided by 7 (number of cups x 1.5), and 15 per large baked potato or cup of rice, corn, bulgur, barley and other grains divided by 7 (number of servings x 2).

- The scoring for question 37 is provided on that question.

- Express each score to one decimal place (3.3, 5.0, etc).

Summary Scores for THE DIET HABIT SURVEY:

The questions have been grouped into 6 summary scores: cholesterol-saturated fat score (questions 1-20), carbohydrate score (questions 21-25), beverage score (questions 26-28), salt score (questions 29-33), restaurant and recipe score (questions 34-38), seafood score (questions 39-40) and a total score.

The summary and total scores are categorized into the present U.S. (37% fat) and four lower fat diets (30% fat, 25% fat, 20% fat, 10% fat).

The nutrient composition associated with these diets is also provided.

Examples are given for two calorie levels: one for 2000 Calories (women/children) and one for 2800 Calories (men/teens).

One example of using scores from THE DIET HABIT SURVEY in a research study. In the Family Heart Study, the diet of each subject was categorized using THE DIET HABIT SURVEY scores as eating 37% fat (the present U.S. diet) or one of three lower fat diets – 30% fat, 25% fat or 20% fat using the cholesterol-saturated fat score. The subject's diet was also categorized using the carbohydrate score. If a subject's cholesterol-saturated fat score placed him/her in the 25% fat diet category and the carbohydrate score placed him/her in the 30% fat diet category, the subject was classified overall as eating a 30% fat diet. The overall score was used in the analyses of the Family Heart Study data reported in the Journal of the American Dietetic Association (92:41-47, 1992).

APPENDIX B

LABORATORY METHODOLOGY

Plasma Apolipoprotein B Manual Procedure
(Sigma Procedure No. 357)

Principle

ApoB in serum combines with a specific antibody present in the reagent and forms an insoluble complex resulting in turbidity of the assay mixture. The amount of turbidity formed is proportional to the ApoB concentration in the sample. The turbidity is measured in a spectrophotometer at 340nm and the concentration of ApoB in the sample is determined from a calibration curve obtained using the multi-level ApoB Calibrators.

Materials in Sigma Kit:

ApoB antibody reagent (Buffered solution containing goat antibodies to human apo B. Sodium azide, 0.1%, added as preservative)
ApoB Calibrators 1-5 (Human serum containing 5 different concentrations of Apo B. Sodium azide, 0.1%, added as preservative)
Apolipoprotein serum controls I and II (Human serum containing Apo B. Sodium azide, 0.1% added as preservative)

Procedure:

1. Make 2 cuvettes for each calibration point and each control. One cuvette will serve as a blank. Mark each blank cuvettes with a dot. Add 5µl calibrator or control to each cuvette using a 10 µl pipet.

2. Make 3 cuvettes for each sample. Add 5 µl of sample to each cuvette.

3. Add 0.5ml saline to each cuvette, including calibrators, controls and samples, to increase the volume.

4. Add 0.5ml distilled water to the first set of tubes for the calibrators, controls, and samples for the blanks (cuvettes with a dot). Add 0.5ml antibody reagent to the rest of the tubes. Time each cuvette for 15 seconds and mix on the vortex.

5. At the end of 7 minutes, read the absorption of each cuvette at 340 nm. Time each reading 15 seconds apart so that each sample incubates exactly 7 minutes. Use distilled water as a reference.

6. Subtract the blank from each calibration point. Plot on graph paper as calibration curve. Set the x-axis as ApoB concentration and y-axis as absorption.

7. Subtract blank from each sample point. Find the ApoB concentration from the calibration curve.

Precision and Accuracy of Apo B Assay

Precision:

Run-to-run precision analysis on the controls I and II (A 7173 and A 7298, respectively, Sigma Diagnostics) yielded the following data:

Controls	I	II
Mean (mg/dL)	50.75	96.50
SD (mg/dL)	5.12	7.05
Coefficient of variation (%)	10.09	7.31
Number of assays ran over a period of 4 days	4	4

Accuracy:

All of the measured values for Control I (A 7173) and Control II (A 7298) fell within the acceptable assigned ranges of 40 - 68 mg/dL and 86 - 122 mg/dL, respectively, set by Sigma Diagnostics (St. Louis, MO).

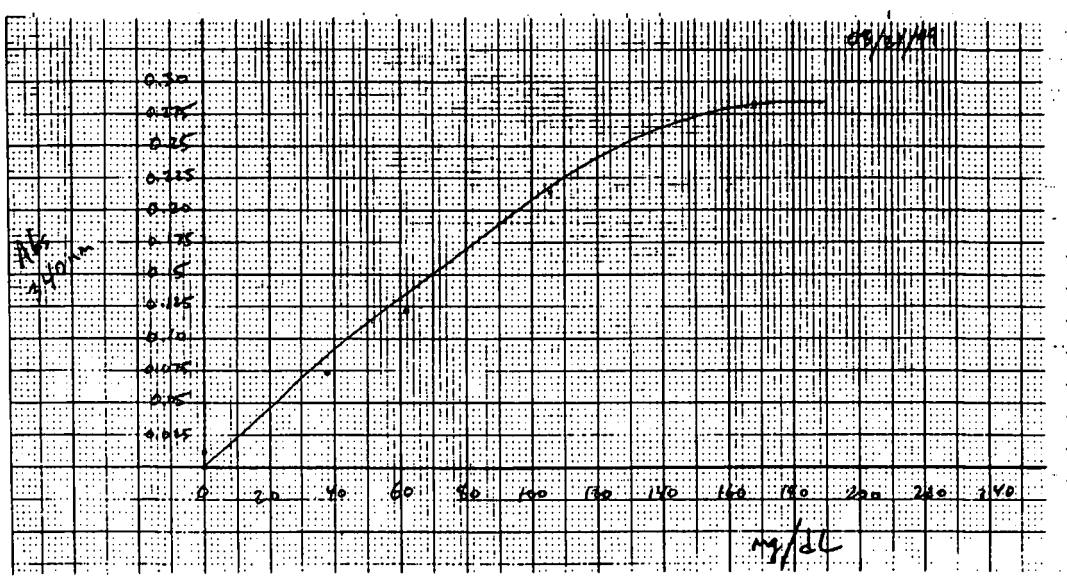

Figure 1. Typical Calibration Curve for Apo B

Apo A1 Basic Manual Procedure

The following is a user-validated protocol for performing the Wako Autokit Apo A1 assay. Each laboratory should validate that the performance criteria listed in the package insert are met.

Preparation of Reagents:

Reconstitute the Apolipoprotein Calibrator (Item: 992-27591) as indicated in the package insert for this product. *Note: the calibrator must be purchased separately as it is not a component of this assay.*

Procedure:

1. Accurately pipette 9 µl of sample, calibrator or saline into labeled cuvettes using a 10 µl pipet. Add 750 µl of Reagent 1 (Buffer) every 20 seconds.

2. Mix thoroughly on a vortex and incubate in a Shimadzu UV160U Spectrophotometer at 37°C for 5 minutes.

3. Measure the absorbance for each cuvette at 700 nm. This will serve as a background turbidity blank.

4. Add 78 µl of Reagent 2 (Antibody) to each cuvette every 20 seconds using a 100 µl pipet.

5. Mix thoroughly on a vortex and incubate at 37°C for 5 minutes.

6. Read at 700 nm at 5 minutes from the addition of Reagent 2.

7. Calculate the blank subtracted absorbance for each sample by subtracting the blank absorbance read in step 3 from the final absorbance in step 6. The blank subtracted absorbance value is used for all further calculations.

8. The concentration of Apo A1 in the unknown samples is calculated via simple proportion analysis against the assigned calibrator value. [(Abs of Sample/Abs of Calibrator) X Value of Calibrator].

9. The Wako Autokit Apo A1 is highly linear across its dynamic range. Samples generating results greater than 220 mg/dL should be diluted 1:1 with saline and repeated. If a standard curve is required, the serial dilutions of the Wako Apolipoprotein High Calibrator (Item: 415-77201) may be used.

Precision and Accuracy of Apo A1 Assay

Precision:

Run-to-run precision analysis on the control A (blood sample from O.S.U female volunteer) yielded the following data:

Control	A
Mean (mg/dL)	159.00
SD (mg/dL)	18.21
Coefficient of variation (%)	11.45
Number of assays over a 4-day period	4

[1]Blood sample was collected in plasma EDTA tubes, then separated by centrifugation for 15 minutes. The plasma was then separated into labeled vials and stored at -40°C for several days and then thawed for analysis.

Accuracy:

Accuracy could not be determined since we did not know the control's expected value.

APPENDIX C

DATA

*** PHOTOMETRIC ***
(CUVETTE VAR.)

λ 700.0

No.	A1
1	0.089
2	0.089
3	0.089
4	0.089
5	0.089
6	0.089
7	0.089
8	0.089
9	0.089
10	0.089
11	0.089
12	0.089
13	0.089
14	0.089
15	0.089
16	0.089
17	0.089
18	0.089

Plastic Disposable Cuvette Variability

Rationale:
We suspected that the disposable cuvettes that we used for the apolipoprotein assays would exhibit greater variability compared to quartz cuvettes.

Results:
No variability between 18 Semi UV Cuvettes (FISHERbrand - No. 14-385-938) in well

#1- measured on a Shimadzu UV160U Spectrophotometer (Columbia, MD) at 700 nm.

Baseline Characteristics of Subjects

(ID = subject no.; PA = physical activity, L = low and H = high; HS = hormone status, NO = no HRT, E = unopposed estrogen and EP = unopposed estrogen + progestin; AGE [years]; MENOP = age at onset of menopause; HT = height in cm; WT = weight in kg)

ID	PA	HS	AGE	MENOP	HT	WT	BMI
4	L	NO	67	50	160	79.5	31
6	L	NO	67	45	162.6	75.5	28.6
7	L	NO	59	49	162.6	62.3	23.6
9	L	NO	61	50	170.2	57.7	19.9
20	L	NO	69	50	162.6	95.5	36.1
24	L	NO	55	46	174.6	80	26.2
27	L	NO	51	48	157.5	55.5	22.4
28	L	NO	53	48	164.5	72.3	26.7
30	L	NO	57	53	177.2	83.6	26.6
57	L	NO	53	52	167.6	62.5	22.2
69	L	NO	69	49	163.8	59	22
70	L	NO	56	49	165.1	55.5	20.4
71	L	NO	58	45	160.7	119.3	46.2
76	L	NO	59	42	157.5	73.5	29.6
80	L	NO	62	53	156.2	75	30.7
83	L	NO	71	50	160	69	26.9
112	L	NO	55	52	172.7	60	20.1
123	L	NO	63	55	172.7	67.3	22.6
1	H	NO	63	26	165.1	54.1	19.8
10	H	NO	75	41	157.5	50.9	20.5
11	H	NO	62	55	165.1	79.1	29
12	H	NO	54	47	165.1	63.2	23.2
13	H	NO	51	45	162.6	51.8	19.6
15	H	NO	63	51	165.1	72.3	26.5
16	H	NO	67	55	165.1	78.6	28.8
18	H	NO	74	52	167.6	60.9	21.7
21	H	NO	69	48	155.6	55.5	22.9
22	H	NO	50	45	163.8	71.4	26.6
25	H	NO	60	46	151.1	47.7	20.9
32	H	NO	70	31	166.4	65.5	23.7
33	H	NO	55	50	157.5	54.1	21.8
79	H	NO	67	50	157.5	67	27
94	H	NO	78	45	158.1	56.2	22.5
100	H	NO	65	52	160	63.2	24.7
120	H	NO	64	55	149.2	43.6	19.6
43	L	E	63	43	167.6	73.5	26.2
53	L	E	50	43	164.5	60	22.2
55	L	E	69	53	160	82.5	32.2
65	L	E	56	30	163.2	85.5	32.1
75	L	E	56	40	167.6	66	23.5
89	L	E	62	47	157.5	76.5	30.8
101	L	E	63	47	149.2	51.8	23.3
106	L	E	71	42	157.5	63	25.4
121	L	E	72	42	156.2	65.5	26.8
124	L	E	72	50	154.9	74.1	30.9

127 L	E	53	49	175.3	110.9	36.1
138 L	E	62	44	163.8	59.1	22
44 H	E	62	45	158.8	56.5	22.4
45 H	E	58	49	170.2	87	30
54 H	E	57	44	172.1	66	22.3
88 H	E	66	56	165.1	65.7	24.1
103 H	E	78	46	156.2	52.3	21.4
130 H	E	68	38	155.6	50.7	20.9
140 H	E	68	53	165.1	63.4	23.3
38 L	EP	61	50	162.6	87	32.9
77 L	EP	64	55	167	66.5	23.8
85 L	EP	63	55	160.7	50	19.4
90 L	EP	77	50	156.8	56.5	23
92 L	EP	72	55	170.2	68	23.5
118 L	EP	62	44	161.9	70.5	26.9
131 L	EP	54	48	163.2	80	30
134 L	EP	58	48	167.6	86.8	30.9
137 L	EP	57	50	165.1	58.6	21.5
139 L	EP	59	56	164.5	77.7	28.7
29 H	EP	57	44	161.9	60.9	23.2
34 H	EP	57	51	158.8	57.7	22.9
41 H	EP	54	50	172.7	63.5	21.3
49 H	EP	69	56	152.4	57	24.5
58 H	EP	52	51	165.1	61.5	22.6
66 H	EP	61	42	159.4	45.5	17.9
72 H	EP	57	40	157.5	49	19.8
96 H	EP	67		149.9	75	33.4
98 H	EP	74	43	160	68	26.6
105 H	EP	64	54	153.7	48.2	20.4
109 H	EP	57	54	149.9	40.5	18
111 H	EP	75	50	154.9	72.7	30.3
132 H	EP	55	54	167.6	59.1	21
133 H	EP	56	46	162.6	68.2	25.8

Apo B and Apo A1 Concentrations

(ID = subject no.; PA = physical activity, L = low and H = high; HS = hormone status, NO = no HRT, E = oral unopposed estrogen and EP = unopposed estrogen + progestin; apo B and apo A1 values are expressed in mg/dL)

ID	PA	HS	APOB	APOA
4	L	NO	95	169.9
6	L	NO	45	143
7	L	NO	58	169.4
9	L	NO	48	126.9
20	L	NO	58.5	162
24	L	NO	53	137.6
27	L	NO	50	175.2
28	L	NO	54	142.2
30	L	NO	87	141.8
57	L	NO	78	174.3
69	L	NO	54	160
70	L	NO	34	155.8
71	L	NO	61	138.9
76	L	NO	94.5	135.5
80	L	NO	73	151.5
83	L	NO	56	134.2
112	L	NO	49	163
123	L	NO	74	161.3
1	H	NO	42	155.6
10	H	NO	47.5	148.2
11	H	NO	69	147.4
12	H	NO	52	146.2
13	H	NO	30	165.4
15	H	NO	69	145.4
16	H	NO	51	171.1
18	H	NO	69	150.3
21	H	NO	58.5	139.3
22	H	NO	77.5	154.1
25	H	NO	48	169.8
32	H	NO	53	199.9
33	H	NO	46	175.2
79	H	NO	59	187.1
94	H	NO	100.5	152.4
100	H	NO	80	160
120	H	NO	63	194.5
43	L	E	46	170.9
53	L	E	46	194.4
55	L	E	64	152.1
65	L	E	68	183.7
75	L	E	56	189.6
89	L	E	100	182.4
101	L	E	75	176.9
106	L	E	68	190.9
121	L	E	68.5	173.8
124	L	E	84	176.8

127 L	E	77	174.2
138 L	E	81	194.1
44 H	E	41.5	185.4
45 H	E	43	180.7
54 H	E	81	179
88 H	E	62.5	210.4
103 H	E	54	198.5
130 H	E	67	211.3
140 H	E	64	168.2
38 L	EP	60.5	187.6
77 L	EP	52.5	201.9
85 L	EP	58.5	184.6
90 L	EP	51.5	190.9
92 L	EP	51.5	190.9
118 L	EP	60.5	217.2
131 L	EP	92	157.4
134 L	EP	80	191
137 L	EP	65	150.9
139 L	EP	86	172.5
29 H	EP	42	159.7
34 H	EP	76	162.4
41 H	EP	43	199.5
49 H	EP	97.5	158.9
58 H	EP	53	164.5
66 H	EP	44	193.1
72 H	EP	46	178.2
96 H	EP	60	179.1
98 H	EP	64.5	180.8
105 H	EP	51	165.9
109 H	EP	37	179.1
111 H	EP	59	176.1
132 H	EP	51	155.7
133 H	EP	59	198.4

Relationship of Self-Reported Physical Activity and/or HRT with Total Cholesterol (TC), LDL-C, HDL-C and Triglyceride (TG) Concentrations[1]

Physical Activity (PA)	Low			High			P-value		
Variable	No HRT (n=18)	E[3] (n=12)	E + P[4] (n=10)	No HRT (n=17)	E (n=7)	E + P (n=14)	PA	HRT	INT[2]
TC (mg/dL)	212±26.6	222±36.6	218±18.2	213±31.9	222±24.9	204±35.3	0.544	0.450	0.639
LDL-C (mg/dL)	128±22.9	117±36.0	117±30.1	129±33.2	120±18.9	113±35.9	0.994	0.218	0.952
HDL-C (mg/dL)	54±8.1	65±14.0	68±24.0	63±11.8	74±11.4	67±12.1	0.047	0.011	0.350
TG (mg/dL)	151±112.9	198±118.8	168±88.3	104±57.5	141±55.4	118±66.5	0.017	0.254	0.980

[1]Values are means ± SD from Williams et al. (Med Sci Sports Exerc 31:S289, 1999)
[2]Interaction between physical activity and HRT
[3]Oral unopposed estrogen group
[4]Oral estrogen + progestin group