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PRINCIPAL INVESTIGATOR: COL(Ret) Marina N. Vernalis, MC, USA

CONTRACTING ORGANIZATION:

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# REPORT DOCUMENTATION PAGE

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| <b>14. ABSTRACT</b><br>The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. A translational research approach will provide the ability to find novel disease markers, optimal prevention and holistic treatment approaches, and a unique venue for future research as the "virtual laboratory" for optimal comprehensive health prevention in the military beneficiary population. This research method also allow us to further hypothesize and define relationships between CVD, other cardio metabolic disease states and maladaptive behavior patterns unique to service members such as pre-diabetes, stress, overweight and sleep disorders with the aim of targeting these disorders in a pre-clinical phase. Using an integrative, interdisciplinary preventive health approach, ICHP has shown that an individual's cluster of CV risk factors can be effectively targeted and improved. |                         |                                |   |  |   |
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**Executive Summary - Integrative Cardiac Health Project**  
**Final Report**  
**Dates: 19 Aug 2010 – 18 Feb 2013**

The Integrative Cardiac Health Project (IHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. IHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. In keeping with this aim, collaborative research efforts have continued between IHP projects at Walter Reed National Military Medical Center (WRNMMC), Windber Research Institute (WRI) and Windber Medical Center (WMC). In this period of performance, the following key accomplishments are noted:

- Total visits at IHP WRNMMC/WMC Prevention Programs: 4743 (includes telephonic follow-up)
- 4 protocols (including 1 sub study) in data analysis phase
- 4 active protocols; 1 submitted for scientific review
- Dissemination of scientific research findings continues
  - 5 manuscripts published; 1 submitted; 7 in preparation
- Intense planning/preparation for relocation to WRNMMC Bethesda which occurred on 29 August 11 to include Clinical Transition Strategy Plan for facilitating a smooth transition and maintaining high quality standards of our DOD Center of Excellence (COE) at WRNMMC to serve all military beneficiaries, including website creation; successful transition to WRNMMC Bethesda
- Data analysis continues. Relevant findings in our population include:
  - BATTLE Study findings suggest knowledge of an abnormal CIMT along with increased CV risk does not improve adherence to a lifestyle program
  - Changes in gene expression mirror changes in many CVD risk factors – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52
  - Most cholesterol and lipid homeostasis genes show a continual decrease in expression throughout the program similar to body weight
  - Medication use clearly does not affect gene expression, thus expression changes may be attributed to the lifestyle change program
  - Genetic variation influences risk factor response
    - Several SNPs show evidence of an influence on triglyceride response
  - Stress plays an unexpectedly prominent role in cardiovascular risk
  - Stress erodes sleep quality and is associated with dysregulation of glucose metabolism
  - Deteriorations of sleep and glucose regulation may serve as mediators of the increased cardiovascular risk in our patient population
  - Increased stress, decreased sleep time, poor sleep quality, glucose dysmetabolism, and increased cardiovascular risk all have a negative impact of military readiness
  - Longer sleep time and improved sleep quality correlate with improved weight control as well as improved cardiovascular risk
- IHP Clinical Database development with informatics architects underway
- Significant innovations in Clinical Flow Plan of CPP, refinement of processes; creation of new clinical positions to support expanding research initiatives
- Identified need for specific track for pre-diabetes and diabetes care; development of pre-diabetes and diabetes clinical track using novel team approach of integrative care

## Introduction

The epidemics of cardiovascular disease (CVD), Type II diabetes, and obesity generate a major share of the preventable costs of American health care. Currently, the American health care market place does not support preventive care that would save lives and costs associated with these problems. Healthcare costs are predicted to rise from 16% of the US GDP in 2005 to 30% of the GDP by 2025 if we fail to invest in prevention. The primary mission of the Integrative Cardiac Health Project (ICHP), a congressionally-supported military-civilian collaboration between Walter Reed National Military Medical Center (WRNMMC) and Windber Medical Center (WMC)/Windber Research Institute (WRI) is to: 1) Teach, implement and study lifestyle changes added to “best” medical practices that promote cardiovascular health; 2) Identify patients at risk earlier by characterizing CVD even at the “molecular” disease stage and identifying biomarkers predictive of subclinical CVD; and 3) Relate genomic/proteomic changes to the evolution of CVD risk factors in response to lifestyle changes in an effort to prevent, arrest or reverse CVD. Within these objectives, ICHP will include: a) a comprehensive and innovative CVD risk factor assessment and prevention program in the military beneficiary population; b) advanced imaging methods for quantifying numerous aspects of heart health in military and other populations; c) an optimal healing environment for CVD patients; and d) an integrated statistical analysis of clinical, biochemical, and molecular data to identify patterns of CVD risk factors that will allow a unique and intensive collection of data at the clinical, biochemical, and molecular levels for heart disease, but with applicability and relevance in patients with other chronic diseases such as cancer, diabetes, metabolic syndrome and obesity. The heart disease data base will provide the ability to find novel disease markers, new or emerging evaluation and treatment approaches, and provide a unique venue for future research. Some overlap in the reporting of the ICHP science may occur it was transferred to Award No: W81XWH-11-2-0227 on 29 Sep 11.

## Body

### **Task #1: “Non-Invasive Coronary Artery Disease Reversal” (CADRe) Study Protocol.**

**Status:** Task complete was actually completed under Award No. W81XWH-05-2-0075, however, a manuscript was published during this period of performance.

### **Task #3: Ongoing data collection for “CADRe Five-Year Follow-up” Protocol.**

This longitudinal observational follow-up study will determine the persistence of healthy lifestyle behavioral changes and CVD risk factor control as results of participation in the original CADRe study. Participants will have yearly follow-up visits at 1, 2, 3, 4, and 5 years after completion or expected completion of the CADRe Study. Specific aims are to determine: 1) Persistence of lifestyle change behaviors in diet, exercise, and stress management; 2) Coronary risk-factor control, and; 3) Quality of Life. We hypothesize that participants who have been exposed to an intensive lifestyle change program will demonstrate long-term carryover of heart healthy characteristics including persistence of favorable lifestyle change behaviors and risk factor control. The plan is to recruit up to

163 male and female CADRe study participants, age 18 years or older, with subsequent completion of Phase 1 of the CADRe Study (3-month data collection) were recontacted and invited to participate in this 5-year follow-up study (post-study completion or expected completion). The primary outcome measure is a composite index of 7 heart healthy characteristics (BMI 18.5 – 25; LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg; regular exercise ≥ 150 min/week, and daily practice of CADRe program stress management techniques) since the main goal of this study is to assess the persistence of lifestyle change behaviors and risk factor control. The Heart Health Index (HHI), presented as a single score (range 0-7), will be assigned to each subject yearly. Additionally, each of the 7 heart healthy characteristics will be assessed independently as a continuous variable. Secondary outcome measures include: Changes in modifiable CVD risk factors (blood pressure, body composition and fitness, lipid levels and glucose); C-reactive protein and, Quality of Life.

**Status:** This study is continued from Award No. W81XWH-05-2-0075 and study methodology and approvals have been reported previously. The Continuing Review was approved by WRNMMC Dept of Research Programs (DRP) on 26 Apr 2012 at which time an updated protocol reflecting the transition to WRNMMC was reflected. The MRMC acknowledgement memorandum was received 13 Nov 2012. This study is closed to accrual and data collection is complete. Statistical analysis needs established for final data analysis and final data analysis is planned to be completed under Award No. W81XWH10-2-0227.

**Preliminary Findings:**

Of the available 163 CADRe study subjects, 102 participants responded (63%) to study mailing: 80 meet eligibility criteria and agreed to make a study visit; 2 were ineligible; 17 declined screening interview / participation; 2 were undecided about participation, and; 1 deceased. Of the 80 eligible former CADRe study participants who agreed to make a study visit, 76 provided informed consent for at least one follow-up visit. Fifty-one participants provided at least one additional follow-up study visit. See Table 1 for actual longitudinal follow-up of study participants by cohort.

**Table 1. Actual Longitudinal Follow-Up of CADRe Study**

| Cohort | # Pts Available | # Replied | # Enrolled | Actual Visits |        |        |        |        | Total Visits |
|--------|-----------------|-----------|------------|---------------|--------|--------|--------|--------|--------------|
|        |                 |           |            | Year 1        | Year 2 | Year 3 | Year 4 | Year 5 |              |
| 1      | 7               | 3         | 3          | 3             | 0      | 0      | 0      | 3      | 3            |
| 2      | 15              | 6         | 3          | 3             | 0      | 0      | 0      | 3      | 3            |
| 3      | 14              | 7         | 7          | 7             | 0      | 0      | 0      | 7      | 7            |
| 4      | 17              | 10        | 9          | 9             | 0      | 0      | 6      | 8      | 14           |
| 5      | 12              | 8         | 7          | 7             | 0      | 0      | 5      | 7      | 12           |
| 6      | 19              | 12        | 6          | 6             | 0      | 0      | 4      | 6      | 10           |
| 7      | 15              | 9         | 5          | 5             | 0      | 4      | 3      | 4      | 11           |
| 8      | 12              | 6         | 4          | 4             | 0      | 4      | 5      | 4      | 13           |
| 9      | 9               | 5         | 5          | 5             | 0      | 4      | 4      | 2      | 10           |
| 10     | 10              | 9         | 9          | 9             | 7      | 8      | 6      | 6      | 27           |

|               |            |           |           | Actual Visits |           |           |           |           |            |
|---------------|------------|-----------|-----------|---------------|-----------|-----------|-----------|-----------|------------|
| 11            | 11         | 5         | 5         |               | 5         | 5         | 4         | 3         | 17         |
| 12            | 10         | 3         | 3         |               | 3         | 3         | 1         | 3         | 10         |
| 13            | 12         | 10        | 10        | 9             | 9         | 10        | 8         | 8         | 44         |
| <b>Totals</b> | <b>163</b> | <b>90</b> | <b>76</b> | <b>9</b>      | <b>24</b> | <b>38</b> | <b>46</b> | <b>64</b> | <b>181</b> |

At study enrollment, participants were 66 yrs old (range 36 to 80), predominantly Caucasian male (79%) and significantly overweight with a BMI of 29.8; similar to their pre-CADRe study BMI of 29.1. Subjects were a mean of 3.2 yrs post CADRe Study completion or anticipated completion. Of the individual CADRe Study lifestyle components, participants were most compliant with exercise (goal  $\geq 180$  minutes/week); mean weekly time =183 minutes of moderate to vigorous physical activity. Although few participants reported a strict vegan dietary pattern following completion of the CADRe Study, dietary fiber and average fruit and vegetable intake was higher than the average U.S intake at 29 grams/day and 9.7 servings per day, respectively. Participants continued to have difficulty in performing 1 hour of stress management daily. Participants reported an average time of 154 minutes/week spent in any of the five CADRe Study techniques with only 33% reporting daily performance of at least 1 stress management technique. Table 2 provides a preliminary descriptive analysis for the major outcome variables as an aggregate sample for the initial study visit. Individual HHI scores (composite score of heart healthy behaviors) has not yet been calculated, however, at least 5 of the 7 heart healthy behaviors are being met in the aggregate (LDL-cholesterol  $< 100$  mg/dL; dietary fiber intake  $\geq 25$  gms/day; consumption of 5 or more fruits and vegetables per day; BP  $< 140/90$  mmHg, and regular exercise  $\geq 150$  min/week).

**Table 2: Major Outcomes Variables at Initial Study Visit**

| Outcome Variable (n=76)            | Mean  | SD    |
|------------------------------------|-------|-------|
| Weight (kg)                        | 88.4  | 22.5  |
| Body Mass Index (BMI)              | 29.8  | 6.3   |
| % Body Fat (n=74)                  | 31.2  | 9.8   |
| Systolic BP (mmHg)                 | 125.6 | 12.6  |
| Diastolic BP (mmHg)                | 71.3  | 6.8   |
| Fasting Glucose (mg/dL)            | 94.8  | 13.4  |
| Total Cholesterol (mg/dL)          | 154.4 | 35.5  |
| HDL Cholesterol (mg/dL)            | 50.2  | 11.4  |
| LDL Cholesterol (mg/dL)            | 86.2  | 30.7  |
| Triglycerides (mg/dL)              | 129.6 | 67.8  |
| C-Reactive Protein (mg/dL) (n=74)* | 0.197 | 0.365 |
| Daily Dietary Fiber (gms )         | 28.6  | 14.1  |
| Daily Fruit/Vegetable Servings     | 9.7   | 5.5   |
| Weekly Exercise Time (minutes)     | 183.0 | 167.0 |
| Weekly Stress Mgt Time (minutes)   | 154.1 | 166.8 |
| Physical Composite Score           | 44.2  | 11.5  |
| Mental Composite Score             | 54.1  | 8.2   |

\*Two outliers (C - reactive protein  $> 6.00$ ) excluded from analysis

Preliminary analysis of changes in modifiable CVD risk factors (BP, body composition and fitness, lipid levels and glucose) can be assessed in comparison to the final CADRe Study visit (Table 3) in those subjects who have completed a 5-year follow-up study visit. In 64 participants with Year 5 data, there were significant increases in both body composition and systolic BP when compared to final CADRe study visit data. Body anthropometrics show an 8% mean weight gain and a 15% increase in body fat despite reporting a mean of 183 minutes per week of moderate physical activity. Systolic BP increased by 3%. No significant change was seen in diastolic BP, glucose, LDL, HDL or CRP. However, significant reductions in TC and TG at Year 5 were 7% and 37%, respectively. Of the 58 participants (91%) on lipid-lowering medications, 40% reported an increase in these medications which may account for the lipid profile improvements from CADRe study completion.

**Table 3. Select Outcome Variables at 5-Year vs. Final CADRe Study Visit (n=64)**

|   | Final CADRe Study Visit | 5 Year Follow-up Visit | Change        | P       |
|---|-------------------------|------------------------|---------------|---------|
| <b>Body Composition/Blood Pressure (BP)</b> |                         |                        |               |         |
| Weight (kg)                                 | 82.1 ± 22.3             | 88.9 ± 25.0            | 6.8 ± 9.7     | <0.0001 |
| BMI (kg/m <sup>2</sup> )                    | 27.5 ± 6.0              | 30.1 ± 6.7             | 2.6 ± 3.5     | <0.0001 |
| % Body Fat *                                | 26.1 ± 9.1              | 30.9 ± 9.1             | 4.8 ± 4.3     | <0.0001 |
| Systolic BP (mmHg)                          | 120.6 ± 12.6            | 125.2 ± 14.5           | 4.6 ± 14.4    | 0.013   |
| Diastolic BP (mmHg)                         | 69.2 ± 7.3              | 71.0 ± 7.6             | 1.9 ± 9.0     | 0.101   |
| <b>Laboratory (mg/dL)</b>                   |                         |                        |               |         |
| Glucose (mg/dL) **                          | 98.0 ± 18.8             | 96.0 ± 17.8            | -2.0 ± 18.9   | 0.411   |
| Total Cholesterol (mg/dL)                   | 158.3 ± 31.7            | 150.7 ± 30.6           | -7.7 ± 29.9   | 0.044   |
| LDL-Cholesterol(mg/dL)                      | 86.8 ± 23.3             | 81.7 ± 24.4            | -5.1 ± 21.5   | 0.065   |
| HDL-Cholesterol(mg/dL)                      | 46.2 ± 10.3             | 48.7 ± 13.0            | 2.5 ± 11.1    | 0.076   |
| Triglycerides (mg/dL)                       | 156.7 ± 87.7            | 130.5 ± 83.6           | -26.2 ± 74.9  | <0.0001 |
| C-reactive protein (mg/dL) #                | 0.222 ± 0.271           | 0.236 ± 0.407          | 0.014 ± 0.373 | 0.386   |

Mean ± SD; \*n=59; \*\*n=63; # n=62.

**Adverse Events:** No adverse events (AEs) occurred during this period of performance. Two adverse events were reported on Final Report for Award No. W81XWH-05-2-0075.

**Task #4: Ongoing enrollment for “Better Adherence to Therapeutic Lifestyle Change Efforts (BATTLE) Trial”.**

The purpose of this study is to determine whether knowledge of abnormal results from a noninvasive test for detection of subclinical atherosclerosis (CIMT), in addition to knowledge of CVD risk factors, enhances adherence to healthy lifestyle behaviors in comparison to only CVD risk factor knowledge. This two-arm, double-blinded study will randomize participants to either receive CIMT results (R-CIMT Group) or have CIMT results withheld (W-CIMT Group) in the setting of a 3-month TLC intervention (Mediterranean-type diet and moderate intensity exercise). After the 3-month TLC intervention period is completed, participants who had CIMT results withheld will receive this information. A composite index of adherence to the TLC intervention was selected as the primary outcome measure since the main goal of this study is to assess the impact of CIMT imaging knowledge on change in lifestyle behaviors.

Secondary outcomes include: 1) adherence to each TLC Program component (diet, exercise, attendance at weekly on-site group sessions); 2) changes in modifiable CVD risk factors and inflammatory makers: blood pressure, body composition and fitness, lipid levels, glucose/insulin resistance, C-reactive protein (CRP); 3) changes in emotional factors (anxiety score, self-efficacy, motivation, and; 4) knowledge assessment in those participants who received their CIMT results. The study will be conducted with individuals at moderate to high risk for cardiovascular events based on CVD risk factor profile and evidence of significant subclinical atherosclerosis (CIMT > 75<sup>th</sup> percentile for age/gender).

**Status:** This study is continued from Award No. W81XWH-05-2-0075 and study methodology and approvals have been reported previously. The Continuing Review was approved by WRNMMC DRP on 28 Nov 2011 and MRMC acknowledgement memorandum was received 14 Feb 2012. Data analysis completed in Dec 2011 when all final data tables received from PREMIER. Formative evaluation of TLC intervention completed and data analysis remained in progress at end of this period of performance.

**The following manuscript was submitted:**

- Saum NS, Halsey JF, Walizer EM, Vernalis MN. Feasibility of “10-Minute” Mindfulness Practice in a Therapeutic Lifestyle Change Program. *Health Education Research*.

**The following manuscript is in preparation:**

- Walizer, EM, Vernalis, MN. Methodology and demographics of the BATTLE Trial (Better Adherence to Therapeutic Lifestyle Change Efforts). (In preparation)

**Findings**

In collaboration with PREMIER Research, data reconciliation, data quality control and analysis were completed during this performance period. Analysis was conducted on both evaluable subjects (study completers=142) and intent-to-treat subjects (subjects who at least one post randomization visit=161). For this report, findings for the completers are provided, although intent-to-treat findings are similar.

Since November 2007, 1068 patients gave permission for a study team member to contact them regarding this study. Approximately 41% of “interested” patients telephonically screened were eligible to initiate the study screening process. Over 48% of those contacted opted out (n=507) of the study primarily for time commitment and travel/distance reasons. The primary reason for ineligibility on the initial telephone screen was low cardiovascular risk profile. Of the 275 consented subjects who were considered screen failures, 60% screened out primarily by CIMT (<75 percentile for gender/age), 14% had an acceptable past medical history, 11% withdrew consent, 6% did not meet diagnostic or severity criteria, 1% had an intercurrent medical event and 8% were categorized as other (deployment, relocation, job conflicts). In summary, approximately 18% of those patients who met initial screening criteria after the telephone screen (n=948) randomized into the main study. The screening phase of the study yielded 166 participants who meet all screening criteria to include completion of a

2 week run-in phase where participants received intensive education and monitoring of the TLC program. After randomization there was a 14.5% drop-out rate (n=24) with 142 participants completing the study. Table 4 provides a summation of study enrollment, disposition by group (received CIMT [R-CIMT] vs. control [W-CIMT]) and total sample.

**Table 4. Subject Enrollment and Study Disposition**

| Parameter Category                               | R-CIMT     | W-CIMT     | Total       |
|--|------------|------------|-------------|
| Initial Telephone Screen Completed               |            |            | 1068        |
| Subjects Screened                                |            |            | 441         |
| Subjects Randomized                              | 83         | 83         | 166         |
| Subjects Included in Evaluable Population        | 69 (83.1%) | 73 (88.0%) | 142 (85.5%) |
| Subjects with Study Completion                   | 69 (83.1%) | 73 (88.0%) | 142 (85.5%) |
| Subjects Who Terminated Early from the Study     | 14 (16.9%) | 10 (12.0%) | 24 (14.5%)  |
| Primary Reason for Early Termination:            |            |            |             |
| -- Adverse Event                                 | 2 (2.4%)   | 2 (2.4%)   | 4 (2.4%)    |
| -- Subject Withdrew Consent                      | 4 (4.8%)   | 3 (3.6%)   | 7 (4.2%)    |
| -- Protocol Non-compliance (After Randomization) | 2 (2.4%)   | 3 (3.6%)   | 5 (3.0%)    |
| -- Lost to Follow-up                             | 2 (2.4%)   | 1 (1.2%)   | 3 (1.8%)    |
| -- Other   | 4 (4.8%)   | 1 (1.2%)   | 5 (3.0%)    |

Study completers were predominately middle aged, overweight (mean BMI=31.5 ± 5.6), Caucasian, females (see Table 5); however, statistical significant differences in the study groups were detected in mean age and gender. The treatment group was older and comprised of more women. No differences between groups was detected in overall reported co-morbid conditions, however, over 50% of the women in the treatment group were postmenopausal. The CVD risk profile of completers is as follows: 53% hypertensive, 82% dyslipidemic, 12% Type 2 diabetes, 4% current smokers, and 56% with family history of CVD; no differences were detected between the treatment groups.

**Table 5: Demographic Characteristics (Study Completers)**

| Parameter Category/Statistics | R-CIMT (N=69) | W-CIMT (N=73) | Total (N=142) | p-value            |
|-------------------------------|---------------|---------------|---------------|--------------------|
| Age (Yr)                      |               |               |               | 0.015 <sup>1</sup> |
| N                             | 69            | 73            | 142           |                    |
| Mean (SD)                     | 56.8 (9.35)   | 52.6 (10.98)  | 54.7 (10.40)  |                    |
| Median                        | 57.0          | 53.0          | 55.0          |                    |
| Min, Max                      | 26, 75        | 30, 78        | 26, 78        |                    |
| Gender                        |               |               |               | 0.018 <sup>2</sup> |
| -- Male                       | 18 (26.1%)    | 33 (45.2%)    | 51 (35.9%)    |                    |
| -- Female                     | 51 (73.9%)    | 40 (54.8%)    | 91 (64.1%)    |                    |
| Race                          |               |               |               |                    |

| Parameter Category/Statistics                | R-CIMT<br>(N=69) | W-CIMT<br>(N=73) | Total<br>(N=142) | p-value            |
|--|------------------|------------------|------------------|--------------------|
| -- American-Indian or Alaska Native          | 3 (4.3%)         | 1 (1.4%)         | 4 (2.8%)         |                    |
| -- Asian                                     | 1 (1.4%)         | 3 (4.1%)         | 4 (2.8%)         |                    |
| -- Black or African-American                 | 29 (42.0%)       | 35 (47.9%)       | 64 (45.1%)       |                    |
| -- Native Hawaiian or Other Pacific Islander | 0                | 0                | 0                |                    |
| -- White or Caucasian                        | 38 (55.1%)       | 30 (41.1%)       | 68 (47.9%)       |                    |
| -- Other                                     | 2 (2.9%)         | 7 (9.6%)         | 9 (6.3%)         |                    |
| <b>Weight (kg)</b>                           |                  |                  |                  |                    |
|  |                  |                  |                  | 0.991 <sup>1</sup> |
| N  | 69               | 73               | 142              |                    |
| Mean (SD)                                    | 90.53 (19.318)   | 90.50 (16.955)   | 90.51 (18.077)   |                    |
| Median                                       | 87.70            | 91.50            | 90.80            |                    |
| Min, Max                                     | 53.1, 127.0      | 58.4, 145.5      | 53.1, 145.5      |                    |

<sup>1</sup>ANOVA model with CIMT group as the factor; if the assumption of normal distribution is violated then Wilcoxon-Mann Whitney test (non-parametric method) will be applied instead; <sup>2</sup>Chi-square or Fisher's exact test as appropriate.

A composite index of adherence to the TLC intervention was selected as the primary outcome measure since the main goal of this study is to assess the impact of CIMT imaging knowledge on change in lifestyle behaviors. A combined measure of adherence, reflecting both aspects of the lifestyle intervention, was chosen that uses accepted measures of diet and exercise adherence reported in the literature. At study closeout, both groups showed marked improvement in both diet and exercise adherence as compared to baseline, however, no difference was detected between the study groups, thereby, confirming the null hypothesis that knowledge of an abnormal CIMT scan did not have a motivational impact on overall adherence to the TLC intervention in this study (see Table 6).

**Table 6: Primary Efficacy Analysis: Mean Adherence Change**

| Parameter                       | Statistics | R-CIMT<br>(N=69) | W-CIMT<br>(N=73) | Total<br>(N=142) | p-value |
|---------------------------------|------------|------------------|------------------|------------------|---------|
| <b>Adherence at Baseline</b>    |            |                  |                  |                  |         |
|                                 | N          | 69               | 73               | 142              |         |
|                                 | Mean (SD)  | 45.447 (16.6740) | 40.678 (17.2143) | 42.995 (17.0623) |         |
|                                 | Median     | 44.762           | 39.722           | 41.111           |         |
|                                 | Min, Max   | 12.14, 82.14     | 7.14, 78.57      | 7.14, 82.14      |         |
|                                 | Q1, Q3     | 32.103, 60.357   | 27.937, 51.786   | 30.357, 57.143   |         |
| <b>Adherence at Week 12 OC*</b> |            |                  |                  |                  |         |
|                                 | N          | 69               | 73               | 142              |         |
|                                 | Mean (SD)  | 65.060 (20.1362) | 63.322 (22.0451) | 64.166 (21.0824) |         |
|                                 | Median     | 62.063           | 66.746           | 63.075           |         |
|                                 | Min, Max   | 28.57, 97.78     | 25.00, 100.00    | 25.00, 100.00    |         |
|                                 | Q1, Q3     | 51.190, 82.143   | 42.421, 85.714   | 45.754, 83.929   |         |

| Parameter            | Statistics | R-CIMT<br>(N=69) | W-CIMT<br>(N=73) | Total<br>(N=142) | p-value                  |
|----------------------|------------|------------------|------------------|------------------|--------------------------|
| Adherence Change (%) | N          | 69               | 73               | 142              | <b>0.519<sup>1</sup></b> |
|                      | Mean (SD)  | 19.613 (24.3395) | 22.644 (24.2286) | 21.171 (24.2440) |                          |
|                      | Median     | 16.746           | 24.365           | 21.429           |                          |
|                      | Min, Max   | -39.29, 73.81    | -50.00, 72.26    | -50.00, 73.81    |                          |
|                      | Q1, Q3     | 3.452, 36.270    | 4.206, 41.627    | 3.532, 38.889    |                          |

Note: P value of change from baseline is obtained by ANCOVA model with CIMT group, gender as factors and age as the covariate; if assumptions are invalid, non-parametric ANCOVA will be applied

<sup>1</sup> indicates that P value is obtained from parametric method;

<sup>2</sup> shows P value is obtained from non-parametric method.

\* OC refers to the measurement observed cases at week 12. Adherence will be calculated by OC of Mediterranean Diet % adherence and OC of Exercise % adherence; adherence is capped at 100%

Although the hypothesis was not supported, study completers did make significant improvements in most of their modifiable risk factors (anthropometrics; total and LDL-cholesterol; triglycerides). Slight increases were seen in systolic and diastolic blood pressure.

Data analysis on these completers, comparing study completion to baseline, was conducted using *t-test* and *Wilcoxon Sign Test* statistics (see Table 7). Measures of obesity including weight, BMI and % body fat were reduced by 5%. Additionally, a 5% reduction in waist circumference and a 7% reduction in abdominal sagittal diameter were seen. Both systolic and diastolic blood pressure increased by 2%.

Levels of total cholesterol were reduced by 6%, LDL-cholesterol decreased by 9% and triglycerides were lowered by 14%. C-reactive protein (CRP) was decreased by 17%. Despite these positive changes, a 1% reduction in HDL-cholesterol was seen.

Serum fasting glucose and insulin were collected and HOMA scores calculated as a measure of insulin resistance (IR). At baseline, 48% of the study completers had HOMA score > 2.8, indicative of IR. At study completion, 19 subjects were able to lower their HOMA scores < 2.8 and reduce their risk of pre-diabetes. Overall, serum glucose was reduced by 4% and fasting insulin was reduced by 23.3%.

**Table 7. Secondary Outcome Variables in Study Completers (n=142)**

|                          | Baseline     | Study Completion | Change     | P                   |
|--------------------------|--------------|------------------|------------|---------------------|
| <b>Body Composition</b>  |              |                  |            |                     |
| Weight (kg)              | 90.5 ± 18.1  | 86.1 ± 17.3      | -4.5 ± 3.8 | <0.001 <sup>1</sup> |
| BMI (kg/m <sup>2</sup> ) | 31.5 ± 5.6   | 29.9 ± 5.5       | -1.5 ± 1.3 | <0.001 <sup>1</sup> |
| % Body Fat               | 37.1 ± 8.5   | 35.3 ± 8.7       | -1.8 ± 2.6 | <0.001 <sup>1</sup> |
| Sagittal Diameter (cm)   | 24.0 ± 3.8   | 22.3 ± 3.7       | -1.7 ± 1.6 | <0.001 <sup>1</sup> |
| Waist Circumference (cm) | 100.6 ± 13.1 | 95.6 ± 13.1      | -5.0 ± 4.1 | <0.001 <sup>1</sup> |

|                            | Baseline      | Study Completion | Change         | P                   |
|----------------------------|---------------|------------------|----------------|---------------------|
| <b>Laboratory (mg/dL)</b>  |               |                  |                |                     |
| Glucose (mg/dL)            | 96.8 ± 19.0   | 93.1 ± 14.1      | -3.7 ± 14.6    | 0.003 <sup>1</sup>  |
| Insulin (uIU/mL)           | 14.6 ± 12.6   | 11.2 ± 8.0       | -3.4 ± 7.6     | <0.001 <sup>1</sup> |
| HOMA (Insulin Resistance)  | 3.9 ± 4.4     | 2.8 ± 2.8        | -1.1 ± 3.3     | <0.001 <sup>2</sup> |
| Total Cholesterol (mg/dL)  | 195.8 ± 40.1  | 184.1 ± 35.5     | -11.8 ± 26.0   | <0.001 <sup>1</sup> |
| LDL-Cholesterol(mg/dL)     | 119.5 ± 35.5  | 108.4 ± 28.8     | -11.2 ± 22.0   | <0.001 <sup>1</sup> |
| HDL-Cholesterol(mg/dL)     | 56.0 ± 15.9   | 55.4 ± 14.5      | -0.61 ± 7.7    | 0.348 <sup>1</sup>  |
| Triglycerides (mg/dL)      | 122.2 ± 62.4  | 105.3 ± 52.6     | -16.9 ± 43.8   | <0.001 <sup>1</sup> |
| C-reactive protein (mg/dL) | 0.425 ± 0.528 | 0.352 ± 0.461    | -0.074 ± 0.318 | 0.007 <sup>2</sup>  |

Values are mean ± SD.

<sup>1</sup> P value is obtained from parametric method.

<sup>2</sup> P value is obtained from non-parametric method.

Although these data do not support the motivational impact of an abnormal CIMT scan on program adherence, more analysis will be conducted to fully describe the study results. It is clear that this data supports participation in a lifestyle modification program which includes education and frequent monitoring does result in substantial CV risk factor improvements. Some of these changes rival what has been observed with pharmacological treatment.

**Preliminary Analysis for Summative Evaluation of Lifestyle Program (Addendum #6):**

This formative evaluation took place between Jan-Mar 2011. Of the 140 surveys mailed to consenting BATTLE Study participants, 49% (n=68) were returned. Additionally, 36 completers from study year 2 and 5 non-completers gave permission to be contacted for a telephone interview. Of these 41 participants, 35 telephonic interviews were conducted over this 2 month period. All telephone interviews have been transcribed. Frequency counts of survey responses and thematic coding of telephone interviews and open ended survey responses will be finalized on Award No. W81XWH10-2-0227..

**Adverse Events:** No adverse events occurred during this period of performance. A summary of adverse events (10 serious and 25 non-serious) were reported on Final Report for Award No. W81XWH-05-2-0075.

**Task #5: Transition away from the traditional Dr. Dean Ornish Program for Reversing Heart Disease protocol.**

**Status:** This study is continued from Award No. W81XWH-05-2-0075 and study methodology and approvals have been reported previously. Enrollment into the Dr. Dean Ornish Program is closed and all active participants have completed their participation in the study. Data analysis is ongoing.

### **Subject Enrollment and Demographics**

This program is closed to enrollment and all active subjects have completed the program. Subject enrollment was 422 participants including 25 cohorts and 4 retreats. 339 participants graduated from the program and 83 participants discontinued participation (20% dropout rate). Demographic characteristics of participants were: average age of 66.1 years, 53% female, 33% veterans or the spouse of a veteran, and 41% had diagnosed coronary heart disease.

### **Outcome Data**

Participants in the Dr. Dean Ornish Program at WMC achieved significant improvement in levels of virtually all of the measured coronary artery disease (CAD) risk factors over the initial 12-week period. Measures of obesity including weight and BMI declined ~7%, levels of total cholesterol were reduced by nearly 13%, blood pressure dropped ~9%, measures of physical fitness increased more than 26%, and levels of depression decreased approximately 47%. These data demonstrate that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease. Over the course of one year, weight and BMI decreased ~9%, diastolic blood pressure decreased ~7%, measures of physical fitness increased 25%, and levels of depression decreased nearly 50%.

### **Task #6: Complete enrollment in Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Subjects in the Dr. Dean Ornish Program protocol.**

#### **Status:**

This study is continued from Award No. W81XWH-05-2-0075 and study methodology and approvals have been reported previously. Enrollment to the global profiling study is closed and all active participants have completed their participation in the study. Enrollment in the sub-study was closed as of July 27, 2007. Data analysis is ongoing.

#### **Subject Enrollment and Demographics**

Subject enrollment was 374. There were 166 participants taking part in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study. Demographic characteristics of the control group were: average age of 63.7 years, 51% were female, 29% were veterans or the spouse of a veteran, and 34% had diagnosed coronary heart disease.

#### **Data:**

##### **Inflammation biomarker panel –**

Statistical analysis was performed on the biomarker panel.

A summary of the results for insulin and leptin is presented below. Table 8 shows levels of insulin and leptin, as well as physiological measures, at baseline. Change over time in Ornish participants and controls for insulin, leptin, and physiological measures is presented in Tables 9 and 10. Table 11 shows medications known to affect plasma

levels of insulin, leptin, and lipids. Table 12 shows the effects of medication use on plasma insulin, leptin, and lipid levels.

Considerable effort was devoted to tabulating the number of brand name medications tracked in our database for each Ornish patient, and to reviewing the effects of medications on plasma insulin and leptin levels. We also collected information on the degree to which leptin and insulin change with other therapeutic or lifestyle regimens.

**Table 8. Insulin, Leptin, and Physiological Measures at Baseline by Case/Control Status**

| Measure (n)            | Controls    | Participants | p Value*          |
|------------------------|-------------|--------------|-------------------|
| Metabolic hormones     |             |              |                   |
| Insulin (150)          | 14.3 ± 7.1  | 18.1 ± 10.2  | 0.01 <sup>†</sup> |
| Leptin (152)           | 19.0 ± 17.2 | 23.5 ± 18.6  | 0.06 <sup>†</sup> |
| Physiological measures |             |              |                   |
| Age (152)              | 60.6 ± 7.6  | 60.6 ± 7.6   | 0.99              |
| BMI (152)              | 28.5 ± 4.5  | 32.9 ± 7.2   | <0.01             |
| SBP (146)              | 132 ± 16    | 136 ± 17     | 0.12 <sup>†</sup> |
| DBP (146)              | 78.6 ± 10.1 | 81.0 ± 10.1  | 0.24 <sup>†</sup> |
| HDL (152)              | 49.4 ± 13.0 | 45.5 ± 13.4  | 0.06              |
| LDL (142)              | 108 ± 34    | 112 ± 39     | 0.59              |
| TCH (152)              | 185 ± 43    | 195 ± 48     | 0.20              |
| TG (152)               | 144 ± 98    | 176 ± 94     | <0.01             |
| EC (122)               | 9.3 ± 2.9   | 6.6 ± 2.1    | <0.01             |
| Fram risk (124)        | 6.8 ± 6.7   | 8.8 ± 7.7    | 0.16 <sup>†</sup> |

Values are presented as mean ± SD. Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCH, total cholesterol; TG, triglycerides; EC, exercise capacity.

\*Tested by 1-factor ANOVA by cohort type.

<sup>†</sup>Tested by a nonparametric Mann-Whitney U test because data was not normally distributed after LN transformation.

**Table 9. Changes in Insulin, Leptin, and Physiological Measures by Case/Control Status**

| Measure                              | Controls (n=76) |                          |                       |          | Participants (n=76) |                          |                          |          | Between Group p Value* |
|--------------------------------------|-----------------|--------------------------|-----------------------|----------|---------------------|--------------------------|--------------------------|----------|------------------------|
|                                      | Baseline        | Week 12                  | Week 52               | % Change | Baseline            | Week 12                  | Week 52                  | % Change |                        |
| Metabolic hormones <sup>†</sup>      |                 |                          |                       |          |                     |                          |                          |          |                        |
| Insulin                              | 14.3 ± 7.1      | 14.9 ± 6.3               | 14.9 ± 6.8            | +4.0     | 18.1 ± 10.2         | 14.8 ± 7.1 <sup>‡</sup>  | 14.6 ± 7.8 <sup>§</sup>  | -19.2    | <0.01                  |
| Leptin                               | 19.0 ± 17.2     | 16.5 ± 14.7              | 20.3 ± 16.8           | +6.6     | 23.5 ± 18.6         | 14.3 ± 11.1 <sup>§</sup> | 15.8 ± 13.6 <sup>§</sup> | -32.9    | <0.01                  |
| Physiological measures <sup>  </sup> |                 |                          |                       |          |                     |                          |                          |          |                        |
| BMI                                  | 28.5 ± 4.5      | 28.3 ± 4.7               | 28.7 ± 4.8            | +0.9     | 32.9 ± 7.2          | 30.5 ± 6.6 <sup>§</sup>  | 29.8 ± 6.8 <sup>§</sup>  | -9.3     | <0.01                  |
| SBP                                  | 132 ± 16        | 126 ± 15 <sup>‡</sup>    | 125 ± 13 <sup>‡</sup> | -5.3     | 136 ± 17            | 122 ± 14 <sup>§</sup>    | 127 ± 17 <sup>§</sup>    | -6.4     | 0.56                   |
| DBP                                  | 78.6 ± 10.1     | 77.1 ± 8.3               | 77.3 ± 9.3            | -1.5     | 81.0 ± 10.1         | 73.0 ± 9.0 <sup>§</sup>  | 75.5 ± 9.5 <sup>§</sup>  | -6.7     | 0.02                   |
| HDL                                  | 49.4 ± 13.0     | 52.0 ± 13.1 <sup>‡</sup> | 47.9 ± 13.3           | -3.0     | 45.5 ± 13.4         | 38.5 ± 9.5 <sup>§</sup>  | 43.1 ± 10.5 <sup>‡</sup> | -5.2     | 0.50                   |
| LDL                                  | 108 ± 34        | 106 ± 35                 | 108 ± 34              | -0.4     | 112 ± 39            | 98 ± 32 <sup>§</sup>     | 109 ± 33                 | -2.8     | 0.54                   |
| TCH                                  | 185 ± 43        | 187 ± 46                 | 185 ± 43              | -0.2     | 195 ± 48            | 170 ± 43 <sup>§</sup>    | 185 ± 44 <sup>‡</sup>    | -5.0     | 0.07                   |
| TG                                   | 144 ± 98        | 156 ± 138                | 146 ± 88              | +2.0     | 176 ± 94            | 163 ± 73                 | 163 ± 93                 | -7.2     | 0.21                   |
| EC                                   | 9.3 ± 2.9       | 9.5 ± 2.8                | 9.3 ± 2.7             | -0.6     | 6.6 ± 2.1           | 8.4 ± 2.2 <sup>§</sup>   | 9.0 ± 2.6 <sup>§</sup>   | +37.6    | <0.01                  |
| Fram risk                            | 6.8 ± 6.7       | 6.3 ± 6.5                | 6.7 ± 7.0             | -2.2     | 8.8 ± 7.7           | 8.3 ± 7.7                | 8.5 ± 7.4                | -3.9     | 0.49                   |

Values are presented as mean ± SD; % change = week 0-52. Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCH, total cholesterol; TG, triglycerides; EC, exercise capacity.

\*From independent samples t-tests (two-tailed) of Baseline to Week 52 changes in intervention participants compared to controls.

<sup>†</sup>There was 1.3% missing data.

<sup>‡</sup>Significantly different from Baseline at p<0.05 based on repeated-measures ANOVA.

<sup>§</sup>Significantly different from Baseline at p<0.001 based on repeated-measures ANOVA.

<sup>||</sup>There was <5.8% missing data.

**Table 10. Changes in Insulin, Leptin, and Selected Physiological Measures by Gender**

| Measure                              | Gender | Controls (n=76) |             |             |          | Participants (n=76) |                          |                          |          | Between Group p Value* |
|--------------------------------------|--------|-----------------|-------------|-------------|----------|---------------------|--------------------------|--------------------------|----------|------------------------|
|                                      |        | Baseline        | Week 12     | Week 52     | % Change | Baseline            | Week 12                  | Week 52                  | % Change |                        |
| Metabolic hormones <sup>†</sup>      |        |                 |             |             |          |                     |                          |                          |          |                        |
| Insulin                              | F      | 13.3 ± 6.3      | 14.3 ± 6.4  | 14.1 ± 6.7  | +5.7     | 18.0 ± 8.6          | 15.3 ± 7.0               | 14.5 ± 7.8 <sup>‡</sup>  | -19.5    | <0.01                  |
|                                      | M      | 15.4 ± 7.7      | 15.6 ± 6.3  | 15.7 ± 6.9  | +2.4     | 18.2 ± 11.8         | 14.3 ± 7.3 <sup>‡</sup>  | 14.8 ± 7.8 <sup>‡</sup>  | -18.9    | <0.05                  |
| Leptin                               | F      | 27.4 ± 19.7     | 23.8 ± 16.7 | 28.9 ± 17.5 | +5.6     | 32.1 ± 19.7         | 19.9 ± 10.6 <sup>§</sup> | 21.4 ± 12.3 <sup>§</sup> | -33.3    | <0.01                  |
|                                      | M      | 10.2 ± 7.0      | 8.8 ± 6.0   | 11.2 ± 9.9  | +9.5     | 14.5 ± 12.1         | 8.4 ± 8.4 <sup>‡</sup>   | 9.9 ± 12.3 <sup>‡</sup>  | -32.0    | <0.01                  |
| Physiological measures <sup>  </sup> |        |                 |             |             |          |                     |                          |                          |          |                        |
| BMI                                  | F      | 29.0 ± 5.0      | 29.0 ± 5.1  | 28.2 ± 5.5  | -0.8     | 33.5 ± 7.1          | 31.1 ± 6.5 <sup>§</sup>  | 30.3 ± 6.9 <sup>§</sup>  | -9.6     | <0.01                  |
|                                      | M      | 27.9 ± 4.0      | 27.6 ± 4.2  | 28.2 ± 4.1  | +1.0     | 32.2 ± 7.3          | 29.8 ± 6.8 <sup>§</sup>  | 29.4 ± 6.7 <sup>§</sup>  | -8.9     | <0.01                  |
| DBP                                  | F      | 80.6 ± 10.4     | 77.9 ± 8.8  | 79.1 ± 9.2  | -1.8     | 81.3 ± 7.2          | 72.6 ± 8.0 <sup>§</sup>  | 76.1 ± 8.6 <sup>‡</sup>  | -6.4     | 0.09                   |
|                                      | M      | 76.2 ± 9.2      | 76.3 ± 7.7  | 75.3 ± 9.1  | -1.2     | 80.7 ± 12.7         | 73.4 ± 10.2 <sup>§</sup> | 74.9 ± 10.6 <sup>‡</sup> | -7.2     | 0.12                   |
| TCH                                  | F      | 202 ± 42        | 206 ± 48    | 199 ± 43    | -1.4     | 209 ± 47            | 183 ± 40 <sup>§</sup>    | 198 ± 43                 | -5.4     | 0.28                   |
|                                      | M      | 168 ± 36        | 168 ± 35    | 170 ± 39    | +1.3     | 179 ± 45            | 156 ± 41 <sup>§</sup>    | 171 ± 40                 | -4.4     | 0.11                   |
| TG                                   | F      | 161 ± 100       | 184 ± 181   | 155 ± 82    | -3.5     | 174 ± 84            | 179 ± 82                 | 183 ± 110                | +5.2     | 0.45                   |
|                                      | M      | 126 ± 94        | 126 ± 58    | 137 ± 95    | +9.5     | 178 ± 105           | 146 ± 57                 | 143 ± 65                 | -19.9    | <0.01                  |
| EC                                   | F      | 8.4 ± 2.9       | 8.7 ± 2.8   | 8.4 ± 2.6   | -0.3     | 6.5 ± 1.9           | 8.0 ± 1.8 <sup>§</sup>   | 8.7 ± 2.3 <sup>§</sup>   | +35.0    | <0.01                  |
|                                      | M      | 10.5 ± 2.3      | 10.6 ± 2.3  | 10.4 ± 2.5  | -1.0     | 6.7 ± 2.4           | 8.9 ± 2.5 <sup>§</sup>   | 9.4 ± 2.9 <sup>§</sup>   | +40.7    | <0.01                  |

Values are presented as mean ± SD; % change = week 0-52. Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; TCH, total cholesterol; TG, triglycerides; EC, exercise capacity.

\*From independent samples t-tests (two-tailed) of Baseline to Week 52 changes in intervention participants compared to controls.

<sup>†</sup>There was <3.2% missing data.

<sup>‡</sup>Significantly different from Baseline at p<0.05 based on repeated-measures ANOVA.

<sup>§</sup>Significantly different from Baseline at p<0.001 based on repeated-measures ANOVA.

<sup>||</sup>There was <7.2% missing data.

**Table 11. Medications Affecting Plasma Levels of Insulin, Leptin, and Lipids**

| Medication Category (n)*       | Insulin        | Leptin         | HDL            | LDL            | TCH            | TG             |
|--------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| ACE inhibitor (16)             | ↑              | ↑ <sup>†</sup> |                | ↓              | ↓              | ↓              |
| Anticoagulant (4)              |                |                | ↑              |                |                |                |
| Beta blocker (15)              | ↑              | ↓              | ↓              | ↓              | ↓              |                |
| Calcium channel blocker (13)   | ↑              | ↓              | ↑              | ↓              | ↓              | ↓              |
| Insulin medication (7)         | ▲ <sup>†</sup> | ↓              |                |                |                |                |
| Diuretic (14)                  |                | ↑ <sup>†</sup> |                | ↑              | ↑              |                |
| Lipid lowering medication (22) |                | ↓ <sup>†</sup> | ↑ <sup>†</sup> | ▼ <sup>†</sup> | ▼ <sup>†</sup> | ▼ <sup>†</sup> |
| Nitrate (4)                    |                |                | ↑ <sup>†</sup> | ↓              | ↓              |                |
| Oral antidiabetic (14)         | ▲ <sup>†</sup> |                |                |                |                |                |

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCH, total cholesterol; TG, triglycerides.

Key to medication effects: ▲ – increase, main effect; ↑ – increase, side effect; ▼ – decrease, main effect; ↓ – decrease, side effect.

\*The number of brand name medications in each category is indicated in parentheses.

<sup>†</sup>Considered a primary medication category in Table 5.

**Table 12. Effects of Medication Changes on Insulin, Leptin, and Selected Physiological Measures**

| Measure           | All Participants         |                          |                        | Composite Medications*   |                          |                        | Primary Medications†     |                          |                        |
|-------------------|--------------------------|--------------------------|------------------------|--------------------------|--------------------------|------------------------|--------------------------|--------------------------|------------------------|
|                   | % Change in One Year (n) |                          |                        | % Change in One Year (n) |                          |                        | % Change in One Year (n) |                          |                        |
|                   | Controls                 | Participants             | Between Group p Value‡ | Controls                 | Participants             | Between Group p Value‡ | Controls                 | Participants             | Between Group p Value‡ |
| Metabolic hormone |                          |                          |                        |                          |                          |                        |                          |                          |                        |
| Insulin           | +4.0 (75)                | -19.2 <sup>  </sup> (75) | <0.01                  | -3.3 (60)                | -14.2 <sup>§</sup> (47)  | <0.01                  | +4.7 (72)                | -16.9 <sup>§</sup> (69)  | <0.01                  |
| Leptin            | +6.6 (76)                | -32.9 <sup>  </sup> (76) | <0.01                  | 3.0 (43)                 | -32.1 <sup>  </sup> (41) | <0.01                  | +9.9 (47)                | -34.5 <sup>  </sup> (50) | <0.01                  |
| Lipid measures    |                          |                          |                        |                          |                          |                        |                          |                          |                        |
| HDL               | -3.0 (76)                | -5.2 <sup>§</sup> (76)   | 0.50                   | -2.1 (41)                | -6.3 <sup>§</sup> (39)   | 0.22                   | -2.5 (49)                | -6.1 <sup>§</sup> (58)   | 0.28                   |
| LDL               | -0.4 (71)                | -2.8 (71)                | 0.54                   | -5.6 (41)                | -3.4 (37)                | 0.03                   | +4.2 (47)                | -0.1 (57)                | 0.26                   |
| TCH               | -0.2 (76)                | -5.0 <sup>§</sup> (76)   | 0.07                   | -3.4 (43)                | -5.3 <sup>§</sup> (40)   | <0.01                  | +3.1 (50)                | -3.3 (61)                | 0.02                   |
| TG                | +2.0 (76)                | -7.2 (76)                | 0.21                   | -4.5 (47)                | -2.9 (48)                | 0.47                   | +7.3 (50)                | -4.9 (61)                | 0.19                   |

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TCH, total cholesterol; TG, triglycerides.

\*Composite medication categories for all measures are shown in Table 4.

†Primary medication categories include: for insulin — insulin medications, oral antiglycemics; leptin — ACE inhibitors, diuretics, lipid lowering medications; HDL — lipid lowering medications, nitrates; LDL — lipid lowering medications; TCH — lipid lowering medications; TG — lipid lowering medications.

‡From independent samples t-tests (two-tailed) of Baseline to Week 52 changes in intervention participants compared to controls.

§Significantly different from Baseline at p<0.05 based on repeated-measures ANOVA.

||Significantly different from Baseline at p<0.001 based on repeated-measures ANOVA.

Based on these results, we have been able to show that the metabolic hormones insulin and leptin decrease significantly in patients involved in the lifestyle modification program compared to controls. There do not appear to be significant differences between males and females in regard to insulin/leptin lowering response following intervention. Likewise, changes in medication use and/or dosage during the program did not have significant effects on insulin and leptin response.

The Ornish program compares favorably with other lifestyle interventions for reducing circulating insulin and leptin levels (Table 13).

**Table 13. Change in insulin and leptin during various lifestyle interventions.**

| Variable       | Units                | Intervention          | Mean follow-up <sup>¶</sup> (weeks) | Baseline value ± SD | Mean change (%) | Reference |
|----------------|----------------------|-----------------------|-------------------------------------|---------------------|-----------------|-----------|
| <b>Insulin</b> | pmol/L               | exercise              | 104                                 | 41.3 ± 1.2          | +17.7           | 13        |
|                | mU/L                 | exercise              | 96                                  | 13.4 ± 2.3          | +16.4           | 56        |
|                | pmol/L <sup>-1</sup> | exercise              | 8                                   | 80.8 ± 40.7         | +13.9           | 74        |
|                | μU/mL                | diet                  | 8                                   | 12.4                | +8              | 35        |
|                | pmol/L               | diet and exercise     | 104                                 | 45 ± 14             | +6.7            | 12        |
|                | pmol/L               | lifestyle             | 8                                   | 61 ± 32             | +6.5            | 23        |
|                | mU/L                 | lifestyle             | 24                                  | 11.9                | +4              | 11        |
|                | mmol/L               | exercise              | 52                                  | 62.6 ± 53.6         | +2.6            | 48        |
|                | pmol/L               | lifestyle             | 52                                  | 141 ± 105 **        | +2.5            | 73        |
|                | IU/L                 | exercise              | 12                                  | 9.26 ± 5.82         | +1.9            | 49        |
|                | pmol/L <sup>-1</sup> | exercise              | 16                                  | 92.3 ± 50.1         | -2.4            | 63        |
|                | μU/mL                | exercise              | 1                                   | 16 ± 2              | -6.3            | 52        |
|                | μU/mL                | exercise              | 24                                  | 24.57 ± 3.85        | -6.7            | 61        |
|                | U/mL                 | diet                  | 12                                  | 18 ± 6              | -7.8            | 34        |
|                | pmol/L               | diet                  | 52                                  | 56.5 ± 3.96         | -8              | 3         |
|                | pmol/L               | lifestyle             | 8                                   | 49 ± 21             | -8              | 23        |
|                | μU/mL                | exercise              | 12                                  | 20.1 ± 3.5          | -9.5            | 77        |
|                | μU/mL                | diet                  | 20                                  | 4.82                | -10.6           | 9         |
|                | IU/L                 | exercise              | 24                                  | 12.3 ± 8.0          | -10.6           | 60        |
|                | mU/L                 | diet (cod)            | 8                                   | 10.1 ± 4.1          | -12             | 28        |
|                | μU/mL                | diet                  | 4                                   | 14.2 ± 5.8          | -12.7           | 33        |
|                | μU/mL                | diet and exercise     | 12                                  | 16.7 ± 1.5          | -13.8           | 47        |
|                | pmol/L               | diet and exercise     | 52                                  | 143 ± 82            | -14             | 29        |
|                | μU/mL                | diet and exercise     | 24                                  | 25 ± 1              | -14             | 81        |
|                | μU/mL                | exercise              | 12                                  | 9.7 ± 0.8           | -14.4           | 36        |
|                | μU/mL                | diet                  | 8                                   | 10.06               | -14.5           | 22        |
|                | μU/mL                | exercise              | 52                                  | 13.8 ± 6.8          | -15             | 59        |
|                | μU/mL                | exercise              | 12                                  | 21.8 ± 2.7          | -16.5           | 82        |
|                | μU/mL <sup>-1</sup>  | diet and exercise     | 12                                  | 21 ± 2.7            | -16.7           | 46        |
|                | pmol/L               | supplement + exercise | 24                                  | 46 ± 11             | -17.4           | 80        |
|                | μU/mL                | lifestyle             | 52                                  | 19.4 ± 10           | -17.5           | 17        |
|                | μU/mL                | exercise              | 52                                  | 11.6 ± 4.9          | -18             | 27        |
| pmol/L         | diet and exercise    | 52                    | 81.3 ± 6.85 **                      | -18.5               | 65              |           |
| μU/mL          | diet and exercise    | 68                    | 4.7 ± 0.4                           | -19                 | 14              |           |
| »              | <b>μU/mL</b>         | <b>Ornish Program</b> | <b>52</b>                           | <b>18.1 ± 10.2</b>  | <b>-19.2</b>    |           |

| Variable      | Units               | Intervention       | Mean follow-up <sup>¶</sup> (weeks) | Baseline value ± SD | Mean change (%) | Reference |
|---------------|---------------------|--------------------|-------------------------------------|---------------------|-----------------|-----------|
|               | μU/mL               | diet               | 13                                  | 10                  | -20             | 15        |
|               | IU/L                | exercise           | 12                                  | 13.8 ± 7.8          | -21             | 53        |
|               | μU/mL               | diet and exercise  | 48                                  | 9.6 ± 7.9           | -21.9           | 41        |
|               | mU/L                | diet (salmon)      | 8                                   | 10.8 ± 5.2          | -22             | 28        |
|               | pmol/L              | exercise           | 24                                  | 102 ± 9             | -22.5           | 54        |
|               | μU/mL               | diet and exercise  | 1                                   | 16.8 ± 1.5          | -22.6           | 71        |
|               | pmol/L              | exercise           | 12                                  | 96.5 ± 49.5         | -22.9           | 70        |
|               | IU/mL               | lifestyle          | 12                                  | 8.2 ± 7.0           | -23.2           | 76        |
|               | mU/L                | diet (fish oil)    | 8                                   | 10.1 ± 4.6          | -23.7           | 28        |
|               | pmol/L              | exercise           | 16                                  | 59                  | -23.7           | 57        |
|               | μU/mL               | diet and exercise  | 20                                  | 11.7 ± 8.4          | -24.8           | 62        |
|               | pmol/L              | diet               | 12                                  | 54.4 ± 33.1         | -26.4           | 19        |
|               | μU/mL               | diet and exercise  | 12                                  | 6.3 ± 4.9           | -27             | 5         |
|               | μU/mL               | exercise           | 16                                  | 10.3 ± 10.4         | -27.2           | 55        |
|               | μU/mL               | exercise           | 16                                  | 10.3 ± 10.4         | -27.2           | 78        |
|               | μU/mL               | diet               | 8                                   | 11.94               | -27.5           | 22        |
|               | μU/mL               | diet and exercise  | 4                                   | 12.8 ± 6.5          | -28.1           | 18        |
|               | mU/L                | yoga               | 6.4                                 | 31.47 ± 17.28       | -29             | 1         |
|               | μU/mL               | diet and exercise  | 3                                   | 33.8 ± 4.0          | -29.6           | 6         |
|               | μU/mL               | diet and exercise  | 3                                   | 30.9 ± 3.3          | -30             | 7         |
|               | mU/L                | exercise and diet* | 8                                   | 7.3 ± 0.8           | -30             | 20        |
|               | μU/mL               | diet and exercise  | 8                                   | 13.55 ± 13.35       | -30             | 64        |
|               | mU/L                | diet and exercise  | 20                                  | 16.0 ± 8.0          | -30.1           | 45        |
|               | μU/mL               | diet and exercise  | 16                                  | 18.1                | -30.9           | 67        |
|               | μU/mL               | diet               | 20                                  | 19.0 ± 12.4         | -31             | 16        |
|               | mU/L                | lifestyle          | 52                                  | 16                  | -31             | 25        |
|               | μU/mL <sup>-1</sup> | exercise           | 15                                  | 19.4 ± 3.4          | -31             | 51        |
|               | IU/mL               | diet and exercise  | 12                                  | 13.2 ± 6.6          | -31.8           | 68        |
|               | pmol/L              | diet               | 4                                   | 8.1 ± 3.3           | -32             | 30        |
|               | μU/mL               | diet               | 104                                 | 11.0 ± 5.0          | -36             | 8         |
|               | μU/mL               | lifestyle          | 104                                 | 14 ± 4              | -36             | 10        |
|               | mU/mL <sup>-1</sup> | exercise           | 36                                  | 29.2                | -36.6           | 50        |
|               | uk                  | diet               | 8                                   | 23.7 ± 12.4         | -37             | 4         |
|               | μU/mL               | exercise           | 12                                  | 11.5 ± 8.4          | -39             | 31        |
|               | μU/mL               | diet               | 6                                   | 12.7 ± 10.1         | -39             | 39        |
|               | IU/L                | lifestyle          | 9                                   | 10.12 ± 3.40        | -40.5           | 72        |
|               | pmol/L              | exercise           | 12                                  | 109.0 ± 68.2        | -42.4           | 66        |
|               | μU/mL               | exercise           | 12                                  | 8.47 ± 1.27         | -43.8           | 69        |
|               | μU/mL               | lifestyle          | 24                                  | 45 ± 6.3            | -46.4           | 58        |
|               | pmol/L              | diet               | 12                                  | 186 ± 65            | -49             | 2         |
| <b>Leptin</b> | ng/ml               | lifestyle          | 52                                  | 18.4 ± 14.3         | +27.2           | 17        |
|               | μg/l                | exercise           | 24 months                           | 7.4 ± 3.8           | +6.7            | 56        |
|               | ng/ml               | exercise           | 16                                  | 10.6 ± 5.3          | 0               | 55        |
|               | ng/ml               | exercise           | 1                                   | 21.5 ± 4.5          | -5.6            | 52        |
|               | ng/ml               | diet               | 8                                   | 30.5 ± 16.3         | -9              | 35        |
|               | ng/ml               | exercise           | 8                                   | 52.0 ± 14.4         | -9              | 43        |

| Variable | Units               | Intervention          | Mean follow-up <sup>†</sup> (weeks) | Baseline value ± SD | Mean change (%) | Reference |
|----------|---------------------|-----------------------|-------------------------------------|---------------------|-----------------|-----------|
|          | mmol/L              | lifestyle             | 52                                  | 675                 | -10.8           | 32        |
|          | ng/ml               | diet and exercise     | 12                                  | 12.20 ± 3.22        | -11             | 42        |
|          | ng/mL <sup>-1</sup> | exercise              | 15                                  | 34.8 ± 5.8          | -12             | 51        |
|          | ng/ml               | diet                  | 12                                  | 9.6 ± 12.8          | -12.5           | 26        |
|          | ng/ml               | exercise              | 9                                   | 8.15 ± 3.75         | -14             | 44        |
|          | ng/ml               | diet                  | 12                                  | 156.9 ± 84          | -17             | 21        |
|          | ng/ml               | diet and exercise     | 12                                  | 15.1 ± 1.2          | -17             | 47        |
|          | ng/ml               | diet                  | 8                                   | 24.1                | -19.9           | 22        |
|          | ng/ml               | diet                  | 12                                  | 149.5 ± 84          | -23             | 21        |
|          | ng/ml               | lifestyle             | 52                                  | 19.6                | -23.5           | 25        |
|          | ng/ml               | exercise              | 52                                  | 6.7 ± 4.0           | -23.9           | 27        |
|          | ng/ml               | diet and exercise     | 52                                  | 9.1 ± 6.2           | -24.2           | 40        |
|          | U/L                 | diet and exercise     | 52                                  | 33.5 ± 11.3         | -26             | 37        |
|          | ng/ml               | diet and exercise     | 48                                  | 63.6 ± 27.0         | -27.8           | 41        |
|          | ng/ml               | diet and exercise     | 4                                   | 7.8 ± 4.4           | -29.1           | 18        |
| »        | <b>ng/ml</b>        | <b>Ornish Program</b> | <b>52</b>                           | <b>23.5 ± 18.6</b>  | <b>-32.9</b>    |           |
|          | pg/mL               | diet                  | 166.4                               | 989                 | -33.5           | 24        |
|          | ng/ml               | exercise and diet*    | 8                                   | 28.3 ± 3.5          | -34             | 20        |
|          | ng/ml               | diet (cod)            | 8                                   | 26.4 ± 18.7         | -34             | 28        |
|          | ng/ml               | diet (salmon)         | 8                                   | 25.6 ± 19.5         | -35             | 28        |
|          | µmol/L              | lifestyle             | 8                                   | 45.5 ± 26.2         | -35.4           | 23        |
|          | ng/ml               | diet (fish oil)       | 8                                   | 28.6 ± 19.3         | -37             | 28        |
|          | ng/ml               | diet                  | 8                                   | 27.5                | -37.5           | 22        |
|          | µmol/L              | lifestyle             | 8                                   | 10.3 ± 5.9          | -37.9           | 23        |
|          | ng/ml               | diet and exercise     | 12                                  | 20.4 ± 4.5          | -38.2           | 38        |
|          | ng/ml               | diet and exercise     | 12                                  | 24 ± 7              | -38.8           | 75        |
|          | ng/ml               | diet and exercise     | 12                                  | 14.7 ± 5.3          | -39.4           | 5         |
|          | ng/ml               | diet                  | 6                                   | 36.5 ± 25.4         | -49.9           | 39        |
|          | µg/ml               | diet                  | 12                                  | 18.6 ± 11.9         | -52.2           | 19        |
|          | ng/ml               | diet and exercise     | 12                                  | 7.2                 | -53             | 79        |
|          | ng/ml               | diet                  | 4                                   | 33.1 ± 10.2         | -67.7           | 30        |

uk - unknown

\*Supplement added to intervention

Inclusion criteria - studies conducted in 2007 or earlier, fasting insulin levels, intervention ≥ 1 week, Baseline measures reported, studies reported in English, studies indexed in PubMed and available

\*\*Separate data for men and women were combined and averaged to get mean % change

<sup>†</sup>Follow up times are approximate and based on 4 weeks per month and 52 weeks per year

»Our data

Inclusion of dietary data in the manuscript in preparation for Nutrition, Metabolism, and Cardiovascular Diseases delayed the above manuscript submission. The dietary data collection software was updated to the newest version available and required a laborious process of manual re-entry of all food diaries. All of this data has now been re-entered and re-analyzed.

Because so much effort was needed to re-enter the Ornish dietary data for the Insulin/Leptin biomarker study, it was decided to extract all dietary data from the food diaries, which will allow us to evaluate the major nutrient components of their diets (calories, carbohydrates, protein, and fat), as well as additional macronutrients, minerals, and vitamins. This data has been collected and preliminary analysis shows that there are significant differences in nutrients between Intervention and Control diets, which may contribute to cardiovascular disease reversal.

This study will evaluate if dietary levels of these nutrients are significant for the prevention or reversal of cardiovascular disease risk. For this purpose, we evaluated the dietary information for several macronutrients, vitamins, and minerals from three-day food diaries at each of three distinct time points (Baseline, Week 12, and Week 52). We collected data on 152 participants (control n=76, intervention n=76) at 456 total time points; however, 22 time points were excluded due to missing food diary entries.

Nutrient variables (n=37) were measured using the ESHA Food Processor software version 8.4.0 (Table 14 below). Twenty-eight of these variables were found to be significantly different between Ornish participants and controls. After a review of the literature and meetings with a registered dietician, we selected six variables of specific interest for this study: cholesterol, vitamin B6, potassium, folate, manganese, and thiamin.

**Cholesterol** – Our results show that an Ornish diet decreases dietary cholesterol compared to control diets. Hypercholesterolemia is a known risk factor for cardiovascular disease, and there is a dramatic decrease in dietary cholesterol when participants follow a strict vegetarian diet compared to controls that eat their normal diet.

**Vitamin B6 and Folate** – Low levels of B vitamins can lead to homocysteine accumulation and increased blood coagulation, which is why increased homocysteine levels have been characterized as an independent risk factor for cardiovascular disease. Dietary vitamin B supplementation is recommended to reduce cardiovascular disease risk. Vitamin B6 and folate levels are significantly higher in Ornish participants compared to controls.

**Potassium** – Potassium is an electrolyte required for normal electrical activity in the heart and for maintaining normal blood pressure. Increasing dietary potassium, as we observed in the Ornish Program, can reduce blood pressure and therefore may reduce cardiovascular risk.

**Manganese** – Manganese is a trace element that plays a role in lipid and carbohydrate metabolism, cholesterol regulation, and ultimately atherosclerosis. Manganese levels were significantly increased in the diet of participants compared to controls.

**Thiamin** – Thiamin is related to diabetes, an independent risk factor for cardiovascular disease. Those with type I and type II diabetes have been shown to have ~75% lower serum thiamin concentrations than non-diabetics. Thiamin deficiency increases

microvascular complications by making vascular cells more susceptible to the adverse effects of hyperglycemia. Our study shows that Ornish participants had a significantly higher amount of thiamin in their diets, which could indirectly lead to a reduction in cardiovascular disease risk.

Based on our preliminary results, an Ornish diet is comprised of a beneficial balance of macronutrients, vitamins, and minerals that may be associated with a reduction in cardiovascular disease risk.

**Table 14. Dietary intake of macronutrients, vitamins, and minerals in participants versus controls**

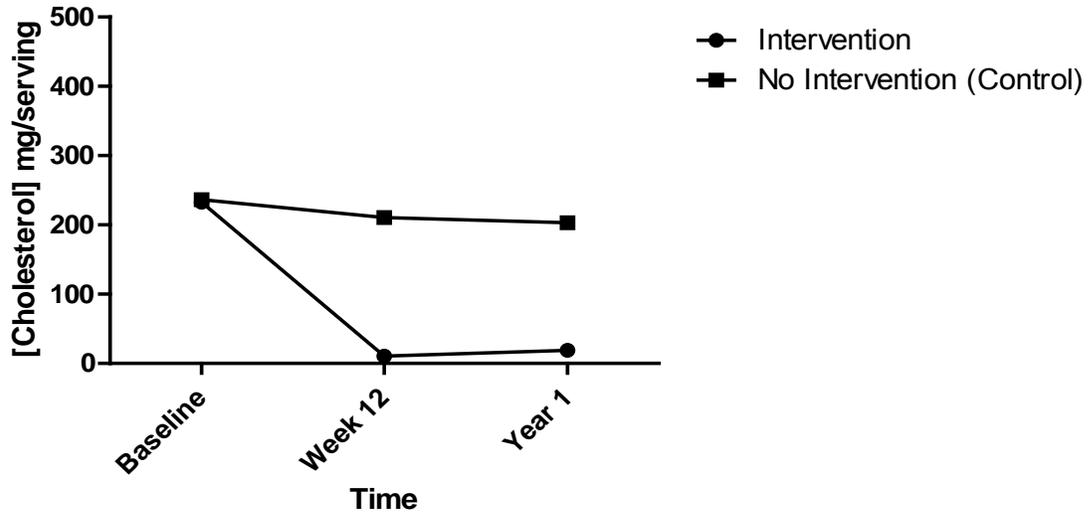
| Macronutrient        | P-value  |
|----------------------|----------|
| Kilocalories         | 0.3854   |
| Protein              | 0.1164   |
| Carbohydrate         | < 0.0001 |
| Fat, Total           | < 0.0001 |
| Alcohol              | 0.0011   |
| Cholesterol          | < 0.0001 |
| Saturated Fat        | < 0.0001 |
| Monounsaturated Fat  | < 0.0001 |
| Polyunsaturated Fat  | 0.0503   |
| Dietary Fiber, total | < 0.0001 |
| Sugar, Total         | < 0.0001 |
| Other Fats           | 0.046    |

| Vitamins                | P Value  |
|-------------------------|----------|
| Vitamin A (RE)          | < 0.0001 |
| Vitamin C               | < 0.0001 |
| Vitamin D (µg)          | 0.0016   |
| Vitamin E (ATE)         | 0.007    |
| Thiamin                 | < 0.0001 |
| Riboflavin              | < 0.0001 |
| Niacin                  | 0.2409   |
| Pyridoxine (Vitamin B6) | < 0.0001 |
| Folate                  | < 0.0001 |
| Cobalamin (Vitamin B12) | 0.7891   |
| Biotin                  | 0.4181   |
| Pantothenic Acid        | 0.0017   |
| Vitamin K               | 0.0004   |

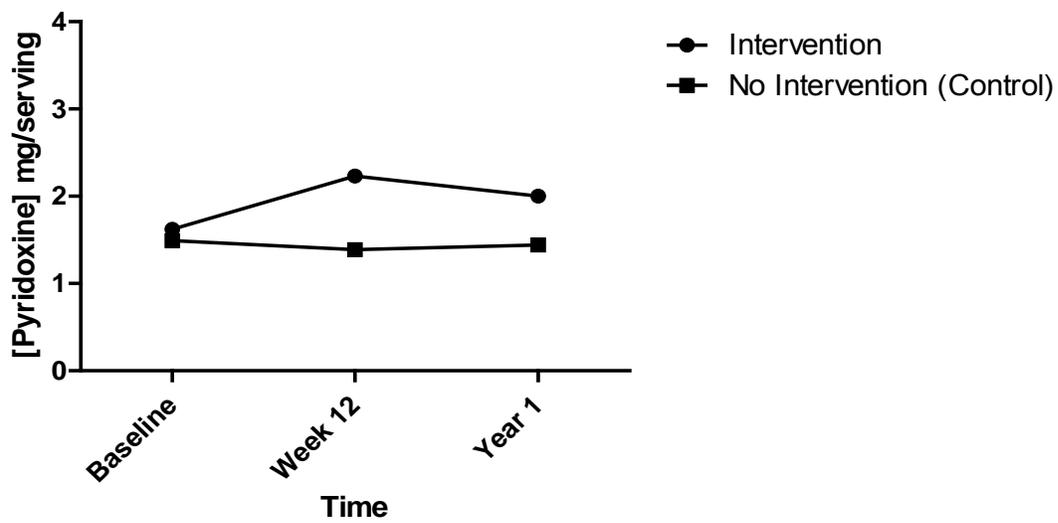
| Minerals   | P Value  |
|------------|----------|
| Sodium     | 0.8394   |
| Potassium  | < 0.0001 |
| Calcium    | < 0.0001 |
| Iron       | < 0.0001 |
| Phosphorus | < 0.0001 |
| Magnesium  | < 0.0001 |

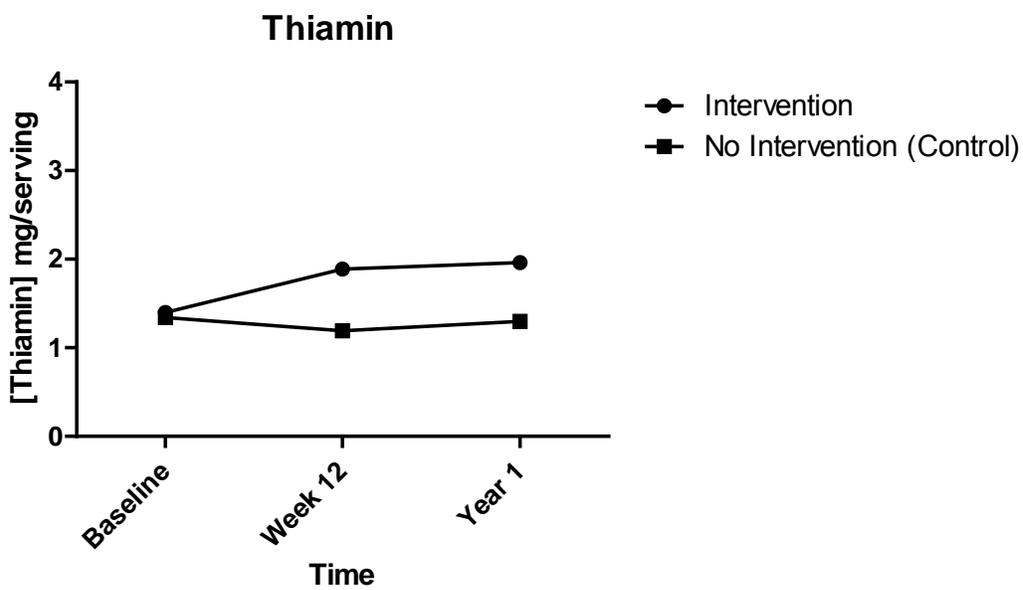
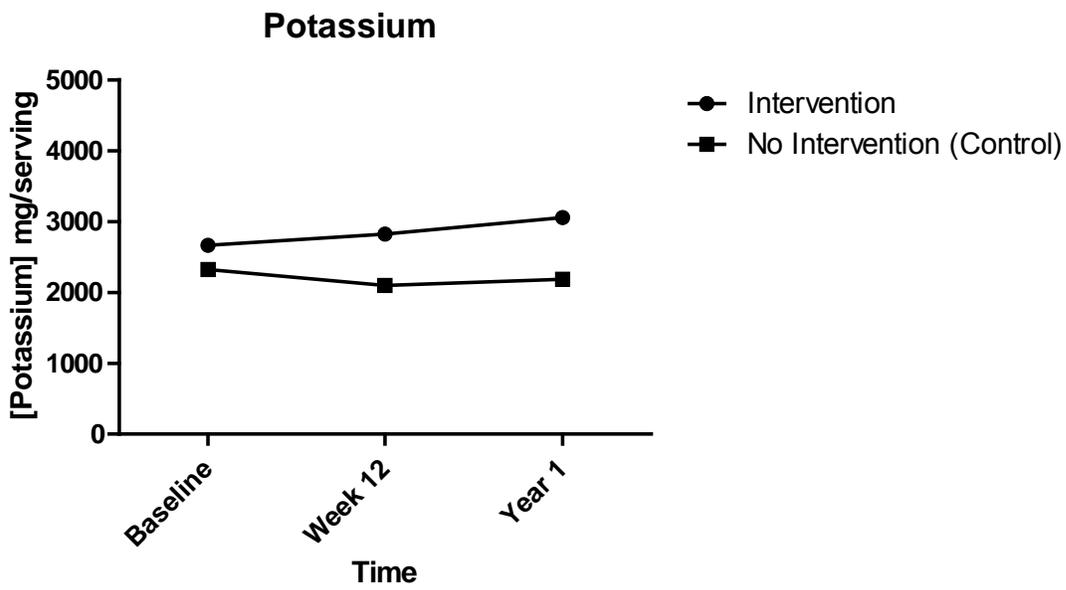
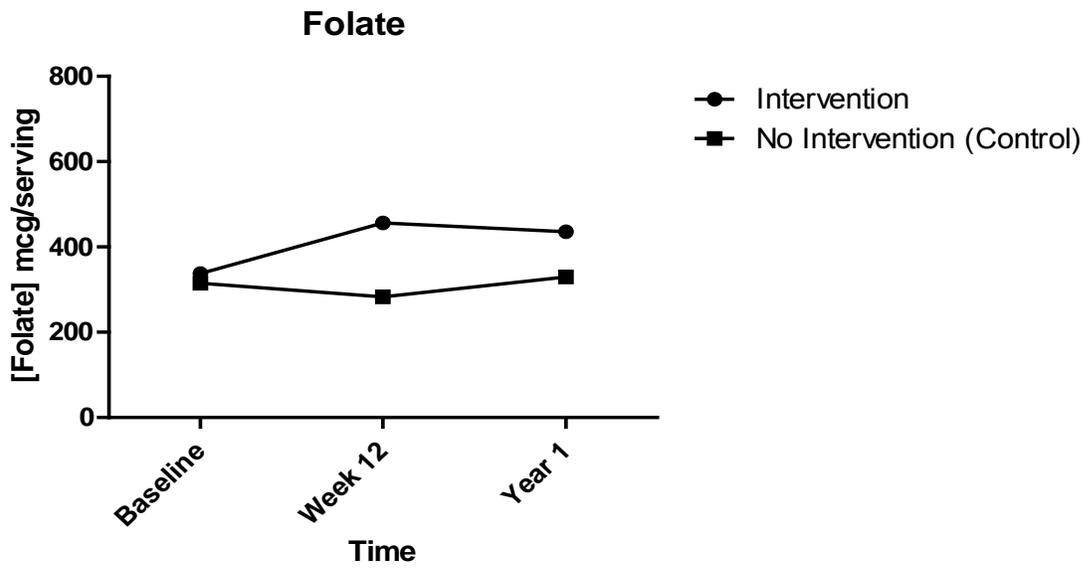
|            |          |
|------------|----------|
| Zinc       | 0.0001   |
| Copper     | < 0.0001 |
| Manganese  | < 0.0001 |
| Selenium   | 0.3515   |
| Chromium   | 0.4381   |
| Molybdenum | 0.0002   |

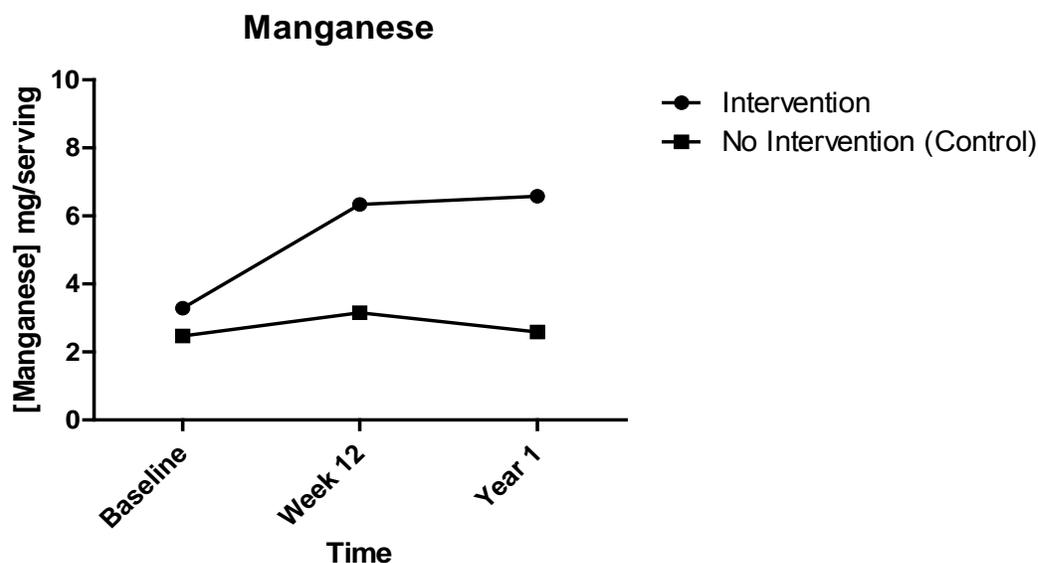
### Cholesterol



### Pyridoxine (Vitamin B6)







**Macrophage migration inhibitory factor (MIF)** – MIF is an inflammatory cytokine that regulates smooth muscle cell migration and proliferation, and thus plays an important role in promoting development of atherosclerotic lesions. MIF has been shown to be an important biomarker for diseases with inflammation, such as CVD, diabetes, obesity, and cancer.

Genotyping of genetic variants in MIF gene that influence circulating levels completed; data analysis complete. MIF levels decreased significantly ( $p < 0.05$ ) in Ornish participants compared to controls at 12 weeks, but no difference in MIF levels between cases and controls at one year (Table 15).

**Table 15. Change in MIF levels in Ornish participants and controls at 12 weeks and one year.**

| Variable | Cohort Type | n  | Baseline | Week 12 | % Change B to 12 Wk | Year 1  | % Change B to 1 Yr | Between Group P Value | B to Y1 P Value |
|----------|-------------|----|----------|---------|---------------------|---------|--------------------|-----------------------|-----------------|
| MIF      | Control     | 85 | 2.9±1.9  | 3.1±2.2 | +6.3%               | 2.9±1.9 | +1.9%              | 0.014                 | 0.939           |
|          | Ornish      | 85 | 2.9±1.9  | 2.6±1.8 | -12.6%              | 2.9±2.1 | +1.3%              |                       |                 |

Stratification by gender (Table 9) indicated that only women participants showed significant ( $p < 0.05$ ) reductions in MIF levels at 12 weeks (-23%). No change in men (Table 16). Transcription of the human MIF gene is regulated by genetic polymorphisms in the MIF promoter, including the -173G/C single-nucleotide polymorphism and a sequence of tetra-nucleotide repeats at -794 (-794CATT<sub>5-8</sub>). These polymorphisms may have relevance to cardiovascular disease, and this area has become a growing area of investigation; however, the tetranucleotide polymorphism and SNP variants were too infrequent for meaningful analysis.

**Table 16. Change in MIF levels in Ornish participants and controls stratified by gender at 12 weeks and one year.**

| Variable | Cohort Type | n       | Baseline | Week 12              | % Change B to 12 Wk | Year 1  | % Change B to 1 Yr | Between Group    |                 |
|----------|-------------|---------|----------|----------------------|---------------------|---------|--------------------|------------------|-----------------|
|          |             |         |          |                      |                     |         |                    | B to W12 P Value | B to Y1 P Value |
| MIF      | F           |         |          |                      |                     |         |                    |                  |                 |
|          | Control     | 40      | 3.3±2.1  | 3.7±2.6              | +12.7%              | 3.2±2.2 | -3.7%              | 0.001            | 0.722           |
|          | Ornish      | 40      | 3.1±1.9  | 2.4±1.6 <sup>b</sup> | -23.0% <sup>b</sup> | 3.1±2.2 | -0.4%              |                  |                 |
|          | M           |         |          |                      |                     |         |                    | 0.907            | 0.680           |
| Control  | 45          | 2.5±1.7 | 2.5±1.5  | -1.2%                | 2.7±1.7             | +8.5%   |                    |                  |                 |
| Ornish   | 45          | 2.8±1.9 | 2.7±2.0  | -2.3%                | 2.9±2.0             | +2.9%   |                    |                  |                 |

Transcription of the human MIF gene is regulated by genetic polymorphisms in the MIF promoter, including the -173G/C single-nucleotide polymorphism and a sequence of tetra-nucleotide repeats at -794 (-794CATT<sub>5-8</sub>). These polymorphisms may have relevance to cardiovascular disease, and this area has become a growing area of investigation.

A number of investigators examining the human MIF promoter genotypes have shown some evidence that MIF genetic variation is associated with coronary artery disease, although the degree of influence may well vary according to specific MIF alleles, populations, ethnicities, and other co-morbidities, such as diabetes mellitus or familial hypercholesterolemia.

During this period, we conducted further analysis on the MIF data, which incorporated genetic information on the -173G/C SNP and -794CATT repeat. First, we evaluated baseline MIF values for the CATT<sub>7</sub> allele versus all other CATT alleles and for the CC-GC versus GG SNP genotypes. Because this data was not normally distributed and could not be log-transformed into a normal distribution, we used a non-parametric Mann-Whitney U test for these analyses. No significant differences in the baseline data were observed for either polymorphism. We then used repeated-measures ANOVA by cohort type (Ornish or control) and CATT polymorphism within cohort type (Table 17). These analyses indicated a significant (p<0.05) change in MIF levels from Baseline to Week 12 in Ornish participants only, not in controls. Interestingly, MIF levels decreased 29% in participants who carried the -794CATT<sub>7</sub> allele, but decreased only ~5% in participants who did not carry at least one CATT 7 allele.

**Table 17. Change in plasma MIF levels by cohort type and -794CATT genotype**

| Cohort Type | CATT7 Value | n  | Baseline <sup>e</sup> | Week 12 <sup>e</sup> | % Change B to 12 Wk | Year 1 <sup>e</sup> | % Change B to 1 Yr. | Between Factor Value <sup>a</sup> |                 |
|-------------|-------------|----|-----------------------|----------------------|---------------------|---------------------|---------------------|-----------------------------------|-----------------|
|             |             |    |                       |                      |                     |                     |                     | B to W12 P Value                  | B to Y1 P Value |
| Control     | No          | 63 | 2.8±1.9               | 3.0±2.3              | 8.3%                | 2.8±1.8             | -0.1%               | 0.501                             | 0.600           |
|             | Yes         | 20 | 3.2±1.8               | 3.2±2.0              | -0.4%               | 3.4±2.4             | 5.9%                |                                   |                 |
| Ornish      | No          | 63 | 2.8±1.7               | 2.7±1.9              | -5.2%               | 2.9±2.0             | 4.3%                | 0.026                             | 0.993           |
|             | Yes         | 18 | 3.5±2.4               | 2.5±1.4              | -29.3%              | 3.6±2.4             | 2.6%                |                                   |                 |

**Gene Expression –**

In 2009-2010 we finalized two data sets consisting of: (1) 63 Ornish participants and 63 matched controls with gene expression data at all three time points, and (2) 89 Ornish participants (matched and unmatched) with gene expression data at all three time points. Statistical analysis was completed on the first gene expression data set.

The gene expression datasets (456 arrays assaying ~18,000 genes each) likely represent the largest gene expression datasets on participants in a CVD lifestyle change program.

Integrity of the microarray gene expression data in the first data set was assessed by rigorous QC. CEL files from all participants and controls were imported into Partek<sup>®</sup> Genomics Suite v6.5 (Partek Incorporated, St. Louis, MO). Probe set intensities were obtained by Robust Multichip Algorithm (RMA) background correction, quantile normalization, median polish summarization, and log<sub>2</sub> transformation. To assess data integrity, the processed intensity data was subjected to standard GeneChip<sup>®</sup> quality control parameters, which evaluated assay performance and ensured suitability for analysis. All arrays passed the quality control assessment and thus were included in further analyses.

Duplicate blood samples were collected from seven randomly-selected participants at each time point and applied to U133A 2.0 arrays as outlined above to evaluate the consistency of gene expression among duplicate assays. Overall repeatability of the array data was first assessed using Pearson correlation coefficients between all pairwise comparisons of RMA normalized intensities. The average correlation between the 21 duplicate samples was 0.992 ± 0.006, range 0.969–0.996), indicating high repeatability of the microarray data. Paired t-tests were then used to identify genes that consistently showed significant differences in expression among the duplicate samples as a filter for exclusion. Comparative analysis identified nine genes that were

differentially expressed based on a false discovery rate (FDR) adjusted p-value <0.05 between the duplicate samples and thus were excluded from further analysis: CKLF-like MARVEL transmembrane domain containing 6 (CMTM6); dehydrogenase/reductase (SDR family) member 9 (DHRS9); guanine nucleotide binding protein (G protein),  $\alpha$ 11 (GNA11); kelch-like 18 (KLHL18); kinesin family member 1A (KIF1A); mitogen-activated protein kinase 1 interacting protein 1-like (MAPK1IP1L); nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (NR3C1); transportin 1 (TNPO1); vesicle-associated membrane protein 1 (synaptobrevin 1) (VAMP1).

Differential gene expression analysis between time points (baseline—12 weeks, baseline—52 weeks) was conducted using ANOVA with participant as the random effects factor and time point as the fixed effects factor. Resulting p-values were adjusted by FDR correction for multiple testing. Stringent gene lists were generated through combined significance (FDR-adjusted p<0.05) and expression change ( $\geq 1.1$ -fold) filtering. For matched controls, gene lists were filtered at FDR-adjusted p-value <0.05 with no fold change filter, because our objective was to identify any differentially expressed genes with greater statistical certainty to serve as a comparison for gene expression changes in the cardiovascular intervention participants.

Functional enrichment analysis was performed on the stringent gene lists using Gene Ontology (GO) annotations to summarize the most enriched biological processes. The GO annotations were ranked by an enrichment p-value, which identified biological processes represented more frequently than expected by chance among genes that changed significantly in expression during the Ornish program.

Significant changes in expression were observed as follows:

1. Changes in expression  $\geq 1.1$ -fold at a False Discovery Rate (FDR) p<0.05

- Lifestyle participants
  - 26 genes at 3 months
  - 162 genes at one year
- Controls
  - 0 genes at 3 months
  - 23 genes at one year

2. Changes in expression  $\geq 1.2$ -fold at a False Discovery Rate (FDR) p<0.05

- Lifestyle participants
  - 20 genes at 3 months
  - 33 genes at one year
- Controls
  - 0 genes at 3 months
  - 0 genes at one year

Many genes were involved in immune and defense response. However, other biological processes such as cell adhesion/migration and carbohydrate/cholesterol metabolism also were altered. Many genes altered at 3 months remained altered at one year. This is

the first study to show that lifestyle change programs can produce fundamental molecular changes that persist up to one year (Tables 18 and 19).

When participants were stratified by gender, different patterns of expression were evident between men and women. Significant changes in expression (Table 20) indicated a clear gender difference in the timing of molecular response:

- Men
  - 12 genes at 3 months
  - 0 genes at one year
- Women
  - 0 genes at 3 months
  - 19 genes at one year

The next year, validation qRT-PCR experiments will be performed to confirm differential gene expression detected by microarray analysis. Total RNA (500 ng) will be reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Resulting cDNA (10 ng) will be subjected to qRT-PCR using TaqMan<sup>®</sup> Gene Expression Assays (Applied Biosystems) according to the manufacturer's protocol on an iCycler iQ<sup>™</sup> Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). All samples will be run in duplicate for each assay and the mean value of the duplicate assays was analyzed by the  $\Delta\Delta C_T$  method, which determines levels of expression for each target gene at each time point.

**Table 18. Genes showing  $\geq 1.25$ -fold change in expression from baseline to three-months in Ornish program participants**

| Probe ID    | Gene Name   | Symbol      | Fold Change | GO Biological Process <sup>a</sup>                                      |
|-------------|---|-------------|-------------|---|
| 202018_s_at | Lactotransferrin  | LTF         | -1.86       | Immune response, ion transport, iron homeostasis                        |
| 206676_at   | Carcinoembryonic antigen-related CAM8                             | CEACAM8     | -1.68       | Immune response   |
| 212531_at   | Lipocalin 2   | LCN2        | -1.55       | Transporter activity; binding <sup>b</sup>                              |
| 207269_at   | Defensin $\alpha$ 4, corticostatin                                | DEFA4       | -1.53       | Defense response  |
| 205033_s_at | Defensin $\alpha$ 1, $\alpha$ 1B, $\alpha$ 3, neutrophil-specific | DEFA1,1B,A3 | -1.49       | Immune response, defense response, chemotaxis                           |
| 212768_s_at | Olfactomedin 4  | OLFM4       | -1.49       | Cell adhesion, protein binding  |
| 207802_at   | Cysteine-rich secretory protein 3                                 | CRISP3      | -1.45       | Immune response, defense response                                       |
| 210244_at   | Cathelicidin antimicrobial peptide                                | CAMP        | -1.35       | Defense response  |
| 203757_s_at | Carcinoembryonic antigen-related CAM6                             | CEACAM6     | -1.33       | Signal transduction, cell-cell signaling                                |
| 207329_at   | Matrix metalloproteinase 8  | MMP8        | -1.30       | Ossification, proteolysis, metabolism, collagen catabolism              |
| 205557_at   | Bactericidal/permeability-increasing protein                      | BPI         | -1.29       | Immune response, (-) regulation of IL6, IL8; lipid binding <sup>b</sup> |
| 208470_s_at | Haptoglobin/haptoglobin-related protein                           | HP/HPR      | -1.28       | Defense response, proteolysis, iron homeostasis                         |
| 206871_at   | Elastase, neutrophil expressed                                    | ELANE       | -1.26       | Proteolysis, inflammatory response, immune response                     |
| 205513_at   | Transcobalamin I (vit B12 binding protein)                        | TCN1        | -1.25       | Cobalt ion transport, cobalamin (vitamin B12) transport                 |
| 206851_at   | Ribonuclease, RNase A family 3                                    | RNASE3      | -1.25       | RNA catabolism, defense response  |
| 203153_at   | Interferon-induced protein with TPRs 1                            | IFIT1       | +1.27       | Binding <sup>b</sup>  |

Abbreviations: CAM, cell adhesion molecule; TPR, tetratricopeptide repeat; GO, Gene Ontology.

CEACAM6 was represented by multiple probes.

<sup>a</sup>Derived from NetAffx™ Analysis Center (<http://www.affymetrix.com/analysis/index.affx>).

<sup>b</sup>GO molecular function.

**Table 19. Genes showing  $\geq 1.25$ -fold change in expression from baseline to one year in Ornish program participants**

| Probe ID         | Gene Name                                  | Symbol      | Fold Change  | GO Biological Process <sup>a</sup>   |
|------------------|--|-------------|--------------|--|
| 202018_s_at      | Lactotransferrin*                          | LTF         | -1.67        | Immune response, ion transport, iron homeostasis                             |
| 221748_s_at      | Tensin 1                                   | TNS1        | -1.55        | Cell migration, cell-substrate junction assembly                             |
| 212531_at        | Lipocalin 2*                               | LCN2        | -1.47        | Transporter activity; binding <sup>b</sup>                                   |
| 206676_at        | Carcinoembryonic antigen-related CAM8*     | CEACAM8     | -1.44        | Immune response  |
| 214407_x_at      | Glycophorin B (MNS blood group)            | GYPB        | -1.41        | Signal transduction; receptor activity <sup>b</sup>                          |
| 206698_at        | X-linked Kx blood group                    | XK          | -1.41        | Amino acid transport   |
| 206665_s_at      | BCL2-like 1                                | BCL2L1      | -1.39        | Response to hypoxia, apoptosis, response to oxidative stress                 |
| 203502_at        | 2,3-bisphosphoglycerate mutase             | BPGM        | -1.37        | Carbohydrate metabolism, glycolysis, respiration                             |
| <b>203115_at</b> | <b>Ferrochelatase</b>                      | <b>FECH</b> | <b>-1.35</b> | <b>Metabolites/energy, porphyrin/heme synthesis, cholesterol metabolism</b>  |
| 207802_at        | Cysteine-rich secretory protein 3*         | CRISP3      | -1.32        | Defense response, immune response  |
| 208470_s_at      | Haptoglobin/haptoglobin-related protein*   | HP/HPR      | -1.30        | Defense response, proteolysis, iron homeostasis                              |
| 212768_s_at      | Olfactomedin 4*                            | OLFM4       | -1.29        | Cell adhesion, protein binding   |
| 213446_s_at      | IQ motif containing GTPase activating pr 1 | IQGAP1      | -1.28        | Small GTPase-mediated signal transduction                                    |
| 208632_at        | Ring finger protein 10                     | RNF10       | -1.28        | Transcription, (-) Schwann cell proliferation, (+) myelination               |
| 221627_at        | Tripartite motif-containing 10             | TRIM10      | -1.28        | Erythrocyte differentiation; protein binding, metal ion binding <sup>b</sup> |
| 218418_s_at      | KN motif and ankyrin repeat domains 2      | KANK2       | -1.28        | —  |
| 217878_s_at      | Cell division cycle 27 homolog             | CDC27       | -1.27        | Mitotic metaphase/anaphase transition, cell proliferation, cell division     |
| 210244_at        | Cathelicidin antimicrobial peptide*        | CAMP        | -1.27        | Defense response   |

**Table 19. Genes showing  $\geq 1.25$ -fold change in expression from baseline to one year in Ornish program participants**

---

|             |  |       |       |  |
|-------------|--|-------|-------|--|
| 200615_s_at | Adaptor-related protein complex 2, $\square 1$ | AP2B1 | -1.26 | Protein transport, defense response                                      |
| 205557_at   | Bactericidal/permeability-increasing protein*  | BPI   | -1.25 | Immune response, (-) regulation of IL6, IL8; lipid binding <sup>b</sup>  |
| 211993_at   | WNK lysine deficient protein kinase 1          | WNK1  | -1.25 | (+) regulation of blood pressure, protein phosphorylation, ion transport |
| 216050_at   |  | —     | +1.48 | —  |

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Abbreviations: CAM, cell adhesion molecule; GO, Gene Ontology.

TNS1, FECH, GYPB, and HP were represented by multiple probes.

<sup>a</sup>Derived from NetAffx™ Analysis Center (<http://www.affymetrix.com/analysis/index.affx>).

<sup>b</sup>GO molecular function.

**Table 20. Genes showing  $\geq 1.2$ -fold change in expression during the lifestyle intervention in participants stratified by gender**

| Probe ID                          | Gene Name                                     | Symbol  | Fold Change | GO Biological Process <sup>a</sup>                                 |
|-----------------------------------|---|---------|-------------|--|
| <b>Men – Baseline to 3 months</b> |   |         |             |  |
| 202018_s_at                       | Lactotransferrin*                             | LTF     | -1.94       | Immune response, ion transport, iron homeostasis                   |
|                                   | Carcinoembryonic antigen-related              |         |             | Immune response  |
| 206676_at                         | CAM8*   | CEACAM8 | -1.80       |  |
| 212768_s_at                       | Olfactomedin 4*                               | OLFM4   | -1.68       | Cell adhesion, protein binding                                     |
| 207802_at                         | Cysteine-rich secretory protein 3*            | CRISP3  | -1.57       | Defense response, immune response                                  |
| 210244_at                         | Cathelicidin antimicrobial peptide*           | CAMP    | -1.39       | Defense response   |
| 207329_at                         | Matrix metalloproteinase 8                    | MMP8    | -1.33       | Ossification, proteolysis, metabolism, collagen catabolism         |
| 220570_at                         | Resistin*                                     | RETN    | -1.30       | Response to insulin, fat cell differentiation                      |
| 209771_x_at                       | CD24 molecule                                 | CD24    | -1.28       | Inflammatory response, immune response, cell-cell adhesion         |
| 211113_s_at                       | ATP-binding cassette, sub-family G            | ABCG1   | +1.21       | Lipid transport, cholesterol/lipid storage, lipoprotein remodeling |
| 203505_at                         | ATP-binding cassette, sub-family A            | ABCA1   | +1.27       | Lipid metabolism, cholesterol metabolism/transport/storage         |
| <b>Women – Baseline to 1 year</b> |   |         |             |  |
| 202018_s_at                       | Lactotransferrin*                             | LTF     | -1.57       | Immune response, ion transport, iron homeostasis                   |
| 217878_s_at                       | Cell division cycle 27 homolog                | CDC27   | -1.35       | Cell proliferation, cell division                                  |
| 200615_s_at                       | Adaptor-related protein complex 2, $\alpha 1$ | AP2B1   | -1.32       | Protein transport, defense response                                |
| 213926_s_at                       | ArfGAP with FG repeats 1                      | AGFG1   | -1.21       | Cell differentiation, organism development, transport              |
| 203609_s_at                       | Aldehyde dehydrogenase 5 family, A1           | ALDH5A1 | -1.20       | Glucose/glycerophospholipid/fatty acid metabolism                  |

Abbreviations: CAM, cell adhesion molecule; GO, Gene Ontology; <sup>a</sup>Derived from NetAffx™ Analysis Center; <sup>b</sup>GO molecular function.

During 2010-2011, we analyzed the second set of gene expression data from 89 Ornish participants (matched and unmatched) who had data at all three time points.

As before, integrity of the microarray gene expression data was assessed by rigorous QC. CEL files from all time points were imported into Partek<sup>®</sup> Genomics Suite v6.5 (Partek Incorporated, St. Louis, MO). Probe set intensities were obtained by Robust Multichip Algorithm (RMA) background correction, quantile normalization, median polish summarization, and log<sub>2</sub> transformation. To assess data integrity, the processed intensity data was subjected to standard GeneChip<sup>®</sup> quality control parameters, which evaluated assay performance and ensured suitability for analysis. All arrays passed the quality control assessment and thus were included in further analyses.

Differential gene expression analysis between time points (baseline—12 weeks, baseline—52 weeks) was conducted using ANOVA with participant as the random effects factor and time point as the fixed effects factor. Resulting p-values were adjusted by FDR correction for multiple testing. Stringent gene lists were generated through combined significance (FDR-adjusted p<0.05) and expression change ( $\geq 1.1$ -fold) filtering.

Functional enrichment analysis was performed on the stringent gene lists using Gene Ontology (GO) annotations to summarize the most enriched biological processes. The GO annotations were ranked by an enrichment p-value, which identified biological processes represented more frequently than expected by chance among genes that changed significantly in expression during the Ornish program.

Data analysis examined differential gene expression in three separate analyses: 1) diabetics, non-diabetics with high insulin, and non-diabetics with low insulin; 2) individuals diagnosed with clinical stress and unstressed participants; and 3) participants with the highest weight loss and participants with the least weight loss. Significant changes in expression were determined as a change in expression from Baseline to Week 12 or Baseline to Week 52 that was  $\geq 1.1$ -fold at a False Discovery Rate (FDR) p<0.05

Below is a summary of results:

1. Diabetics (n=25), non-diabetics with high insulin (n=29), non-diabetics with low insulin (n=33)

- Diabetics  
0 genes at 12 weeks; 0 genes at 52 weeks
- Non-diabetics, high insulin  
30 genes at 12 weeks; 39 genes at 52 weeks
- Non-diabetics, low insulin  
0 genes at 12 weeks; 0 genes at 52 weeks

All of the changes in gene expression were restricted to the non-diabetics with high insulin. Functional enrichment analysis showed enrichment in defense response genes at 12 weeks and genes regulating symbiosis at 52 weeks.

For cross-group comparisons, we saw:

At 12 weeks

- Diabetics vs high insulin: 0
- Diabetics vs low insulin: 201
- High insulin vs low insulin: 0

At 52 weeks

- Diabetics vs high insulin: 0
- Diabetics vs low insulin: 4
- High insulin vs low insulin: 0

2. High stress (n=29; PSS  $\geq$ 18), low stress (n=60, PSS <18)

-- High stress

6 genes at 3 months; 0 genes at one year

-- Low stress

26 genes at 3 months; 29 genes at one year

Most of the changes in gene expression were observed in the low-stress group. Similar to the diabetics vs. non-diabetics analysis, functional enrichment analysis showed enrichment in defense response genes and genes regulating symbiosis at 12 weeks and 52 weeks.

3. Successful weight loss (top two percentiles, n=35), unsuccessful weight loss (bottom two percentiles, n=36)

-- Successful weight loss

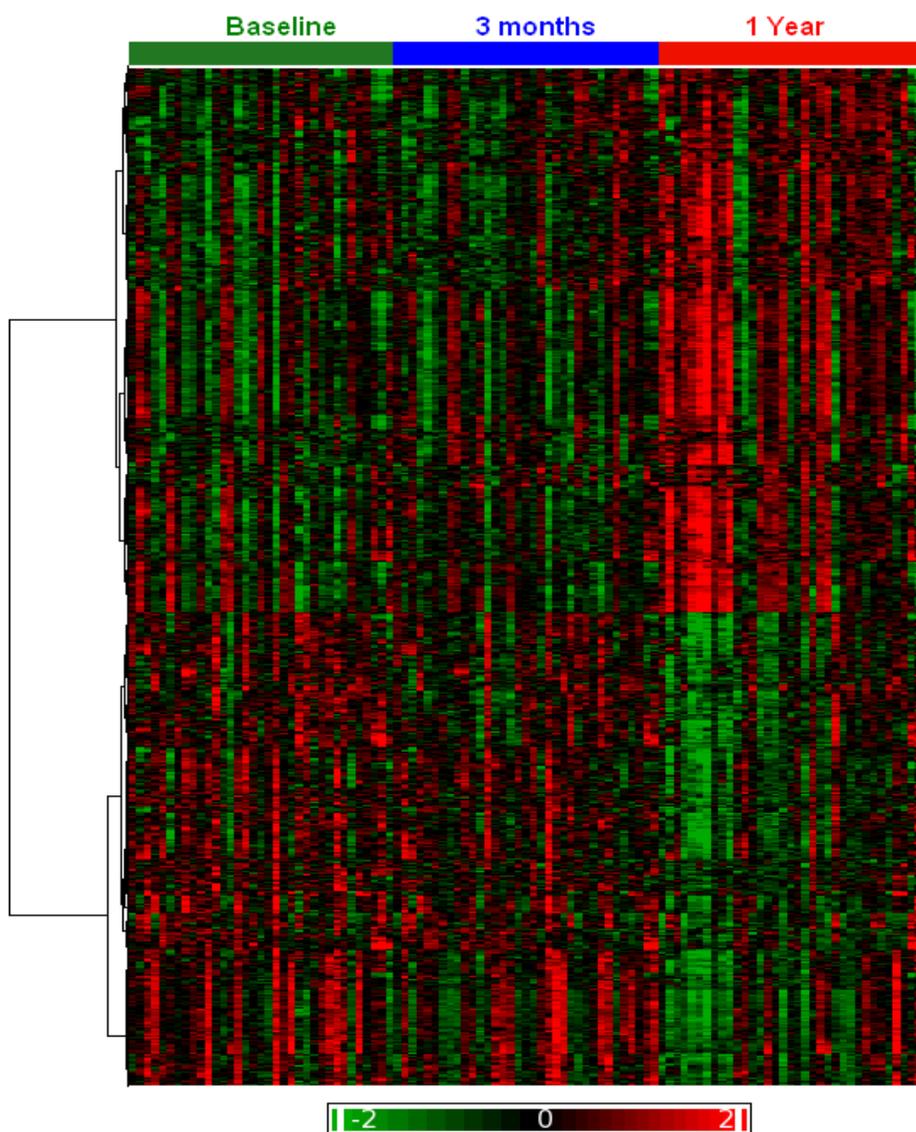
41 genes at 3 months; 3223 genes at one year

-- Unsuccessful weight loss

0 genes at 3 months; 0 genes at one year

Clearly, individuals who lost the most weight showed dramatic changes in gene expression. Many genes were involved in immune and defense response as well as endosymbiosis at 12 weeks. At 52 weeks, most genes affected carbohydrate and cholesterol metabolism. A heat map depicting the degree of molecular change is presented in Figure 1 below.

In the coming year, validation qRT-PCR experiments will be performed to confirm differential gene expression detected by microarray analysis. Total RNA (500 ng) will be reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA). Resulting cDNA (10 ng) will be subjected to qRT-PCR using TaqMan<sup>®</sup> Gene Expression Assays (Applied Biosystems) according to the manufacturer's protocol on an iCycler iQ<sup>™</sup> Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). All samples will be run in duplicate for each assay and the mean value of the duplicate assays was analyzed by the  $\Delta\Delta C_T$  method, which determines levels of expression for each target gene at each time point.

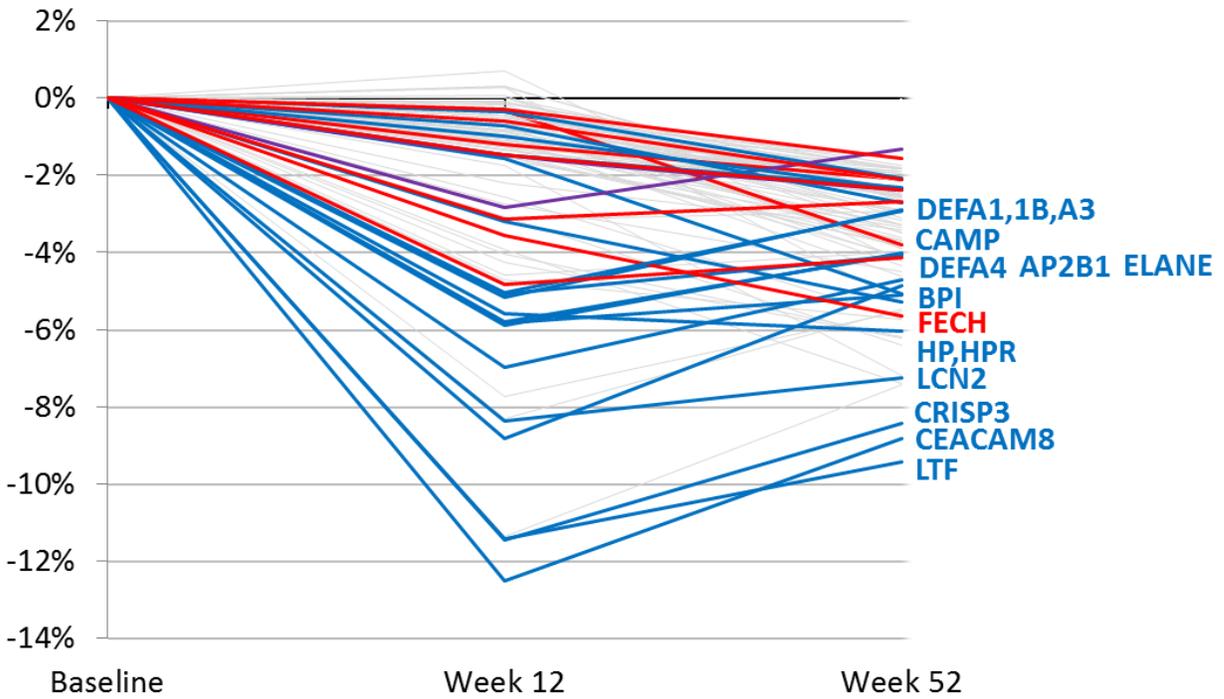
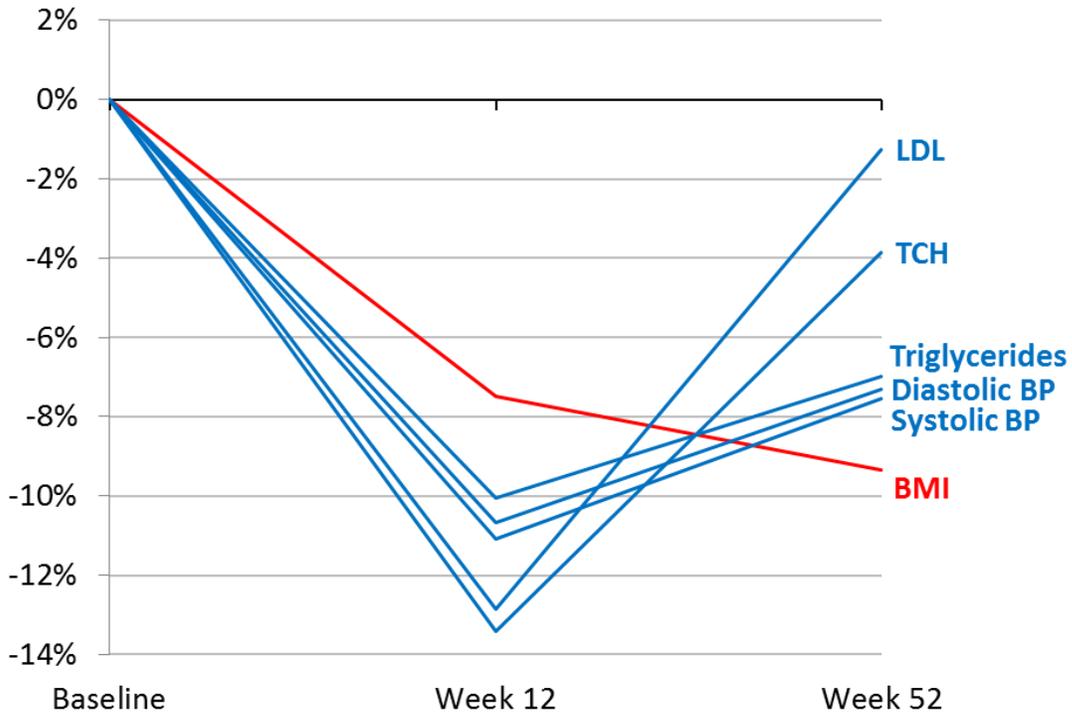


**Figure 1. Heat map showing gene expression changes with weight loss**

In 2011-2012, pathway analysis was performed on Ornish vs Control datasets, comparing each time point vs control, using three different pathway databases, KEGG, BioCarta, and Broad Molecular Signature Data Base. For the BioCarta database, this analysis identified 3 pathways differentially expressed at 3 months and 5 pathways differentially expressed at 1 year in Ornish participants. The KEGG pathways are:

| <b>Baseline – Week 12</b> |                                  | <b>Genes</b> |   |
|---------------------------|----------------------------------|--------------|---|
| hsa00640                  | Propanoate metabolism            | 26           | Carboxylic acid metabolism; related to carbohydrate metabolism          |
| hsa04912                  | GnRH signaling pathway           | 64           | Synthesis/release of gonadotropins; gene expression, cell proliferation |
| hsa05120                  | Epithelial cell signaling in Hpy | 53           | Human diseases; Infectious diseases                                     |
| <b>Baseline – Week 52</b> |                                  |              |   |
| hsa00150                  | Androgen and estrogen metabolism | 19           | Inactivation/catabolism of androgen & estrogen in target tissues        |
| hsa04810                  | Regulation of actin cytoskeleton | 136          | Cellular processes; Cell motility                                       |
| hsa00563                  | GPI-anchor biosynthesis          | 18           | Metabolism; Glycan biosynthesis/metabolism                              |

Manuscript is complete and will be submitted for publication soon. Additional analysis showed that changes in gene expression mirrored changes in many CVD risk factors (Fig. 2) – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52 (Fig. 3). Most cholesterol and lipid homeostasis genes showed a continual decrease in expression throughout the program similar to body weight (Fig. 3). Medication use clearly did not affect gene expression, thus expression changes may be attributed to the lifestyle change program (Table 21). For the Cardiovascular Risk Clinic (CRC), total RNA was isolated from 20 participants over various time points.



Figures 2 and 3. Change in traditional CVD risk factors (Fig. 2) and changes in gene expression (Fig. 3) during intensive lifestyle change.

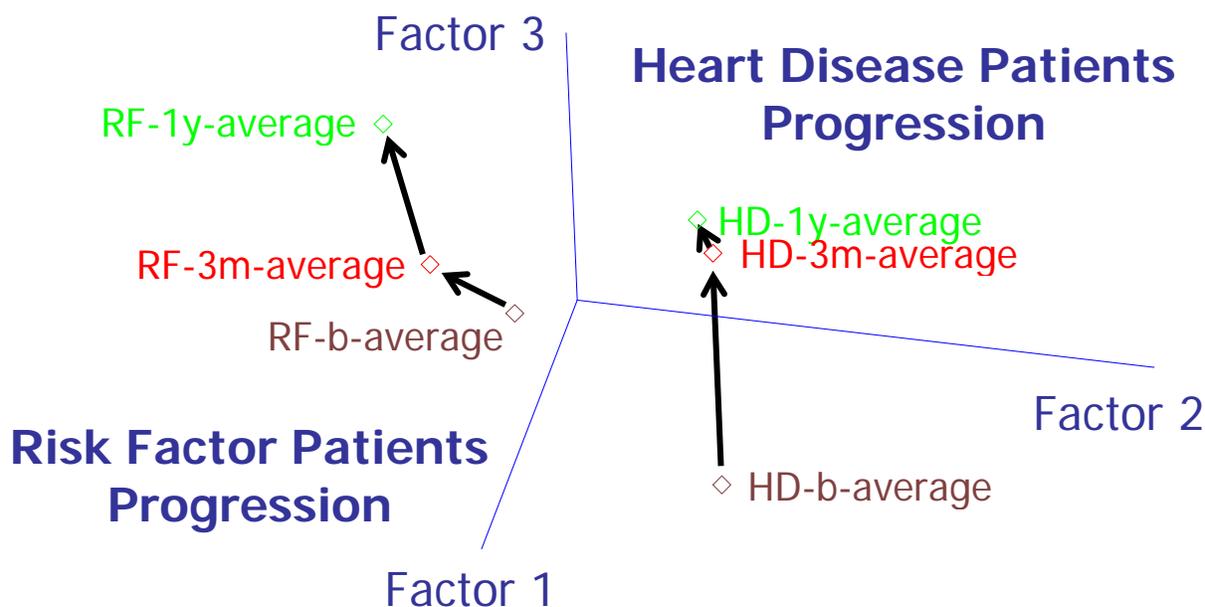
**Table 21. Effects of medications on gene expression from Baseline to Week 52**

| Probe ID    | Symbol  | Fold Change All Participants (n=63) | Fold Change Lipid Lowering Medications* (n=51) | Fold Change All Medications <sup>†</sup> (n=34) |
|-------------|---------|-------------------------------------|--|---|
| 202018_s_at | LTF     | -1.67                               | -1.67  | -1.70   |
| 221748_s_at | TNS1    | -1.55                               | -1.51  | -1.43   |
| 212531_at   | LCN2    | -1.47                               | -1.44  | -1.48   |
| 206676_at   | CEACAM8 | -1.44                               | -1.48  | -1.68   |
| 214407_x_at | GYPB    | -1.41                               | -1.34  | -1.26   |
| 206698_at   | XK      | -1.41                               | -1.43  | -1.36   |
| 206665_s_at | BCL2L1  | -1.39                               | -1.35  | -1.31   |
| 203502_at   | BPGM    | -1.37                               | -1.40  | -1.41   |
| 203115_at   | FECH    | -1.35                               | -1.31  | -1.28   |
| 207802_at   | CRISP3  | -1.32                               | -1.32  | -1.43   |
| 208470_s_at | HP/HPR  | -1.30                               | -1.31  | -1.24   |
| 212768_s_at | OLFM4   | -1.29                               | -1.20  | -1.23   |
| 213446_s_at | IQGAP1  | -1.28                               | -1.25  | -1.22   |
| 208632_at   | RNF10   | -1.28                               | -1.25  | -1.18   |
| 221627_at   | TRIM10  | -1.28                               | -1.23  | -1.21   |
| 218418_s_at | KANK2   | -1.28                               | -1.22  | -1.21   |
| 217878_s_at | CDC27   | -1.27                               | -1.26  | -1.22   |
| 210244_at   | CAMP    | -1.27                               | -1.26  | -1.27   |
| 200615_s_at | AP2B1   | -1.26                               | -1.24  | -1.22   |
| 205557_at   | BPI     | -1.25                               | -1.22  | -1.29   |
| 211993_at   | WNK1    | -1.25                               | -1.23  | -1.17   |

**Plasma Metabolites** – Collaboration with Dr. Dean Jones and Dr. Quinlyn Soltow at Emory University to profile plasma metabolites associated with CVD development continuing. We have analyzed 17 Ornish patients and 17 matched controls (all at three time points) by liquid chromatography-Fourier transform mass spectrometry (LC-FTMS). All assays were run in duplicate.

Metabolomic profiling identified 12,859 metabolite features in plasma; 4,432 features were present in more than 90% of the 102 samples analyzed. False discovery rate (FDR, 10%) analysis detected changes in 19 metabolites after 3 months and 7 metabolites after 1 year in participants, but changes in only 1 metabolite at 3 months in controls. At the 1-year

examination, 87 differences in metabolite profiles distinguished participants from controls. Metabolites changing significantly in abundance were matched to primarily plant-derived compounds associated with inflammation and platelet aggregation in metabolomics databases (METLIN and Madison Metabolomics Consortium Database). Principal component analysis (PCA) showed clear differences in metabolite abundance during the program and distinct profiles of metabolite change in participants with diagnosed heart disease compared to those with only elevated risk factors (Figure 4 below).



**Figure 4. Principle Component Analysis of metabolomic data for participants in the Ornish program. Top panel: PCA grouping of participants stratified by overt heart disease (HD) or risk factors only (RF) at each time point; Bottom panel: Progression in metabolomic profiles over time in participants from the heart disease and risk factor groups.**

A manuscript is in preparation on these initial findings.

### **Structural and Functional Measures of Cardiovascular Health**

endpoints measured include ejection fraction and wall motion, coronary artery calcification Specific scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and tissue perfusion and viability. Work continues on the quantification and interpretation of the huge volumes of imaging data we have acquired; collaboration ongoing with Dr. Edward Miller, Boston University to provide clinical insight into data.

### **Proteomics**

As the proteomics core facility at WRI was disbanded in 2011, no updates on our proteomics research are available at this time.

## **Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Previous Subjects in the Dr. Dean Ornish Program for Reversing Heart Disease.**

The primary objective of this study is to examine associations between DNA variation (in the form of 500,000+ single nucleotide polymorphisms) and participant response to the program. We are examining the influence of innate genetic variation on overall response, quantified as the risk of future cardiac events (Framingham risk), as well as response of specific cardiovascular disease risk factors. The main hypothesis is that innate variation in genes associated with lipid metabolism, protein biosynthesis, protein modification, transcription regulation and/or cell surface receptors (or other genes) will correlate positively with response to intensive lifestyle changes involving diet, exercise, meditation, yoga and group support, which may lead to improved CHD risk factor profiles and genetic markers of coronary artery disease reversal or stabilization. Participants in this study were recruited from previous cohorts of the Dr. Dean Ornish Program for Reversing Heart Disease at WMC (prior to implementation of the primary Molecular Profiling Protocol described above).

### **Status:**

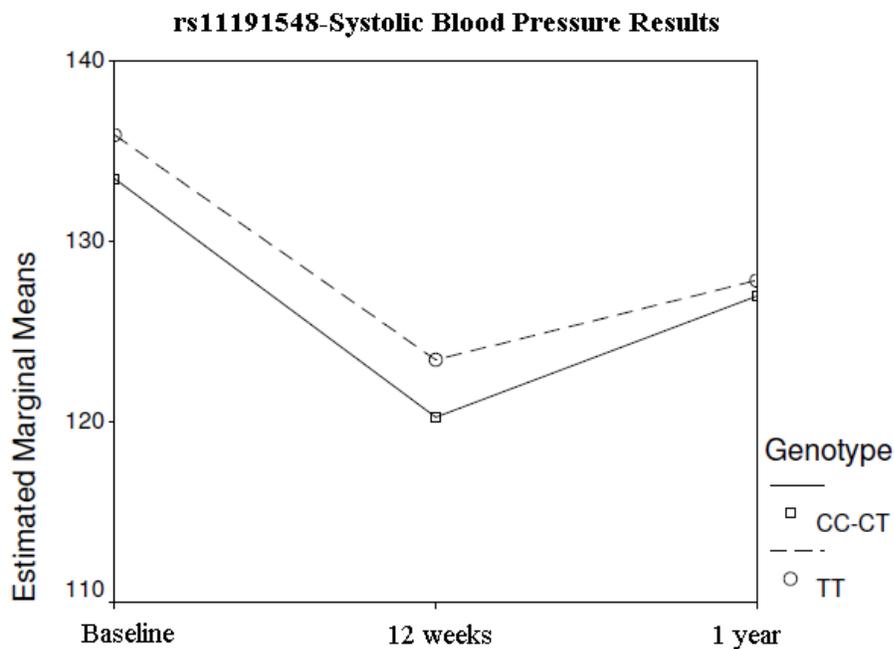
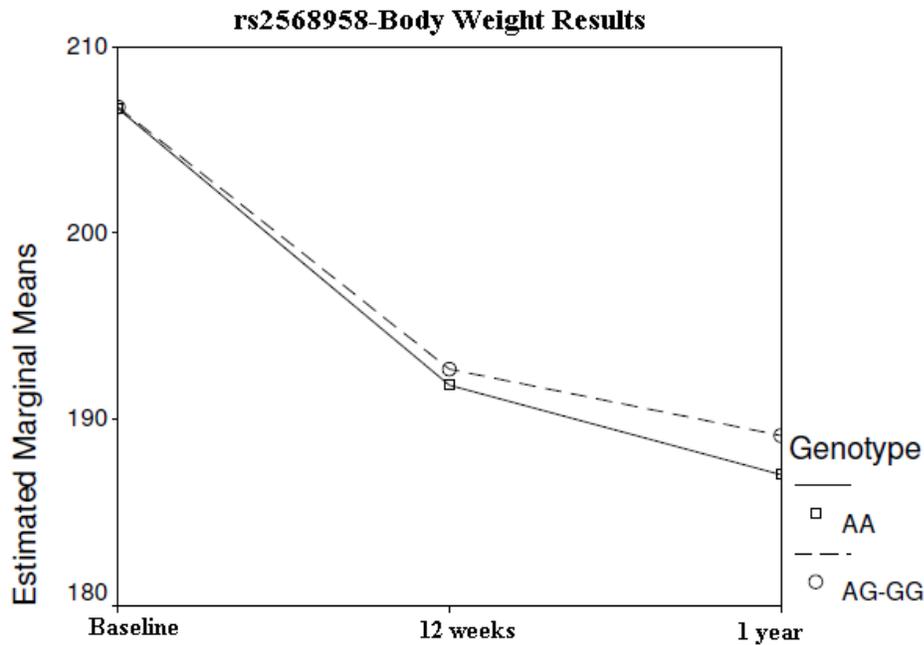
This study is continued from Award No. W81XWH-05-2-0075 and study methodology and approvals have been reported previously. During 2009-2010, we profiled individual SNPs defined in recent genome-wide association studies to have an impact on CVD (or associated risk factors) development. All of the SNPs examined this year were selected from previously published genome-wide association studies and were related to specific traits such as BMI, blood pressure, and lipid levels in the general population. We are determining if SNPs that have been shown to influence CVD-related traits in the general population influence how these traits respond during participation in the Ornish program.

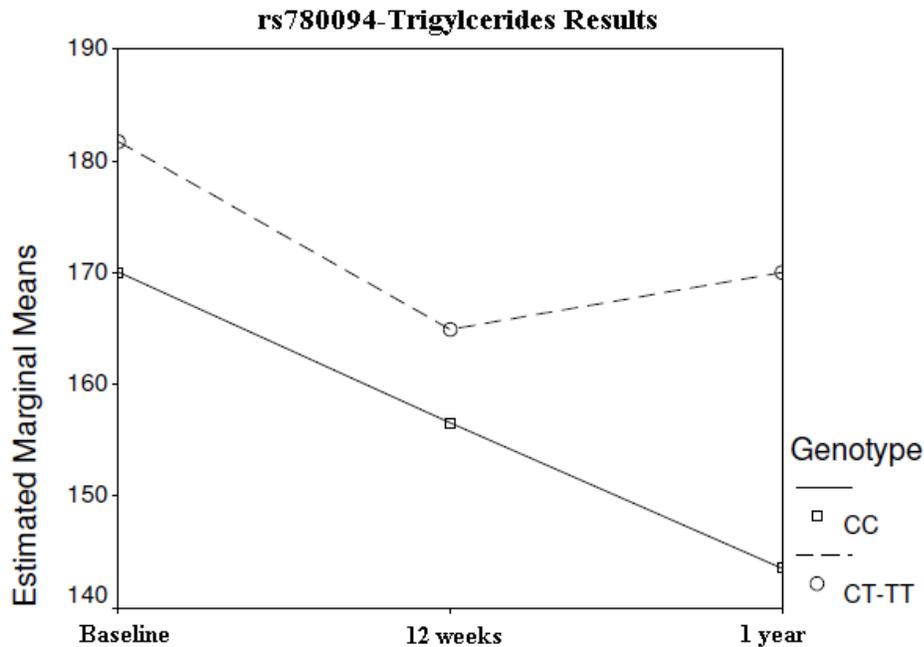
Our summer intern, Marisa Hicks, did an excellent job completing TaqMan<sup>®</sup> genotyping on 186 Ornish participants (117 Ornish samples and 69 Ornish Sub-Study samples) for 13 SNPs using the ABI 7000 platform (rs6548238, rs1378942, rs2568958, rs9939609, rs17782313, rs7498665, rs17367504, rs16998073, rs646776, rs780094, rs3846662, rs3905000, rs12272004). In total, Marisa generated 2418 genotypes. Genotyping data also was completed on three additional SNPs (rs1530440, rs11191548, rs12946454). The results were confirmed for rs1530440 and rs11191548 by performing restriction fragment length polymorphism (RFLP) analysis with the restriction enzymes *Nde* I and *Hpy*CH4IV, respectively. These analyses genotyped 558 samples by TaqMan<sup>®</sup> assays and 372 samples by RFLP.

The following SNPs were analyzed for the corresponding traits: rs11191548 — systolic blood pressure; rs12946454 — systolic blood pressure; rs1378942 — diastolic blood pressure; rs1530440 — diastolic blood pressure; rs16998073 — diastolic blood pressure; rs2568958 — BMI and body weight; rs3846662 — HDL, LDL, total cholesterol, and triglycerides; rs3905000 — HDL, LDL, total cholesterol, and triglycerides; rs6548238 — BMI and body weight; rs780094 — HDL, LDL, total cholesterol, and triglycerides; rs17367504 — systolic blood pressure; rs17782313 —

BMI and body weight; rs7498665 — BMI and body weight; rs9939609 — BMI and body weight; rs646776 — HDL, LDL, total cholesterol, and triglycerides; rs12272004 — HDL, LDL, total cholesterol, and triglycerides.

Results of the statistical analysis showed that genotype did not have an effect on change in BMI/weight, systolic or diastolic blood pressure, or HDL, LDL, or total cholesterol. However, response of triglycerides to the program was significantly influenced by several SNPs (Figure 5).





**Figure 5. Change in selected CVD risk factors by SNP genotype.**

During the 2010-2011 year, we profiled individual SNPs defined in recent genome-wide association studies to have an impact on plasma lipids other than triglycerides [high density lipoprotein (HDL-) cholesterol, low density lipoprotein (LDL-) cholesterol, and total cholesterol]. All of the SNPs were selected from previously published genome-wide association studies. We are determining if SNPs that have been shown to influence lipid traits in the general population influence how these traits respond during participation in the Ornish program.

A total of 2,778 DNA samples were genotyped, with an additional 76 reruns. As all genotypes were assayed in duplicate, a total of 5,708 genotypes were generated. Genotypes were generated for 16 SNPs using the new Applied Biosystems ViiA 7™ Real-Time PCR System. The following SNPs were analyzed for the corresponding traits: rs11206510 – LDL cholesterol, rs12740374 – LDL cholesterol, rs599839 – LDL cholesterol, rs515135 – LDL cholesterol, rs12654264 – LDL cholesterol, rs1501908 – LDL cholesterol, rs12670798 – LDL cholesterol and total cholesterol, rs4149268 – HDL cholesterol, rs2338104 – HDL cholesterol, rs1532085 – HDL cholesterol, rs1800588 – HDL cholesterol, rs3764261 – HDL cholesterol, rs255052 – HDL cholesterol, rs4939883 – HDL cholesterol, rs688 – LDL cholesterol, rs157580 – LDL cholesterol and total cholesterol.

In addition to the above SNPs, over this past period of performance we profiled 27 SNPs defined in recent genome-wide association studies to have an impact on CVD development or associated risk factors such as weight, blood pressure, or lipids. We observed no relationship between SNPs influencing BMI and blood pressure and response to the Ornish program. Numerous SNPs influencing triglycerides showed a

differential response to the program (manuscript in preparation). Below in Table 22 is a summary of the 19 SNPs from recent genome-wide association and confirmation studies with robust statistical evidence for association with plasma triglyceride levels in the general population and a minor allele frequency (MAF)  $\geq 5\%$  in populations of European descent in the National Center for Biotechnology Information (NCBI) Entrez SNP database.

Of the 19 SNPs examined, two SNPs (rs442177 and rs17145738) appeared to influence plasma triglycerides prior to enrollment in the program, as triglyceride levels for both SNPs were significantly different ( $p < 0.05$ ) between genotypes at baseline (Table 23). Sixteen SNPs showed evidence of an influence on triglyceride response throughout the program — triglyceride levels changed significantly from Baseline to Week 12 and/or Baseline to Week 52 in participants with one genotype but not in those carrying the alternate genotype. For 3 SNPs, change in triglyceride levels during the program was significantly different between genotype groups at Week 12 (rs442177 and rs17145738) or at Week 52 (rs3846662 and rs17145738).

We investigated potential interactive effects of gender and genotype on triglyceride response by two-factor repeated measures ANOVA. At 4 SNPs (rs10889353, rs442177, rs3846662, and rs16996148) response differed significantly by gender for one genotype, but not the other genotype (Table 24). In all cases, triglycerides showed a much greater decrease in men compared to women.

**Table 22. SNPs associated with plasma triglyceride levels in recent GWAS**

| SNP <sup>a</sup>  | Chromosome    | Position <sup>b</sup> | Location   | Gene(s) <sup>c</sup>                   | Alleles (MAF) <sup>d</sup> | Effect size (mg/dl) <sup>e</sup> | References                  |
|-------------------|---------------|-----------------------|------------|--|----------------------------|----------------------------------|-----------------------------|
| rs10889353        | 1p31.3        | 63118196              | Intron 4   | <u>DOCK7</u> /ANGPTL3-ATG4C            | A/c (0.33)                 | -4.9 <sup>f</sup>                | 967,968,975,979,982         |
| rs12130333        | 1p31.3        | 63191777              | Intergenic | <u>DOCK7</u> /ANGPTL3-ATG4C            | C/t (0.16)                 | -4.9 <sup>f</sup>                | 965,968,974,975             |
| rs4846914         | 1q41-q42      | 230295691             | Intron 1   | URB2- <u>GALNT2</u> -PGBD2             | A/g (0.43)                 | +2.8                             | 965,968                     |
| rs673548          | 2p24-p23      | 21237544              | Intron 23  | APOB                                   | G/a (0.25)                 | -6.0 <sup>g</sup>                | 967,968,976                 |
| rs1260326         | 2p23          | 27730940              | Leu446Pro  | GCKR                                   | C/t (0.44)                 | +10.3                            | 966,968,974,975,976,978,979 |
| rs780094          | 2p23          | 27741237              | Intron 16  | IFT172-FNDK4- <u>GCKR</u>              | G/a (0.45)                 | +8.6                             | 965,966,967,969,971,975,976 |
| rs442177          | 4q21          | 88030261              | Intron 12  | SLC10A6- <u>AFF1</u> -KLHL8            | A/c (0.38)                 | -2.3                             | 968,980                     |
| rs3846662         | 5q13.3-q14    | 74651084              | Intron 13  | ANKRD31- <u>HMGCR</u> -COL4A3BP        | T/c (0.49)                 | —                                | 967                         |
| rs17145738        | 7q11.23       | 72982874              | Intergenic | BCL7B- <u>TBL2</u> -MLXIPL             | C/t (0.11)                 | -8.2                             | 965,966,968,975,976,978,982 |
| rs328             | 8p22          | 19819724              | Ser474X    | LPL                                    | C/g (0.10)                 | -9.4 <sup>h</sup>                | 965,969,970,974,976,978,983 |
| <u>rs17321515</u> | 8q24.13       | 126486409             | Intergenic | KIAA0196-NSMCE2- <u>TRIB1</u>          | A/g (0.47)                 | -6.4                             | 965,966,975,978,982         |
| <u>rs2954029</u>  | 8q24.13       | 126490972             | Intergenic | KIAA0196-NSMCE2- <u>TRIB1</u>          | A/t (0.46)                 | -6.4                             | 966,968,975,979,980         |
| rs3905000         | 9q31.1        | 107657070             | Intron 2   | NIPSNAP3A/B- <u>ABCA1</u> -SLC44A1     | G/a (0.13)                 | —                                | 967                         |
| rs174547          | 11q12.2-q13.1 | 61570783              | Intron 9   | FEN1- <u>FADS1</u> -FADS2-FADS3        | T/c (0.34)                 | -16.4 <sup>i</sup>               | 975,982                     |
| rs12272004        | 11q23.3       | 116603724             | Intergenic | <u>BUD13</u> -ZNF259-APO(A5/A4/C3/A1)  | C/a (0.07)                 | +18.1 <sup>j</sup>               | 967                         |
| <u>rs964184</u>   | 11q23.3       | 116648917             | Intergenic | BUD13- <u>ZNF259</u> -APO(A5/A4/C3/A1) | C/g (0.13)                 | +18.1                            | 966,968,975,979             |
| <u>rs10401969</u> | 19p13.11      | 19407718              | Intron 8   | TM6SF2- <u>SUGP1(SF4)</u> -MAU2        | T/c (0.06)                 | -12.3                            | 966,968                     |
| rs16996148        | 19p13.11      | 19658472              | Intergenic | YJEFN3- <u>CILP2</u> -PBX4             | G/t (0.05)                 | -6.1                             | 965,966,975                 |
| rs439401          | 19q13.2       | 45414451              | Intergenic | TOMM40- <u>APOE</u> -APOC1             | C/t (0.36)                 | -5.5                             | 967,968                     |

<sup>a</sup> Underlined SNPs also have been associated (P<0.001) with increased risk of coronary artery disease (#966, 968).

<sup>b</sup> National Center for Biotechnology Information human genome reference assembly, Build 37.1.

<sup>c</sup> Nearest annotated genes and nearby biological candidate genes. For intergenic and intronic SNPs, the closest gene is underlined.

<sup>d</sup> Major allele, minor allele, and minor allele frequency (MAF) in this study. Alleles are designated with respect to the “+” strand.

<sup>e</sup> For SNPs rs1260326, rs780094, rs17145738, rs17321515, rs2954029, rs964184, rs10401969, and rs16996148 effect sizes were measured as additive effects corresponding to the average change in triglyceride levels when one major allele was replaced with one minor allele (#966). For SNPs rs4846914, rs442177, and rs439401 effect sizes were estimated as percent changes in triglyceride levels, from a mean triglyceride level of 137.9 mg/dl, due to a single copy of the minor allele (#968).

<sup>f</sup> Effect size, estimated as a percent change in triglyceride levels, from nearby SNP rs2131925 not examined in this study.

<sup>g</sup> Effect size, estimated as a percent change in triglyceride levels, from nearby SNP rs1042034 not examined in this study.

<sup>h</sup> See #983.

<sup>i</sup> See #982.

<sup>j</sup> Effect size, corresponding to actual change in triglyceride levels, from nearby SNP rs964184 examined in this study.

**Table 23. Triglyceride levels at Baseline, Week 12, and Week 52 by SNP genotype.**

| SNP <sup>a</sup> | Genotype | n   | Baseline (SD) | Week 12 (SD)              | % Change           | Between genotype p-value <sup>b</sup> | Week 52 (SD)              | % Change           | Between genotype p-value <sup>c</sup> |
|------------------|----------|-----|---------------|---------------------------|--------------------|---------------------------------------|---------------------------|--------------------|---------------------------------------|
| rs10889353 [1]   | AA       | 77  | 190.3 (96.5)  | 168.4 (75.4) <sup>d</sup> | −11.5 <sup>d</sup> | 0.381                                 | 161.7 (72.7) <sup>d</sup> | −15.0 <sup>d</sup> | 0.093                                 |
|                  | CC-CA    | 93  | 170.9 (90.2)  | 159.1 (76.6)              | −6.9               |                                       | 162.4 (94.9)              | −5.0               |                                       |
| rs12130333 [1]   | TT-TC    | 51  | 159.3 (60.7)  | 155.7 (67.6)              | −2.3               | 0.142                                 | 148.2 (59.0)              | −7.0               | 0.475                                 |
|                  | CC       | 119 | 188.4 (103.2) | 166.6 (79.4) <sup>d</sup> | −11.6 <sup>d</sup> |                                       | 168.0 (94.0) <sup>d</sup> | −10.8 <sup>d</sup> |                                       |
| rs4846914 [1]    | AA       | 65  | 183.6 (90.5)  | 160.7 (79.8) <sup>d</sup> | −12.5 <sup>d</sup> | 0.308                                 | 158.4 (73.9) <sup>d</sup> | −13.7 <sup>d</sup> | 0.229                                 |
|                  | GG-GA    | 112 | 173.6 (93.4)  | 162.3 (73.5)              | −6.5               |                                       | 162.7 (90.8)              | −6.3               |                                       |
| rs673548 [2]     | AA-AG    | 72  | 172.2 (84.8)  | 159.6 (66.0)              | −7.3               | 0.570                                 | 155.2 (84.8)              | −9.9               | 0.928                                 |
|                  | GG       | 98  | 185.2 (99.2)  | 166.0 (82.8) <sup>d</sup> | −10.3 <sup>d</sup> |                                       | 167.1 (85.7)              | −9.8               |                                       |

**Table 23. Triglyceride levels at Baseline, Week 12, and Week 52 by SNP genotype.**

| SNP <sup>a</sup> | Genotype | n   | Baseline (SD)              | Week 12 (SD)              | % Change           | Between genotype p-value <sup>b</sup> | Week 52 (SD)              | % Change           | Between genotype p-value <sup>c</sup> |
|------------------|----------|-----|----------------------------|---------------------------|--------------------|---------------------------------------|---------------------------|--------------------|---------------------------------------|
| rs1260326 [2]    | TT       | 37  | 189.6 (95.0)               | 157.0 (59.3) <sup>d</sup> | -17.2 <sup>d</sup> | 0.108                                 | 158.1 (76.2) <sup>d</sup> | -16.6 <sup>d</sup> | 0.186                                 |
|                  | CC-CT    | 136 | 175.9 (91.7)               | 165.2 (79.4)              | -6.1               |                                       | 163.3 (87.4)              | -7.2               |                                       |
| rs780094 [2]     | CC       | 60  | 170.0 (90.8)               | 156.6 (73.6)              | -7.9               | 0.774                                 | 143.5 (71.2) <sup>d</sup> | -15.6 <sup>d</sup> | 0.223                                 |
|                  | TT-TC    | 118 | 181.7 (93.0)               | 164.9 (76.7) <sup>d</sup> | -9.3 <sup>d</sup>  |                                       | 170.0 (89.6)              | -6.4               |                                       |
| rs442177 [4]     | CC       | 23  | 141.9 (58.3) <sup>e</sup>  | 160.7 (85.1)              | +13.2              | 0.014                                 | 143.7 (74.6)              | +1.2               | 0.198                                 |
|                  | AA-AC    | 147 | 185.6 (96.5) <sup>e</sup>  | 163.7 (74.8) <sup>d</sup> | -11.8 <sup>d</sup> |                                       | 165.0 (86.7) <sup>d</sup> | -11.1 <sup>d</sup> |                                       |
| rs3846662 [5]    | AA       | 39  | 170.0 (80.6)               | 154.6 (78.8)              | -9.0               | 0.978                                 | 174.5 (109.5)             | +2.7               | 0.049                                 |
|                  | GG-GA    | 137 | 179.6 (95.9)               | 164.6 (75.3)              | -8.4               |                                       | 156.8 (76.6) <sup>d</sup> | -12.7 <sup>d</sup> |                                       |
| rs17145738 [7]   | TT-TC    | 38  | 208.2 (110.7) <sup>e</sup> | 171.2 (70.1) <sup>d</sup> | -17.8 <sup>d</sup> | 0.039                                 | 165.2 (84.6) <sup>d</sup> | -20.7 <sup>d</sup> | 0.018                                 |
|                  | CC       | 139 | 169.5 (85.4) <sup>e</sup>  | 160.0 (77.3)              | -5.6               |                                       | 159.5 (85.0)              | -5.9               |                                       |
| rs328 [8]        | GG-GC    | 31  | 166.3 (77.9)               | 153.7 (63.4)              | -7.5               | 0.747                                 | 156.5 (65.0)              | -5.9               | 0.533                                 |
|                  | CC       | 139 | 182.7 (96.4)               | 165.4 (78.6) <sup>d</sup> | -9.5 <sup>d</sup>  |                                       | 163.3 (89.4) <sup>d</sup> | -10.6 <sup>d</sup> |                                       |
| rs17321515 [8]   | GG       | 41  | 170.3 (73.0)               | 149.2 (66.0)              | -12.4              | 0.589                                 | 143.5 (69.6)              | -15.7              | 0.338                                 |
|                  | AA-AG    | 137 | 180.0 (97.3)               | 166.0 (78.1)              | -7.8               |                                       | 166.3 (88.1)              | -7.6               |                                       |
| rs2954029 [8]    | TT       | 37  | 166.7 (67.0)               | 152.7 (68.5)              | -8.4               | 0.833                                 | 145.9 (72.3)              | -12.5              | 0.699                                 |
|                  | AA-AT    | 133 | 182.6 (99.0)               | 166.2 (77.9) <sup>d</sup> | -9.0 <sup>d</sup>  |                                       | 167.3 (88.6)              | -8.3               |                                       |
| rs3905000 [9]    | AA-AG    | 45  | 180.7 (99.6)               | 168.8 (77.6)              | -6.6               | 0.690                                 | 154.8 (68.9)              | -14.3              | 0.353                                 |

**Table 23. Triglyceride levels at Baseline, Week 12, and Week 52 by SNP genotype.**

| SNP <sup>a</sup> | Genotype | n   | Baseline (SD) | Week 12 (SD)              | % Change           | Between genotype p-value <sup>b</sup> | Week 52 (SD)              | % Change           | Between genotype p-value <sup>c</sup> |
|------------------|----------|-----|---------------|---------------------------|--------------------|---------------------------------------|---------------------------|--------------------|---------------------------------------|
| rs174547 [11]    | GG       | 133 | 176.8 (89.9)  | 159.8 (75.1) <sup>d</sup> | -9.6 <sup>d</sup>  |                                       | 163.2 (89.4)              | -7.7               |                                       |
|                  | TT       | 71  | 184.2 (92.7)  | 167.6 (74.7)              | -9.0               | 0.982                                 | 166.7 (93.4)              | -9.5               | 0.984                                 |
|                  | CC-CT    | 99  | 176.5 (94.1)  | 160.2 (77.2)              | -9.2               |                                       | 158.8 (79.3)              | -10.1              |                                       |
| rs12272004 [11]  | AC       | 25  | 172.2 (88.3)  | 170.8 (70.2)              | -0.8               | 0.298                                 | 172.8 (122.0)             | +0.4               | 0.392                                 |
|                  | CC       | 152 | 178.7 (93.4)  | 161.0 (76.7) <sup>d</sup> | -9.9 <sup>d</sup>  |                                       | 158.7 (77.2) <sup>d</sup> | -11.2 <sup>d</sup> |                                       |
| rs964184 [11]    | GG-GC    | 45  | 175.7 (98.0)  | 166.6 (79.0)              | -5.2               | 0.480                                 | 165.7 (99.8)              | -5.7               | 0.577                                 |
|                  | CC       | 128 | 178.5 (91.6)  | 160.3 (75.5) <sup>d</sup> | -10.1 <sup>d</sup> |                                       | 159.1 (80.4) <sup>d</sup> | -10.8 <sup>d</sup> |                                       |
| rs10401969 [19]  | CC-CT    | 18  | 158.4 (69.1)  | 159.6 (59.5)              | +0.8               | 0.302                                 | 167.6 (66.5)              | +5.8               | 0.137                                 |
|                  | TT       | 152 | 181.6 (95.4)  | 163.7 (77.8) <sup>d</sup> | -9.8 <sup>d</sup>  |                                       | 162.1 (87.7) <sup>d</sup> | -10.7 <sup>d</sup> |                                       |
| rs16996148 [19]  | TT-TG    | 16  | 161.0 (72.2)  | 173.6 (66.3)              | +7.8               | 0.098                                 | 168.6 (68.4)              | +4.7               | 0.173                                 |
|                  | GG       | 154 | 181.7 (95.2)  | 162.2 (77.1) <sup>d</sup> | -10.7 <sup>d</sup> |                                       | 161.4 (87.0) <sup>d</sup> | -11.1 <sup>d</sup> |                                       |
| rs439401 [19]    | CC       | 74  | 193.0 (115.0) | 168.3 (82.6) <sup>d</sup> | -12.8 <sup>d</sup> | 0.200                                 | 181.1 (104.7)             | -6.1               | 0.395                                 |
|                  | TT-TC    | 96  | 169.5 (71.3)  | 159.5 (70.7)              | -5.9               |                                       | 147.4 (63.4) <sup>d</sup> | -13.0 <sup>d</sup> |                                       |

<sup>a</sup> Chromosome location in brackets.

<sup>b</sup> From independent samples t-test (two-tailed) comparing genotypes at Baseline and Week 12.

<sup>c</sup> From independent samples t-test (two-tailed) comparing genotypes at Baseline and Week 52.

<sup>d</sup> Significantly different from Baseline at  $p < 0.05$ .

<sup>e</sup> Baseline values significantly different ( $p < 0.05$ ) between genotypes.

**Table 24. Triglyceride levels at Baseline, Week 12, and Week 52 for selected SNPs by genotype and gender.**

| SNP <sup>a</sup> | Genotype | Gender | n  | Baseline (SD) | Week 12 (SD)              | % Change           | Between gender p-value <sup>b</sup> | Week 52 (SD)              | % Change           | Between gender p-value <sup>c</sup> |
|------------------|----------|--------|----|---------------|---------------------------|--------------------|-------------------------------------|---------------------------|--------------------|-------------------------------------|
| rs10889353 [1]   | AA       | F      | 34 | 197.7 (105.6) | 179.1 (91.8)              | -9.4               | 0.742                               | 173.9 (85.7)              | -12.1              | 0.611                               |
|                  |          | M      | 43 | 184.4 (89.4)  | 160.0 (59.2)              | -13.3              |                                     | 152.1 (59.9) <sup>d</sup> | -17.6 <sup>d</sup> |                                     |
|                  | CC-CA    | F      | 48 | 159.4 (73.2)  | 165.2 (72.9)              | +3.7               | 0.013                               | 168.0 (106.2)             | +5.4               | 0.029                               |
|                  |          | M      | 45 | 183.2 (104.9) | 152.5 (80.7) <sup>d</sup> | -16.8 <sup>d</sup> |                                     | 156.4 (81.8)              | -14.7              |                                     |
| rs442177 [4]     | CC       | F      | 14 | 142.1 (48.4)  | 171.8 (81.0)              | +20.9              | 0.328                               | 143.9 (63.4)              | +1.3               | 0.991                               |
|                  |          | M      | 9  | 141.7 (74.4)  | 143.3 (93.4)              | +1.2               |                                     | 143.2 (93.7)              | +1.1               |                                     |
|                  | AA-AC    | F      | 68 | 182.1 (94.6)  | 170.8 (81.6)              | -6.2               | 0.106                               | 175.9 (102.9)             | -3.4               | 0.040                               |
|                  |          | M      | 79 | 188.6 (98.6)  | 157.6 (68.3) <sup>d</sup> | -16.4 <sup>d</sup> |                                     | 155.5 (69.3) <sup>d</sup> | -17.5 <sup>d</sup> |                                     |
| rs3846662 [5]    | AA       | F      | 23 | 176.9 (86.2)  | 159.8 (83.9)              | -9.6               | 0.849                               | 181.7 (127.8)             | +2.7               | 0.979                               |
|                  |          | M      | 16 | 160.1 (73.3)  | 147.1 (72.9)              | -8.1               |                                     | 164.2 (78.8)              | +2.5               |                                     |
|                  | GG-GA    | F      | 62 | 173.7 (89.7)  | 175.8 (78.7)              | +1.2               | 0.016                               | 165.8 (83.3)              | -4.6               | 0.029                               |
|                  |          | M      | 75 | 184.4 (101.2) | 155.3 (71.5) <sup>d</sup> | -15.8 <sup>d</sup> |                                     | 149.4 (70.2) <sup>e</sup> | -19.0 <sup>e</sup> |                                     |
| rs17145738 [7]   | CC       | F      | 71 | 170.2 (88.5)  | 168.0 (80.8)              | -1.3               | 0.212                               | 169.8 (98.1)              | -0.3               | 0.138                               |
|                  |          | M      | 68 | 168.7 (82.6)  | 151.6 (73.0)              | -10.1              |                                     | 148.8 (67.7)              | -11.8              |                                     |
|                  | TT-TC    | F      | 14 | 196.7 (86.5)  | 188.9 (75.8)              | -4.0               | 0.083                               | 171.7 (93.1)              | -12.7              | 0.223                               |
|                  |          | M      | 24 | 214.9 (124.0) | 160.8 (66.0) <sup>d</sup> | -25.2 <sup>d</sup> |                                     | 161.4 (81.0) <sup>d</sup> | -24.9 <sup>d</sup> |                                     |
| rs16996148 [19]  | GG       | F      | 77 | 178.0 (91.7)  | 173.1 (82.9)              | -2.8               | 0.012                               | 172.0 (99.5)              | -3.3               | 0.022                               |

**Table 24. Triglyceride levels at Baseline, Week 12, and Week 52 for selected SNPs by genotype and gender.**

| SNP <sup>a</sup> | Genotype | Gender | n  | Baseline (SD) | Week 12 (SD)              | % Change           | Between gender p-value <sup>b</sup> | Week 52 (SD)              | % Change           | Between gender p-value <sup>c</sup> |
|------------------|----------|--------|----|---------------|---------------------------|--------------------|-------------------------------------|---------------------------|--------------------|-------------------------------------|
|                  |          | M      | 77 | 185.3 (99.1)  | 151.4 (69.6) <sup>e</sup> | -18.3 <sup>e</sup> |                                     | 150.8 (71.5) <sup>e</sup> | -18.6 <sup>e</sup> |                                     |
|                  | TT-TG    | F      | 5  | 134.0 (8.6)   | 138.8 (32.6)              | +3.6               | 0.811                               | 146.6 (65.5)              | +9.4               | 0.861                               |
|                  |          | M      | 11 | 173.3 (85.3)  | 189.5 (72.7)              | +9.3               |                                     | 178.6 (70.3)              | +3.0               |                                     |

<sup>a</sup> Chromosome location in brackets.

<sup>b</sup> From independent samples t-test (two-tailed) comparing gender within genotypes at Baseline and Week 12.

<sup>c</sup> From independent samples t-test (two-tailed) comparing gender within genotypes at Baseline and Week 52.

<sup>d</sup> Significantly different from Baseline at p<0.05.

<sup>e</sup> Significantly different from Baseline at p<0.001.

Then in the year of 2011-2012, we profiled 39 SNPs defined in recent genome-wide association studies to have an impact on CVD development or associated risk factors; influence of 23 SNPs on triglyceride response has been evaluated; manuscript nearly complete, data analysis is ongoing.

Four additional SNPs were genotyped, 3 in close proximity to rs17145738 and one near rs3846662. rs17145738 and rs3846662 showed significant differences between genotypes in triglyceride response in previous analyses. Of the 4 new SNPs, 2 SNPs (rs12916 and rs714052) also showed a significant difference in triglyceride response to the Ornish program between genotypes.

**Dietary Factors in the Ornish and CRC Programs** – Brianne Seitz our summer intern compared dietary outcomes of CRC participants to the Ornish program and controls. Ornish participants showed improvement in most dietary factors, but there was little change in controls. Ornish participants significantly lowered their daily fat intake by more than 60% ( $P < 0.001$  versus controls), while increasing carbohydrate intake by 30% ( $P < 0.001$  versus controls). Due to the stringency of the program, initial changes among Ornish participants were larger than those in CRC participants, but there was evidence that CRC participants showed less regression over time. Manuscript in preparation.

**Task #10: Continuation of the “Comprehensive Cardiovascular Risk Assessment and Prevention Program (CPP)”.**

This program serves as a platform for ongoing translational research activities, a “virtual laboratory” based on scientific findings for the development of best personalized preventive practices. In other words, the platform allows ICHP to gather an expansive number of data points for each patient or subgroup of patients (eventually combined with data at a molecular level) that when leveraged will result in the creation of new tools in technology to define wellness, predict and prevent disease, and empower patients and providers to transform their healthcare.

The CPP platform has a dual purpose and is multifunctional. This platform 1) allows for multiple research protocols to be conducted as it sets the stage for recruitment, enrollment and hypothesis generation, advanced data modeling and simultaneously 2) provides a venue where research findings from these protocols can then be tested, validated and translated into application for clinical practice. Our protocols within the CPP are specifically designed to examine the effects of our military’s high op tempo which predisposes our service members to accelerated atherosclerotic risk resulting from high stress, PTSD, depression, sleep insufficiency, overweight, pre-diabetes and pre-hypertension among other traditional disease risk factors.

This program was established to address the unique needs of military beneficiaries at risk for CV disease. It includes conventional and novel CV risk profiling (health assessments, labs, markers, wearable monitors) and tailored personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a cardiologist, sleep specialist, nurse practitioners, nutritionists,

stress management instructors and exercise physiologists. Validated tools to screen for and measure CV risk are part of this inclusive package. Report cards for the patient and provider as well as email notifications are utilized. The program is an adjunct to the best medical practices provided by their primary care provider. Up to 1000 patients may be enrolled each year. Some of the patients (such as nurses or medical holdovers etc) may be in subgroup programs because of unique needs. The CPP serves as a platform for ongoing translational research activities, a “virtual laboratory” for the development of best preventive practices and for CV educational and marketing materials.

**Status:**

Total patient visits: 1,458 + 757 telephonic visits = 2,215 from Aug 10 – Aug 11. Patient visits after Aug 11 reported on Award No. W81XWH-11-2-0227.

Despite the transition to WRNMMC Bethesda during this performance period, customer satisfaction surveys continued to average a score of 3.95 out of 4.0, demonstrating high patient satisfaction even in the face of multiple barriers encountered addressed on previous quarterly reports.

We have systematically analyzed relevant clinical data through the protocol entitled “Outcomes of the Cardiovascular Prevention Program (CPP)”. Continuing review approval was received from WRNMMC DRP on 28 Mar 12. Conclusions to date include: 1) Findings that suggest stress plays an unexpectedly prominent role in cardiovascular risk; 2) Stress erodes sleep quality and is associated with dysregulation of glucose metabolism; 3) Deteriorations of sleep and glucose regulation, probably serve as mediators of the increased cardiovascular risk in our patient population; 4) Increased stress, decreased sleep time, poor sleep quality, glucose dysmetabolism, and increased cardiovascular risk all have a negative impact of military readiness, and; 5) Longer sleep time and improved sleep quality correlate with improved weight control as well as improved cardiovascular risk. Abstracts submitted for presentation at numerous national conferences outline these findings and can be found in Appendix A.

**During this period of performance, we have accomplished the following:**

- Clinical Transition Strategy Plan for smooth transition and to re-establish high standards of our DOD COE at WRNMMC to serve all military beneficiaries.
- Analysis of Active Duty (AD) subgroups: Total Soldier Concept
  - Refined process for workshop, appointments, data collection, coaching, follow-up and aggregate report to Senior Enlisted Advisor
  - Refined tracking system for admin staff to follow flow of pts
- Data management of subgroups for preclinical states (pre-hypertension, pre-diabetes, subclinical hypothyroidism)
- Creation of new track: Prevention Empowerment/Education Plan for Pre-diabetic and Diabetic patients as an adjunct to our already established four dimensional approach to prevention.
- Designed and re-enforced of new “Empowerment Train” schematic used to guide program flow, appointment and behavioral compliance

- Continued CPP Optimization Initiative (clinical & administrative)
  - Designed and implemented new Interactive Educational Workshop
  - Re-established customer service training for Admin staff and re-enforced training
  - Designed and implemented motivational incentive sequence for patients
  - Tracked productivity of clinical and admin staff with new system and tracked productivity with new system
  - Refined Clinical Review process and data capture of metrics
- Performed 5% chart reviews quarterly- provided recommendations to clinical staff  
Continued clinical enhancement of adding CIMT on all pts
- Revision of clinical guidelines to update CV health screening practices
- Continue to validate our successful model of care while alumni return to take part in our Healthy Cooking Demonstrations using our *Healthy Cooking Guide* designed to provide AD with practical tips for healthy living.
- Heart Health Month (Feb 11): nurses provided blood pressure screening and education for Active Duty and other beneficiaries based on recent AHA blood pressure reduction initiatives
- Investigated new approaches to behavior modification sustainment through multimedia approaches to learning.
- Investigating possible statistical support for analysis of data, maintenance of ICHP publication plan, and generation of more detailed patient outcome clinical reports.
- Creation of new ICHP Website in order to support transition to the new WRNMMC location and to maintain communication with research participants.
- Creation of new clinical positions to support expanding research initiatives: Integrative Health Coach, Data Outcomes Specialist, Clinical Outcomes Registered Nurse

ICHP is implementing a Clinical Information Management System with the objective to refine individualized CVD prevention strategies through statistical data modeling to define the most cost-effective and sustainable approaches in promoting cardiovascular health throughout the military lifecycle. ICHP desires to implement a web-based Clinical Information Management System to support program operations and to obtain, update, store and report on participant data collected by all members of the ICHP clinical team and to support its patient management, diagnostic testing, clinical monitoring and clinical research for all protocols. Database features include:

- Secure, web-based software solution to support protocol management
- Role-based workflow and data capture to support protocol process
- Unique and integrated modules provide 'end-to-end' solution
- Provider Portal, Patient Portal, & Scheduling Module
- Clinical Report Form (CRF) generation, validation, and reporting
- Data capture including surveys, lab values, diagnosis data, vital signs, and medications
- Form and recommendation outputs for patient and PCP distribution ·Supports multiple protocols and studies

**Database status:**

- Functional requirements discussed with HJF
- Vendor selected
- Data variables outlined and streamlined for database creation
- Personnel responsible for management of database identified, new job description written
- Database and platform creation remains in progress

**Sub Task #10.1 Continue “Validation of the ICHP Cardiovascular Risk Score” protocol.**

Data previously collected on patients enrolled in the Prospective Army Coronary Calcium (PACC) and PACC Rescan projects were reviewed. Specific information was gathered and analyzed to give each patient a CV disease risk score according to a formula developed by the ICHP. This ICHP formula uses the Framingham model of risk prediction and adds historical factors and biochemical markers to produce a novel score predictive of CV disease risk in military beneficiaries. The goal of the study was to validate the utility of this novel ICHP scoring system by comparing the predicted risk with outcomes in this well characterized population. The primary objective of the project was to validate the predictive utility and accuracy of the ICHP CV risk score (or ICHP score). Specifically, the goals: a) to determine if the ICHP score correlates with cross-sectional prevalence of coronary calcium as measured in the PACC project and b) with the development of CHD events such as angina, myocardial infarction, or need for CV intervention such as coronary stenting, angioplasty, or bypass surgery. A third goal: c) to determine the correlation of the ICHP score with coronary calcium progression as measured in the PACC rescan project.

**Status:** This study is continued from Award No. W81XWH-05-2-0075 and study methodology and approvals have been reported previously. After statistical analysis of data from the PACC project, ICHP score performed successfully in the linear model with a coefficient of 0.003 ( $p=0.004$ ), indicating that an increase of one point in ICHP score was associated with increasing CIMT 0.3%. In the logistic model, the odds ratio for the ICHP score was 1.04 ( $p=0.01$ ), signifying that a one point increase in ICHP score increases odds by 4% of having a top quartile “atherosclerosis score”. In conclusion, incorporating novel risk factors such as those proposed in the ICHP score and considering the value of family history may significantly improve the predictive accuracy of CVD risk assessment and may reveal appropriate targets for therapeutic intervention.

QA of data to ensure integrity of data collected according to ICHP CV Score parameters completed. We further utilized the principles and equations of the ICHP CV Score to analyze new data sets to demonstrate the clinical and practical use of this validated scoring tool as seen in BATTLE Study sub analysis. This sub analysis demonstrated that the ICHP Risk Score dramatically improves CV disease risk prediction in this cohort of women with subclinical atherosclerosis. These findings emphasize the need for improved CV disease risk identification in women. Family history and other novel risk

factors add predictive value to current risk models and identify potential therapeutic targets.

During this reporting period, we have analyzed the existing data set with new approaches utilizing skills of statistician at Department of Research Programs. Manuscript preparation is underway.

**Sub Task #10.2 Continue “Caregivers Optimizing Readiness thru Prevention Strategies” programs (subgroup of CPP).**

This proposal provides a comprehensive health program for the WRAMC nursing staff, including prescriptions for therapeutic lifestyle change.

Project participants complete questionnaires and lab tests to evaluate individual CV risk and identify emotional/behavioral triggers of stress. A dedicated workshop at ICHP delivers comprehensive instruction on diet, exercise, sleep, and stress management. Follow-up over 12 weeks includes facilitated group support sessions, coaching on coping skills, tension tamer techniques, and scheduled group exercise sessions. Participants are engaged by telephone and email to track progress and deliver pertinent instruction and encouragement. At the end of the 12-week program, measures are repeated to determine progress in stress reduction and changes in the CV health profile. Subsequently, participants continue to be engaged by telehealth and those who report setbacks are offered re-enrollment in the program. Data gathered on participants undergo dynamic statistical modeling to yield predictive information on best lifestyle change strategies to employ for future participants. This dynamic statistical modeling will provide a more precise intervention strategy for incoming participants and allow for improved outcomes, greater efficiencies, and cost savings.

**Status:** Future pilots will be conducted pending funding.

**Sub Task #10.4 Initiate ultrapersonalized CPP Pilot to determine if targeted allocation of resources (guided by CV risk score stratification and tailored by actigraphy/survey results) may yield a cost-effective preventive care model.**

**Status:** Currently in progress under CPP task. Reported under Task #10 (CPP).

**Sub Task #10.5: Initiate “Digital Thermal Monitoring of Vascular Function” in an Integrative Cardiovascular Prevention Program (CPP) – renamed to ZENITH (randomiZed Evaluation of a Novel comprehensive prevention program on aTherosclerosis progression) Trial.**

The purpose of this study is to investigate the impact of an innovative cardiovascular disease (CVD) risk factor assessment and prevention program, Integrative Cardiac Health Project-Cardiovascular Prevention Program (ICHP-CPP), on vascular health, atherosclerosis progression and left-ventricular relaxation (diastolic function) among patients with increased lifetime CVD risk, but low short term coronary heart disease

(CHD) risk (according to the Framingham Risk Score, FRS) as compared to receiving usual care (UC). This will be a single-center study, prospective, randomized, controlled, interventional trial at the Walter Reed National Military Medical Center (WRNMMC-B) where participants will be randomized in a 1:1 fashion to either a multi-modality ICHP-CPP or to UC. Up to 170 male and female patients between 18-50 years of age with low (<10%) 10-year FRS for CHD but estimated lifetime risk (to age 95 years) of coronary death or myocardial infarction (MI) of  $\geq 39\%$  without clinically manifest CVD [MI, coronary or peripheral arterial revascularization, obstructive coronary artery disease (CAD), heart failure or cerebrovascular event] will be randomized to participation in the currently ongoing ICHP-CPP, an established, comprehensive CVD prevention program, or to UC. The primary endpoint is the between-group differences in change of vascular endothelial function, a validated CVD surrogate endpoint, as measured using Digital Thermal Monitoring (DTM). Additionally, the impact on change in carotid intima-media thickness (CIMT) and cardiac diastolic function will be explored.

**Status:** Protocol prepared in collaboration with WRNMMC Cardiology staff and WRI. Statistical needs identified in collaboration with CLINIRX. Protocol was submitted to WRNMMC Department of Research Programs on 8 Mar 2012 for scientific review.

**Sub Task #10.6: Validation of CPP model of preventive care utilizing Coronary CT Angiography to measure Plaque Volume changes resulting from an integrative prevention program (protocol title to be determined).**

**Status:** No progress to report and will be removed on future SOW.

**Task #11: Cardiovascular Risk Assessment and Prevention Program.**

This program, now called the Cardiovascular Risk Clinic (CRC), was established as a platform to address the unique needs of retired military beneficiaries at risk for CV disease. The program mirrors the WRNMMC ICHP Cardiac Prevention Program (CPP). It includes conventional and novel CV risk profiling and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a nurse case manager, psychologist, nurse practitioners, dietitians, stress management instructors, and exercise physiologists. Validated tools to screen for and measure CV risk, stress, sleep health, compliance with dietary recommendations and exercise are standard of care. The program is an adjunct to the best medical practices provided by their primary care provider.

Phase I of the program involves each participant undergoing a comprehensive health risk assessment that is completed by a physician, followed by a four- hour "Pearls for your Heart" workshop and participants then schedule individual appointments with each modality specialist to receive education and counseling in nutrition, exercise, stress management and mind/body health. These are monthly appointments to be completed over a 4-6 month period.

Phase II of the program begins after the completion of the healthy lifestyle intervention (Phase I). During this phase each participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the Program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase 2 for five years, during which time they will come to the center for re-assessments every six months.

**Status:** Study is currently ongoing.

**Subject Enrollment and Demographics:**

Total subject enrollment in the CRC is 247 participants; (144 intervention; 103 controls); 203 remain active; 44 drop-outs; 34 control participants have transferred to the intervention arm after one year as a control. Demographic characteristics of participants are: average age 58.1 years, 60% female, 21% veterans or the spouse of a veteran, and 19% with diagnosed coronary heart disease.

Since the last reportable quarter (1 November 2012- 28 February 2013), there were a total of 766 participant visits including periodic follow up phone calls to participants enrolled in the intervention arm of the study and 102 visits by participants enrolled in the control arm of the study.

**Outcome Data**

The intervention cohorts have shown change in the desired direction for virtually all of the measured coronary artery disease (CAD) risk factors over the initial 4-6 month period (Table 25A). Measures of obesity including weight and BMI declined ~3.5%. Levels of total cholesterol were reduced by ~2%, and triglycerides dropped by 13%. Dietary analysis shows marked improvement in daily total and saturated fat intake, two main dietary components that contribute to plaque formation. Systolic and diastolic blood pressure decreased by nearly 7%. Measures of carotid intima-medial thickness (CIMT) also were significantly lower after the intervention phase. In addition, psychometric measures also significantly decreased, specifically, depression by 30% and hostility by 15%. Similarly, sleep quality improved by 25%. This data demonstrates that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease.

Results from the first long-term follow up time point (6 months after completion of the intervention) are shown in Table 25B. Over the course of approximately 8-10 months, weight, BMI, total cholesterol, triglycerides, blood pressure, and CIMT measurements all maintained statistical significance proving that the positive improvements in these traditional risk factors for CAD can be maintained over a longer period of time. In addition, at this time point, depression and hostility remained significantly improved as well sleep quality.

In Table 25C, results 1 year after completion of the intervention are shown. Weight, BMI, total cholesterol, LDL cholesterol, CIMT measurements as well as psychosocial

and sleep factors continue to maintain statistical significance. Most variables continue to trend in the desirable direction.

Tables 25D and 25E show the furthest time points reached thus far, 18 months and 2 years respectively after completion of the intervention. Although a relatively small sample size risk factors continue to show positive improvements.

**Table 25A. Comparison of “Baseline” to “Intervention Complete” (4-6 months) data for participants in the intervention arm of the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Intervention Complete Value (SD) | Average Change | P-Value  |
|---------------------------------------|----|-----------------------------|--|----------------|----------|
| Weight (lbs.)                         | 91 | 194.98 (45.6)               | 190.48 (42.8)                            | -4.5           | <0.00001 |
| Body Mass Index                       | 88 | 30.92 (6.1)                 | 30.16 (6.0)                              | -0.8           | <0.00001 |
| Total Cholesterol (mg/dl)             | 85 | 187.59 (39.4)               | 184.28 (37.5)                            | -3.3           | 0.2845   |
| High Density Lipids (mg/dl)           | 85 | 48.42 (12.6)                | 48.74 (11.1)                             | 0.3            | 0.7003   |
| Low Density Lipids (mg/dl)            | 85 | 112.49 (32.0)               | 111.98 (31.4)                            | -0.5           | 0.8370   |
| Triglycerides (mg/dl)                 | 85 | 135.40 (72.3)               | 117.69 (51.0)                            | -17.7          | <0.001   |
| Systolic Blood Pressure               | 85 | 131.48 (17.9)               | 125.06 (17.0)                            | -6.4           | <0.0001  |
| Diastolic Blood Pressure              | 85 | 80.64 (11.1)                | 75.48 (9.2)                              | -5.2           | <0.00001 |
| Depression Scale [CES-D]              | 85 | 10.32 (9.3)                 | 7.11 (6.9)                               | -3.2           | <0.00001 |
| Hostility Scale [Cook-Medley]         | 85 | 7.26 (4.7)                  | 6.20 (4.2)                               | -1.1           | <0.001   |
| Perceived Stress Scale [PSS]          | 85 | 13.09 (5.9)                 | 10.73 (5.4)                              | -2.4           | <0.0001  |
| Daily Total Fat (grams)               | 79 | 64.05 (32.6)                | 52.43 (22.6)                             | -11.6          | <0.01    |
| Daily Saturated Fat (grams)           | 79 | 19.85 (10.7)                | 16.38 (8.8)                              | -3.5           | <0.01    |
| Avg. CCA/Mean IMT                     | 85 | 0.748 (0.1608)              | 0.700 (0.1407)                           | -0.048         | <0.00001 |
| Avg. CCA / Max IMT                    | 85 | 0.862 (0.1856)              | 0.805 (0.1506)                           | -0.1           | <0.00001 |
| Fasting Glucose (mg/dl)               | 85 | 104 (33.6)                  | 101 (24.4)                               | -3.0           | 0.2996   |
| HgbA1c                                | 85 | 6.0 (1.07)                  | 6.0 (1.10)                               | -0.1           | 0.2433   |
| Cortisol                              | 84 | 11.9 (3.93)                 | 13.7 (3.98)                              | 1.8            | <0.001   |
| TSH                                   | 85 | 1.94 (1.063)                | 2.14 (1.305)                             | 0.2            | 0.1285   |
| Epworth Sleepiness Scale (0-24)       | 84 | 8 (4.5)                     | 7 (4.2)                                  | -0.9           | <0.01    |
| Pittsburgh Sleep Quality Index (0-21) | 83 | 8 (4.3)                     | 6 (3.9)                                  | -1.3           | <0.0001  |

**Table 25B. Change in outcome variables 6 months after completion of the intervention for participants in the Cardiovascular Risk Clinic**

| Category / Metrics          | N  | Average Baseline Value (SD) | Average 10 month value (SD) | Average Change | P-Value  |
|-----------------------------|----|-----------------------------|-----------------------------|----------------|----------|
| Weight (lbs.)               | 60 | 190.84 (45.1)               | 184.07 (41.2)               | -6.8           | <0.00001 |
| Body Mass Index             | 60 | 30.47 (6.0)                 | 29.59 (5.7)                 | -0.9           | <0.001   |
| Total Cholesterol (mg/dl)   | 61 | 189.41 (42.4)               | 178.25 (38.1)               | -11.2          | <0.05    |
| High Density Lipids (mg/dl) | 61 | 49.28 (13.7)                | 48.44 (12.8)                | -0.8           | 0.3427   |
| Low Density Lipids (mg/dl)  | 61 | 112.38 (32.8)               | 105.56 (31.5)               | -6.8           | 0.0896   |

**Table 25B. Change in outcome variables 6 months after completion of the intervention for participants in the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average 10 month value (SD) | Average Change | P-Value  |
|---------------------------------------|----|-----------------------------|-----------------------------|----------------|----------|
| Triglycerides (mg/dl)                 | 61 | 141.64 (72.7)               | 122.25 (65.6)               | -19.4          | <0.01    |
| Systolic Blood Pressure               | 60 | 131.03 (18.0)               | 126.40 (17.3)               | -4.6           | <0.05    |
| Diastolic Blood Pressure              | 60 | 80.50 (11.0)                | 74.97 (9.1)                 | -5.5           | <0.001   |
| Depression Scale [CES-D]              | 59 | 9.37 (9.3)                  | 7.12 (9.1)                  | -2.3           | <0.05    |
| Hostility Scale [Cook-Medley]         | 59 | 7.00 (4.6)                  | 6.56 (4.3)                  | -0.4           | 0.2956   |
| Perceived Stress Scale [PSS]          | 59 | 12.47 (6.1)                 | 10.44 (6.6)                 | -2.0           | <0.01    |
| Daily Total Fat (grams)               | 55 | 64.33 (33.0)                | 51.42 (20.8)                | -12.9          | <0.05    |
| Daily Saturated Fat (grams)           | 55 | 19.56 (10.6)                | 15.79 (8.1)                 | -3.8           | <0.05    |
| Avg. CCA/Mean IMT                     | 60 | 0.772 (0.1446)              | 0.699 (0.1407)              | -0.073         | <0.00001 |
| Avg. CCA / Max IMT                    | 60 | 0.891 (0.1680)              | 0.798 (0.1508)              | -0.1           | <0.00001 |
| Fasting Glucose (mg/dl)               | 62 | 102 (20.0)                  | 102 (25.5)                  | 0.5            | 0.8710   |
| HgbA1c                                | 61 | 6.1 (0.94)                  | 6.0 (1.19)                  | -0.1           | 0.1140   |
| Cortisol                              | 59 | 11.8 (3.96)                 | 13.6 (4.02)                 | 1.8            | <0.01    |
| TSH                                   | 61 | 1.99 (1.180)                | 2.31 (1.527)                | 0.3            | <0.05    |
| Epworth Sleepiness Scale (0-24)       | 57 | 8 (4.4)                     | 7 (4.3)                     | -1.1           | <0.01    |
| Pittsburgh Sleep Quality Index (0-21) | 57 | 7 (4.0)                     | 6 (3.6)                     | -1.4           | <0.001   |

**Table 25C. Change in outcome variables 1 year after completion of the intervention for participants in the Cardiovascular Risk Clinic**

| Category / Metrics            | N  | Average Baseline Value (SD) | Average 10 month value (SD) | Average Change | P-Value  |
|-------------------------------|----|-----------------------------|-----------------------------|----------------|----------|
| Weight (lbs.)                 | 27 | 191.01 (41.7)               | 183.30 (38.3)               | -7.7           | <0.0001  |
| Body Mass Index               | 26 | 30.47 (5.7)                 | 29.24 (5.1)                 | -1.2           | <0.001   |
| Total Cholesterol (mg/dl)     | 26 | 187.96 (41.0)               | 171.38 (37.9)               | -16.6          | <0.01    |
| High Density Lipids (mg/dl)   | 26 | 52.50 (15.6)                | 50.46 (13.2)                | -2.0           | 0.2047   |
| Low Density Lipids (mg/dl)    | 26 | 109.81 (30.8)               | 98.65 (27.6)                | -11.2          | <0.01    |
| Triglycerides (mg/dl)         | 26 | 131.12 (70.1)               | 111.81 (54.6)               | -19.3          | 0.0563   |
| Systolic Blood Pressure       | 26 | 132.15 (14.0)               | 125.85 (14.1)               | -6.3           | 0.0591   |
| Diastolic Blood Pressure      | 26 | 79.54 (10.2)                | 75.54 (7.8)                 | -4.0           | 0.0686   |
| Depression Scale [CES-D]      | 26 | 10.00 (9.7)                 | 6.92 (7.4)                  | -3.1           | <0.05    |
| Hostility Scale [Cook-Medley] | 26 | 7.96 (4.5)                  | 6.69 (4.2)                  | -1.3           | <0.05    |
| Perceived Stress Scale [PSS]  | 26 | 12.77 (7.1)                 | 10.23 (6.3)                 | -2.5           | <0.01    |
| Daily Total Fat (grams)       | 26 | 69.24 (32.2)                | 45.08 (13.8)                | -24.2          | <0.001   |
| Daily Saturated Fat (grams)   | 26 | 20.91 (10.9)                | 13.31 (4.8)                 | -7.6           | <0.01    |
| Avg. CCA/Mean IMT             | 26 | 0.860 (0.1249)              | 0.719 (0.1172)              | -0.141         | <0.00001 |
| Avg. CCA / Max IMT            | 26 | 0.996 (0.1510)              | 0.828 (0.1365)              | -0.2           | <0.00001 |
| Fasting Glucose (mg/dl)       | 26 | 101 (13.7)                  | 104 (22.3)                  | 2.7            | 0.4426   |
| HgbA1c                        | 26 | 6.2 (1.05)                  | 6.0 (1.21)                  | -0.2           | <0.01    |
| Cortisol                      | 26 | 12.1 (4.57)                 | 13.9 (3.07)                 | 1.8            | <0.05    |
| TSH                           | 26 | 2.34 (1.387)                | 2.39 (1.371)                | 0.0            | 0.8242   |

**Table 25C. Change in outcome variables 1 year after completion of the intervention for participants in the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average 10 month value (SD) | Average Change | P-Value |
|---------------------------------------|----|-----------------------------|-----------------------------|----------------|---------|
| Epworth Sleepiness Scale (0-24)       | 25 | 8 (4.3)                     | 7 (3.6)                     | -1.1           | 0.1034  |
| Pittsburgh Sleep Quality Index (0-21) | 25 | 9 (5.0)                     | 6 (3.2)                     | -2.2           | <0.01   |

**Table 25D. Change in outcome variables 18 months after completion of the intervention for participants in the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average 10 month value (SD) | Average Change | P-Value  |
|---------------------------------------|----|-----------------------------|-----------------------------|----------------|----------|
| Weight (lbs.)                         | 14 | 188.96 (42.7)               | 182.93 (38.0)               | -6.0           | <0.05    |
| Body Mass Index                       | 14 | 29.56 (4.2)                 | 28.44 (3.8)                 | -1.1           | <0.01    |
| Total Cholesterol (mg/dl)             | 14 | 179.14 (46.2)               | 175.29 (43.6)               | -3.9           | 0.6836   |
| High Density Lipids (mg/dl)           | 14 | 52.36 (10.8)                | 49.21 (7.2)                 | -3.1           | 0.2554   |
| Low Density Lipids (mg/dl)            | 14 | 105.50 (35.6)               | 105.71 (32.6)               | 0.2            | 0.9780   |
| Triglycerides (mg/dl)                 | 14 | 112.71 (52.7)               | 101.00 (41.5)               | -11.7          | 0.2346   |
| Systolic Blood Pressure               | 14 | 128.29 (14.5)               | 129.86 (20.1)               | 1.6            | 0.7470   |
| Diastolic Blood Pressure              | 14 | 77.86 (11.9)                | 74.00 (8.5)                 | -3.9           | 0.1917   |
| Depression Scale [CES-D]              | 14 | 11.00 (11.2)                | 10.36 (10.7)                | -0.6           | 0.7789   |
| Hostility Scale [Cook-Medley]         | 14 | 7.14 (4.6)                  | 6.57 (4.2)                  | -0.6           | 0.5616   |
| Perceived Stress Scale [PSS]          | 14 | 12.21 (8.1)                 | 12.36 (8.7)                 | 0.1            | 0.9482   |
| Daily Total Fat (grams)               | 14 | 58.69 (30.7)                | 63.48 (52.2)                | 4.8            | 0.7661   |
| Daily Saturated Fat (grams)           | 14 | 18.75 (12.2)                | 24.69 (27.5)                | 5.9            | 0.4872   |
| Avg. CCA/Mean IMT                     | 13 | 0.853 (0.1176)              | 0.698 (0.1431)              | -0.156         | <0.00001 |
| Avg. CCA / Max IMT                    | 13 | 0.982 (0.1184)              | 0.809 (0.1548)              | -0.2           | <0.00001 |
| Fasting Glucose (mg/dl)               | 14 | 101 (12.3)                  | 103 (33.7)                  | 2.4            | 0.7658   |
| HgbA1c                                | 14 | 6.3 (1.16)                  | 6.1 (1.21)                  | -0.2           | <0.01    |
| Cortisol                              | 14 | 13.0 (4.66)                 | 13.2 (4.60)                 | 0.2            | 0.9239   |
| TSH                                   | 14 | 2.18 (1.366)                | 2.24 (1.724)                | 0.1            | 0.8444   |
| Epworth Sleepiness Scale (0-24)       | 14 | 7 (4.6)                     | 6 (4.4)                     | -1.0           | 0.2544   |
| Pittsburgh Sleep Quality Index (0-21) | 14 | 8 (4.9)                     | 6 (3.0)                     | -2.1           | <0.05    |

**Table 25E. Change in outcome variables 2 years after completion of the intervention for participants in the Cardiovascular Risk Clinic**

| Category / Metrics          | N  | Average Baseline Value (SD) | Average 10 month value (SD) | Average Change | P-Value |
|-----------------------------|----|-----------------------------|-----------------------------|----------------|---------|
| Weight (lbs.)               | 14 | 188.96 (42.7)               | 183.63 (39.7)               | -5.3           | <0.05   |
| Body Mass Index             | 14 | 29.56 (4.2)                 | 28.71 (4.0)                 | -0.8           | <0.05   |
| Total Cholesterol (mg/dl)   | 14 | 179.14 (46.2)               | 174.64 (48.2)               | -4.5           | 0.7302  |
| High Density Lipids (mg/dl) | 14 | 52.36 (10.8)                | 50.21 (8.0)                 | -2.1           | 0.3804  |
| Low Density Lipids (mg/dl)  | 14 | 105.50 (35.6)               | 106.57 (37.8)               | 1.1            | 0.9158  |

**Table 25E. Change in outcome variables 2 years after completion of the intervention for participants in the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average 10 month value (SD) | Average Change | P-Value  |
|---------------------------------------|----|-----------------------------|-----------------------------|----------------|----------|
| Triglycerides (mg/dl)                 | 14 | 112.71 (52.7)               | 89.86 (51.3)                | -22.9          | 0.0637   |
| Systolic Blood Pressure               | 14 | 128.29 (14.5)               | 126.00 (16.2)               | -2.3           | 0.6571   |
| Diastolic Blood Pressure              | 14 | 77.86 (11.9)                | 73.29 (8.6)                 | -4.6           | 0.1246   |
| Depression Scale [CES-D]              | 14 | 11.00 (11.2)                | 7.93 (9.8)                  | -3.1           | 0.0673   |
| Hostility Scale [Cook-Medley]         | 14 | 7.14 (4.6)                  | 6.07 (4.3)                  | -1.1           | 0.1969   |
| Perceived Stress Scale [PSS]          | 14 | 12.21 (8.1)                 | 9.43 (7.2)                  | -2.8           | 0.0565   |
| Daily Total Fat (grams)               | 14 | 58.69 (30.7)                | 63.44 (18.8)                | 4.8            | 0.6025   |
| Daily Saturated Fat (grams)           | 14 | 18.75 (12.2)                | 19.69 (8.1)                 | 0.9            | 0.8048   |
| Avg. CCA/Mean IMT                     | 14 | 0.846 (0.1166)              | 0.650 (0.1460)              | -0.196         | <0.00001 |
| Avg. CCA / Max IMT                    | 14 | 0.974 (0.1180)              | 0.750 (0.1617)              | -0.2           | <0.00001 |
| Fasting Glucose (mg/dl)               | 14 | 101 (12.3)                  | 105 (31.5)                  | 4.1            | 0.5713   |
| HgbA1c                                | 14 | 6.3 (1.16)                  | 5.7 (1.07)                  | -0.6           | <0.001   |
| Cortisol                              | 14 | 13.0 (4.66)                 | 13.8 (2.98)                 | 0.7            | 0.5582   |
| TSH                                   | 14 | 2.18 (1.366)                | 2.83 (2.280)                | 0.6            | 0.0592   |
| Epworth Sleepiness Scale (0-24)       | 14 | 7 (4.6)                     | 5 (3.5)                     | -1.9           | <0.05    |
| Pittsburgh Sleep Quality Index (0-21) | 14 | 8 (4.9)                     | 6 (3.7)                     | -2.1           | <0.05    |

In subjects randomized to the control arm of the study, who do not participate in the lifestyle change intervention showed no significant changes in risk factors, except for CIMT and cortisol at the first 6 month time point (Table 26A). Subsequent follow up time points (Table 26B: 6 month time point; Table 26C: 1 year; Table 26D: 18 months; and Table 3E: 2 years) continue to show that most risk factors did not improve and those risk factors that did not maintain improvement at the next time point, perhaps this improvement could be attributed to small sample size and large variability among the participant's results. This lack of consistent improvement within the control arm further proves the benefits of a team-base, patient-centered lifestyle change model in improving risk for developing heart disease.

**Table 26A. Change in outcome variables from baseline to “waiting period complete” period for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics          | N  | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value |
|-----------------------------|----|-----------------------------|--|----------------|---------|
| Weight (lbs.)               | 67 | 189.08 (43.0)               | 188.63 (42.5)                              | -0.4           | 0.6694  |
| Body Mass Index             | 67 | 30.97 (6.4)                 | 30.84 (6.2)                                | -0.1           | 0.6791  |
| Total Cholesterol (mg/dl)   | 68 | 190.87 (37.0)               | 186.60 (35.9)                              | -4.3           | 0.2413  |
| High Density Lipids (mg/dl) | 68 | 49.85 (14.5)                | 49.07 (12.3)                               | -0.8           | 0.3747  |
| Low Density Lipids (mg/dl)  | 68 | 115.50 (31.2)               | 110.56 (31.0)                              | -4.9           | 0.1406  |
| Triglycerides (mg/dl)       | 68 | 130.40 (65.5)               | 133.00 (68.5)                              | 2.6            | 0.6591  |
| Systolic Blood Pressure     | 68 | 129.85 (17.8)               | 130.59 (21.0)                              | 0.7            | 0.7205  |

**Table 26A. Change in outcome variables from baseline to “waiting period complete” period for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value |
|---------------------------------------|----|-----------------------------|--|----------------|---------|
| Diastolic Blood Pressure              | 68 | 79.06 (10.2)                | 77.85 (10.2)                               | -1.2           | 0.2927  |
| Depression Scale [CES-D]              | 67 | 11.61 (10.2)                | 10.61 (8.9)                                | -1.0           | 0.2772  |
| Hostility Scale [Cook-Medley]         | 67 | 7.60 (4.9)                  | 7.30 (5.1)                                 | -0.3           | 0.3442  |
| Perceived Stress Scale [PSS]          | 67 | 13.33 (7.1)                 | 13.10 (7.7)                                | -0.2           | 0.7121  |
| Daily Total Fat (grams)               | 57 | 72.24 (32.3)                | 68.37 (32.7)                               | -3.9           | 0.4075  |
| Daily Saturated Fat (grams)           | 57 | 22.25 (10.2)                | 21.14 (11.2)                               | -1.1           | 0.4542  |
| Avg. CCA / Mean IMT                   | 67 | 0.801 (0.2040)              | 0.746 (0.1778)                             | -0.055         | <0.001  |
| Avg. CCA / Max IMT                    | 67 | 0.924 (0.2406)              | 0.850 (0.1990)                             | -0.1           | <0.0001 |
| Fasting Glucose (mg/dl)               | 68 | 108 (37.4)                  | 109 (41.2)                                 | 0.3            | 0.9243  |
| HgbA1c                                | 67 | 6.0 (1.32)                  | 6.0 (1.10)                                 | 0.0            | 0.8779  |
| Cortisol                              | 68 | 12.0 (4.31)                 | 13.6 (4.56)                                | 1.6            | <0.001  |
| TSH                                   | 66 | 1.99 (1.253)                | 2.07 (1.088)                               | 0.1            | 0.5620  |
| Epworth Sleepiness Scale (0-24)       | 66 | 8 (4.3)                     | 8 (3.9)                                    | -0.4           | 0.4011  |
| Pittsburgh Sleep Quality Index (0-21) | 66 | 7 (3.6)                     | 7 (3.5)                                    | -0.2           | 0.5784  |

**Table 26B. Change in outcome variables at the 6 month time point for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics            | N  | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value  |
|-------------------------------|----|-----------------------------|--|----------------|----------|
| Weight (lbs.)                 | 50 | 194.80 (43.7)               | 194.68 (45.7)                              | -0.1           | 0.9370   |
| Body Mass Index               | 49 | 31.63 (6.7)                 | 31.41 (6.8)                                | -0.2           | 0.4642   |
| Total Cholesterol (mg/dl)     | 50 | 194.94 (33.8)               | 183.84 (34.0)                              | -11.1          | <0.01    |
| High Density Lipids (mg/dl)   | 50 | 51.06 (15.2)                | 49.30 (17.6)                               | -1.8           | 0.3313   |
| Low Density Lipids (mg/dl)    | 50 | 117.80 (27.7)               | 110.54 (27.8)                              | -7.3           | <0.05    |
| Triglycerides (mg/dl)         | 50 | 133.98 (65.0)               | 128.32 (57.8)                              | -5.7           | 0.4946   |
| Systolic Blood Pressure       | 50 | 130.48 (16.2)               | 131.00 (24.8)                              | 0.5            | 0.8496   |
| Diastolic Blood Pressure      | 50 | 79.16 (10.4)                | 77.76 (11.8)                               | -1.4           | 0.4013   |
| Depression Scale [CES-D]      | 48 | 10.98 (9.8)                 | 11.02 (9.9)                                | 0.0            | 0.9692   |
| Hostility Scale [Cook-Medley] | 48 | 7.40 (5.2)                  | 7.06 (4.6)                                 | -0.3           | 0.3528   |
| Perceived Stress Scale [PSS]  | 48 | 12.48 (7.2)                 | 12.21 (7.0)                                | -0.3           | 0.7484   |
| Daily Total Fat (grams)       | 47 | 71.80 (34.2)                | 65.51 (28.4)                               | -6.3           | 0.2617   |
| Daily Saturated Fat (grams)   | 47 | 22.03 (10.8)                | 22.19 (10.5)                               | 0.2            | 0.9265   |
| Avg. CCA / Mean IMT           | 48 | 0.847 (0.1892)              | 0.740 (0.1719)                             | -0.107         | <0.00001 |
| Avg. CCA / Max IMT            | 48 | 0.976 (0.2225)              | 0.855 (0.1923)                             | -0.1           | <0.0001  |
| Fasting Glucose (mg/dl)       | 50 | 115 (41.5)                  | 114 (41.6)                                 | -0.6           | 0.8686   |
| HgbA1c                        | 50 | 6.2 (1.43)                  | 6.2 (1.19)                                 | -0.1           | 0.4052   |
| Cortisol                      | 50 | 12.0 (4.08)                 | 13.1 (3.98)                                | 1.1            | 0.0686   |
| TSH                           | 50 | 1.96 (1.267)                | 2.12 (0.935)                               | 0.2            | 0.3041   |

**Table 26B. Change in outcome variables at the 6 month time point for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value |
|---------------------------------------|----|-----------------------------|--|----------------|---------|
| Epworth Sleepiness Scale (0-24)       | 48 | 8 (4.2)                     | 8 (3.9)                                    | -0.1           | 0.8213  |
| Pittsburgh Sleep Quality Index (0-21) | 48 | 6 (3.5)                     | 6 (3.4)                                    | 0.1            | 0.7277  |

**Table 26C. Change in outcome variables at year 1 time point for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value  |
|---------------------------------------|----|-----------------------------|--|----------------|----------|
| Weight (lbs.)                         | 22 | 199.55 (41.6)               | 194.65 (42.3)                              | -4.9           | <0.05    |
| Body Mass Index                       | 22 | 31.75 (5.9)                 | 30.68 (6.1)                                | -1.1           | <0.05    |
| Total Cholesterol (mg/dl)             | 21 | 196.86 (39.3)               | 182.33 (37.8)                              | -14.5          | <0.05    |
| High Density Lipids (mg/dl)           | 21 | 54.38 (19.0)                | 49.86 (17.0)                               | -4.5           | <0.01    |
| Low Density Lipids (mg/dl)            | 21 | 116.43 (32.6)               | 107.43 (30.9)                              | -9.0           | 0.2162   |
| Triglycerides (mg/dl)                 | 21 | 138.71 (81.7)               | 124.76 (77.1)                              | -14.0          | 0.1953   |
| Systolic Blood Pressure               | 21 | 131.24 (14.8)               | 129.62 (19.6)                              | -1.6           | 0.6637   |
| Diastolic Blood Pressure              | 21 | 80.76 (9.8)                 | 77.81 (12.5)                               | -3.0           | 0.1814   |
| Depression Scale [CES-D]              | 21 | 9.48 (9.4)                  | 9.43 (10.4)                                | 0.0            | 0.9682   |
| Hostility Scale [Cook-Medley]         | 21 | 7.76 (5.5)                  | 7.95 (4.7)                                 | 0.2            | 0.7558   |
| Perceived Stress Scale [PSS]          | 21 | 11.52 (8.0)                 | 12.24 (6.6)                                | 0.7            | 0.5529   |
| Daily Total Fat (grams)               | 16 | 80.18 (33.7)                | 67.22 (35.2)                               | -13.0          | 0.1789   |
| Daily Saturated Fat (grams)           | 16 | 24.46 (10.0)                | 21.61 (10.9)                               | -2.8           | 0.3257   |
| Avg. CCA / Mean IMT                   | 21 | 0.914 (0.1655)              | 0.774 (0.1364)                             | -0.141         | <0.00001 |
| Avg. CCA / Max IMT                    | 21 | 1.057 (0.1763)              | 0.882 (0.1520)                             | -0.2           | <0.00001 |
| Fasting Glucose (mg/dl)               | 21 | 121 (46.6)                  | 111 (47.1)                                 | -9.9           | 0.1832   |
| HgbA1c                                | 21 | 6.4 (1.46)                  | 6.1 (1.00)                                 | -0.4           | 0.0886   |
| Cortisol                              | 21 | 13.7 (4.34)                 | 13.3 (4.50)                                | -0.4           | 0.7584   |
| TSH                                   | 21 | 1.84 (1.132)                | 2.03 (0.822)                               | 0.2            | 0.2164   |
| Epworth Sleepiness Scale (0-24)       | 21 | 9 (4.7)                     | 7 (4.1)                                    | -1.1           | 0.1530   |
| Pittsburgh Sleep Quality Index (0-21) | 21 | 7 (4.1)                     | 6 (3.6)                                    | -0.2           | 0.6863   |

**Table 26D. Change in outcome variables 18 month time point for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value |
|---------------------------------------|----|-----------------------------|--|----------------|---------|
| Weight (lbs.)                         | 13 | 204.22 (40.8)               | 198.86 (47.3)                              | -5.4           | 0.3780  |
| Body Mass Index                       | 13 | 33.15 (6.0)                 | 32.19 (7.0)                                | -1.0           | 0.3696  |
| Total Cholesterol (mg/dl)             | 13 | 211.00 (35.7)               | 201.31 (40.9)                              | -9.7           | 0.4169  |
| High Density Lipids (mg/dl)           | 13 | 56.77 (21.2)                | 54.92 (20.4)                               | -1.8           | 0.4082  |
| Low Density Lipids (mg/dl)            | 13 | 129.00 (31.0)               | 120.69 (35.6)                              | -8.3           | 0.5152  |
| Triglycerides (mg/dl)                 | 13 | 136.08 (67.7)               | 127.92 (98.0)                              | -8.2           | 0.6172  |
| Systolic Blood Pressure               | 9  | 134.89 (17.0)               | 127.33 (24.5)                              | -7.6           | 0.3164  |
| Diastolic Blood Pressure              | 9  | 81.33 (10.5)                | 79.78 (9.6)                                | -1.6           | 0.6560  |
| Depression Scale [CES-D]              | 12 | 8.00 (5.0)                  | 9.00 (7.6)                                 | 1.0            | 0.5124  |
| Hostility Scale [Cook-Medley]         | 12 | 6.92 (5.1)                  | 7.00 (5.2)                                 | 0.1            | 0.9030  |
| Perceived Stress Scale [PSS]          | 12 | 10.58 (5.6)                 | 11.75 (6.3)                                | 1.2            | 0.5632  |
| Daily Total Fat (grams)               | 10 | 72.35 (27.3)                | 80.50 (31.1)                               | 8.1            | 0.4831  |
| Daily Saturated Fat (grams)           | 10 | 21.82 (6.9)                 | 25.60 (8.5)                                | 3.8            | 0.2724  |
| Avg. CCA / Mean IMT                   | 13 | 0.935 (0.1549)              | 0.751 (0.1475)                             | -0.183         | <0.0001 |
| Avg. CCA / Max IMT                    | 13 | 1.069 (0.1786)              | 0.862 (0.1681)                             | -0.2           | <0.0001 |
| Fasting Glucose (mg/dl)               | 13 | 113 (38.6)                  | 104 (21.8)                                 | -9.2           | 0.2277  |
| HgbA1c                                | 13 | 6.4 (1.31)                  | 5.9 (0.69)                                 | -0.5           | 0.0673  |
| Cortisol                              | 13 | 13.3 (4.52)                 | 11.4 (4.93)                                | -1.9           | 0.2866  |
| TSH                                   | 13 | 1.65 (0.836)                | 1.92 (0.564)                               | 0.3            | 0.1088  |
| Epworth Sleepiness Scale (0-24)       | 12 | 9 (4.8)                     | 7 (4.0)                                    | -1.4           | 0.1857  |
| Pittsburgh Sleep Quality Index (0-21) | 12 | 7 (4.7)                     | 7 (4.3)                                    | -0.5           | 0.6595  |

**Table 26E. Change in outcome variables at Year 2 time point for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics            | N | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value |
|-------------------------------|---|-----------------------------|--|----------------|---------|
| Weight (lbs.)                 | 8 | 192.63 (43.1)               | 186.48 (38.6)                              | -6.2           | <0.05   |
| Body Mass Index               | 9 | 32.43 (5.9)                 | 30.80 (5.3)                                | -1.6           | 0.0917  |
| Total Cholesterol (mg/dl)     | 8 | 209.50 (36.3)               | 182.75 (48.2)                              | -26.8          | 0.1127  |
| High Density Lipids (mg/dl)   | 8 | 62.75 (25.4)                | 56.88 (22.9)                               | -5.9           | 0.1441  |
| Low Density Lipids (mg/dl)    | 8 | 122.38 (25.2)               | 99.50 (35.3)                               | -22.9          | 0.2084  |
| Triglycerides (mg/dl)         | 8 | 137.50 (82.4)               | 131.75 (64.5)                              | -5.8           | 0.7400  |
| Systolic Blood Pressure       | 8 | 135.50 (17.3)               | 135.50 (25.8)                              | 0.0            | 1.0000  |
| Diastolic Blood Pressure      | 8 | 82.75 (10.3)                | 73.13 (10.3)                               | -9.6           | <0.01   |
| Depression Scale [CES-D]      | 6 | 5.00 (3.2)                  | 4.00 (3.6)                                 | -1.0           | 0.4466  |
| Hostility Scale [Cook-Medley] | 6 | 7.50 (4.1)                  | 9.00 (4.4)                                 | 1.5            | 0.2264  |
| Perceived Stress Scale [PSS]  | 6 | 9.50 (7.0)                  | 8.50 (6.4)                                 | -1.0           | 0.6884  |

**Table 26E. Change in outcome variables at Year 2 time point for participants in the control arm of the Cardiovascular Risk Clinic**

| Category / Metrics                    | N | Average Baseline Value (SD) | Average Waiting Period Complete Value (SD) | Average Change | P-Value |
|---------------------------------------|---|-----------------------------|--|----------------|---------|
| Daily Total Fat (grams)               | 3 | 58.89 (19.6)                | 79.45 (16.9)                               | 20.6           | 0.0850  |
| Daily Saturated Fat (grams)           | 3 | 17.28 (6.3)                 | 29.19 (11.2)                               | 11.9           | 0.1616  |
| Avg. CCA / Mean IMT                   | 8 | 0.915 (0.1560)              | 0.694 (0.1393)                             | -0.221         | <0.001  |
| Avg. CCA / Max IMT                    | 8 | 1.037 (0.1771)              | 0.775 (0.1518)                             | -0.3           | <0.001  |
| Fasting Glucose (mg/dl)               | 8 | 121 (45.9)                  | 107 (20.2)                                 | -14.3          | 0.2917  |
| HgbA1c                                | 8 | 6.4 (1.27)                  | 5.8 (0.65)                                 | -0.7           | 0.0740  |
| Cortisol                              | 8 | 12.8 (5.25)                 | 12.4 (3.65)                                | -0.5           | 0.8034  |
| TSH                                   | 8 | 1.61 (0.804)                | 2.54 (1.447)                               | 0.9            | 0.2154  |
| Epworth Sleepiness Scale (0-24)       | 6 | 9 (4.0)                     | 8 (4.2)                                    | -1.2           | 0.3522  |
| Pittsburgh Sleep Quality Index (0-21) | 6 | 5 (2.1)                     | 6 (3.4)                                    | 0.3            | 0.7497  |

**Adverse Events:** No adverse events reported in the past quarter. All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There were no adverse events in either arm of the study during the last quarter. To date there have been a total of 16 adverse events, 8 in the intervention and 8 in the control arm of the study, all deemed serious events, not related and not expected. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, all 16 events were considered serious due to inpatient hospitalizations. There were 7 non-cardiac and 1 cardiac adverse events in the intervention arm of the study. No deaths occurred and none of these adverse events were deemed to be study related. There were 5 non-cardiac and 3 cardiac adverse events in the control arm of the study. No deaths occurred and none of these adverse events were deemed to be study related.

### **NMR Lipid Panel and Biomarkers**

This year for the CRC program, approximately 5,570 aliquots have been made summarized by the following:

PAXGene Tubes                    296  
RBCs                                        586

#### Plasma samples

NMR lipids                                293  
Leptin                                        293  
CRP    293  
Resistin                                       293

#### Serum samples

Adiponectin                                298  
Serum amyloid A                        298  
Vitamin D                                    298  
Lp(a)     298

|              |     |             |      |
|--------------|-----|-------------|------|
| Insulin      | 293 | Extra Serum | 1165 |
| Extra plasma | 866 |             |      |

**Task #11a: Initiate Stress Therapy Empowering Prevention (STEP) component to the Cardiovascular Risk Assessment program outlined in Task #11.**

This is a collaborative study involving researchers from WRI and WRNMMC and is modeled after the Caretakers Optimizing Readiness through Preventive Strategies (CORPS), designed by the Integrative Cardiac Health Program (ICHP) at WRNMMC, except that it targets participants with chronic disease. The purpose of this task is to determine the degree of stress, sleep disturbance, and cardiovascular disease risk in patients who have been diagnosed with breast cancer or are at high risk of developing breast disease.

In the first part of the intervention, patients will be randomized to a 12 week Healthy Lifestyle intervention group or a non-intervention group. During this phase, each intervention participant undergo a comprehensive health risk assessment that is completed by a physician, followed by mandatory attendance to on-site group sessions in which they will participate in 1 hour of stress management, 30 minutes of nutrition education every week, and 30 minutes of exercise alternated with 30 minutes of mind/body health every other week. In addition, the nurse will provide educational lectures on various health topics during 4 sessions. After completing Phase I, patients will participate in a five year healthy lifestyle intervention or control group.

During phase II each intervention participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase II for five years, during which time they will come to the center for re-assessments every six months.

We hypothesize that the 12 week healthy lifestyle interventions will significantly reduce stress, sleep disturbances, and cardiovascular risk in patients at risk for, or already diagnosed with, breast cancer.

**Status:** Study is currently being closed for enrollment but will remain open for data analysis.

**Subject Enrollment and Demographics:**

Total subject enrollment was 18 (intervention only); 10 active; 8 dropouts. Demographic characteristics of participants were: average age 65.6 years, 28% veterans or the spouse of a veteran, 6% have diagnosed coronary heart disease, and 61% have diagnosed breast cancer. Due to the lack of public interest we were unable to recruit a sufficient number of participants to keep this protocol open. The protocol was closed for enrollment on September 1, 2012 but will remain open for data analysis.

In the last quarter (July 2012- 15 Sept 2012) there were a total of 8 participant visits including periodic follow up phone calls made to participants.

**Outcomes Data:**

Overall participants showed change in the desired direction for most of the measured coronary artery disease (CAD) risk factors over the 2 years of the program (see Tables 27A-27D below). No participants were enrolled into the control arm of the study and lack of statistically significant levels of improvement in some measures may be attributable to small sample size and wide variability in some measures.

**Table 27A. Comparison of baseline to Week 12 data for participants in the STEP Program**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Week 12 Value (SD) | Average Change | P Value |
|---------------------------------------|----|-----------------------------|----------------------------|----------------|---------|
| Weight (lbs.)                         | 16 | 182.57 (35.9)               | 179.30 (33.0)              | -3.3           | <0.01   |
| Body Mass Index                       | 16 | 32.83 (6.3)                 | 32.04 (5.9)                | -0.8           | <0.01   |
| Total Cholesterol (mg/dl)             | 16 | 198.38 (36.4)               | 196.69 (44.2)              | -1.7           | 0.7954  |
| High Density Lipids (mg/dl)           | 16 | 54.44 (12.4)                | 52.25 (12.8)               | -2.2           | 0.0928  |
| Low Density Lipids (mg/dl)            | 16 | 114.50 (28.7)               | 118.63 (38.4)              | 4.1            | 0.5290  |
| Triglycerides (mg/dl)                 | 16 | 155.13 (90.6)               | 132.81 (73.4)              | -22.3          | 0.0926  |
| Systolic Blood Pressure               | 16 | 134.75 (18.8)               | 124.50 (14.1)              | -10.3          | 0.0763  |
| Diastolic Blood Pressure              | 16 | 80.63 (11.3)                | 73.75 (8.1)                | -6.9           | <0.05   |
| Depression Scale [CES-D]              | 16 | 15.31 (10.2)                | 11.44 (10.4)               | -3.9           | 0.0914  |
| Hostility Scale [Cook-Medley]         | 16 | 7.06 (4.4)                  | 5.25 (3.3)                 | -1.8           | 0.0720  |
| Daily Total Fat (grams)               | 8  | 58.62 (39.1)                | 44.48 (5.8)                | -14.1          | 0.3394  |
| Daily Saturated Fat (grams)           | 8  | 19.77 (19.5)                | 11.77 (3.4)                | -8.0           | 0.2853  |
| Perceived Stress Scale [PSS]          | 16 | 17.00 (7.2)                 | 12.88 (6.5)                | -4.1           | <0.05   |
| Avg. CCA/Mean IMT                     | 16 | 0.735 (0.1488)              | 0.810 (0.1677)             | 0.075          | <0.01   |
| Avg. CCA / Max IMT                    | 16 | 0.865 (0.1556)              | 0.928 (0.2046)             | 0.1            | <0.05   |
| Fasting Glucose (mg/dl)               | 16 | 107 (28.8)                  | 109 (25.7)                 | 2.4            | 0.6604  |
| HgbA1c                                | 16 | 6.3 (0.87)                  | 6.5 (0.77)                 | 0.2            | 0.3545  |
| Cortisol                              | 16 | 12.8 (3.83)                 | 16.5 (5.44)                | 3.7            | 0.0507  |
| TSH                                   | 16 | 1.71 (1.342)                | 2.07 (1.674)               | 0.4            | 0.2887  |
| Epworth Sleepiness Scale (0-24)       | 16 | 9 (4.5)                     | 8 (4.2)                    | -0.9           | 0.4320  |
| Pittsburgh Sleep Quality Index (0-21) | 16 | 10 (4.8)                    | 8 (4.4)                    | -2.5           | 0.0512  |

**Table 27B. Comparison of baseline to Year 1 data for participants in the STEP program**

| Category / Metrics          | N  | Average Baseline Value (SD) | Average Year 1 Value (SD) | Average Change | P Value |
|-----------------------------|----|-----------------------------|---------------------------|----------------|---------|
| Weight (lbs.)               | 14 | 180.49 (35.5)               | 177.30 (32.7)             | -3.2           | <0.05   |
| Body Mass Index             | 14 | 32.49 (6.4)                 | 31.66 (6.0)               | -0.8           | <0.05   |
| Total Cholesterol (mg/dl)   | 14 | 201.07 (37.3)               | 200.21 (45.8)             | -0.9           | 0.9083  |
| High Density Lipids (mg/dl) | 14 | 54.64 (13.1)                | 52.14 (13.7)              | -2.5           | 0.0715  |
| Low Density Lipids (mg/dl)  | 14 | 116.79 (30.0)               | 121.57 (40.2)             | 4.8            | 0.5252  |
| Triglycerides (mg/dl)       | 14 | 157.21 (95.4)               | 136.71 (76.1)             | -20.5          | 0.1721  |

**Table 27B. Comparison of baseline to Year 1 data for participants in the STEP program**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Year 1 Value (SD) | Average Change | P Value |
|---------------------------------------|----|-----------------------------|---------------------------|----------------|---------|
| Systolic Blood Pressure               | 14 | 134.00 (18.4)               | 125.14 (15.0)             | -8.9           | 0.1451  |
| Diastolic Blood Pressure              | 14 | 79.57 (11.3)                | 72.86 (8.3)               | -6.7           | 0.0838  |
| Depression Scale [CES-D]              | 14 | 13.71 (9.7)                 | 11.29 (11.0)              | -2.4           | 0.1056  |
| Hostility Scale [Cook-Medley]         | 14 | 6.36 (4.2)                  | 4.79 (3.2)                | -1.6           | 0.3330  |
| Perceived Stress Scale [PSS]          | 14 | 16.29 (7.4)                 | 12.71 (6.8)               | -3.6           | <0.05   |
| Daily Total Fat (grams)               | 10 | 61.64 (36.5)                | 54.64 (25.4)              | -7.0           | 0.4039  |
| Daily Saturated Fat (grams)           | 10 | 21.49 (18.2)                | 15.26 (8.5)               | -6.2           | 0.1507  |
| Avg. CCA/Mean IMT                     | 14 | 0.745 (0.1557)              | 0.826 (0.1718)            | 0.081          | <0.01   |
| Avg. CCA / Max IMT                    | 14 | 0.879 (0.1607)              | 0.948 (0.2110)            | 0.1            | <0.05   |
| Fasting Glucose (mg/dl)               | 14 | 109 (30.6)                  | 111 (27.4)                | 2.0            | 0.7535  |
| HgbA1c                                | 14 | 6.4 (0.91)                  | 6.6 (0.77)                | 0.2            | 0.3356  |
| Cortisol                              | 14 | 12.4 (3.61)                 | 16.9 (5.61)               | 4.5            | <0.05   |
| TSH                                   | 14 | 1.66 (1.419)                | 2.19 (1.766)              | 0.5            | 0.1559  |
| Epworth Sleepiness Scale (0-24)       | 14 | 8 (4.5)                     | 8 (4.5)                   | -0.6           | 0.6020  |
| Pittsburgh Sleep Quality Index (0-21) | 14 | 10 (5.1)                    | 8 (4.6)                   | -2.7           | 0.0639  |

**Table 27C. Comparison of baseline to 18 month data for participants in the STEP program**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Year 1 Value (SD) | Average Change | P Value |
|---------------------------------------|----|-----------------------------|---------------------------|----------------|---------|
| Weight (lbs.)                         | 10 | 189.23 (36.5)               | 188.36 (33.3)             | -0.9           | 0.7195  |
| Body Mass Index                       | 10 | 33.66 (7.0)                 | 33.37 (6.2)               | -0.3           | 0.4956  |
| Total Cholesterol (mg/dl)             | 10 | 200.50 (40.4)               | 211.50 (60.3)             | 11.0           | 0.4066  |
| High Density Lipids (mg/dl)           | 10 | 53.50 (13.3)                | 52.00 (13.2)              | -1.5           | 0.3974  |
| Low Density Lipids (mg/dl)            | 10 | 113.40 (31.1)               | 126.60 (43.3)             | 13.2           | 0.1605  |
| Triglycerides (mg/dl)                 | 10 | 180.80 (103.6)              | 163.20 (115.1)            | -17.6          | 0.3961  |
| Systolic Blood Pressure               | 10 | 135.80 (20.2)               | 137.40 (18.0)             | 1.6            | 0.8362  |
| Diastolic Blood Pressure              | 10 | 79.40 (12.6)                | 75.20 (11.8)              | -4.2           | 0.2921  |
| Depression Scale [CES-D]              | 10 | 13.20 (9.6)                 | 6.80 (7.1)                | -6.4           | <0.05   |
| Hostility Scale [Cook-Medley]         | 10 | 7.40 (4.4)                  | 5.90 (3.6)                | -1.5           | 0.1604  |
| Perceived Stress Scale [PSS]          | 10 | 16.00 (8.2)                 | 8.90 (6.8)                | -7.1           | <0.01   |
| Daily Total Fat (grams)               | 10 | 61.64 (36.5)                | 37.96 (12.0)              | -23.7          | 0.0766  |
| Daily Saturated Fat (grams)           | 10 | 21.49 (18.2)                | 10.20 (4.2)               | -11.3          | 0.0943  |
| Avg. CCA/Mean IMT                     | 10 | 0.751 (0.1695)              | 0.775 (0.1719)            | 0.024          | 0.4697  |
| Avg. CCA / Max IMT                    | 10 | 0.888 (0.1815)              | 0.919 (0.2275)            | 0.0            | 0.4407  |
| Fasting Glucose (mg/dl)               | 10 | 110 (34.8)                  | 112 (39.7)                | 1.8            | 0.6648  |
| HgbA1c                                | 10 | 6.4 (0.98)                  | 6.7 (1.52)                | 0.3            | 0.3126  |
| Cortisol                              | 10 | 12.8 (3.83)                 | 13.1 (4.67)               | 0.3            | 0.8983  |
| TSH                                   | 10 | 1.64 (1.577)                | 1.44 (1.243)              | -0.2           | 0.5141  |
| Epworth Sleepiness Scale (0-24)       | 10 | 8 (4.7)                     | 7 (4.0)                   | -0.7           | 0.5496  |
| Pittsburgh Sleep Quality Index (0-21) | 10 | 10 (5.0)                    | 7 (3.4)                   | -3.1           | 0.1121  |

**Table 27D. Comparison of baseline to year 2 data for participants in the STEP program**

| Category / Metrics                    | N  | Average Baseline Value (SD) | Average Year 1 Value (SD) | Average Change | P Value |
|---------------------------------------|----|-----------------------------|---------------------------|----------------|---------|
| Weight (lbs.)                         | 10 | 189.23 (36.5)               | 184.84 (31.3)             | -4.4           | 0.1403  |
| Body Mass Index                       | 10 | 33.66 (7.0)                 | 32.58 (5.7)               | -1.1           | 0.1971  |
| Total Cholesterol (mg/dl)             | 10 | 200.50 (40.4)               | 194.60 (53.8)             | -5.9           | 0.5141  |
| High Density Lipids (mg/dl)           | 10 | 53.50 (13.3)                | 48.70 (11.9)              | -4.8           | <0.001  |
| Low Density Lipids (mg/dl)            | 10 | 113.40 (31.1)               | 114.70 (43.3)             | 1.3            | 0.8637  |
| Triglycerides (mg/dl)                 | 10 | 180.80 (103.6)              | 162.20 (118.8)            | -18.6          | 0.4207  |
| Systolic Blood Pressure               | 10 | 135.80 (20.2)               | 132.60 (15.3)             | -3.2           | 0.4981  |
| Diastolic Blood Pressure              | 10 | 79.40 (12.6)                | 76.40 (10.6)              | -3.0           | 0.3412  |
| Depression Scale [CES-D]              | 10 | 13.20 (9.6)                 | 12.00 (11.9)              | -1.2           | 0.7045  |
| Hostility Scale [Cook-Medley]         | 10 | 7.40 (4.4)                  | 6.40 (5.3)                | -1.0           | 0.3765  |
| Perceived Stress Scale [PSS]          | 10 | 16.00 (8.2)                 | 13.00 (9.1)               | -3.0           | 0.2401  |
| Daily Total Fat (grams)               | 10 | 61.64 (36.5)                | 56.12 (20.1)              | -5.5           | 0.7037  |
| Daily Saturated Fat (grams)           | 10 | 21.49 (18.2)                | 17.05 (6.0)               | -4.4           | 0.5093  |
| Avg. CCA/Mean IMT                     | 10 | 0.751 (0.1695)              | 0.738 (0.1484)            | -0.013         | 0.7605  |
| Avg. CCA / Max IMT                    | 10 | 0.888 (0.1815)              | 0.845 (0.1683)            | 0.0            | 0.3379  |
| Fasting Glucose (mg/dl)               | 10 | 110 (34.8)                  | 111 (30.9)                | 0.6            | 0.8474  |
| HgbA1c                                | 10 | 6.4 (0.98)                  | 6.4 (0.94)                | 0.0            | 0.5987  |
| Cortisol                              | 10 | 12.8 (3.83)                 | 14.7 (4.31)               | 1.8            | 0.1363  |
| TSH                                   | 10 | 1.64 (1.577)                | 2.22 (1.635)              | 0.6            | 0.2716  |
| Epworth Sleepiness Scale (0-24)       | 10 | 8 (4.7)                     | 7 (4.5)                   | -0.7           | 0.6380  |
| Pittsburgh Sleep Quality Index (0-21) | 10 | 10 (5.0)                    | 7 (4.3)                   | -3.1           | 0.0895  |

During this year, we received data on the following variables for CRC participants.

Analysis will be forthcoming.

|                 |     |               |     |
|-----------------|-----|---------------|-----|
| NMR lipid panel | 296 | Insulin       | 106 |
| CRP             | 148 | Adiponectin   | 0   |
| Leptin          | 106 | Serum Amyloid | 0   |
| Lipoprotein (a) | 0   | Resistin      | 106 |
| Vitamin D       | 69  |               |     |

**Adverse Events:** All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There was one adverse event during the last quarter, the event was deemed serious, not related and unexpected due to testing that revealed terminal metastasis to the bone and adrenal gland. To date, there have been 5 adverse events, 4 were deemed serious and 1 event was not serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Three of the events

were considered serious due to inpatient hospitalizations and one due to poor prognosis related disease progression. No deaths occurred and none of these adverse events were deemed to be study related.

**Task #12: Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Military Service Women.**

This study will identify genes that affect susceptibility to heart attack in young military service personnel who have had a heart attack before the age of 55. Patients will be selected from the Department of Defense Serum Repository, which has millions of serum samples in storage. Cutting-edge technology will be used to isolate very small amounts of DNA that can be found in serum. More than 1,000,000 variations in the DNA will be tested. The ultimate objective is to identify new genes that increase risk for heart attack at an early age – such genes represent new targets for preventive or therapeutic interventions.

**Status:**

We have revised the study protocol, which will be initiated as a feasibility study. This modification in the study design will determine the feasibility of isolating and genotyping quality DNA from serum samples in the Department of Defense Serum Repository (DoDSR). For this proof-of-principal study we aim to: (1) assess the quantity and quality of DNA isolated from serum samples obtained from the DoDSR and (2) evaluate the performance of the obtained DNA on Affymetrix 6.0 SNP arrays containing 1.6 million markers. These preliminary studies will determine if we can use DoDSR DNA on high-density genetic marker arrays for future studies.

We continued our research and development work on whole-genome amplification of DNA samples and large-scale genomic research on these samples. Using laboratory samples that should be similar to the repository samples, call rates for all genomic DNA samples were all >97.90% (Table 28). Call rates for DNA isolated from serum were >93.00% and for DNA isolated from heparin plasma were >95.7%. Samples from EDTA tubes that were whole-genome amplified did not perform well (~69-89% call rates). Serum samples from the DoDSR will be compared to these samples in the next period.

**Table 28. Call rates on Affymetrix 6.0 arrays for DNA from various sources.**

| Sample               | P/S   | CQC  | Call Rate |
|----------------------|-------|------|-----------|
| #1 Genomic           | N/A   | 3.04 | 98.78     |
| #2 Genomic           | N/A   | 2.63 | 97.9484   |
| #3 Genomic           | N/A   | 2.43 | 97.9153   |
| #5 Genomic           | N/A   | 2.67 | 98.0477   |
| #1 Serum Unamplified | Serum | 0.72 | 93.051    |
| #2 Serum Unamplified | Serum | 2.32 | 97.7498   |

|                        |        |       |         |
|------------------------|--------|-------|---------|
| #3 Serum Unamplified   | Serum  | 2.21  | 98.2793 |
| #5 Serum Unamplified   | Serum  | 2.25  | 97.85   |
| #1 Serum WGA           | Serum  | 2.19  | 96.1946 |
| #2 Serum WGA           | Serum  | -0.05 | 84.71   |
| #3 Serum WGA           | Serum  | 2.15  | 96.1284 |
| #5 Serum WGA           | Serum  | 0.72  | 90.7346 |
| #1 EDTA Unamplified    | Plasma | 0.47  | 89.74   |
| #2 EDTA Unamplified    | Plasma | 3.31  | 99.01   |
| #3 EDTA Unamplified    | Plasma | -0.46 | 87.7895 |
| #5 EDTA Unamplified    | Plasma | 0.43  | 91.4295 |
| #1 EDTA WGA            | Plasma | -0.07 | 77.9616 |
| #2 EDTA WGA            | Plasma | 0.01  | 88.88   |
| #3 EDTA WGA            | Plasma | -0.03 | 68.63   |
| #5 EDTA WGA            | Plasma | -0.06 | 73.0311 |
| #1 Heparin Unamplified | Plasma | 1.67  | 95.7313 |
| #2 Heparin Unamplified | Plasma | 2.6   | 97.1873 |
| #3 Heparin Unamplified | Plasma | 2.6   | 98.84   |
| #5 Heparin Unamplified | Plasma | 2.41  | 98.1469 |
| #1 Heparin WGA         | Plasma | 2.02  | 94.143  |
| #2 Heparin WGA         | Plasma | 2.75  | 98.5109 |
| #3 Heparin WGA         | Plasma | 2.21  | 97.3527 |
| #5 Heparin WGA         | Plasma | 2.61  | 97.9815 |

**Task #13: Initiate “Lifestyle Education and Nutrition (LEAN) Program pilot project.**

**Status:** Pilot conducted. Findings suggest future research study not to be feasible. Task completed.

**Task #14: Initiate “Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Military Service Women” protocol.** (Task in collaboration with WRI)

**Status:** Proof of principle feasibility to be conducted first – see Task #12.

**Task #15: Initiate certification of ICHP as optimal healing environment**

**Status:** No progress and is not feasible at this time.

**Task #16: Begin pilot study to assess the utility of direct tissue protein profiling for identifying new markers of heart disease.**

**Status:** Study not feasible at this time.

## Key Research Accomplishments

- CADRe Five-Year Follow-Up protocol
  - Statistical analysis needs established for final data analysis
- Better Adherence to Therapeutic Lifestyle Change Efforts (BATTLE) Trial
  - Data analysis complete
  - One manuscript submitted; manuscript in preparation
  - Two abstract presented at the 2011 Preventive Cardiovascular Nurses Association Annual Conference; one abstract published from this meeting
  - One abstract presented as oral presentation at the 2010 American College of Physicians, Army Region meeting
  - Findings suggest knowledge of an abnormal CIMT and increased CV risk does not improve adherence to a lifestyle program
- Dr. Dean Ornish Program for Reversing Heart Disease protocol
  - Subject enrollment over 25 cohorts is complete – 422 participants were enrolled, 339 participants graduated, 83 participants dropped out
  - Age/gender/disease status matched control group established to compare risk factor changes
- Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal
  - Subject enrollment was 374 – 166 participants in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study
  - One abstract presented at Obesity 2012 Annual Scientific Meeting in San Antonio, TX.
  - Two abstracts presented at the Association for Molecular Pathology (AMP) 2011 Annual Meeting in Dallas, TX
  - Two abstracts accepted to upcoming meetings: American Heart Association Scientific Sessions 2012 in Los Angeles, CA and the American Society of Human Genetics Meetings in San Francisco, CA.
  - Participation in the Program reduces levels of important biochemical risk factors for CAD, such as CRP and MIF (manuscripts in progress).
  - Changes in gene expression mirror changes in many CVD risk factors – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52.
  - Most cholesterol and lipid homeostasis genes show a continual decrease in expression throughout the program similar to body weight.
  - Medication use clearly does not affect gene expression, thus expression changes may be attributed to the lifestyle change program
  - Genetic variation influences risk factor response
    - Several SNPs show evidence of an influence on triglyceride response
- Comprehensive Cardiovascular Risk Assessment and Prevention Program (CPP)
  - Clinical Database development with informatics architects underway

- Integrative approach applied to specific prevention tracks for optimal impact and improved clinical outcomes for military beneficiaries
  - Refinement of clinical research model based on recent findings.
  - New program track for pre-diabetics established
  - Data management and analysis underway
  - Publication plan in progress
  - 9 peer-reviewed abstracts and 1 manuscript from the CPP have been generated
  - 7 abstracts presented as poster presentation at the following meeting venues:
    - American Thoracic Society
    - Associated Professional Sleep Societies
    - National Sleep Foundation
    - American College of Physicians, Army Region
    - American College of Nurse Practitioners
    - Force Health Protection Conference
  - 2 abstracts presented as oral presentations at the American College of Physicians' Army Region meeting and the American College of Chest Physicians
  - Data analysis is suggesting the following:
    - Stress plays an unexpectedly prominent role in cardiovascular risk
    - Stress erodes sleep quality and is associated with dysregulation of glucose metabolism
    - Deteriorations of sleep and glucose regulation may serve as mediators of the increased cardiovascular risk in our patient population
    - Increased stress, decreased sleep time, poor sleep quality, glucose dysmetabolism, and increased cardiovascular risk all have a negative impact of military readiness
    - Longer sleep time and improved sleep quality correlate with improved weight control as well as improved cardiovascular risk.
- Validation of the ICHP Cardiovascular Risk Score protocol
    - ICHP CV Risk score revalidated with new data set of women from BATTLE Study (see Task #4)
    - 1 abstracts presented and published
  - The Cardiovascular Risk Clinic (CRC)
    - Subject enrollment is 132; 114 participants remain active
  - The Stress Therapy Empowering Prevention (STEP) program
    - Two abstracts presented at the National Consortium of Breast Centers Interdisciplinary Breast Center Conference in Las Vegas
  - ZENITH (randomiZed Evaluation of a Novel comprehensive prevention program on atherosclerosis progression) Trial
    - Protocol submitted for scientific review
    - Statistical support needs identified

## **Reportable Outcomes (See Appendix A)**

### **Manuscripts in Scientific Journals**

#### **Attributed to completed Task #1 (Award No. W81XWH-05-2-0075)**

Marshall D, Walizer E, & Vernalis M. Effect of a one-year lifestyle intervention program on carotid intima media thickness, *Mili Med* 2011;176(7):798-804.

#### **Task #6:**

Voegtly LM, Neatrou DM, Decewicz DJ, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. Cardiometabolic risk factors during an intensive cardiovascular lifestyle intervention. *Nutr Metab Cardiovasc Dis* 2012 May 25. [Epub ahead of print].

#### **Sub Task #10.2**

Kashani M, Eliasson A, Chrosniak L, Vernalis M. Taking aim at nurse stress: a call to action. *Milit Med* 2010;175:96-100.

#### **Task #10:**

Kashani M, Eliasson A, Vernalis M. Perceived stress correlates with disturbed sleep—a link connecting stress and cardiovascular disease. *Stress* 2011;19 June [epub ahead of print]. (In print - 2012;15(1):45-51.

#### **Task #11a:**

Burke AM, Ellsworth DL, Vernalis MN. Stress Therapy Empowers Prevention (STEP): A healthy-lifestyle program for breast cancer patients. *J Oncol Navig Surviv* 2012;3:8-14.

### **Abstracts in Scientific Journals**

#### **Task #4:**

Walizer E, Kashani M, Eliasson A, Vernalis M. Integrative Cardiac Health Project risk score improves cardiovascular risk assessment in women with subclinical atherosclerosis. *J Cardiovasc Nurs* 2011; 26(4):265A.

#### **Task #6:**

Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. *Circ* 2012;126:A14216.

Ellsworth DL, Decewicz DJ, Neatrou DM, Burke A, Haberkorn MJ, Patney HL, Vernalis MN. Intensive lifestyle modification for CAD reversal successfully reduces circulating levels of metabolic hormones insulin and leptin. *Arterioscler Thromb Vasc Biol* 2010;30:e245.

#### **Task #10:**

Kashani M, Eliasson A, Bailey K, Vernalis M. Novel stress reduction technique improves sleep and fatigue. *Chest* 2012;142(4\_MeetingAbstracts):1052A.

Eliasson A, Kashani M, Vernalis M. Fatigued on Venus, sleepy on Mars? *Am J Respir Crit Care Med* 2012; 185:A5033.

Kashani M, Eliasson A, Bailey K, Vernalis M. Novel tool improves CV risk stratification and guides therapy. *Circ Cardiovasc Qual Outcomes* 2011;4(6):AP88.

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**Task #11a:**

Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Vernalis MN, Ellsworth DL. Stress Therapy Empowers Prevention (STEP): A healthy-lifestyle program for breast cancer patients. *Am J Clin Oncol* 2011;34(5):551.

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**Task #12:**

Croft DT Jr, Voegtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. *J Mol Diag* 2011;13(6):781.

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## **Presentations (Oral & Poster)**

### **Task #4:**

Saum N, Walizer E, Vernalis, M. Feasibility of including limited mindfulness training in an existing therapeutic lifestyle change (TLC) program. Prevention Cardiovascular Nurses Association (PCNA), Mar 2011, Orlando, FL. (poster)

Walizer E, Kashani M, Eliasson A, Vernalis M. Integrative cardiac health project risk score improves cardiovascular risk assessment in women with subclinical atherosclerosis. Prevention Cardiovascular Nurses Association (PCNA), Mar 2011, Orlando, FL. (poster/published abstract)

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**Task #10:**

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Eliasson A, Kashani M, Hoffman J, Vernalis M. Racial differences in perceived stress, sleep habits, and daytime symptoms. Associated Professional Sleep Societies, Jun 2011, Minneapolis, MN. (poster/published abstract)

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Eliasson A, Kashani M, Vernalis M. Longer sleep confers cardiovascular health benefit. American Thoracic Society, May 2010 (poster/published abstract)

**Sub Task #10.1:**

Kashani M, Eliasson A, Bailey K, Vernalis M. Novel tool improves CV risk stratification and guides therapy. American Heart Association - Quality of Care and Outcomes Research in CV Disease and Stroke, May 2011, Washington DC. (poster/published abstract)

**Task #11a:**

Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Vernalis MN, Ellsworth DL. Stress Therapy Empowers Prevention (STEP): A healthy-lifestyle program for breast cancer patients. National Consortium of Breast Centers Conference, Mar 2011, Las Vegas, NV. (poster/published abstract)

Ellsworth D, Patney HL, Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Neatrour DM, Vernalis MN. Improvement in cardiovascular risk factors in breast cancer patients participating in the Stress Therapy Empowers Prevention (STEP) Program. National Consortium of Breast Centers Conference, Mar 2011, Las Vegas, NV. (poster/published abstract)

**Task #12:**

Croft DT Jr, Voegtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. Association for Molecular Pathology (AMP) 2011 Annual Meeting, 17-19 Nov 11, Grapevine, TX. (poster/published abstract)

Voegtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. Association for Molecular Pathology (AMP) 2011 Annual Meeting, 17-19 Nov 11, Grapevine, TX. (poster/published abstract)

## **Conclusions**

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits could nearly eliminate the development of CHD and substantially decrease CHD morbidity and mortality. We have demonstrated that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. We also have shown that molecular change occurs during lifestyle modification, but this change may be transient and may be dependent on maintaining a healthy lifestyle. Future research endeavors from this project will provide new information regarding strategies to improve adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make cardiovascular health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

## **Appendices**

- **Appendix A** : Published Manuscripts  
Published Abstracts in Scientific Journals  
Oral and Poster Presentation Abstracts
- **Appendix B** : Gantt Charts

## **Attachments**

- **Attachment 1**: ICHP Personnel
- **Attachment 2**: WRI Personnel

**Appendix A**  
Published Manuscripts  
Published Abstracts in Scientific Journals  
Oral and Poster Presentation Abstracts

## The Effect of a One-Year Lifestyle Intervention Program on Carotid Intima Media Thickness

Debra Marshall, MD\*; LTC Elaine Walizer, NC USA (Ret.)†; COL Marina Vernalis, MC USA (Ret.)‡

**ABSTRACT** This study assesses the impact of a year long lifestyle intervention program on carotid intima media thickness (CIMT) in 60 subjects, at-risk for or with coronary artery disease. We calculated mean CIMT at baseline ( $0.731 \pm 0.151$  mm) and 1 year ( $0.720 \pm 0.129$  mm), overall CIMT change and the relationship of CIMT change to the number (0–5) of achieved Heart Health Index (HHI) measures (body mass index  $< 25$  kg/m<sup>2</sup>, exercise  $\geq 150$  min/wk, blood pressure  $< 140/90$  mm Hg, LDL-Cholesterol  $< 100$  mg/dL, fiber intake  $> 25$  g/d). CIMT was unchanged ( $-0.011 \pm 0.118$  mm;  $p = 0.48$ ); however, there was a trend toward CIMT decrease ( $-0.025 \pm 0.120$  mm vs.  $+0.033 \pm 0.102$  mm;  $p = 0.10$ ) between subjects with HHI Score  $\geq 3$  ( $n = 45$ ) compared to those with an HHI Score  $< 3$  ( $n = 15$ ) at 1 year. These findings suggest atherosclerosis progression can be blunted with a lifestyle intervention that fully leverages nonpharmacologic approaches to cardiovascular risk reduction.

### INTRODUCTION

Lifestyle interventions that include a heart healthy diet, regular physical activity, weight maintenance/reduction, smoking cessation, and stress management have been shown to prevent or reduce cardiovascular disease (CVD) risk factors.<sup>1,2</sup> The efficacy of these nonpharmacologic measures can be comparable to pharmacologic therapies, particularly when several health behaviors are adopted together.<sup>3–9</sup>

Large, controlled trials on lifestyle interventions that assess morbidity and mortality endpoints have not been performed because of their expense and difficulties in preventing carry-over effects between experimental and control groups during a long-term trial. Therefore, use of surrogate markers that predict the likelihood of CVD events is becoming more accepted as an approach to improve clinical trial efficiency, duration and cost. Measurement of carotid intima media thickness (CIMT) by B-mode ultrasonography is among the imaging tools for noninvasive assessment of atherosclerosis and has been validated as a predictor of cardiovascular (CV) events in several studies.<sup>10–13</sup> However, studies assessing the effect of lifestyle interventions on atherosclerosis are limited.

The Coronary Artery Disease Reversal study was conducted in military health care beneficiaries, with or at-risk for coronary artery disease (CAD), to determine the feasibility and efficacy of an intensive, multicomponent lifestyle intervention. This article presents the findings of the CIMT sub-study, which assessed the impact of this intervention and the

number of CV health measures achieved on atherosclerosis progression over one year.

### METHODS

#### Study Population and Design

This is a prospective, single-arm study modeled after the Dean Ornish Program for Reversing Heart Disease<sup>8</sup> that was conceived to determine the feasibility and efficacy of this specific lifestyle intervention in a nonresidential military population. Volunteer subjects were self-referred military health care beneficiaries, age 18 or older with known coronary risk factors or CAD, willing to make comprehensive lifestyle changes for one year. This protocol was approved by the Department of Clinical Investigation/Human Use Committee of the Walter Reed Army Medical Center (Washington, DC) and Institutional Review Board at the Uniformed Services University for the Health Sciences (Bethesda, Maryland).

The Coronary Artery Disease Reversal study has been previously described.<sup>7,14</sup> Briefly, subjects participated in a 5-day residential retreat for instruction and initial monitoring of the multicomponent lifestyle change intervention that included: ultralow fat diet ( $\leq 10\%$  total calories as fat, 5–10 mg cholesterol/d, soy and legumes as the protein source, limited nonfat dairy products, 35–50-g of fiber, and  $\geq 5$  servings of fruit and vegetables daily), aerobic exercise ( $\geq 180$  min/wk), and stress management (Hatha yoga poses, deep relaxation, meditation, guided imagery for 60 min/d). During the first 3 months, subjects were on-site twice weekly, 4 hours each visit, for supervised exercise and yoga, meals with educational lectures and group support led by a psychologist. During months 3 through 9 on-site visits were decreased to once weekly. After 9 months, the on-site visits were replaced by weekly telephone monitoring by study nurses and subjects were invited, but not required, to continue subject-directed, group support with their entry cohort.

\*Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46825.

†Integrative Cardiac Health Project, 6900 Georgia Avenue, Walter Reed Army Medical Center, Washington, DC 20307-5001.

The views, opinions and/or findings contained in this article are those of the authors and do not necessarily reflect the views of the Department of the Army, Department of Defense, or U.S. Government and should not be construed as an official DoD/Army position, policy or decision unless so designated by other documentation. No official endorsement should be made.

Subjects voluntarily provided written-informed consent before eligibility screening, which included a complete medical history, physical examination, and treadmill testing. Exclusion criteria included: high-risk treadmill test, unstable coronary artery disease/revascularization procedure within 3 months of study entry, symptomatic congestive heart failure with ejection fraction < 35%, inability/unwillingness to fully participate in all study intervention components, or substance abuse, including tobacco, within 3 months of study entry. A total of 714 patients were referred for recruitment between February 2000 and March 2004 from which 200 subjects enrolled and 186 subjects subsequently initiated the lifestyle program in 13 study cohorts. The final cohort completed the one-year study in April 2005. There was a 23% dropout rate after one year in those subjects who enrolled or initiated the lifestyle intervention, with 166 subjects (89%) completing the 3-month milestone and 144 subjects (77%) completing the year long study. The major reasons for discontinuing study participation were dissatisfaction with specific aspects of the study intervention, time constraints, and relocation away from the study site. The CIMT substudy began in April 2000. Of 130 subjects with baseline carotid ultrasonography, 93 completed the study. Both baseline and 1-year CIMT measures were available for 60 subjects because of missing or noninterpretable images.

### Data Collection

#### Carotid B-Mode Ultrasound

Carotid ultrasonography was performed at baseline and 1 year by study nurses and sonographers specifically trained to perform the research study examinations. Images were obtained on a single ultrasound machine (SonoHeart Elite; SonoSite, Bothell, Washington) using a linear array 5 to 10-MHz transducer with standardized image settings, including resolution mode, depth of field, and gain and transmit focus. All sonograms were obtained with subjects in the supine position and head turned toward the contralateral side. Digital images from a diastolic frame of the cine-loop recording were electronically stored and transferred to an off-line workstation for later analysis. Each ultrasound scan was performed as an independent study, without knowledge of the earlier CIMT result, and a subject's earlier scan was not used to guide the follow-up examination. A single independent observer, who was blinded to the study phase of image acquisition and trained in the measurement of CIMT, performed the analyses with commercially available software (ProSolv Echo Analyzer; Problem Solving Concepts, Indianapolis, Indiana). CIMT was determined from images of the far wall of the distal common carotid arteries (immediately proximal to the carotid bulb) and reported as the mean value for the bilateral measurement. The near (intimal-luminal interface) and far (medial-adventitial interface) field arterial wall borders were manually traced for measurement of mean CIMT (mm) across a 10-mm arterial segment. The high precision and reliability of the ultrasound method and

reproducibility of the CIMT measurements (>0.90 correlation coefficient) have been previously reported.<sup>15</sup>

### Laboratory, Body Composition/Fitness, Blood Pressure, and Nutritional Analyses

Variables measured at baseline and 1 year included: blood pressure (BP) by standard auscultatory methods, weight and body mass index (BMI) by a factory-calibrated Tanita Body Composition Analyzer (Model TBF-300A; Tokyo, Japan), % body fat (3-site skin-fold caliper analysis as described by Pollock<sup>16</sup>), fitness (peak metabolic equivalent [MET] level achieved on maximal treadmill exercise test), fasting plasma lipids (total cholesterol, LDL cholesterol [LDL-C], HDL cholesterol [HDL-C], and triglycerides), and high-sensitivity C-Reactive Protein. Total cholesterol, LDL-C, HDL-C, and triglyceride values were directly measured on a COBAS INTEGRA analyzer using reagents from Roche Diagnostics (Indianapolis, IN). C-Reactive Protein (CRP) was measured with a high-sensitivity, commercially available immunoturbidimetric assay that uses monoclonal anti-CRP antibodies (Roche COBAS INTEGRA, Basel, Switzerland). Nutrient composition was determined at baseline and at the visit closest to week 52 with 3-day food records that were analyzed with Nutritionist V software (Version 2.2; First DataBank, San Bruno, California). Medications were assessed at baseline and any changes in medications or dosage were queried on a weekly basis by study nurses.

### Adherence

Intervention adherence was determined from daily personal adherence logs. Overall adherence was calculated as the arithmetic average of adherence to each of the intervention components. Diet adherence was capped at 100% and calculated with a scoring system on the basis of essential elements of the vegan dietary pattern (avoidance of meat/poultry/fish and added oils, intake of specified servings of whole grains, fruits, vegetables, legumes, and soy protein). Exercise (weekly minutes of structured exercise activity) and stress management (combined weekly minutes of previously described techniques) adherence were not capped at 100%, but calculated as the percentage of goal achieved, which was 180 minutes and 420 minutes, respectively. Logs from weeks 39 to 52 were used to calculate adherence at 1 year.

### Statistical Methods and Analysis

The 1-year change in mean CIMT across the substudy population (1-year-Baseline CIMT) was evaluated with a paired *t* test. An investigator developed Heart Health Index (HHI) score (range, 0-5), modeled after several healthy lifestyle index scores<sup>17,18</sup> was calculated for 1-year completer subjects (*n* = 144) with 1 point given for each criteria met in the 5-component index: Fiber intake > 25g/d; exercise ≥ 150 min/wk; LDL-C < 100 mg/dL; BMI < 25 kg/m<sup>2</sup>; BP < 140/90 mm Hg. The effect of the number of CV health measures achieved (HHI Score) on CIMT changes over 1 year was determined using one-way analysis of variance for paired comparisons.

Analyses on other continuous outcome variables between baseline and 1 year also utilized a one-way analysis of variance for paired or independent comparisons, as appropriate. Fisher's Exact test was used for analyses of all categorical variables. The Wilcoxon Signed Rank test was used for the variables not normally distributed (CRP and triglycerides). Sample size varied slightly across some of these analyses because of missing data on some variables. Values are reported as mean  $\pm$  SD, except where indicated.

Power estimation was not done for this specific sub analysis. A 2-sided probability value of  $\leq 0.05$  was considered as statistically significant. Statistical analyses were performed using SAS statistical software (Version 8.2; SAS Institute, Cary, North Carolina) and SPSS statistical software (Version 14.0; SPSS, Chicago, Illinois).

## RESULTS

Subjects were predominantly older Caucasian men with chronic CAD and/or CVD risk factors (Table I). The CIMT substudy population was comparable with all study completers except that they were slightly younger. Study completers having baseline ultrasonography ( $n = 93$ ) and the CIMT substudy completers also did not differ in their baseline CIMT values. Overall study intervention adherence was approximately 90%. Exercise and dietary adherence were well-maintained at 1 year ( $\geq 90\%$  study goals), whereas the time reported for stress management was 57% of goal.

Medication use was relatively stable throughout the study. At baseline, subjects with hypertension (HTN) were taking antihypertensive medications. Proportion of medications use (baseline to 1 year) was  $\beta$ -blocker (70–73%), angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (63–70%). Despite an increase in the proportion of types of medications used at study completion, dose comparisons showed that 50% of subjects with HTN experienced no change, 23% decreased medication dosage, and 28% had an increase in medication use. From baseline to 1 year, medication use in persons with diabetes increased from 80 to 90%. No change

or a decrease in glucose-lowering medications were seen in 70% of the diabetic subjects; however, the proportion of combination therapy (insulin plus oral agent use) decreased from 10 to 0%, whereas oral agent use increased from 70 to 90% of diabetic subjects. Cholesterol-lowering medication use in subjects with dyslipidemia increased from 86 to 88% at 1 year; however, 59% reported no change, 15% a decrease, and 26% an increase in their cholesterol-lowering therapy. Statin therapy increased slightly (76–79%) as well the use of niacin (14–17%), and fish oil (12–16%).

Over one year there was significant improvement in body composition, fitness, BP, and lipid profiles (Table II). BMI decreased by nearly 7% and there was a 10% decrease in percent body fat. Fitness improved 25% as measured by an increase of 2.3 METs. BP was well-controlled at baseline but further improvements in systolic ( $-4.0\%$ ) and diastolic ( $-3.6\%$ ) were demonstrated. On a background of relatively stable lipid medications, a further reduction of 6% occurred in total cholesterol and 8% reductions in both LDL-C and triglycerides. The inflammatory marker, CRP, also improved significantly, decreasing by 9%. The estimated energy consumption and protein intake remained stable, whereas other nutrient composition changed significantly. Subjects reported a baseline low-fat diet and maintained dietary fat intake at 10% of total calories with adherence to a vegan diet. The proportion of dietary carbohydrate increased substantially, largely through a 136% increase in fiber intake.

At study entry,  $<55\%$  of study subjects met HHI score components, except for BP (Figure 1). Thirty percent of subjects exercised for at least 150 minutes per week, nearly 77% were overweight, and only 35% reported a dietary fiber intake  $>25$  g. Almost 55% had an LDL-C under 100 mg/dL. After one year of study participation, subjects significantly improved achievement of individual HHI score components. At least 62% of subjects reached the individual index criteria for fiber intake, exercise, BP, and LDL-C. Although subjects demonstrated significant weight loss, the proportion of subjects able to achieve BMI in the normal range was less

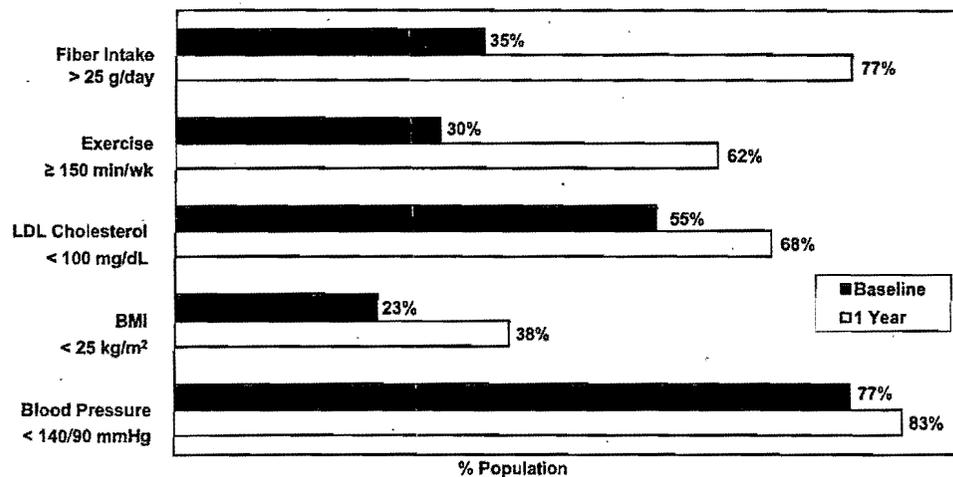
**TABLE I.** Demographics, Baseline Characteristics and Study Intervention Adherence: CIMT vs. All 1-Year Completer Subjects

|   | CIMT Subjects ( $n = 60$ ) | All 1-Year Completers ( $n = 144$ ) | <i>p</i> Value |
|---|----------------------------|-------------------------------------|----------------|
| Age (Years)                             | 58.6 $\pm$ 9.5             | 60.6 $\pm$ 9.7                      | 0.03           |
| Female (%)                              | 21.7                       | 28.5                                | 0.14           |
| Caucasian (%)                           | 81.7                       | 84.0                                | 0.88           |
| BMI (kg/m <sup>2</sup> )                | 30.3 $\pm$ 5.7             | 29.8 $\pm$ 5.8                      | 0.39           |
| CAD (%)                                 | 60.0                       | 68.1                                | 0.10           |
| HTN (%)                                 | 65.0                       | 67.4                                | 0.72           |
| Diabetes (%)                            | 15.0                       | 18.1                                | 0.51           |
| Hyperlipidemia (%)                      | 96.7                       | 95.8                                | 1.00           |
| Baseline CIMT (mm)                      | 0.731 $\pm$ 0.151          | 0.729 $\pm$ 0.159*                  | 0.94           |
| Adherence (Overall, %) <sup>b</sup>     | 91.9 $\pm$ 20.6            | 92.4 $\pm$ 22.0                     | 0.88           |
| Diet (% Specified Pattern) <sup>b</sup> | 90.5 $\pm$ 9.8             | 89.5 $\pm$ 14.1                     | 0.61           |
| Exercise (% Time $\geq$ 150 min/wk)     | 95.4 $\pm$ 35.5            | 94.8 $\pm$ 44.6                     | 0.93           |
| Yoga (% Time $\geq$ 420 min/wk)         | 50.7 $\pm$ 27.1            | 57.9 $\pm$ 37.0                     | 0.18           |

\* $n = 93$  for completers with baseline CIMT. <sup>b</sup> $n = 143$  for all 1-Year completers due to missing dietary records.

**TABLE II.** Serology, Body Composition/Fitness, BP, and Nutrition in CIMT Population

|  | Baseline       | 1 Year         | Change         | p Value |
|--|----------------|----------------|----------------|---------|
| <b>Body Composition and Fitness (n = 60)</b> |                |                |                |         |
| Weight (lbs)                                 | 204.5 ± 45.1   | 190.6 ± 45.4   | -14.0 ± 18.9   | <0.0001 |
| BMI (kg/m <sup>2</sup> )                     | 30.3 ± 5.7     | 28.2 ± 5.7     | -2.1 ± 2.8     | <0.0001 |
| % Body Fat                                   | 27.3 ± 7.5     | 24.8 ± 7.9     | -2.6 ± 3.3     | <0.0001 |
| MET Level                                    | 9.7 ± 2.7      | 12.0 ± 3.6     | 2.3 ± 2.1      | <0.0001 |
| <b>BP (n = 60)</b>                           |                |                |                |         |
| Systolic (mm Hg)                             | 125.6 ± 14.5   | 120.4 ± 14.6   | -5.1 ± 14.4    | 0.007   |
| Diastolic (mm Hg)                            | 73.2 ± 10.2    | 70.1 ± 9.5     | -3.2 ± 9.8     | 0.015   |
| <b>Serology (n = 60)</b>                     |                |                |                |         |
| Total Cholesterol (mg/dL)                    | 170.9 ± 40.0   | 159.7 ± 37.9   | -11.2 ± 25.2   | 0.001   |
| LDL-C (mg/dL)                                | 98.2 ± 29.0    | 89.2 ± 27.2    | -9.0 ± 21.8    | 0.002   |
| HDL-C (mg/dL)                                | 48.3 ± 12.9    | 47.3 ± 11.8    | -1.0 ± 6.8     | 0.256   |
| Triglycerides (mg/dL)                        | 147.0 ± 90.5   | 143.1 ± 67.8   | -3.8 ± 67.8    | 0.506   |
| C-Reactive Protein (mg/L)                    | 3.4 ± 4.2      | 2.3 ± 2.5      | -1.1 ± 3.4     | 0.003   |
| <b>Nutritional Values (n = 44)</b>           |                |                |                |         |
| Total Kcal                                   | 1919.6 ± 488.2 | 1780.6 ± 388.4 | -139.0 ± 554.9 | 0.104   |
| % Fat  | 25.2 ± 9.6     | 10.1 ± 2.6     | -15.2 ± 9.7    | <0.0001 |
| % Carbohydrate                               | 55.5 ± 12.0    | 71.9 ± 5.2     | 16.4 ± 11.6    | <0.0001 |
| % Protein                                    | 17.1 ± 3.8     | 15.9 ± 2.6     | -1.2 ± 3.8     | 0.043   |
| Fiber (g/d)                                  | 26.1 ± 11.9    | 51.2 ± 15.0    | 25.1 ± 17.2    | <0.0001 |



**FIGURE 1.** Individual heart health characteristics at baseline and 1 year. Changes in distribution for fiber, exercise and BMI are statistically significant at  $p < 0.004$  vs. baseline.

dramatic, with 38% of subjects meeting this goal compared with 23% at study entry. The HHI score at study entry was  $2.2 \pm 1.4$  and improved to  $3.3 \pm 1.2$  by 1 year compared with baseline ( $p < 0.0001$ ). An HHI score  $\geq 3$  was found in only 38% of subjects at baseline, whereas at 1 year 75% achieved this category ( $p < 0.0001$ ). Only 7% of the study population met all 5 HHI criteria at baseline, but improved to 13% at 1 year.

Given that CIMT values exceeding the 75th percentile for age and gender are generally considered abnormal<sup>19,20</sup>; 68% of the population (41/60) was abnormal at baseline with a numerical increase to 77% at 1 year ( $p = 0.20$ ). No difference was detected in mean change in CIMT ( $-0.011 \pm 0.118$  mm) in this population, though there is wide variability seen in the

individual CIMT values. Decreases, increases, and no change in CIMT were seen in 57%, 43%, and 0% of the population, respectively.

The number of achieved HHI measures correlated with 1-year CIMT change. In subjects with an HHI score  $\geq 3$  ( $n = 45$ ) CIMT decreased ( $-0.025 \pm 0.120$  mm) compared to an increase ( $0.033 \pm 0.102$  mm) in HHI score  $< 3$  ( $n = 15$ ) subjects (95% Class Interval =  $-0.04$  to  $0.012$ ;  $p = 0.10$ ) (Figure 2). There was a trend for lower baseline CIMT in the HHI  $< 3$  group ( $0.682 \pm 0.159$  mm vs.  $0.747 \pm 0.147$  mm;  $p = 0.16$ ). No differences were detected in within group comparisons of CIMT change; however, there was a trend toward atherosclerotic progression in the HHI  $< 3$  group

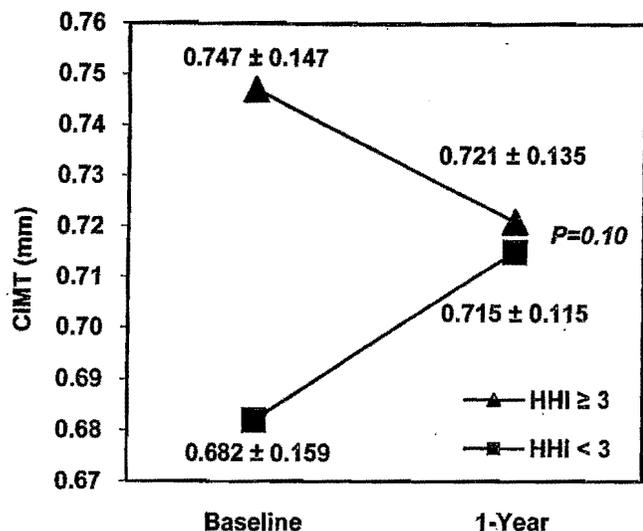


FIGURE 2. Baseline to 1-year CIMT change: comparison between HHI ≥ 3 and HHI < 3 subjects.

TABLE III. Individual HHI Measures and CRP: Comparison Between HHI ≥ 3 and HHI < 3 Subjects (% Change from Baseline to 1 Year)

|                      | HHI < 3 Group<br>(n = 15) | HHI ≥ 3 Group<br>(n = 45) | Between Group,<br>p Values |
|----------------------|---------------------------|---------------------------|----------------------------|
| BMI                  | -2.6 ± 13.2               | -8.2 ± 5.9*               | 0.028                      |
| Exercise             | 110.7 ± 158.7             | 67.2 ± 99.9*              | 0.297                      |
| BP                   |                           |                           |                            |
| Systolic             | 1.0 ± 10.6                | -5.7 ± 10.3*              | 0.036                      |
| Diastolic            | 1.2 ± 14.6                | -5.3 ± 12.1*              | 0.093                      |
| LDL-C                | 2.1 ± 25.9                | -11.0 ± 14.8*             | 0.019                      |
| Dietary Fiber Intake | 55.9 ± 45.8               | 142.1 ± 127.3*            | 0.254                      |
| C-Reactive Protein   | 16.0 ± 70.5               | -17.2 ± 39.8*             | 0.027                      |

\*Within group p < 0.01.

(p = 0.23) compared to a regression trend in the HHI ≥ 3 group (p = 0.16). Although differences were not found between or within groups for CIMT change, the sub analysis was not specifically powered to rule out significant differences with this number of subjects enrolled.

Of the individual HHI measures (Table III), the most significant differences between HHI ≥ 3 and < 3 subjects were in LDL-C (-11% vs. +2.1%; p = 0.02), systolic BP (-6% vs. +1.0%; p = 0.04), BMI (-8% vs. -2.6%; p = 0.03), and CRP (-17% vs. +16%; p = 0.03). Within group comparisons of HHI measures demonstrated no differences from baseline in the HHI < 3 subjects, whereas all measures, except BMI, improved significantly in the HHI ≥ 3 subjects.

**DISCUSSION**

This study demonstrates that military health care beneficiaries with chronic CAD or CVD risk factors who fully participate in a multicomponent lifestyle intervention program can realize not only substantial improvement in body composition, fitness, BP, lipids, and inflammation, but also an absence

of atherosclerosis progression as measured by CIMT, a validated marker of atherosclerosis progression.<sup>21</sup> This finding is likely attributable to the lifestyle intervention as subjects were on relatively stable drug treatment throughout the study, including lipid-lowering therapies. CIMT did not change significantly for the total study population, despite the overall beneficial changes in CVD risk factors. Only the number of commonly recognized CV health goals achieved, correlating with significant reductions in BMI, BP, LDL-C, and CRP, differentiated those subjects with a trend toward CIMT regression vs. progression.

Unlike for pharmacologic therapies<sup>15,22,23</sup>, there are no large trials that have evaluated the impact of lifestyle interventions on atherosclerosis progression. The Lifestyle Heart Trial, from which the intervention regimen in our study was adapted, used quantitative coronary angiography to demonstrate a 4.5% coronary stenosis improvement in the experimental group compared with a 5.4% worsening in the control group after 1 year.<sup>8,9</sup> Although our study had no control group and a small substudy group, the magnitude of CIMT change in the group with the most (-2%) compared with least (+7%) CVD risk factor reduction is similar to that study. More recent studies have assessed the impact of various lifestyle changes on CIMT. Using a similar lifestyle change program to ours, Fields et al<sup>24</sup> demonstrated a significant CIMT decrease (-0.15 mm/yr) in 20 intervention subjects, although there was no difference between them and subjects in the comparison control groups. When comparing participants in an Ornish lifestyle program (n = 46) to those in a traditional cardiac rehabilitation program (n = 47), Aldana et al<sup>25</sup> was also unable to demonstrate a significant reduction in CIMT. A 6-month diet, exercise and behavior modification program in type 2 diabetics significantly reduced CIMT compared with control subjects (-0.04 mm vs. 0.083).<sup>26</sup> Other studies have reported reduction in CIMT progression,<sup>27</sup> but not regression. Weight loss after bariatric surgery was associated with three-fold less CIMT progression (0.024 vs. 0.068) compared with obese controls.<sup>28</sup> Reduction of dietary fat intake along with smoking cessation, and BMI decrease of 5 units was associated with a 0.13-mm/yr CIMT reduction in progression.<sup>29</sup> In menopausal women, a dietary and physical activity intervention slowed CIMT progression compared with control subjects (0.008 vs. 0.004 mm/yr), the lower magnitude of effect consistent with a less intense intervention and smaller change in CVD risk factors than seen in our study.<sup>30</sup> CIMT progression was lower in subjects with the greatest vs. least reduction in saturated fat (0.03 vs. 0.10 mm/yr).<sup>31</sup> Some lifestyle intervention studies have not demonstrated any effect on CIMT.<sup>32-35</sup> The small study populations and the magnitude of CVD risk factor change likely explain the variability of CIMT effect reported. Lipid improvement is an important factor, as the extent of CIMT change has been significantly related to LDC-C changes in pharmacologic studies.<sup>36</sup>

There is potential for lifestyle change to have a favorable impact on morbidity and mortality. A recent review suggests

that about a 40% reduction in all-cause mortality might be realized by CAD patients who practice a healthy lifestyle.<sup>37</sup> In The Health Professionals Follow-up Study, men who adopted  $\geq 2$  lifestyle practices over 16 years had a 27% lower risk of CAD and 62% of their CV events might have been prevented with the best adherence to recommended lifestyle practices.<sup>18</sup> Individuals achieving four diet and lifestyle factors in the HALE project had a 64% lower rate of CAD death.<sup>38</sup> Large-scale epidemiologic studies have found a significant association between CIMT progression and CV events. The Rotterdam Study, Cardiovascular Health Study and The Atherosclerosis Risk in Communities Study demonstrated 1.3- to 1.7-fold higher risk of myocardial infarction for approximately each 0.2-mm CIMT increase.<sup>10,13,39</sup> Prospective data from the Carotid Atherosclerosis Progression Study confirms these earlier findings across a wide age range.<sup>12</sup> In Carotid Atherosclerosis Progression Study, each 0.16-mm CIMT increase was significantly predictive of a 1.45-fold higher incidence of myocardial infarction, stroke, or death. Thus, the reduction of CIMT among subjects achieving the greatest number of CV health measures in our study supports the potential of lifestyle intervention to reduce future CV morbidity and mortality. Larger clinical trials of lifestyle interventions that assess CIMT as an endpoint are needed to provide convincing evidence in this regard. In the interim, our findings may be utilized to motivate better adherence in lifestyle change programs to maximize their benefit.

### Study Limitations

Our study is not a randomized trial and, thus, is subject to the limitations common to all observational studies. The relatively small sample size, along with a self-referred, highly motivated, predominantly male, nonsmoking population increases the referral bias. The relationships analyzed between CV risk factor changes and CIMT progression were performed in a smaller subgroup, thus adding to bias or confounding from unmeasured factors.

### CONCLUSIONS

Lifestyle intervention can lead to a delay in atherosclerosis progression, but may depend on the extent of CV health measures achieved. This finding supports an intensive, case-managed program to fully leverage nonpharmacologic approaches for CV risk reduction. Prospective studies are needed to improve understanding of the effects of lifestyle intervention on atherosclerotic progression.

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## Cardiometabolic risk reduction in an intensive cardiovascular health program

L.M. Voegtly<sup>a</sup>, D.M. Neatrour<sup>b</sup>, D.J. Decewicz<sup>a</sup>, A. Burke<sup>b</sup>,  
M.J. Haberkorn<sup>b</sup>, F. Lechak<sup>b</sup>, H.L. Patney<sup>a</sup>, M.N. Vernalis<sup>c</sup>,  
D.L. Ellsworth<sup>a,\*</sup>

<sup>a</sup> Integrative Cardiac Health Program, Windber Research Institute, Windber, PA, USA

<sup>b</sup> Windber Medical Center, Windber, PA, USA

<sup>c</sup> Integrative Cardiac Health Program, Walter Reed National Military Medical Center, Bethesda, MD, USA

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### KEYWORDS

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disease;  
Cardiometabolic risk;  
Insulin;  
Leptin;  
Risk reduction;  
Lifestyle  
modification;  
Low-fat diet;  
Exercise

**Abstract** *Background and aims:* Insulin and leptin are important markers of insulin resistance and vascular inflammation in metabolic and cardiovascular diseases. This study evaluated changes in circulating levels of insulin and leptin during a cardiovascular health program to improve our understanding of cardiometabolic risk reduction.

*Methods and results:* Participants ( $n = 76$ ) completed a prospective, nonrandomized program designed to stabilize or reverse progression of coronary artery disease through dietary changes, exercise, stress management, and group support. Controls ( $n = 76$ ) were matched to participants based on age, gender, and disease status. Traditional cardiovascular risk factors were assessed at baseline, 12 weeks, and 52 weeks by standard methods. Dietary data were collected by 72-h recall and evaluated by Food Processor<sup>®</sup> v8.4.0. Ultra-sensitive insulin and leptin levels were measured by radioimmunoassay. Participants successfully reduced their total caloric intake from >2000 calories per day to ~1700 calories per day ( $p < 0.05$  compared to controls), lowered daily fat intake by >60% ( $p < 0.001$  compared to controls), and increased carbohydrate intake by >30% ( $p < 0.001$ ). Repeated-measures ANOVA indicated significant beneficial changes ( $p < 0.001$  compared to controls) in plasma insulin (−19%) and leptin (−33%) during the lifestyle program, as well as improvement in traditional cardiovascular risk factors. Response was similar between men and women for most risk factors and was not markedly influenced by medication use. *Conclusion:* Lifestyle changes focusing on diet, physical activity, and stress reduction can successfully modify both cardiovascular and metabolic risk factors, with the potential to mediate cardiometabolic risk through beneficial anti-inflammatory and anti-oxidative effects on the vasculature.

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\* Corresponding author. Tel.: +1 814 361 6911; fax: +1 814 467 6334.  
E-mail address: [d.ellsworth@wriwindber.org](mailto:d.ellsworth@wriwindber.org) (D.L. Ellsworth).

## Introduction

Insulin and leptin represent two important and well-characterized markers of insulin resistance and vascular inflammation in metabolic and cardiovascular diseases (CVD). Insulin is a polypeptide hormone that affects the vascular endothelium by modulating glucose homeostasis and glycogen synthesis [1]. Fasting insulin levels have increased dramatically in non-diabetic adults over the past two decades, often developing as a consequence of resistance to the action of insulin in peripheral tissues [2]. Hyperinsulinemia has been linked to dyslipidemia, impaired glucose regulation, and hypertension [3], as well as overall risk for cardiovascular mortality [4].

Leptin is an adipocytokine secreted by white adipose tissue that functions mainly in energy balance and metabolism, but plays an important role in vascular physiology through interactions with the vascular endothelium [5,6]. High circulating levels of leptin may accelerate atherosclerosis and contribute to CVD risk by inducing oxidative stress on endothelial cells [7] and impairing arterial reactivity [8]. Clinical studies have shown that high leptin contributes to CVD risk in the general population and is associated with myocardial infarction and coronary events, independent of traditional cardiovascular risk factors [9,10].

Insulin resistance, vascular inflammation, and oxidative stress play important roles in endothelial dysfunction. Pharmacologic therapies to improve endothelial function show marked variability in their ability to lower circulating markers of inflammation [11], and are often used in combination to be most effective in reducing inflammation and oxidative stress. An alternative approach for treating patients with high cardiovascular risk involves lifestyle modification to reduce traditional CVD risk factors and slow or reverse progression of coronary atherosclerosis [12]. Lifestyle programs focusing on nutrition and exercise can improve endothelial function and enhance insulin sensitivity, in part by reducing markers of systemic vascular inflammation and insulin resistance [13].

Insulin and leptin have important effects on vascular biology, but may function through different molecular pathways – insulin through metabolic pathways and leptin through inflammatory and thrombogenic factors [14]. We investigated the impact of an intensive cardiovascular health program on circulating levels of insulin and leptin to improve our understanding of cardiometabolic risk factor reduction by (1) measuring changes in physiological risk factors for CVD throughout a year-long cardiac health program and (2) assessing response of insulin and leptin and relating changes in these inflammatory markers to improvement in vascular health.

## Methods

### Study population

The intervention group consisted of 76 white men and women who completed a prospective, nonrandomized program to stabilize or reverse progression of coronary artery disease (CAD) through dietary changes, exercise, stress management,

and group support. Eligibility criteria were (1) a diagnosis of CAD, including acute myocardial infarction, bypass surgery, stent placement, stable angina, angioplasty, or evidence of  $\geq 50\%$  luminal narrowing on coronary angiogram; or (2) two or more CAD risk factors such as high blood pressure (BP) defined as systolic pressure  $> 140$  mm Hg or diastolic pressure  $> 90$  mm Hg, high total cholesterol ( $> 200$  mg/dL), physician diagnosed diabetes, obesity – body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>, or family history of heart disease in parents or siblings. Physician approval, motivation to commit to following the guidelines of the program, and successful abstinence from smoking for at least three months prior to enrollment also were part of the acceptance criteria.

Controls ( $n = 76$  white men and women) were matched to participants based on gender, age at baseline within five years, and CAD status (overt CAD or risk factors) using a prospective individual matching strategy to achieve a balanced distribution of risk factors between intervention participants and controls in nonrandomized clinical trials [15]. Controls receiving only standard care from their primary physicians underwent identical examinations at baseline, 12 weeks, and 52 weeks, but did not participate in the program or receive healthy lifestyle information.

This study was approved by the Institutional Review Board at Windber Medical Center. All participants voluntarily enrolled in the program and provided written informed consent.

### Intervention

The lifestyle program included four components: (1) low-fat vegetarian diet ( $< 10\%$  of calories from fat); (2) 180 min/week of moderate aerobic exercise; (3) 1 h of stress management each day; and (4) two 1-h group support sessions per week for the first 12 weeks and one group session per week during the remainder of the year [16]. Adherence was self-reported by summarizing diet (fat, carbohydrate, protein intake), exercise (frequency and duration), stress management (frequency and duration), and group support (frequency of meeting attendance) for each day. Program staff reviewed compliance forms weekly and provided immediate feedback to participants on progress and guidance for improving adherence.

From January 2004 to February 2009, approximately 35 participants or controls were enrolled each year in separate cohorts of  $\sim 12$  individuals per cohort. The dropout rate was  $\sim 32\%$  ( $n = 53$ ) among participants in the program, likely attributable to the magnitude of lifestyle changes required.

### Physiological measures

Data collection and reporting followed recommendations of the Transparent Reporting of Evaluations with Non-randomized Designs (TREND) group [17]. Clinical examinations conducted by physicians or trained personnel at baseline, 12 weeks, and 52 weeks collected information on age, gender, ethnicity, smoking status, cardiovascular history, and medication use. Height and weight measurements were used to calculate BMI. Blood pressure was recorded using a mercury sphygmomanometer on the arm of

seated, relaxed subjects. General endurance was determined by a graded treadmill exercise test that estimated the volume of oxygen each participant could consume ( $\text{VO}_2$  max; ml/kg/min) based on exercise intensity, duration, and body weight (Bruce score) [18]. Assays for standard high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, and triglycerides were conducted using the AEROSET™ clinical chemistry system (Abbott Laboratories, Abbott Park, IL).

### Insulin and leptin measurements

Fasting blood samples for standard insulin and leptin analysis were obtained at each examination and placed directly on ice. Within 1 h of collection, plasma aliquots were isolated by centrifugation and stored at  $-80^\circ\text{C}$ . Ultrasensitive insulin ( $\mu\text{U/ml}$ ) and leptin ( $\text{ng/ml}$ ) levels were measured in duplicate on freshly thawed plasma samples by radioimmunoassay (Millipore, Billerica, MA) at the Johns Hopkins Bayview Clinical Research Unit. Inter-assay coefficients of variation (CV%) were 3.27 for insulin and 3.81 for leptin.

### Dietary composition

Participants and controls completed a self-reported 72-h dietary recall questionnaire at each examination, recording their total dietary intake for breakfast, lunch, dinner, and snacks over three consecutive days. Participants reported specific food items and drinks consumed, portion sizes, preparation methods, and location (home or away). Food Processor® v8.4.0 (ESHA Research, Salem, OR) was used to determine daily caloric intake and nutrient composition.

### Statistical analysis

Statistical analyses were conducted using SPSS version 15.0 and JMP® version 9.0;  $p$  values  $<0.05$  were considered significant. Prior to analysis, normality of the outcome data was determined by Lilliefors test, and natural log-transformations were used for variables with non-normal distributions. Potential differences in baseline measures among participant cohorts and among control cohorts were examined by analysis of variance (ANOVA). As no significant cohort-to-cohort variability at baseline was detected, all intervention and all control cohorts were, respectively, combined in subsequent analyses.

An independent samples  $t$ -test, or nonparametric Mann-Whitney  $U$  test if data remained non-normally distributed after natural log transformation, was used to compare baseline characteristics between intervention participants and controls. Repeated-measures ANOVA was used to compare changes in CVD risk factors at 12 weeks and 52 weeks between intervention and control groups. Independent samples  $t$ -tests (two-tailed) then identified differences in risk factor response from baseline to week 52 between the intervention and control groups. For each variable, differences in response between men and women were assessed by two-factor repeated-measures ANOVA using a Bonferroni adjustment. As above,  $t$ -tests compared baseline to week 52 changes between groups, by gender. To examine the potential confounding effects of medications

on insulin and leptin response, sub-group analyses were conducted that excluded participants who changed the brand or dosage of any medication known to affect insulin, leptin, and/or lipid levels through (1) main (intended) effects or secondary (side) effects, or (2) main effects only.

## Results

### Baseline measures

At baseline, participants showed higher plasma insulin, % carbohydrate intake, BMI, and triglycerides, but lower % fat consumption and exercise capacity than controls despite the prospective matching strategy (Table 1). Insulin values did

Table 1 Cardiometabolic risk factors, dietary components, and physiological measures at baseline for participants and controls in the cardiac lifestyle program.

| Measures                            | n   | Controls         | Participants     | $P^a$              |
|-------------------------------------|-----|------------------|------------------|--------------------|
| <b>Cardiometabolic risk factors</b> |     |                  |                  |                    |
| Insulin ( $\mu\text{U/ml}$ )        | 150 | 14.3 $\pm$ 7.1   | 18.1 $\pm$ 10.2  | 0.012 <sup>b</sup> |
| Leptin (ng/ml)                      | 152 | 19.0 $\pm$ 17.2  | 23.5 $\pm$ 18.6  | 0.059 <sup>b</sup> |
| <b>Dietary components</b>           |     |                  |                  |                    |
| Calories (kcal/day)                 | 114 | 1736 $\pm$ 582   | 2095 $\pm$ 776   | 0.056              |
| % Carbohydrate intake               | 114 | 49.5 $\pm$ 9.2   | 54.0 $\pm$ 12.2  | 0.010 <sup>b</sup> |
| % Fat intake                        | 114 | 32.5 $\pm$ 8.5   | 28.8 $\pm$ 10.2  | 0.037              |
| % Protein intake                    | 114 | 16.7 $\pm$ 3.9   | 16.8 $\pm$ 6.3   | 0.591 <sup>b</sup> |
| <b>Physiological measures</b>       |     |                  |                  |                    |
| Age (years)                         | 152 | 60.6 $\pm$ 7.6   | 60.6 $\pm$ 7.6   | 0.992              |
| BMI ( $\text{kg/m}^2$ )             | 152 | 28.5 $\pm$ 4.5   | 32.9 $\pm$ 7.2   | $<0.001$           |
| Systolic BP (mm Hg)                 | 146 | 132.0 $\pm$ 16.3 | 136.2 $\pm$ 16.9 | 0.119 <sup>b</sup> |
| Diastolic BP (mm Hg)                | 146 | 78.6 $\pm$ 10.1  | 81.0 $\pm$ 10.1  | 0.238 <sup>b</sup> |
| HDL cholesterol (mg/dl)             | 152 | 49.4 $\pm$ 13.0  | 45.5 $\pm$ 13.4  | 0.057              |
| LDL cholesterol (mg/dl)             | 142 | 108.1 $\pm$ 33.8 | 112.5 $\pm$ 39.1 | 0.590              |
| Total cholesterol (mg/dl)           | 152 | 185.3 $\pm$ 42.7 | 194.8 $\pm$ 48.2 | 0.200              |
| Triglycerides (mg/dl)               | 152 | 143.5 $\pm$ 97.8 | 175.9 $\pm$ 94.2 | 0.004              |
| Exercise capacity (Bruce score)     | 122 | 9.3 $\pm$ 2.9    | 6.6 $\pm$ 2.1    | $<0.001$           |

Data are presented as mean  $\pm$  SD; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

<sup>a</sup> Tested by 1-factor ANOVA by cohort type.

<sup>b</sup> Tested by a nonparametric Mann-Whitney  $U$  test because data was not normally distributed after natural log transformation.

not differ significantly between participants who completed the program (graduates) and those who dropped out; however, leptin levels were higher ( $30.0 \pm 18.4$  versus  $23.5 \pm 18.6$ ,  $p < 0.05$ ) among dropouts. Dropouts also tended to be younger ( $55.1 \pm 11.0$  versus  $60.6 \pm 7.6$ ,  $p < 0.01$ ) and have lower systolic BP ( $130.4 \pm 18.7$  versus  $136.1 \pm 16.7$ ,  $p < 0.05$ ) than graduates. None of the risk factors differed between participants excluded from the analysis due to non-matching and those included in the study.

### Changes in cardiometabolic risk factors

Participants in the cardiovascular health program experienced significant beneficial changes in plasma insulin and leptin (Table 2). Insulin levels declined  $\sim 19\%$  in participants ( $p < 0.001$  versus controls), while leptin levels decreased  $33\%$  ( $p < 0.001$  versus controls). In contrast, both insulin ( $+4\%$ ) and leptin ( $+6\%$ ) increased in controls over one year.

### Changes in dietary composition

Controls showed no significant change in any dietary component; whereas, participants reduced total caloric intake from  $>2000$  calories/day to  $\sim 1700$  calories/day ( $-18\%$ ) ( $p < 0.05$  versus controls). Similarly, participants lowered daily fat intake by  $>60\%$  ( $p < 0.001$  versus controls) and, on average, maintained a total fat intake of  $\sim 11\%$  of calories (Table 2). Carbohydrate intake increased by  $>30\%$  among participants ( $p < 0.001$  versus controls), while dietary protein remained unchanged.

### Response of traditional CVD risk factors

Participants achieved a  $9\%$  reduction in BMI by the end of the year ( $p < 0.001$  versus controls), a  $6\%$  reduction in diastolic BP ( $p < 0.05$ ), and a  $37\%$  increase in physical fitness ( $p < 0.001$ ), all significant improvements. Systolic BP and total cholesterol improved significantly from baseline to 52 weeks, but the degree of change did not differ between participants and controls. HDL decreased significantly by the end of the year, but overall change was not significantly different from controls.

### Gender differences in response

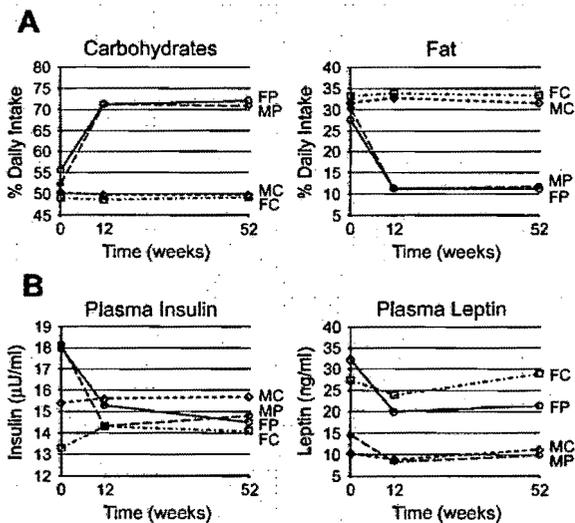
Gender was not a significant factor for changes in any CVD risk factor from baseline to 52 weeks among controls. Men and women participating in the program showed similar significant improvement for insulin and leptin, nearly identical changes in diet (Fig. 1), and equivalent changes in BMI and physical fitness ( $p < 0.001$ ) compared to controls after one year. Response for diastolic BP also was similar between genders – significantly different from baseline at 12 weeks and 52 weeks in participants, but the magnitude of change was not significantly different from controls. Triglyceride levels dropped significantly ( $-20\%$ ) among male participants ( $p < 0.05$  versus controls), but in women, triglyceride response did not differ between participants and controls, and actually increased  $\sim 5\%$  among female participants from baseline to the end of the year.

**Table 2** Cardiometabolic risk factors, dietary components, and physiological measures for participants and controls in the cardiac lifestyle program at baseline, 12 weeks, and 52 weeks.

| Measures                            | Controls (n = 76) |                      |                   |          | Participants (n = 76) |                       |                       |          | Between group $P^a$ |
|-------------------------------------|-------------------|----------------------|-------------------|----------|-----------------------|-----------------------|-----------------------|----------|---------------------|
|                                     | Baseline          | Week 12              | Week 52           | % Change | Baseline              | Week 12               | Week 52               | % Change |                     |
| <b>Cardiometabolic risk factors</b> |                   |                      |                   |          |                       |                       |                       |          |                     |
| Insulin                             | $14.3 \pm 7.1$    | $14.9 \pm 6.3$       | $14.9 \pm 6.8$    | +4.0     | $18.1 \pm 10.2$       | $14.8 \pm 7.1^{**}$   | $14.6 \pm 7.8^{***}$  | -19.2    | <0.001              |
| Leptin                              | $19.0 \pm 17.2$   | $16.5 \pm 14.7$      | $20.3 \pm 16.8$   | +6.6     | $23.5 \pm 18.6$       | $14.3 \pm 11.1^{***}$ | $15.8 \pm 13.6^{***}$ | -32.9    | <0.001              |
| <b>Dietary components</b>           |                   |                      |                   |          |                       |                       |                       |          |                     |
| Calories                            | $1736 \pm 582$    | $1736 \pm 604$       | $1633 \pm 493$    | -5.9     | $2095 \pm 776$        | $1545 \pm 333^{***}$  | $1709 \pm 497^{***}$  | -18.5    | 0.028               |
| % Carbs                             | $49.5 \pm 9.2$    | $49.0 \pm 7.0$       | $49.4 \pm 8.9$    | -0.3     | $54.0 \pm 12.2$       | $71.3 \pm 3.5^{***}$  | $71.5 \pm 3.2^{***}$  | +32.4    | <0.001              |
| % Fat                               | $32.5 \pm 8.5$    | $33.2 \pm 6.6$       | $32.4 \pm 7.2$    | -0.1     | $28.8 \pm 10.2$       | $11.2 \pm 2.0^{***}$  | $11.4 \pm 2.8^{***}$  | -60.3    | <0.001              |
| % Protein                           | $16.7 \pm 3.9$    | $16.5 \pm 4.0$       | $17.1 \pm 4.6$    | +2.4     | $16.8 \pm 6.3$        | $17.3 \pm 2.5$        | $16.5 \pm 2.4$        | -1.7     | 0.501               |
| <b>Physiological measures</b>       |                   |                      |                   |          |                       |                       |                       |          |                     |
| BMI                                 | $28.5 \pm 4.5$    | $28.3 \pm 4.7$       | $28.7 \pm 4.8$    | +0.9     | $32.9 \pm 7.2$        | $30.5 \pm 6.6^{***}$  | $29.8 \pm 6.8^{***}$  | -9.3     | <0.001              |
| SBP                                 | $132 \pm 16$      | $126 \pm 15^{**}$    | $125 \pm 13^{**}$ | -5.3     | $136 \pm 17$          | $122 \pm 14^{***}$    | $127 \pm 17^{***}$    | -6.4     | 0.562               |
| DBP                                 | $78.6 \pm 10.1$   | $77.1 \pm 8.3$       | $77.3 \pm 9.3$    | -1.5     | $81.0 \pm 10.1$       | $73.0 \pm 9.0^{***}$  | $75.5 \pm 9.5^{***}$  | -6.7     | 0.022               |
| HDL                                 | $49.4 \pm 13.0$   | $52.0 \pm 13.1^{**}$ | $47.9 \pm 13.3$   | -3.0     | $45.5 \pm 13.4$       | $38.5 \pm 9.5^{***}$  | $43.1 \pm 10.5^*$     | -5.2     | 0.497               |
| LDL                                 | $108 \pm 34$      | $106 \pm 35$         | $108 \pm 34$      | -0.4     | $112 \pm 39$          | $98 \pm 32^{***}$     | $109 \pm 33$          | -2.8     | 0.536               |
| TCH                                 | $185 \pm 43$      | $187 \pm 46$         | $185 \pm 43$      | -0.2     | $195 \pm 48$          | $170 \pm 43^{***}$    | $185 \pm 44^{**}$     | -5.0     | 0.066               |
| TG                                  | $144 \pm 98$      | $156 \pm 138$        | $146 \pm 88$      | +2.0     | $176 \pm 94$          | $163 \pm 73$          | $163 \pm 93$          | -7.2     | 0.213               |
| EC                                  | $9.3 \pm 2.9$     | $9.5 \pm 2.8$        | $9.3 \pm 2.7$     | -0.6     | $6.6 \pm 2.1$         | $8.4 \pm 2.2^{***}$   | $9.0 \pm 2.6^{***}$   | +37.6    | <0.001              |

Data are presented as mean  $\pm$  SD; % change is from baseline to week 52; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to baseline by repeated-measures ANOVA; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; EC, exercise capacity.

<sup>a</sup> From independent samples t-tests (two-tailed) of baseline to week 52 changes in program participants compared to controls.



**Figure 1** Changes in dietary composition (panel A) and cardiometabolic risk factors (panel B) among men and women participating in a year-long cardiovascular health program. FP, female participants; MP, male participants; FC, female controls; MC, male controls.

### Effects of medications

Review of patient medical charts identified 114 prescription medications used by participants and controls at baseline. Medications ( $n = 67$ ) known to influence circulating levels of insulin, leptin, and/or lipids as the primary intended effect, or as a secondary effect, were then partitioned into 8 categories based on function (Table 3). Separate analyses were used to assess the effects of (1) all medications in these 8 categories (composite medications), and (2) only medications influencing insulin, leptin, and lipids as a primary

effect (primary medications). Because no medications were deemed to alter leptin or HDL as a primary effect, medications with the strongest secondary effects on these variables were examined.

Results of the sub-group analyses showed that composite and primary medications did not have significant effects on biomarker responses to the lifestyle change program (Table 4). Changes in insulin and leptin in participants and controls not taking medications known to influence these biomarkers or whose medication levels did not change during the study were similar to analyses encompassing all participants. Response for lipids was attenuated slightly when the effects of medications were considered. The largest effect was evident among controls, where LDL, total cholesterol, and triglyceride levels increased more in subjects with no medication changes.

### Discussion

Participants who completed the year-long lifestyle change program reduced total caloric intake from  $>2000$  calories/day to  $\sim 1700$  calories/day, increased carbohydrate consumption by 30%, and decreased daily fat intake by 60%. The lifestyle intervention improved circulating levels of insulin ( $-19\%$ ) and leptin ( $-33\%$ ), which contribute to cardiometabolic risk, as well as traditional cardiovascular risk factors. Changes in circulating insulin and leptin were comparable to, or superior to, responses reported in other dietary or exercise interventions (Web Appendix), and were not significantly influenced by medication use. Men and women showed similar beneficial changes for most risk factors.

The term "cardiometabolic risk" for developing coronary atherosclerosis encompasses risk factors such as age, gender, high cholesterol, hypertension, smoking, and obesity plus additional contributing factors including insulin resistance, vascular inflammation, atherogenic dyslipidemia, and poor lifestyle behaviors. Leptin may contribute to

**Table 3** Medications used by participants and controls in the cardiac lifestyle program known to affect plasma levels of insulin, leptin, and lipids.

| Medication Category ( $n$ ) <sup>a</sup> | Insulin        | Leptin         | HDL            | LDL            | TCH            | TG             |
|--|----------------|----------------|----------------|----------------|----------------|----------------|
| ACE inhibitors (13)                      | ↓              | ↓              | ↑ ns           | ↓ ns           | ↓ ns           | ↓              |
| Anticoagulants (2)                       |                |                |                |                |                |                |
| Platelet aggregation inhibitors          |                |                |                |                | ↑              |                |
| Beta blockers (10)                       | ↑              | ↑              | ↓              | ↓ ns           | ↑ ns           | ↑              |
| Calcium channel blockers (9)             | ↑              | ↓              | ↑              | ↑              | ↑              | ↓              |
| Insulin medications (3)                  | ▲ <sup>b</sup> | ↑ <sup>b</sup> |                |                |                |                |
| Diuretics (6)                            |                |                | ↓ ns           | ↑ ns           | ↑              | ↑              |
| Lipid lowering medications (16)          |                |                | ↑ <sup>b</sup> | ▼ <sup>b</sup> | ▼ <sup>b</sup> | ▼ <sup>b</sup> |
| Oral antihyperglycemics (8)              |                |                |                |                |                |                |
| Thiazolidinediones                       |                |                | ↓              | ↑              | ↑              | ↑              |
| Biguanides                               | ↓              | ↓              | ↑              | ↓              | ↓              | ↓              |
| Sulfonylureas                            | ↑              |                |                |                |                |                |

Abbreviations: HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides; ACE, angiotensin converting enzyme; ns, not statistically significant; —, effect may be neutral. Adapted from [29].

Key to medication effects: ▲ — increase, main effect; ↑ — increase, secondary effect; ▼ — decrease, main effect; ↓ — decrease, secondary effect; \* — dose dependent.

<sup>a</sup> The number of brand name medications in each category is indicated in parentheses.

<sup>b</sup> Considered a primary medication in Table 4.

**Table 4** Effects of medication changes on cardiometabolic risk factors and lipid measures for participants and controls in the cardiac lifestyle program.

| Measures                            | All participants        |               |                              | Composite medications <sup>a</sup> |               |                              | Primary medications <sup>b</sup> |               |                              |
|-------------------------------------|-------------------------|---------------|------------------------------|------------------------------------|---------------|------------------------------|----------------------------------|---------------|------------------------------|
|                                     | % Change at week 52 (n) |               | Between group P <sup>c</sup> | % Change at week 52 (n)            |               | Between group P <sup>c</sup> | % Change at week 52 (n)          |               | Between group P <sup>c</sup> |
|                                     | Controls                | Participants  |                              | Controls                           | Participants  |                              | Controls                         | Participants  |                              |
| <b>Cardiometabolic risk factors</b> |                         |               |                              |                                    |               |                              |                                  |               |                              |
| Insulin                             | +4.0 (75)               | -19.2*** (76) | <0.001                       | +3.0 (61)                          | -16.0** (54)  | 0.005                        | +4.4 (74)                        | -20.3*** (75) | <0.001                       |
| Leptin                              | +6.6 (76)               | -32.9*** (76) | <0.001                       | +7.9 (61)                          | -32.7*** (48) | <0.001                       | +9.1* (75)                       | -33.6*** (75) | <0.001                       |
| <b>Lipid measures</b>               |                         |               |                              |                                    |               |                              |                                  |               |                              |
| HDL                                 | -3.0 (76)               | -5.2* (76)    | 0.497                        | -2.7 (45)                          | -6.0* (54)    | 0.372                        | -2.8 (50)                        | -5.9* (60)    | 0.375                        |
| LDL                                 | -0.4 (71)               | -2.8 (71)     | 0.536                        | +4.3 (49)                          | -0.2 (58)     | 0.230                        | +4.3 (49)                        | -0.2 (58)     | 0.230                        |
| TCH                                 | -0.2 (76)               | -5.0** (76)   | 0.066                        | +2.6 (42)                          | -2.0 (44)     | 0.126                        | +3.1 (50)                        | -3.4* (60)    | 0.013                        |
| TG                                  | +2.0 (76)               | -7.2 (76)     | 0.213                        | +4.5 (44)                          | +0.8 (43)     | 0.798                        | +7.3 (50)                        | -4.9 (60)     | 0.214                        |

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to baseline by repeated-measures ANOVA; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TCH, total cholesterol; TG, triglycerides.

<sup>a</sup> Composite medication categories are described in Table 3.

<sup>b</sup> Primary medication categories include: insulin and leptin = insulin medications; HDL, LDL, TCH, and TG = lipid-lowering medications.

<sup>c</sup> From independent samples  $t$ -tests (two-tailed) of baseline to week 52 changes in intervention participants compared to controls.

cardiometabolic risk through atherogenic effects on the vasculature, stimulating production of reactive oxygen species and proinflammatory cytokines, which leads to oxidative stress, vascular inflammation, and atherosclerotic lesion formation [19]. Similarly, insulin stimulates the actions of various growth factors within the vasculature leading to inflammation and endothelial dysfunction [20].

Lifestyle modification can delay or prevent progression of atherosclerotic disease and significantly reduce risk of CVD mortality [21,22]. Therefore, interventions that modify both cardiovascular and metabolic risk factors may have the greatest potential to mediate cardiometabolic risk. In this study, participants in a cardiovascular lifestyle program showed significant reductions in plasma insulin and leptin, which may have beneficial anti-inflammatory and anti-oxidative effects on the vasculature.

Despite evidence that lifestyle modification can lead to significant improvements in overall cardiovascular risk profiles, gender differences in response of plasma insulin and leptin to exercise training [23] and a combination of diet and physical activity [24] have been reported. Men and women in our program showed similar reductions in plasma insulin and leptin, likely caused by changes in dietary composition and increased physical activity. These behaviors resulted in significant weight loss in both men and women over one year. Although gender differences may exist in the physiological action of insulin and leptin within the vasculature, fasting insulin and leptin are strongly correlated with percent body fat. Thus, through diet, exercise, and weight loss, both men and women may have derived similar benefit in terms of cardiometabolic risk reduction.

High consumption of fruits, vegetables, and whole grains has been associated with a favorable CVD biomarker profile, including lower fasting insulin and leptin concentrations [25]. Likewise, physical activity sustained for at least four weeks has a meaningful effect on insulin, leptin, and several other blood biomarkers implicated in CVD [26]. At baseline, program participants and controls consumed a high fat diet normally associated with obesity, insulin

resistance, and atherosclerosis. Participants in the lifestyle program successfully transitioned to a low-fat diet and dramatically increased their level of physical activity, which may have been important for reducing plasma insulin and leptin levels.

One incidental benefit experienced by some participants in the cardiac lifestyle program is a reduction in the number and/or dosage of prescription medications, which has the potential to influence changes in metabolic and cardiovascular risk factors. To remove the influence of medications on risk factor response during the program, we conducted a sub-group analysis that excluded participants who changed the brand or dosage of any medication known to affect insulin, leptin, and/or lipid levels. These analyses indicate that medications did not have significant effects on biomarker response and suggest that changes in cardiometabolic risk factors during the program are primarily attributable to lifestyle changes.

### Strengths and limitations

Cardiometabolic risk factors are rarely examined simultaneously in cardiac lifestyle modification programs with validated protocols and data collection methods. The prospective, longitudinal nature of this study and availability of matched controls minimized sources of bias and confounding and improved our ability to assess treatment benefits. Participants remained under the care of their primary physician, who may have prescribed changes in medications affecting plasma insulin or leptin levels. Removing pharmacological influences on risk factor modification strengthened the conclusion that participants derived meaningful metabolic and cardiovascular benefit from the program.

The Ornish Program is an established treatment alternative for CVD patients involving demanding lifestyle changes that requires motivation and significant time commitment. Baseline differences between cases and matched controls indicate that participants have

particularly atherogenic CVD risk factor profiles and would benefit most from cardiovascular risk reduction. Because careful screening is essential to identify motivated patients who would adhere to program guidelines, it was impractical to use a randomized study design. However, well-designed case-control studies are highly similar to randomized trials for estimating treatment effects [27,28]. We analyzed the data using a per-protocol approach, which included only patients who completed the program, rather than an intent-to-treat analysis. The lifestyle intervention included multiple modalities over one year, thus we were able to evaluate only short-term changes in cardiometabolic risk factors and were not able to define the relative contribution of each program component. Further, we could not assess applicability to the general public and whether results observed here are achievable outside of a controlled clinical environment. Future research will determine if improvements in cardiometabolic risk continue after participation and translate into improved clinical outcomes and develop less-rigorous cardiac interventions to maximize adherence and cardiovascular/metabolic benefit.

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### Authors' contribution

LV drafted the paper and performed dietary analysis, DN managed the patient database and performed statistical analysis; DD partitioned name brand medications into functional categories, defined primary and secondary effects, and determined comparable dosages; AB and MH conducted the lifestyle intervention; FL collected dietary data and directed dietary analysis; HP collected and processed blood samples, coordinated biomarker assays; MV reviewed the paper and provided oversight as PI of ICHP; DE conceived and supervised the study and drafted the paper, which was reviewed critically by all authors.

### Competing interests

The authors report no conflicts of interest with this study.

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### Appendix. Supplementary data

Supplementary data related to this article can be found in the online version at doi:10.1016/j.numecd.2012.01.012.

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## Taking Aim at Nurse Stress: A Call to Action

Mariam Kashani, MS, CRNP\*; COL Arn Eliasson, USA MC (Ret.)\*;  
Linda Chrosniak, PhD†; COL Marina Vernalis, USA MC (Ret.)\*

**ABSTRACT** The study investigates stress levels and related behaviors of nurses in a military medical center during war-time. In 2007, nurses completed a questionnaire survey with objective validation of data in a subsample using actigraphy over 12 weeks. Of 270 nurses, 255 (94%) returned surveys. A total of 81% reported moderately high or high stress with sources listed as work (66%) and fatigue (39%). Many reported coming to work despite feeling ill and/or stressed (13.6 days/3 months). In contrast, morale was high or moderately high in 71%. Nurses reported an average of 5.5 hours of sleep/night, 8.8 h/wk taken for self, and 3.8 h/wk for exercise. Actigraphy data showed an average of 6.0 hours of sleep/night. These findings highlight a mismatch between stress levels and coping perceptions indicating an inability to properly care for self. To manage the effects of chronic stress, nurse leaders should implement systems targeting healthy life balance.

### INTRODUCTION

The central mission of any medical organization is to care for ill and injured patients. The health and welfare of the nursing staff is of paramount importance to accomplish that mission. The work of caring for sick and dying patients along with the high operational tempo of modern and technically advanced medical practice, have a profound impact on the nursing caregivers themselves.<sup>1-3</sup> The stressful work environment often leads to compassion fatigue, potential patient-care errors, absenteeism, and unhealthy behaviors of the caregiver.<sup>4</sup> These unhealthy behaviors can erode physical fitness and exacerbate weight problems, which elevate cardiovascular disease risk.<sup>5,6</sup> Working in this environment may damage the morale of the nursing staff ultimately leading to burnout. From the organization's point of view, recruitment and retention of nurses may be adversely affected.<sup>7,8</sup>

Our medical facility is a large tertiary care military medical center with the mission of caring for soldiers who are ill and wounded in the course of their military duty. The nursing leadership at our institution has been mindful of the increased burden placed on nurses during time of war. The leadership therefore requested an assessment of the emotional state and morale of the members of the staff. The aim of our study was to investigate the state of the nursing staff during war time. We developed a questionnaire to identify pertinent issues placing increased burden on the staff members. In response to the findings of the questionnaire, we sought to capture objective data and actigraphy on a subset of the original population.

### METHODS

#### Study Sample and Setting

We administered a questionnaire to the nursing staff at our acute care military medical center in 2007. The questionnaire

was distributed to all nurses working on inpatient wards and in outpatient clinics for adult medicine and surgery, gynecology, pediatrics, psychiatry and psychology, intensive care, emergency services, as well as specialty services and clinics where specialized nurses worked. In short, every nurse employed at our medical center was given an opportunity and was encouraged to participate in completing the questionnaire survey. Completion of the questionnaire was voluntary and members of the nursing staff were given privacy and anonymity. The number of questionnaires distributed and collected was tabulated.

#### Questionnaire

The questionnaire asked nurses to note on a scale from 1 to 5 how their morale was, how high their perceived stress was, and to list their sources of stress. Participants were further asked to recall how many days in the previous 3 months they missed work because of illness and to estimate how many days they came to work despite feeling ill. They were also queried on whether they felt they had the tools necessary to cope with stress, tools necessary to follow a healthy diet, and to estimate how much time per week they spent in leisure activities, exercise, and sleep.

The questionnaire was administered to a pilot group of nurses asking for feedback on question clarity and question and answer format issues. Adjustments in the questionnaire were implemented on the basis of feedback from the pilot group leading to a one-page tool with 14 questions.

#### Subsample Survey

To obtain objective data to verify self-reported information from the questionnaire, a 14-member convenience sample of the study population volunteered to wear actigraphy armbands (BodyMedia Sensewear, Pittsburgh, PA) continuously for 12 weeks. The armbands measured body temperature, ambient temperature, position sense, and accelerometry and were programmed to calculate total sleep time (TST), recumbent time, and sleep efficiency (SE). Data downloaded from the actigraphs were averaged and compared with self-reported data.

\*Integrative Cardiac Health Project, 6900 Georgia Avenue, Walter Reed Army Medical Center, Washington, DC 20307-5001.

†Psychology Department, George Mason University, Arlington, VA 22201.

The 14-member subgroup of the study population also completed four other validated survey instruments, the Perceived Stress Scale, the Pittsburgh Sleep Quality Index, the Epworth Sleepiness Scale, and a fatigue scale.

The Perceived Stress Scale<sup>9</sup> (PSS)-14 is one of the most widely accepted of measurements of stress. Validation studies show that the PSS-14 has an internal consistency reliability of 0.85 by Cronbach  $\alpha$  and a test-retest reliability of 0.85. This 14-item questionnaire asks the subject how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0 to 56 with higher scores correlating with higher stress. The PSS is designed for use in community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Scores in the low 20s reveal moderate stress levels whereas scores approaching 30 are substantial and concerning.

The Pittsburgh Sleep Quality Index (PSQI)<sup>10</sup> is a self-rated questionnaire that assesses sleep quality and disturbances over a 1-month interval. Nineteen individual items generate seven component scores whose sum yields one global score with a range of 0 to 21. The psychometric and clinical properties of the PSQI suggest its utility both in clinical practice and research activities. A PSQI greater than 5 has a diagnostic sensitivity of 89.6% and specificity of 86.5% ( $\kappa > 0.75, p < 0.001$ ). Essentially, a global score of greater than 5 indicates a poor sleeper. Sleep perturbations can be categorized by the following scores: 0 to 5 is a good sleep score, 6 to 10 shows mild sleep difficulty, 11 to 15 moderate sleep difficulty, and 16 to 21 severe sleep difficulty.

The Epworth Sleepiness Scale (ESS)<sup>11</sup> is the most widely used tool to estimate the subjective symptom of daytime sleepiness. The ESS has a sensitivity of 93.5% and a specificity of 100% for detecting excessive daytime sleepiness. Subjects are asked to use a scale of 0 to 3 to estimate their likelihood of dozing in seven different situations in recent weeks. The individual scores are summed and possible scores range from 0 to 21. Sleepy subjects score 10 or higher and sleepiness can be categorized by the following scores: 10 to 13 mild sleepiness, 14 to 17 moderate sleepiness, and 18 to 21 severe sleepiness.

The Fatigue Visual Numeric Scale is borrowed from the Stanford Patient Education Research Center where it was tested on 122 subjects, with mean value of 4.89 and standard deviations of 2.71.<sup>12</sup> This fatigue scale asks subjects to express their experience of fatigue from 0 to 10 for the previous 2-week period. Subjects who circle 5 to 6 express mild fatigue, 7 to 8 moderate fatigue, and 9 to 10 severe fatigue.

**DATA ANALYSIS**

Data were analyzed with Microsoft Office Excel 2007 (Redmond, Washington). Variables are expressed as means

with standard deviation (SD) or with range. Intergroup differences were analyzed using Student's *t*-test for continuous variables and  $\chi^2$  test for discrete variables. The significance level was set at  $p \leq 0.05$ .

**RESULTS**

Of 270 nurses who received a questionnaire, 255 (94%) returned surveys. The population was composed of 69% women, 49% married, and racial/ethnic distribution of 51% white, 25% black, 9% Asian, 8% Hispanic, and 7% other.

The salient findings included reports of very high stress in 55% of respondents and moderately high stress in 26% (see Fig. 1). Sources of stress ranked by order of frequency were: work (66%), fatigue (39%), finances (33%), home (25%), and health (18%). These numbers add to a sum greater than 100% because multiple stressors could be noted by each participant. Few nurses reported missed work days for illness and/or stress (average 1.4 days over 3 months), but many reported coming to work despite feeling ill and/or stressed (average 13.6 days over 3 months). In contrast however, morale was scored as high in 47% of respondents and moderately high in 24% (see Fig. 2).

Regarding having necessary tools to cope with stress, 55% affirmed strongly and 21% affirmed moderately strongly that they believed they possessed necessary and appropriate tools to cope with stress (see Fig. 3). In the same vein, 40% of nurses were very confident they could maintain a healthy diet and 26% were moderately confident they could do so. However, respondents averaged 5.5 hours of sleep per night, 8.8 hours per week taken for stress-reducing leisure activities, and 3.8 hours per week taken for exercise. It deserves note that a substantial number of the nursing staff members are serving in active military service and are required to maintain specified fitness levels including passing scores on biannual physical fitness tests.

Actigraphy data were downloaded from armbands worn by the 14-member subgroup of nurses (see Table I). The average

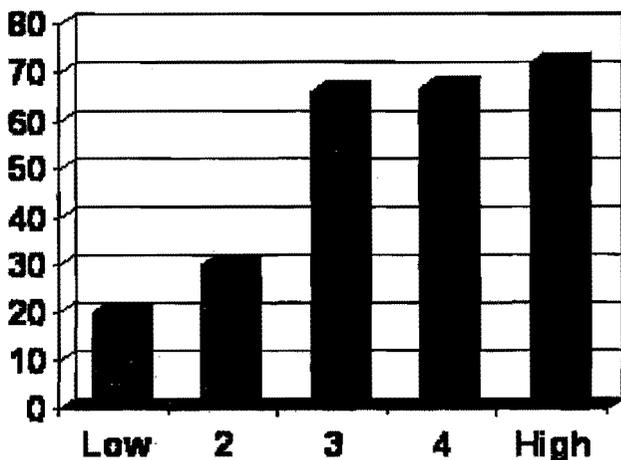


FIGURE 1. Self-reported stress levels in 255 respondents.

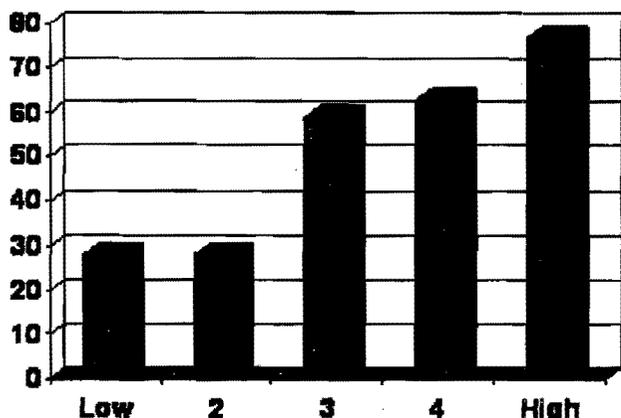


FIGURE 2. Self-report on belief in having tools to cope with stress in 255 respondents.

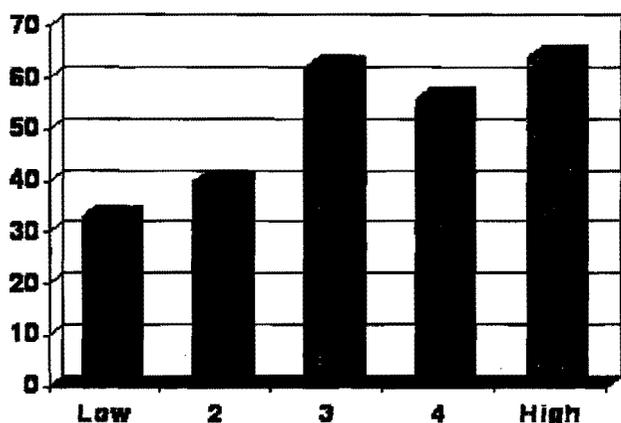


FIGURE 3. Self-reported morale in 255 respondents.

age of the 14 nurses (4 men) showed that they were middle-aged. Their self-reported sleep time by questionnaire was substantially less than 7 to 8 hours commonly recommended for most adults. This self-reported sleep time was somewhat less than the objectively measured sleep time but the difference did not reach a statistically significant difference ( $p = 0.12$ ). Mean recumbent time for the group was  $7.5 \pm 0.7$  hours with a calculated sleep efficiency of  $80 \pm 5\%$ .

The 14-member subgroup of nurses also showed high levels of stress (Table I). Sleep difficulties were evident in 11 of the 14 subjects (79%) with 7 of 14 (50%) in the mild category, 2 of 14 (14%) moderate, and 1 of 14 (7%) severe. Data analysis from the 14-member subgroup of nurses revealed interesting trends (Table II). Five nurses with PSS-14 scores greater than 28 (high stress group) were compared with nine nurses with PSS-14 scores less than 27 (low and moderate stress group). Nurses in the high stress group showed a trend toward being sleepier by the Epworth Score ( $p = 0.06$ ), more fatigued ( $p = 0.09$ ), and with a tendency toward overweight ( $p = 0.17$ ), though these differences did not reach statistical significance.

TABLE I. Data From Subgroup Wearing Actigraphy Armbands

| Gender                   | Age (yrs) | Stress Level | PSQI | Self-Reported Sleep (hr/24 hr) | Sleep (hr/24 hr) Actigraphy |
|--------------------------|-----------|--------------|------|--------------------------------|-----------------------------|
| F                        | 43        | 30           | 5    | 5.0                            | 5.1                         |
| F                        | 38        | 26           | 6    | 5.0                            | 6.7                         |
| M                        | 51        | 24           | 12   | 6.0                            | 5.6                         |
| F                        | 36        | 29           | 10   | 7.5                            | 6.5                         |
| F                        | 57        | 24           | 16   | 4.0                            | 6.6                         |
| M                        | 35        | 32           | 10   | 5.0                            | 6.2                         |
| F                        | 24        | 23           | 10   | 5.0                            | 6.6                         |
| M                        | 42        | 26           | 13   | 6.0                            | 5.5                         |
| F                        | 31        | 33           | 6    | 4.0                            | 5.7                         |
| F                        | 42        | 22           | 7    | 6.5                            | 6.3                         |
| F                        | 42        | 33           | 9    | 5.5                            | 6.4                         |
| M                        | 21        | 26           | 5    | 6.0                            | 5.2                         |
| F                        | 23        | 22           | 5    | 5.0                            | 4.8                         |
| F                        | 32        | 24           | 9    | 6.0                            | 6.5                         |
| Average<br><i>n</i> = 14 | 36.9      | 26.7         | 8.8  | 5.5                            | 6.0                         |
| Standard Deviation       | 10.4      | 4.0          | 3.4  | 0.9                            | 0.7                         |

$p = 0.12$

Stress level measured using the Perceived Stress Scale (PSS-14, see Methods). PSQI (Pittsburgh Sleep Quality Index) is a global measure of sleep quality and quantity formulated to produce a single score. (See Methods section for details.)

## DISCUSSION

Caring for others is central to the definition of nursing. While nurses show commitment to others, including concern for immediate and extended family,<sup>13</sup> they frequently neglect to care for themselves. With the focus on others, nurses often forego self-assessment<sup>14,15</sup> and the corresponding benefit from renewal activities.<sup>16</sup> Ultimately, nurses tend to overestimate their ability to sustain a productive pace.

The most prominent findings of our questionnaire survey were that stress levels, largely from work, were dramatically elevated in this nurse population in curious counterpoint to remarkably high morale and a strong degree of confidence in their ability to cope. This mismatch is underscored by the low amount of time spent on themselves per week and the few hours of sleep obtained at night. These data indicate an unsustainable lifestyle schedule and are similar to previously reported findings in this population.<sup>17</sup>

The mismatch observed in our nurse population mirrors the physiological mechanism of a type-II diabetic patient whose chronic disease renders cells insensitive to insulin leading to an unhealthy metabolic state. Similarly, nurses under chronic stress and on a path toward burnout may be unable to mount a healthy stress response.<sup>18</sup> Much like a physiological environment lacking proper homeostasis, our nurse data reflect an inability to self-regulate demonstrated by unhealthy lifestyle choices in a chaotic work place.

The presence of high morale may mislead supervisors in the chain of command at military institutions to overlook signals of personnel distress. In the military, the culture is different than that of a civilian facility. The military medical team is

TABLE II. Comparison Between Stress Levels, Daytime Symptoms, and BMI

| Group <i>n</i> = 14        | Gender (% Male) | Age (yrs)   | Stress Level | Epworth Sleepiness | Fatigue Scale <sup>a</sup> | BMI (kg/m <sup>2</sup> ) |
|----------------------------|-----------------|-------------|--------------|--------------------|----------------------------|--------------------------|
| More Stressed <i>n</i> = 5 | 20%             | 37.4 ± 5.0  | 31.4 ± 1.8   | 14.0 ± 6.0         | 6.4 ± 2.1                  | 28.5 ± 6.1               |
| Less Stressed <i>n</i> = 9 | 30%             | 36.7 ± 12.7 | 24.1 ± 1.6   | 7.3 ± 5.9          | 4.1 ± 2.3                  | 24.9 ± 3.3               |
| <i>p</i> value             | 0.62            | 0.90        | <0.001       | 0.06               | 0.09                       | 0.17                     |

For details on measurements of stress level, Epworth Sleepiness Scale, and fatigue scale, see Methods section.

<sup>a</sup>Fatigue Visual Numeric Scale.

imbued with a unique allegiance to accomplish the mission at hand despite personal obstacles such as fatigue and burnout. It is critically important that the leadership recognize the long-term effects of this military culture of self-sacrifice for the greater good. Leaders must survey their charges for unsustainable personal lifestyle habits before burnout occurs.

A unique aspect of our study is that the subjective self-reported information was substantiated with data from validated questionnaires and objective information from actigraphy in a subset of the study population. Question 1 of the Needs Assessment Survey asked subjects to rate their stress level on a Likert Scale from 1 to 5 (high stress levels are graphically reported in Fig. 1). This was substantiated in the 14-member subgroup where PSS scores averaged 26.7, values that demonstrate a concerning elevation in stress levels. Similarly for sleep, question 10 asked "On average, how many hours do you sleep each night?" The nurses in the subgroup self-reported their sleep time as 5.5 hrs/night, not statistically different from 6.0 hrs/night as measured with actigraphy.

Of importance, our study showed interesting trends to support the notion that nurses who feel more stressed, also feel sleepier, are more fatigued, and tend to be more overweight. These findings add to our understanding of the cascade effect of poor lifestyle choices: that stress begets poor sleep that leads to more impairing daytime symptoms that in turn lead to dysfunctional lifestyle choices. This self-reinforcing negative cascade may create other vulnerabilities such as cardiovascular disease, chronic sleep disruption, depression, and a negative self-image. Research has shown that risky behaviors tend to cluster with other risk-promoting choices.<sup>19</sup> It is never too early to intervene upon this negative sequence, previously described as loss spirals.<sup>20</sup>

The utility of actigraph recordings derives from the very nature of sleep, a state of unconsciousness with a lack of awareness of the timing of sleep onset and wakefulness. These facts make estimations of sleep time vulnerable to inaccuracies increasing the value of objective measures of sleep. Actigraphy provides an objective measure of total sleep time. Because actigraphs can be worn for a number of sequential days, estimates of total sleep time include daytime napping behaviors in addition to nocturnal sleep. Furthermore, actigraphy can capture data to describe sleep variability that occurs in weekday and weekend schedules.

The original actigraphs developed in the early 1970s were essentially activity monitors worn on the arm or leg.

Actigraphs have since passed through generations of developmental changes. The modern validated actigraph measures a variety of variables including position (e.g., recumbence versus upright posture), motion (including intensity and frequency of movement), body temperature and ambient temperature (to detect drop in body temperature associated with sleep onset). These variables can be utilized to calculate a variety of outputs of interest, most notably highly accurate estimates of total sleep time, sleep efficiency, resting energy expenditure, and exercise energy expenditure. The reliability of actigraphy versus EEG for distinguishing wake from sleep depends on the population studied but in adults with non-pathological sleep, reliability coefficients have ranged from 0.89 to 0.98.<sup>21,22</sup>

The use of actigraphy in clinical medicine and research has flourished. Actigraphy is especially useful in evaluation of insomnia, shift-worker syndrome, and disorders of circadian rhythm where unraveling the particulars of sleep behaviors can be invaluable for diagnosis and management of the condition. Actigraphy has been particularly useful in epidemiological studies where polysomnography is too expensive or impractical in large populations.

Poor behavioral choices on the part of individual nurses have an impact on nursing staff retention. Nurses living and working at such an unsustainable pace are prone to burnout, leading to increased nursing staff turnover.<sup>3</sup> The cost of turnover is felt in terms of economics and quality of care.<sup>23</sup> Nurse turnover costs have been monetarily estimated to be an additional 30% over baseline salary expenditures.<sup>8</sup> Quality of care suffers when institutional memory is depleted and understanding and experience with standard procedures diminish. Team work on a nursing unit is eroded through loss of staff members. In fact, hospital nurse shortages have been linked to higher 30-day patient mortality and higher "failure-to-rescue" rates. Nurses working in this environment are more likely to experience burnout and job dissatisfaction.<sup>4</sup>

High stress levels can potentially paralyze the staff at a military medical facility. Leaders should hear our call to attention as they have the authority and responsibility to safeguard their most important asset, their personnel. Populationwide health systems that use an integrative approach to improve self-care among nurses are successful.<sup>23</sup> Programs that protect medical personnel from the toxicity of wartime must be applied to multiple populations such as chaplains, physicians, physical therapists, and medics to optimize caregiver sustainment.

Previous obstacles to care in the military have included the stigma associated with mental health services. A program that is labeled "stress reduction" or that is housed in mental health services may be poorly subscribed to. The integration of stress reduction with sleep improvement, exercise promotion, and healthful eating naturally weaves together the mainstays of healthy living and opens doors to preventive care. Stress reduction under the nonstigmatized umbrella of a healthy lifestyle program may not only encourage participation but also build toward a cascade of institutional success.

Limitations of this study include a questionnaire that deserves further work to establish its reliability and validity. Furthermore, until we validate our questionnaire and demonstrate its utility in a civilian population, it would not be proper to generalize our findings to nonmilitary populations because of the unique setting in which our nurses work.

We believe that the stressful healthcare environment, nurse burnout, and the consequent impact on work force retention are problems of growing urgency.<sup>15</sup> The nursing work force is aging even as the complexity of providing care and the speed of technological change are increasing.<sup>24</sup> It is time to raise awareness of the effects of stress in military healthcare workers, especially in time of war, and to take systemwide action to establish integrative interventions to sustain the fighting force.

## CONCLUSIONS

In summary, we take aim at nurse stress with a call to action for nurses to engage in more vigilant and effective self-care. Sustainable schedules along with balance between work and home life must be sought. Achieving this balance depends on the ability of nurses to adopt better self-regulatory strategies that will build resilience. Furthermore, it makes good economic and managerial sense for institutions to invest in their nursing staff by developing and implementing accessible programs to assess and manage stress and to promote a pace of life that can be maintained. Perhaps the Golden Rule for nurses should be, "Care for Yourself as You Care for Others."

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## Perceived stress correlates with disturbed sleep: A link connecting stress and cardiovascular disease

MARIAM KASHANI, ARN ELIASSON, & MARINA VERNALIS

*Integrative Cardiac Health Project, Walter Reed Army Medical Center, Washington, DC, USA*

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### Abstract

The association between stress and cardiovascular disease (CVD) risk is becoming established. A mechanistic link clarifying the intermediate steps between the experience of stress and the development of CVD would support this association. We sought to examine the role of perceived stress as a factor associated with disturbed sleep with the goal of providing an explanation for the stress–CVD connection. We performed a cross-sectional analysis of data recorded by subjects at entry to our CVD prevention program. Data collection included questionnaire surveys, anthropometrics, and a CVD-relevant laboratory panel. Of 350 consecutively enrolled subjects (mean age  $54.4 \pm 12.4$  [SD] years, 138 men, 39%), 165 (47%) scored above the mean for stress measures. These high-stress subjects displayed an increased cardiovascular risk profile including elevated body mass index (mean  $\pm$  SD  $31.1 \pm 5.9$  vs.  $29.0 \pm 5.9$ ,  $r_s = 0.175$ ), increased waist circumference ( $102 \pm 17$  cm vs.  $98 \pm 14$ ,  $r_s = 0.135$ ), and elevated high-sensitivity serum C-reactive protein ( $0.384$  mg/dl vs.  $0.356$ ,  $r_s = 0.109$ ). High-stress subjects also demonstrated greater daytime sleepiness (Epworth Sleepiness Scale:  $10.4 \pm 5.0$  vs.  $7.8 \pm 4.8$ ,  $r_s < 0.316$ ), greater fatigue (fatigue scale:  $5.4 \pm 2.2$  vs.  $3.4 \pm 2.4$ ,  $r_s = 0.484$ ), poorer sleep quality (Pittsburgh Sleep Quality Index:  $8.5 \pm 4.4$  vs.  $5.9 \pm 4.0$ ,  $r_s = 0.416$ ), and shorter sleep duration (20 min less/24 h,  $r_s =$  negative  $0.177$ ) with a higher risk for sleep apnea (60% at high risk vs. 40%,  $p = 0.003$ ) than low-stress subjects. High stress was associated with significant disturbances in sleep duration and sleep quality. Stress levels also correlated with daytime consequences of disturbed sleep. The stress–sleep connection may be an important mechanistic mediator of the association between stress and CVD.

**Keywords:** *Cardiovascular disease, perceived stress, risk factors, sleep, sleep quality, stress*

### Introduction

An emerging body of evidence substantiates the observation that there is an association between stress and the occurrence of cardiovascular disease (CVD; Belkic et al. 2004; Rosengren et al. 2004). While the stress–CVD connection has been promoted and taught for decades, a number of difficulties have slowed a productive line of investigation in this area.

The impediments include how to define and measure stress (Cohen et al. 1983; Kocalevent et al. 2007), how to assess stress levels in a reproducible fashion over time (Kocalevent et al. 2009), uncertainty regarding causation between stress and CVD (Tindle et al. 2010), and the expense of performing a study to follow large numbers of subjects over a substantial period of time (Kadojić et al. 1999;

Hamer et al. 2008). Furthermore, some studies appear to contradict the association between stress and CVD (Greenlund et al. 1995; Riese et al. 2000; Heslop et al. 2002a,b; Belkic et al. 2004). Nevertheless, the preponderance of studies to date supports the conclusion that stress, variously defined in a variety of approaches, does correlate with increased cardiovascular risk, both for heart disease (Melamed et al. 1992; Belkic et al. 2004; Rosengren et al. 2004; Brborović et al. 2009; Holden et al. 2010; Puustinen et al. 2010) and stroke (Everson et al. 2001; Surtees et al. 2008; Tsutsumi et al. 2009). Moreover, studies of depressive behaviors in female primates subjected to social stressors over a 4-year period have demonstrated significant acceleration of coronary artery atherosclerosis, suggesting a causal relationship between stress and CVD (Shively et al. 2008).

To substantiate and explain the clinical observations correlating stress and CVD, it would be useful to clarify the underlying pathophysiology to outline mechanisms that link stress and CVD. It is clear that the hypothalamus–pituitary–adrenal axis plays a major role, by stimulating cortisol secretion, as do increased aldosterone and catecholamine levels, with a resulting detrimental effect on the cardiovascular system (Kubzansky and Adler 2010). It remains less clear what maladaptive conditions initiate the cascade of mediators that trigger these responses.

One mechanism was proposed in the Massa Lombarda Project, an epidemiological study including 7000 northern Italian adults (Bove et al. 2010). In a subset of 106 men and women, selected for older age, psycho-emotional stress and depression disorder were associated with the development of metabolic syndrome, a cluster of multiple cardiovascular risk states. Another study examined whether self-reported job strain was associated with early, potentially modifiable cardiovascular (CVD)-related health behaviors (Hellerstedt and Jeffery 1997). This study of 3843 randomly selected men and women employees of 32 worksites in Minnesota showed that work stress, defined as high demand and low latitude, was positively associated with smoking, smoking intensity, and high fat intake in men, and with body mass index (BMI) and smoking intensity in women. In 2008, the most sophisticated studies to date were published to describe the mechanistic links between stress and CVD (Chandola et al. 2008; Hamer et al. 2008). These studies used statistical models to assess the relative contributions of potential mediators of stress and CVD events. The Whitehall II study followed over 10,000 male and female civil servants for an average of 12 years (Chandola et al. 2008). The study showed that two factors, health behaviors and metabolic syndrome, accounted for around 32% of the effect of work stress on CVD. Another study that used statistical modeling was prospective and included 6576 healthy men and women followed over an average of 7.2 years (Hamer et al. 2008). Psychological distress was measured with the validated General Health Questionnaire, and actual CVD events (hospitalization for nonfatal myocardial infarction, coronary artery bypass, angioplasty, stroke, heart failure, and CVD-related mortality) were used as the main outcome. The investigators reported that behavioral factors explained the largest proportion of variance (approximately 65%), whereas pathophysiological factors accounted for a modest amount (C-reactive protein approximately 5.5%; hypertension approximately 13%).

The mechanisms proposed by these studies, while supported by objective data, fail to fully account for the observed relationship between stress and CVD. An often overlooked contributor to ill health and bad medical outcomes is sleep, with important

sleep parameters including sleep duration and sleep quality. Failure to include the role of sleep in the stress–CVD connection is especially surprising in view of the substantial personal experience that all humans have of the ill effects of sleep deprivation and disrupted quality of sleep. Understanding the role of sleep as a possible link between stress and CVD is especially appealing because sleep behaviors can be taught and improved. Furthermore, it has been shown that improving sleep quality through the implementation of behavior modification does lower perceived stress levels (Eliasson et al. 2010). Disrupted sleep is thus a modifiable risk factor for stress levels and may therefore be, in extension, a modifiable risk factor for CVD.

We therefore hypothesized that high levels of perceived stress would correlate with disturbed sleep parameters. Such mechanistic link is indicated by substantial prior research showing that short sleep and disrupted sleep are associated with high risk for CVD (Heslop et al. 2002a,b).

## Methods

The investigation was conducted with the approval of our institutional review board. The study design is a retrospective analysis of data collected on consecutive patients participating in a CVD prevention program at the Walter Reed Army Medical Center Integrative Cardiac Health Project (ICHP). ICHP is a cardiovascular prevention research center for the US Department of Defense. All data were retrospectively gathered and no blood samples were taken specifically for this study. The institutional review board, therefore, did not request informed consent from the study subjects.

Patients were self-referred or referred to the program by a health-care provider to improve habits of diet, exercise, sleep, and stress management. ICHP is accessible to military health-care beneficiaries including active duty service members, retirees, and dependents. The program, therefore, enrolls a broad spectrum of subjects including a variety of races and ethnic backgrounds, both genders, and a range of ages from 18 to 90 years. The typical patient entering the program is found to have two to four risk factors for CVD.

Upon entry, subjects are asked to complete a series of questionnaires to gather information on demographics, current symptoms, past and current medical conditions including medications and lifestyle habits. Among the questionnaires are the validated surveys to assess stress levels, sleep behaviors, sleep quality, and daytime symptoms from inadequate sleep. Data from the questionnaires are reviewed during a medical interview with a nurse practitioner who also performs a physical examination to include anthropomorphic measures.

### Laboratory studies

Subjects gave blood for cardiac-relevant biochemical studies. For all blood samples, subjects were instructed to present to the laboratory between 06:00 and 08:00h having fasted from 20:00h the previous evening. The biochemical measurements on blood samples included a standard lipid panel with total cholesterol concentration, low-density lipoprotein (LDL) cholesterol concentration, high-density lipoprotein (HDL) cholesterol concentration, triglyceride concentration, as well as lipoprotein (a) and lipoprotein PLA2 concentrations. Measures of glucose metabolism include fasting plasma glucose concentration, insulin concentration, and hemoglobin A1C %. High-sensitivity C-reactive protein concentration (hsCRP) was also measured.

The laboratory studies were performed in the institution's certified central laboratory. The lipid panel was measured on a Roche Cobas c501 with appropriate reagents. The technique has documented traceability to the National Reference System for Cholesterol by performing a direct comparison with the cholesterol reference method using fresh human specimens, which cover the National Cholesterol Education Program (NCEP) medical decision points. The system has demonstrated the ability to meet the NCEP's performance criteria for accuracy and precision.

### Perceived Stress Scale (PSS)

The PSS is one of the most widely accepted measures of stress (Cohen et al. 1983). This validated 14-item questionnaire asks the subject how often certain experiences of stress occurred in the last month and is designed to measure the degree to which situations in one's life are appraised as stressful. With item responses from 0 to 4, the range of possible scores is 0–56 with higher scores correlating with higher stress. The PSS is designed for use in community samples with at least a junior high school education. The items are easy to understand and the response alternatives are simple to grasp. Moreover, the questions are quite general in nature and hence relatively free of content specific to any subpopulation group. Score in the low 20s reveal moderate stress levels, while scores approaching 30 are substantial and concerning.

### Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances over a 1-month time interval (Buysse et al. 1989). Nineteen individual items generate seven component scores whose sum yields one global score with a range of 0–21. The psychometric and clinical properties of the PSQI suggest its utility both

in clinical practice and research activities. A global score of greater than 5 indicates a poor sleeper. Sleep perturbations can be categorized by scores: 0–5 is a good sleep score; 6–10 shows mild sleep difficulty; 11–15 moderate sleep difficulty; and 16–21 severe sleep difficulty.

### Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) is the most widely used tool to estimate the subjective symptom of daytime sleepiness (Johns 1992). Subjects were asked to use a scale of 0–3 to estimate their likelihood of dozing in eight different situations in recent weeks. The individual scores were summed and possible scores range from 0 to 24. Sleepy subjects score 10 or higher and sleepiness can be categorized by scores: 10–14 as mild sleepiness, 15–19 as moderate sleepiness, and 20–24 as severe sleepiness.

### Fatigue Scale

The Fatigue Visual Numeric Scale is borrowed from the Stanford Patient Education Research Center (see <http://patienteducation.stanford.edu/research/vnsfatigue.html>, accessed 1 July 2010). This fatigue scale asks subjects to express their experience of fatigue from 0 to 10 for the previous 2-week period. Subjects who circle 5–6 express mild fatigue, 7–8 moderate fatigue, and 9–10 severe fatigue.

### Berlin Questionnaire

Of questionnaires available to screen patients for sleep apnea, the Berlin Questionnaire is one of the most commonly utilized and best validated (Netzer et al. 1999). As measured by the questionnaire, patients with persistent and frequent symptoms are considered to be at high risk for sleep apnea. Questions about symptoms demonstrated internal consistency (Cronbach correlations, 0.86–0.92). With a positive Berlin Questionnaire, sleep apnea was predicted with a sensitivity of 0.86, a specificity of 0.77, a positive predictive value of 0.89, and a likelihood ratio of 3.79.

### Statistical analysis

Data are presented as mean  $\pm$  SD. Two sample *t*-tests were used to compare continuous variables between groups, and categorical data were compared between groups using Fisher's exact test. Body habitus, sleep variables, and hsCRP did not satisfy assumptions of normality (as tested by the Shapiro–Wilk statistic) therefore, Spearman's correlation coefficient; ( $r_s$ ) was used to examine the association of these variables with the PSS. All tests were two-tailed and *p* values < 0.05 were presumed to represent statistical

Table I. Characteristics of the subjects according to perceived stress levels.

|                           | All subjects<br>( <i>n</i> = 350) | Low stress<br>( <i>n</i> = 185) | High stress<br>( <i>n</i> = 165) | <i>z</i> statistic | Degrees of<br>freedom | <i>p</i> value     |
|---------------------------|-----------------------------------|---------------------------------|----------------------------------|--------------------|-----------------------|--------------------|
| Age, years (mean ± SD)    | 54.4 ± 12.4                       | 57.4 ± 11.5                     | 51.1 ± 12.6                      | 4.9                | 348                   | <0.001*            |
| Race (%)                  |                                   |                                 |                                  |                    |                       |                    |
| Caucasian                 | 134 (38%)                         | 73 (39%)                        | 60 (36%)                         |                    |                       | 0.673 <sup>†</sup> |
| African American          | 105 (30%)                         | 53 (29%)                        | 52 (31%)                         |                    |                       |                    |
| Hispanic                  | 14 (4%)                           | 5 (3%)                          | 9 (5%)                           |                    |                       |                    |
| Asian                     | 2 (1%)                            | 1 (1%)                          | 1 (1%)                           |                    |                       |                    |
| Others                    | 96 (27%)                          | 53 (29%)                        | 43 (26%)                         |                    |                       |                    |
| Gender, male (% male)     | 138 (39%)                         | 78 (42%)                        | 60 (36%)                         |                    |                       | 0.28 <sup>†</sup>  |
| PSS score (56 points max) | 22.4 ± 8.1                        | 16.3 ± 4.7                      | 29.3 ± 5.0                       | -25.0              | 348                   | <0.001*            |

Notes: Values are mean ± SD or actual number of subjects in a category (with proportion). Statistical comparisons are between low-stress and high-stress subjects using the two sample *z*-test (or Fisher exact test as noted) with *p* values less than 0.05 representing statistical significance. Low-stress subjects were defined by a Perceived Stress Score less than the mean of 23 points, while high-stress subjects were defined by a score equal to or greater than the mean of 23 points; \* denotes two sample *z*-test between low-stress and high-stress subjects; <sup>†</sup> denotes Fisher's exact test between low-stress and high-stress subjects.

significance. Data were analyzed using SPSS for Windows (v. 17.0, SPSS, Inc., (IBM), Chicago, IL, USA).

## Results

We studied data from 350 participants entering ICHP's CVD prevention program. The mean age (±SD) of our participants was 54.4 ± 12.4 years; consistent with a spectrum of lifestyles from actively working to semi-retired and fully retired adults. Heavily represented racial categories were Caucasian and African American, but a substantial number of subjects identified themselves as mixed race or declined to pick a category. There was a majority of women (61%) in our study sample (see Table I).

As the mean PSS score was 22.4 points, we elected to define subjects with PSS scores of 23 or more points as belonging to the "high-stress" group and subjects with PSS less than 23 as the "low-stress" group. This allowed for analysis of data for nearly equal sized cohorts of high- and low-stress groups. While there are no defined ranges of "normality" or published degrees

of severity based upon the PSS scores, the cut point of 23 does conform to a threshold value above which stress becomes a concerning issue from a clinical point of view in our experience within our program.

As summarized in Table I, there were no significant differences with regard to race or gender for high-stress and low-stress groups, though high-stress subjects were somewhat younger (*p* < 0.001).

As summarized in Table II, the cohort of subjects with high stress had a higher BMI (obese indices vs. merely overweight, *p* = 0.001) and larger measured waist circumferences. The biochemical measurement of hsCRP showed a positive correlation with perceived stress. The high-stress group also showed shorter total sleep times (20 min less per 24 h), poorer sleep quality, higher likelihood of sleep apnea diagnosis, greater sleepiness, and greater fatigue. The correlation between perceived stress and sleep quality is illustrated in Figure 1.

Several measurements (*n* = 350), not presented in the tables, showed no correlation with levels of perceived stress by Spearman's rank correlation. The lipid panel including total serum concentrations of

Table II. Correlations between perceived stress levels vs. anthropometrics, behavior scores, symptom scores, and laboratory values.

|  | Low stress<br>( <i>n</i> = 185) | High stress<br>( <i>n</i> = 165) | Correlation<br>coefficient* | <i>p</i> value<br>two-tailed |
|--|---------------------------------|----------------------------------|-----------------------------|------------------------------|
| BMI (kg/m <sup>2</sup> )                           | 29.0 ± 5.9                      | 31.1 ± 5.9                       | 0.175                       | 0.0011                       |
| Waist circumference (cm)                           | 98 ± 14                         | 102 ± 17                         | 0.135                       | 0.012                        |
| Total sleep time (hours/24 h)                      | 6.4 ± 1.2                       | 6.1 ± 1.5                        | -0.177                      | 0.0011                       |
| Pittsburgh Sleep Quality Index (21-point scale)    | 5.9 ± 4.0                       | 8.5 ± 4.4                        | 0.416                       | <0.001                       |
| Berlin Questionnaire (% high risk for sleep apnea) | 63/152 (41)%                    | 72/120 (60%)                     |                             | 0.003 <sup>†</sup>           |
| Epworth Sleepiness Scale (24-point scale)          | 7.8 ± 4.8                       | 10.4 ± 5.0                       | 0.316                       | <0.001                       |
| Fatigue scale (10-point scale)                     | 3.4 ± 2.4                       | 5.4 ± 2.2                        | 0.484                       | <0.001                       |
| hsCRP (mg/dl)                                      | 0.356                           | 0.384                            | 0.109                       | 0.045                        |

Notes: Values are mean ± SD or proportion. Statistical comparisons are between low-stress and high-stress subjects using the two sample *z*-test (or Fisher exact test as noted) with *p* values less than 0.05 representing statistical significance. Correlation coefficients are derived from the Spearman's rank correlation coefficient (also called Spearman's rho) using a two-tailed test with *p* < 0.05 as the predetermined threshold of statistical significance. BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; \* denotes Spearman's rho (*r<sub>s</sub>*); <sup>†</sup> denotes Fisher's exact test.

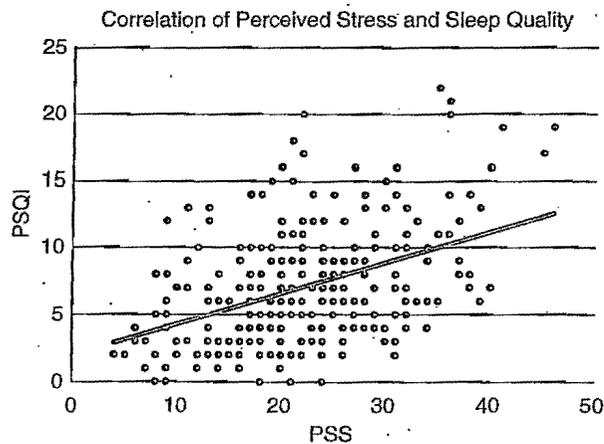


Figure 1. Using the Spearman correlation, there is a significant positive relationship between perceived stress scores (PSS) and scores on the PSQI,  $r_s = 0.43$ ,  $n = 274$ ,  $p < 0.0005$ .

cholesterol ( $r_s = 0.04$ ), LDL cholesterol ( $r_s = 0.004$ ), HDL cholesterol ( $r_s = 0.015$ ), triglyceride ( $r_s = -0.045$ ), and Lp (a) ( $r_s = -0.013$ ) did not correlate with PSS. Likewise, parameters of glucose metabolism did not correlate with PSS, including fasting plasma glucose concentration ( $r_s = 0.057$ ), HgA1C percentage ( $r_s = 0.013$ ), and the homeostatic assessment model or HOMA ( $r_s = 0.093$ ).

When sorted by gender, important differences were revealed. By  $t$ -tests ( $n = 350$ ), women were slightly younger ( $54 \pm 11$  years vs.  $58 \pm 12$  years,  $F = 1.63$ ,  $df = 348$ ,  $p = 0.04$ ), had higher perceived stress scores (PSS =  $24 \pm 8$  vs.  $20 \pm 8$ ,  $F = 0.44$ ,  $df = 348$ ,  $p = 0.04$ ), had higher total serum cholesterol concentration ( $200 \pm 37$  vs.  $176 \pm 72$  mg/dl,  $F = 0.58$ ,  $df = 343$ ,  $p = 0.009$ ), and higher serum HDL cholesterol concentration ( $64 \pm 22$  vs.  $48 \pm 12$  mg/dl,  $F = 15.21$ ,  $df = 343$ ,  $p < 0.001$ ).

## Discussion

The salient findings of this study are that increased levels of perceived stress were correlated with shortened total sleep time, worse scores for sleep quality, higher likelihood of sleep apnea, and worse daytime symptoms of sleepiness and fatigue. It is important to note that there were no concomitant correlations between perceived stress and lipid abnormalities or measures of glucose metabolism, two common risk factors for heart disease. It is known that normal values for lipids and glucose metabolism do not preclude an increased CVD risk. The finding that glucose and lipids did not correlate with stress in our study places greater weight on the role of sleep disruption in the development of CVD. In combination with numerous prior studies that connect short sleep and disturbed sleep with CVD (Heslop et al. 2002a,b), our correlations provide

a mechanistic link to support the observed association between stress and CVD.

It is important to define stress and what is actually being measured with the PSS as it pertains to the current investigation. Because the PSS questions are general and free of content specificity, the instrument assesses subjectively experienced stress independent of an objective external stimulus or situation (Cohen et al. 1983). Personality aspects and resources of the subjects contribute to the total perceived stress score. The PSS correlates closely with trait neuroticism rather than the state of stress imposed. It therefore follows that trait neuroticism may be a pre-morbid characteristic of some good sleepers, who nonetheless manifest hyperarousal in response to stress and thus develop stress-induced insomnia (Basta et al. 2007; Fernandez-Mendoza et al. 2010).

The tools used to measure sleep in this study evaluate both sleep quality and sleep quantity. The high-stress group got an average of 20 min less sleep per night compared to the low-stress group. This may initially appear to be an inconsequential difference in sleep quantity. However, after only a few days or weeks, a substantial sleep debt can accrue, sufficient to affect mood, performance, and sense of well-being (Dinges et al. 1997; Drake et al. 2001). Furthermore, fatigue-inducing pro-inflammatory cytokines (interleukin-6 and tumor necrosis factor alpha) are negatively influenced by the quantity and quality of sleep (Vgontzas et al. 1999). CVD is a disease state stimulated and exacerbated by systemic inflammation. Prior research has also shown that insomnia with objective short sleep duration is associated with a higher risk for hypertension (Vgontzas et al. 2009a,b) and for type 2 diabetes mellitus (Vgontzas et al. 2009a,b), both major risk factors for CVD.

The Berlin Questionnaire focuses on an aspect of sleep quality. It is a validated instrument to quantify high vs. low risk for sleep apnea. The high-stress group with substantially higher BMI also has much higher odds of having sleep apnea. This finding is consistent with prior research that correlates increasing BMI with higher risk for sleep apnea (Newman et al. 2005). Explanations of these associations may include alternative theoretical models. Stress may stimulate maladaptive eating, leading to weight gain and subsequent development of sleep apnea. Alternatively, sleep apnea may disrupt the restorative functions of sleep (experienced as higher stress levels) and simultaneously disrupt hormonal regulation of hunger leading to greater calorie consumption and weight gain. These pathways toward greater risk of CVD warrant corrective attention at a time early in the cycle to preclude a downward spiral of health indicators.

Worse sleep quality as measured by the PSQI correlated with higher stress levels (Figure 1). Similarly, the ESS and fatigue scale, consequences

of the impact of poor sleep quality, correlated with higher stress levels. The finding that different tools showed worse scores with higher stress levels gives credibility to the observation linking poor sleep quality with high stress. Of course the challenge will be finding effective ways to improve sleep quality and consequent daytime symptoms, translating to improvements in CVD risk.

A novel aspect of our research is the use of the PSS and PSQI as tools to measure stress and sleep quality. There are few other studies that link perceived stress with poor sleep quality. There is one publication that utilized both the PSS and the PSQI in the same study (Strange et al. 2009). These coauthors investigated 220 pregnant women and found that PSS did not predict preterm birth and that preterm births were associated with lower daytime dysfunction scores on the PSQI. A PSS-PSQI connection was not reported in the study.

CVD is the leading cause of death in women, despite the cardiovascular protection afforded by their endogenous hormones and increased levels of HDL cholesterol (Wasserthiel-Smoller 2010). In our study, women were found to have significantly higher perceived stress scores than men. This finding may indicate that stress levels, specifically the measured PSS score, may be an important gender-relevant risk factor to survey, especially as a preventive strategy for improving women's health.

What cannot be determined in a cross-sectional study is causality. It cannot be inferred whether or not perceived stress causes deranged sleep or if poor sleep habits cause increases in perceived stress. It is possible that both perceived stress and sleep habits are worsened by another stimulus and that they respond in parallel to that stimulus. The relationship of perceived stress and disturbed sleep deserves further clarification, perhaps with a study providing an intervention aimed at stress or at sleep alone.

One limitation of the current study is that several indices were measured using subjective self-reports. Self-reported data included perceived stress levels, sleep quality, daytime sleepiness, and fatigue. However, the tools utilized to gather these indices were validated instruments with known performance characteristics and some of the data sought have no alternative ways of being measured. It may be useful in future studies to utilize objective measures such as a polysomnogram instead of the Berlin Questionnaire for sleep apnea and actigraphy as an objective measure of sleep quantity. Furthermore, strength of the current study is that actual measurements of height, weight, and waist circumference were used in place of self-reported values.

Our finding of correlation of perceived stress levels with sleep disruption adds to the growing body of evidence that stress may play an important role as a risk factor for CVD. Certainly the evidence to date is

worthy of follow-up studies. A justifiable next study could examine the impact of stress management strategies and sleep improvement on incident CVD. Assessing maladaptive behaviors and physiological abnormalities associated with stress may allow for targeted intervention to promote vascular health.

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## Stress Therapy Empowering Prevention (STEP): A Healthy Lifestyle Program for Breast Cancer Patients

By Amy M. Burke, RN, BSN

*Joyce Murtha Breast Care Center at Windber Medical Center, Windber, PA*

Darrell L. Ellsworth, PhD

*Windber Research Institute, Windber, PA*

Col (Ret) Marina N. Vernalis, DO, FACC

*Walter Reed National Military Medical Center*

*Integrative Cardiac Health Project (ICHP), Bethesda, MD*

**Purpose:** Develop and implement a comprehensive program for lifestyle change, empowering breast cancer patients to manage stress effectively and improve their mental and physical health.

**Method:** Women with breast disease (or those at high risk) are offered a program of lifestyle change, consisting of a Healthy Lifestyle intervention for 3 months followed by monthly contact with a health coach. Instruction and demonstration provide information on exercise, nutrition, stress reduction, and mind/body health. Examinations are conducted at baseline, after completion of the intervention (3 months), at 1 year, and every 6 months for a period of 5 years.

**Conclusion:** Breast cancer has a significant emotional, psychological, and social impact and is often associated with high levels of stress that promote unhealthy behaviors causing weight gain, decreased physical fitness, and an increased risk for cardiovascular disease (CVD). Similar to CVD, research shows breast cancer susceptibility is also influenced in part by modifiable risk factors, suggesting that a healthy lifestyle program may lead to reductions in cancer risk and recurrence as well as improvements in mental health and quality of life. Through the Stress Therapy Empowering Prevention (STEP) program, breast cancer and high-risk patients are empowered with tools to focus on health promotion and optimization and maintenance of quality of life. Patients can improve physical and psychosocial factors in as little as 3 months, but long-term follow-up will determine if lifestyle changes result in improved clinical outcomes over time.

Extensive reports have documented the relationship between lifestyle changes and morbidity/mortality associated with cardiovascular disease (CVD). In particular, diet, physical activity, and stress are known to be associated with cardiovascular morbidity and mortality.<sup>1,3</sup> Similar to CVD, evidence has been mounting that breast cancer susceptibility is influenced in part by modifiable risk factors, such as body weight, diet, and physical activity, suggesting that a healthy lifestyle program may lead to a reduction in risk factors for CVD and breast cancer. Improving health and quality of life in patients with CVD and breast cancer will result in improved outcomes of care over the long term.

Early diagnosis and treatment are still vital to surviving breast cancer. Although an estimated 192,370 new cases of invasive breast cancer were expected in 2009, with approximately 40,170 deaths from the disease, incidence rates actually decreased by 2.0% per year,<sup>4</sup> likely due to

advanced screening and early detection. In an effort to continue to lower incidence rates and improve long-term outcomes, studies of behavior modification in breast cancer patients are providing new information about how lifestyle factors affect survivorship as well as knowledge to help develop new, effective intervention programs to decrease breast cancer risk.<sup>5-11</sup>

The Stress Therapy Empowering Prevention (STEP) program is an innovative approach based on the concept that comprehensive lifestyle changes may have a meaningful impact on the risk for developing breast and cardiac disease. Given the advantages of a healthy lifestyle on both physical and emotional outcomes, cancer patients as well as those at high risk should be urged to address unhealthy behaviors. Our STEP model utilizes a specialized team comprising physicians, nurses, dietitians, licensed therapists, exercise physiologists, and stress management specialists who provide comprehensive strategies

that empower the participant to make healthier choices at an individual level. The program is an adjunct to treatment and care that participants receive from their personal healthcare providers. This combined effort allows for closer monitoring of each participant and coordination of care across the healthcare spectrum to achieve optimal health and quality of life.

## METHODS

The overall goal is to recruit and evaluate approximately 500 women diagnosed with, or at high risk for, both breast and cardiac disease. The objectives of the study are to 1) test the efficacy of a healthy lifestyle intervention on reducing stress, sleep disturbances, and cardiovascular risk factors in both high-risk patients and patients diagnosed with breast disease; 2) evaluate the long-term benefit of an enhanced health coach intervention in promoting sustained wellness behaviors; and 3) examine molecular markers common to atherosclerosis and cancer to assess longitudinal changes and their relationship to disease development.

The STEP program has a 3-month healthy lifestyle intervention period during which participants meet once a week to learn the program guidelines, which include a low-fat, whole food nutrition plan based on the Mediterranean diet, aerobic and strength training exercises, stress management, and weekly mind/body health sessions. After the initial 3-month period, participants are contacted on a monthly basis by a health coach to ensure that program compliance is being maintained and to assist with long-term adherence. Participants are required to return to the center at the 1-year time point, and every 6 months thereafter for a period of 5 years, for testing and evaluation. Information collected includes perceived stress, sleep disturbance, psychosocial measurements, carotid ultrasound to measure carotid intima-media thickness, traditional risk factors (weight, blood pressure, body mass index [BMI], body composition), and biochemical assays.

To be eligible to participate, women must be 18 years of age or older with a diagnosis of breast disease (atypical hyperplasia, in situ carcinoma, or invasive breast cancer) or significant risk factors for developing breast disease such as previous biopsy, family history of breast disease, first pregnancy after the age of 30, early menstruation or

late onset of menopause, or high risk of developing coronary artery disease (CAD) as indicated by having 1 or more of the following: family history of CAD, hypertension, diabetes, smoking, elevated blood lipids, sedentary lifestyle and obesity, or established clinically stable coronary disease.

Participants begin the program with an extensive physician visit to conduct a comprehensive risk assessment and develop a realistic lifestyle change plan. Participants are interviewed to assess sleep patterns, smoking status, cardiovascular and breast history, and medication use. The clinical exam includes height and weight measurements to calculate BMI ( $\text{kg}/\text{m}^2$ ); blood profiles including thyroid-stimulating hormone, comprehensive metabolic panel, and fasting glucose and lipid panel; systolic and diastolic blood pressures; and psychological screening to evaluate mental health. Assessments are repeated at the end of the Healthy Intervention, at year 1, and every 6 months thereafter for a period of 5 years.

**“Our STEP model utilizes a specialized team comprising physicians, nurses, dietitians, licensed therapists, exercise physiologists, and stress management specialists...”**

Following the initial examinations, participants attend an educational workshop designed to provide further instruction regarding the recommended lifestyle changes, followed by once-a-week sessions over a 3-month period. These sessions are tailored to ensure that each individual receives the appropriate education and experience needed to achieve success. Participants are required to complete a personal awareness log each week, which includes documentation of diet, exercise, and stress management frequency and duration, and a self-report of their mind/body session experience.

Blood samples are obtained from each consenting individual at baseline, at completion of the healthy lifestyle intervention, at 1 year, and every 6 months thereafter for a period of 5 years. From the blood samples, the following biochemical assays are analyzed: 1) lipoprotein subclass distributions determined by nuclear magnetic reso-

nance (NMR) spectroscopy; 2) stress/CVD biomarker panel: serum cortisol, insulin, leptin, highly sensitive C-reactive protein, lipoprotein(a), adiponectin, resistin, serum amyloid A, and vitamin D; and 3) breast disease-related panel: HER2/*neu*, tumor necrosis factor (TNF) alpha, and estradiol. In addition, blood is collected for isolating messenger RNA to determine changes in gene expression over the course of the study and identify new molecular markers associated with improved CVD biomarker risk profiles.

**“Upon completion of the healthy lifestyle intervention (3 months), participants (n = 14) showed change in the desired direction for many risk factors.”**

#### RESULTS

Recruitment is being conducted primarily through newspaper and radio ads; distribution of patient information brochures; and speaking engagements at various community education events, physician offices, and support groups. Of 43 women who initially expressed interest in the program, 18 have enrolled thus far. Average age of participants was 65 years. Of the 18 participants enrolled in the program, 11 women had diagnosed breast disease (61%). In addition, of these same 18 women, 17 (94%) were also considered at high risk for developing CVD by having at least 1 documented CAD risk factor. Overall attendance was 88% during the initial 3-month on-site sessions. Four participants (22%) discontinued participation in the program, 3 due to personal, nonmedical reasons, and 1 due to breast cancer progression.

Upon completion of the healthy lifestyle intervention (3 months), participants (n = 14) showed change in the desired direction for many risk factors. Body weight (-1.8%,  $P < .05$ ), BMI (-2.5%,  $P < .05$ ), and perceived stress (-22.1%,  $P < .05$ ) decreased significantly. Diastolic blood pressure (-8.4%,  $P < .08$ ) and sleep quality (-26.5%,  $P < .06$ ) showed near-significant changes. Most importantly, at the 1-year time point, perceived stress (n = 10, 8.2%,  $P < .05$ ) and sleep quality (n = 9, -4.9%,  $P < .05$ ) improvements were maintained, showing that these positive changes could

be maintained over a longer period of time. In addition, though lacking statistical significance with our current sample size, triglycerides, systolic blood pressure, hostility, and depression all decreased at both time points (Table).

Based on self-reported exercise frequency and duration data, at 3 months participants on average were able to increase vigorous activity (heavy lifting, digging, aerobics, or fast bicycling) by 1.13 days/week, moderate activity requiring the participant to breathe somewhat harder than normal (carrying light loads or bicycling at a regular pace) by 1.56 days/week, and walking activity (including walking at work or home for recreation, sport, exercise, or leisure) by 1.63 days/week. At the 1-year time point, participants continued to show increased levels of activity for all measured categories; vigorous activity remained increased by 1.13 days/week, moderate activity by 1.13 days, and walking activity by 0.82 days when compared with baseline activity.

Lipoprotein subclass profiles will be assessed by NMR spectroscopy, which will quantify low-density lipoprotein particle number and size, and provide direct measurement of high-density lipoprotein and very low-density lipoprotein subclasses. Biochemical variables of interest regarding CVD risk, including insulin, leptin, lipoprotein(a), adiponectin, resistin, serum amyloid A, and TNF alpha will permit correlation of traditional CVD risk factors with nontraditional biomarkers to provide more information on the prevention and treatment of CVD. Vitamin D, HER2/*neu*, and estradiol will be analyzed to provide further insight into breast disease development and progression. Lower serum 25 (OH) D (vitamin D) concentrations may be associated with poorer overall survival and distant disease-free survival in postmenopausal breast cancer patients.<sup>12</sup> HER2/*neu* blood levels have potential as a tumor marker in breast cancer. Many studies have monitored circulating levels after surgery and reported that increasing HER2/*neu* levels can indicate recurrence of breast cancer earlier than clinical diagnosis.<sup>13,14</sup> Estrogens are believed to play a critical role in the etiology of breast cancer, and considerable evidence suggests that lifetime exposure to endogenous hormones, notably estrogens, promotes breast carcinogenesis.<sup>15</sup> Finally, cortisol levels, considered a major indicator of altered psychological states in response to stress, may provide information on short- and long-term stressors.<sup>16</sup>

| Outcome                        | Baseline | 3 Months | %Δ    | P*   | 1 Year | %Δ    | P*   |
|--------------------------------|----------|----------|-------|------|--------|-------|------|
| Weight                         | 180.5    | 177.3    | -1.8  | <.05 | 175.6  | -2.7  | .10  |
| Body mass index                | 32.5     | 31.7     | -2.5  | <.05 | 31.5   | -3.1  | .38  |
| Total cholesterol              | 201.1    | 200.2    | -0.5  | .90  | 203.4  | +1.1  | .73  |
| Triglycerides                  | 157.2    | 136.7    | -13.0 | .17  | 147.6  | -6.1  | .52  |
| Systolic blood pressure        | 134.0    | 125.1    | -6.6  | .15  | 126.4  | -5.7  | .23  |
| Diastolic blood pressure       | 79.6     | 72.9     | -8.4  | .08  | 76.3   | -1.3  | .28  |
| Glycosylated Hgb               | 6.4      | 6.6      | +3.1  | .34  | 6.2    | -3.1  | .30  |
| Fasting glucose                | 108.6    | 110.6    | +1.8  | .75  | 112.4  | +3.5  | .51  |
| Depression                     | 13.7     | 11.3     | -17.5 | .23  | 8.5    | -38.0 | .11  |
| Hostility                      | 6.4      | 4.8      | -25.0 | .16  | 5.7    | -10.9 | .33  |
| Perceived stress               | 16.3     | 12.7     | -22.1 | <.05 | 11.7   | -28.2 | <.05 |
| Pittsburgh sleep quality index | 10.2     | 7.5      | -26.5 | .06  | 9.7    | -4.9  | <.05 |

\* P value based on repeated-measures analysis of variance.

## DISCUSSION

There are no proven substitutes for conventional cancer treatments such as surgery, chemotherapy, radiation, and immunotherapy; however, one approach to gaining a better understanding of how lifestyle change can enhance breast cancer survival is to develop studies that address several behavior and lifestyle factors within the same program. Research has shown that among women with breast cancer who had surgery and conventional treatment, those who learned to change their lifestyle through education focused on better nutrition, more exercise, and stress reduction were 68% less likely to die from disease over an 11-year period than those who did not.<sup>17</sup> Although the STEP study currently lacks long-term follow-up data, our program is examining the importance of helping breast cancer patients eat better, lose weight, improve strength and endurance, develop coping skills, and ultimately to improve their overall health and well-being. Participants in a STEP-style program feel better, both physically and emotionally. These observations suggest that the program has potential to improve their long-term overall risk profiles.

An important finding in our study was the struggle encountered in recruiting participants into the program. Obstacles to recruitment included out-of-pocket expenses, lack of local physician referrals, participant time constraints, and lack of knowledge among patients about the benefits of lifestyle change on quality of life or clinical outcomes. However, once women made

the commitment to participate, surveys indicated a high degree of satisfaction with the program. Ultimately, issues encountered with recruitment affected our sample size, leading to difficulties in being able to effectively interpret preliminary data. In the future, we will continue to use best clinical judgment on when to approach appropriate patients based on past experience, to repeatedly offer to assist patients with addressing risk factors, and to educate healthcare providers about the STEP program to increase our sample size and provide additional data for analysis of the effects of lifestyle change on breast disease.

## NUTRITION

Although the relationship between diet and breast cancer remains unclear, studies have shown that improved nutrition reduces the risk of several chronic diseases, such as obesity, diabetes, and heart disease, and that a healthy lifestyle improves overall quality of life.<sup>18,19</sup> Breast cancer patients who practice better nutrition are likely to derive benefit in terms of total mortality, similar to the general population. The Women's Healthy Eating and Living study showed that women who consumed a healthy diet and were physically active increased survival after diagnosis.<sup>20</sup> Patients who reported eating at least 5 servings of fruits and vegetables per day and performing 30 minutes of moderate walking 6 days a week reduced the probability of death by 50%.

The STEP program nutrition plan is based on

the Mediterranean diet and recommends eating vegetables; fruits; whole grains; lean protein sources such as fish, nuts, and olive oil; and minimizing the amount of red meat consumed. Participants are counseled to focus on eating more naturally occurring and fewer highly processed foods. Involvement of a registered dietitian helps to guide this process and provides the education, support, and long-term follow-up needed to meet the challenges of sustaining the recommended dietary changes.

The majority of studies of diet and breast cancer have examined the impact of body weight on survival. Most have observed that obesity at diagnosis is associated with poor prognosis.<sup>21</sup> Similarly, weight gain after diagnosis is common and is associated with mortality, disease recurrence, and development of comorbid conditions including diabetes and CVD.<sup>22</sup> Although some studies have shown that following a prudent diet alone, without adding physical activity, may not be associated with breast cancer survival,<sup>5,23</sup> a healthy diet has been shown to have beneficial effects on overall survival in conditions such as diabetes and heart disease, which are frequently seen in breast cancer patients.<sup>24</sup>

Participants in the STEP program were able to significantly decrease measures of obesity such as weight and BMI within the first 3 months of the program. Although these measures were not statistically significant at 1 year, they continue to remain lower than at baseline, suggesting that participants were successful in meeting or exceeding dietary compliance targets, thus preventing weight gain and promoting weight loss, which has been proven to be an effective strategy for improving overall quality of life and survival.

## EXERCISE

Physical activity is as important as diet for achieving optimal weight and maintaining a healthy lifestyle. In studies examining the relationship between physical activity and the risk of breast cancer, a decrease in risk of approximately 25% was found among the most physically active women.<sup>25</sup> Similarly, in studies examining the effect of physical activity on breast cancer survival, some studies suggest that postdiagnosis physical activity may have great benefit. One study showed that after diagnosis, physical activity equivalent to walking 3 to 5 hours per week reduced mortality by as much as 50%.<sup>26</sup> Although

the risk of developing comorbid conditions, including CVD, type 2 diabetes, fatigue, lymphedema, psychological distress, and poor quality of life, often persists in breast cancer survivors, recent studies have shown that physical activity can lower breast cancer risk and provide additional health benefits, such as decreased risk of stroke and type 2 diabetes, and improved longevity and quality of life.<sup>27</sup>

Most STEP participants achieved improvement in physical activity during the initial 3-month period, and many maintained these initial gains or continued to improve by the end of the first year. While most research demonstrates beneficial effects between physical activity and overall health, it is important to recognize that there is a risk-benefit ratio to exercise that may be different for each breast cancer patient. Utilizing a personalized plan might be most effective because it can be customized for different time periods, from prediagnosis through cancer treatment, based on individual needs and abilities. The STEP program develops each participant's activity plan based on an individual assessment completed by an exercise physiologist, but generally participants are encouraged to exercise aerobically for a minimum of 30 minutes per day, for a total of 3 hours of aerobic exercise each week. More intense exercise is permitted if medically appropriate and desired by the participant. Resistive or strength training exercise also is important, and if medically appropriate, participants were instructed to engage in strength training exercises 2 to 3 times per week. During the healthy lifestyle intervention portion of the study, hour-long supervised exercise sessions were scheduled.

The objectives of our exercise modality are to fully understand the importance and benefits of regular physical activity, to create a safe environment for exercise, and to encourage participants to properly monitor their own exercise program outside of the STEP program. These activities will assist with long-term adherence and allow the participant to achieve her own physical activity goals.

## STRESS MANAGEMENT

Working with participants in the STEP program presents some unique challenges. These women have faced their mortality and live with the ongoing psychological stress of possible cancer recurrence.<sup>28</sup> A recent meta-analysis of 10 randomized controlled trials found that cancer patients who

participated in yoga interventions showed significant improvement in several psychological measures, including anxiety, distress, depression, and stress compared with wait-list controls.<sup>29</sup> For breast cancer survivors in particular, yoga has been shown to improve quality of life and emotional functioning.<sup>30</sup>

A mild form of physical activity, such as yoga or tai chi, may help to promote regular participation in physical activity. The therapeutic application of yoga enables participants to move slowly and safely, concentrating on relaxing their body while building flexibility, strength, and balance, which is especially important in breast cancer patients who may face additional barriers to more vigorous physical activity.<sup>31</sup> As emotional stress has been associated with decreased survival in breast cancer patients,<sup>32</sup> possibly by muting immune functions and accelerating the inflammatory response, stress management may offer a real survival advantage to cancer patients in addition to emotional benefits.

The STEP program's stress management specialist is a certified yoga therapist trained in techniques to provide participants with healthier ways to deal with the stress of living with a potentially life-threatening disease. The practice of yoga relies on physical postures to stretch muscles, focused breathing and meditation to minimize stress through visualization techniques, and guided imagery. Throughout the initial intervention, stress management sessions are held once a week. During these sessions, participants receive education and training in performing these techniques. The result is a relaxed body and a peaceful state of mind. Daily stress management practice was encouraged in the STEP program so that these techniques would be routine when patients are faced with a stressful situation.

#### MIND/BODY HEALTH

Women with breast cancer often exhibit emotional distress similar to posttraumatic stress disorder (PTSD).<sup>33,34</sup> In a recent study, among women who were recruited an average of 47 months following diagnosis of breast cancer, 38% had moderate to high anxiety, 22% had moderate to high depression, and PTSD was observed in 12%.<sup>35</sup> These findings show that the emotional impact of breast cancer can last for years following diagnosis. In addition, women lacking a social

network had a significantly higher risk of breast cancer mortality than women with strong social ties to relatives, friends, and neighbors. Breast cancer patients often experience social isolation due to treatment, body image issues, or fatigue, which can have significant detrimental effects on psychological well-being by increasing levels of anxiety and depression. Therefore, it is important to recognize the signs of psychological distress in breast cancer patients and develop programs that effectively manage stress and mental health.<sup>36</sup>

The mind/body sessions in the STEP program are facilitated by a licensed therapist. These sessions are designed to create an atmosphere in which participants feel comfortable expressing their feelings and personal experiences. Since all STEP participants share common ground, individuals who self-disclose their experiences in dealing with breast disease encourage other participants to share their experiences as well. The overall purpose of the mind/body session is to create an environment where participants can experience belonging and the feeling of being connected. It is important to understand that these sessions are not group therapy—they are intended to facilitate making and sustaining healthy behaviors every day. Most of us know what we need to do to lead healthier lifestyles, but change is difficult to attain and sustain without ongoing support. This component upholds accountability, and the participants come to depend on each other for ongoing support.

#### CONCLUSION

In summary, lifestyle change interventions have proven to be beneficial to the vast majority of participants, but there are a limited number of studies that have examined the effect of combining several lifestyle behaviors into one comprehensive program to benefit breast cancer patients. The STEP program is a pioneer program that has combined the efforts of conventional treatment regimens with simple lifestyle changes, empowering breast cancer patients to actively manage their disease. As well-powered randomized controlled trials continue to define the effectiveness of lifestyle modification, hopefully more comprehensive programs will become available and eventually translate into improved care for breast cancer patients.

## ACKNOWLEDGMENTS

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## Novel Stress Reduction Technique Improves Sleep and Fatigue

Mariam Kashani, Arn Eliasson, Karla Bailey, Marina Vernalis

**Purpose:** A growing body of evidence substantiates the important roles of stress and sleep in cardiovascular disease. We sought to determine the effect of a brief, portable stress reduction technique, the ten-minute Tension Tamer on improvement of stress levels and sleep parameters in a heart health program.

**Methods:** Adult men and women self-referred or referred to the Integrative Cardiac Health Project were assessed for levels of perceived stress and sleep quality using validated surveys. Subjective stress was measured using the Perceived Stress Scale (PSS14, total possible points 56); sleep quality was evaluated with the Pittsburgh Sleep Quality Index (PSQI, total possible points 21); fatigue was assessed using the 10 point fatigue scale. After a 30-minute introductory workshop, subjects were given instruction and guided opportunities to practice ten-minute Tension Tamers over the course of four 30-minute visits with a stress management specialist. This brief technique, encouraged at bedtime, involves deep breathing and imagery using the subject's personal preference. Upon completion of the four visit practice sequence, validated surveys were reassessed and compared with baseline values using t-tests.

**Results:** Of 334 subjects (mean age 55.7 years, 135 men, 200 Caucasian, 117 African-American, 14 Latino, 3 other), 218 (65%, mean age 56.6 years, 40% men) improved their perceived stress by 6.6 points ( $p < 0.001$ ) using the Tension Tamer technique. Non-improvers, 116 subjects (34%, mean age 59.7, 41% men) showed worsened stress levels by 4.6 points. Comparing Improvers with Non-Improvers showed significant differences in sleep quality (PSQI improved 1.78 vs worsened 0.89 points,  $p < 0.001$ ), decreased sleep latency (decreased 4 vs increased 1.9 minutes,  $p = 0.04$ ), and decreased fatigue (decreased 0.89 vs increased 0.27 points,  $p < 0.001$ ).

**Conclusion:** A novel stress reduction technique, the ten-minute Tension Tamer, can reduce perceived stress levels in a majority of subjects resulting in improved sleep quality, decreased sleep latency and improved fatigue.

**Clinical Implications:** Using a portable stress reduction technique in short intervals may be a unique approach to improve cardiovascular risk through sleep improvement.

Citation: Kashani M, Eliasson A, Bailey K, Vernalis M. Novel stress reduction technique improves sleep and fatigue. *Chest* 2012;142(4\_MeetingAbstracts):1052A.

## Fatigued On Venus, Sleepy On Mars?

Arn Eliasson, Mariam Kashani, Marina Vernalis

Integrative Cardiac Health Project  
Walter Reed National Military Medical Center  
Bethesda, Maryland

### Rationale

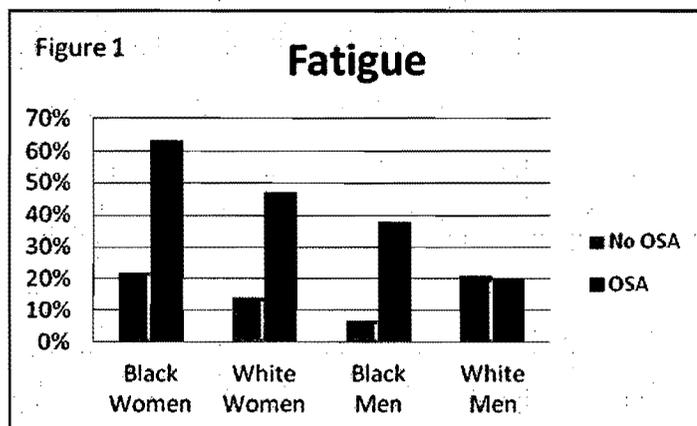
Subjective sleepiness and fatigue are recognized as separate symptoms which may occur singly, together, or may both be absent in subjects with obstructive sleep apnea (OSA). The inter-individual experiences of sleepiness and/or fatigue have recently been shown to be stable and trait-like with potential genetic causes. We sought to examine the vulnerabilities for sleepiness and fatigue in subjects with and without sleep apnea with special attention to the role of gender and race.

### Methods

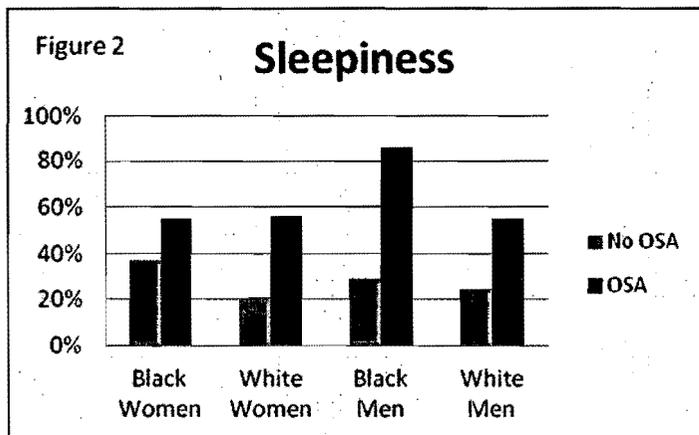
Consecutive subjects entering our heart health program completed a series of validated questionnaires. Thyroid function was tested in every subject. Sleepiness was defined by Epworth Sleepiness Scale  $\geq 10$  of 24 points. Fatigue was defined by the Stanford Fatigue Visual Analog Scale  $\geq 5$  of 10 points. The Berlin Questionnaire identified subjects as high or low likelihood for OSA. The two groups were compared using Fisher's exact test and two sample t-test as appropriate. For data analysis by race, comparisons were limited to White and Black categories as there were too few subjects for other comparisons.

### Results

Of 295 consecutive subjects, 172 women (58%), there were 172 Whites, 105 Blacks, 13 Hispanics, 2 Asians and 3 others, with average age  $57.4 \pm 12.7$  years. Sleepiness was found in 129 subjects (44%) and fatigue in 90 subjects (31%). Berlin Questionnaires identified 159 subjects (54%) as high likelihood for OSA. There was no difference in thyroid function between subjects with and without a positive Berlin score ( $p=0.52$ ). Without OSA present, numbers of subjects with fatigue were similar in women (15%) and men (20%),  $p=0.63$ . With OSA, fatigue was much more common in women (57%) compared to men (26%),  $p<0.001$ .



For sleepiness, there was no significant difference between the genders,  $p=0.43$ , but Black men did demonstrate a significant increase in subjects with sleepiness when comparing those with no OSA (29%) to those with OSA (86%),  $p=0.05$ .



**Conclusions**

Symptoms of fatigue and sleepiness are reported with different prevalence according to gender and race. Overall, women report fatigue more commonly in association with OSA than men. Black men experience sleepiness more commonly with OSA than other groups. These differences are not related to thyroid function. These findings deserve explanation with research that incorporates an objective measure of sleepiness and includes a broader range of variables such as the effects of total sleep time, co-morbid conditions, and medications.

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Citation:

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## Novel Tool Improves CV Risk Stratification and Guides Therapy

Mariam Kashani MS, CRNP, Arn Eliasson MD, Karla Bailey BS, RDMS,  
Marina Vernalis DO

**Background:** Accurate risk assessment is of critical importance to any cardiovascular (CV) disease prevention program. Risk stratification tools enable providers to implement appropriate therapies.

**Objective:** We sought to compare the performance of the Framingham Risk Score (FRS) with a CV Score previously validated by the Integrative Cardiac Health Project (ICHP) in a cohort of subjects with known subclinical atherosclerotic disease by abnormal carotid intima-media thickness (CIMT) measurement.

**Methods:** Consecutive subjects (n=93) identified with subclinical atherosclerosis by abnormal CIMT ( $\geq 75^{\text{th}}$  percentile by age/gender) were enrolled in a 6-month CV risk reduction program. Subjects were assessed for past medical history, family history of CV events, anthropometrics and a cardiac-relevant lab panel. FRS and ICHP CV Risk Score were calculated for each individual and were compared. The ICHP CV Risk Score incorporates additional factors such as family history of CV events as well as novel risk factors. All scores were categorized as low, medium and high for CV risk.

**Results:** In 93 consecutive subjects, mean age was  $53.1 \pm 11.13$  yrs, 59% women, 47% African-American, 46% Caucasian, 3% Latina, 1% other. Diagnosis of diabetes was present in 13% of subjects. Means: BMI= $31.2 \pm 5.3$  kg/m<sup>2</sup>, WC= $100.2 \pm 13.6$  cm, fasting glc= $99.1 \pm 35.9$  mg/dL, insulin  $15.6 \pm 14.4$  ug/dL, Tchol= $194.9 \pm 42.3$  mg/dL, LDL  $114.5 \pm 34.0$  mg/dL, HDL  $56.2 \pm 18.5$  mg/dL, TG  $117.3 \pm 66.3$  mg/dL, Lp(a)= $86.5 \pm 92.3$  mg/dL, CRP  $0.4 \pm 0.6$  mg/dL.

By FRS, 12 (14%) subjects scored high risk, 11 (12%) scored medium and 70 (75%) scored low risk. By ICHP CV Risk Score, 4 (36%) of the FRS medium upscored to high risk and 47 (67%) of the FRS low upscored to medium risk. In total, 63% upscored to an appropriately higher risk category by using the ICHP CV Risk Score.

**Conclusion:** In a population with documented subclinical atherosclerosis and unremarkable conventional risk factor profiles, the ICHP CV Risk Score appeared to be more sensitive in identifying subjects at risk. The ICHP CV Risk Score may be a more discerning tool to guide risk reduction therapy in a prevention program.

Citation: Kashani M, Eliasson A, Bailey K, Vernalis M. Novel tool improves CV risk stratification and guides therapy. *Circ Cardiovasc Qual Outcomes* 2011;4(6):AP88.

## Prediabetics Improve CV Risk Profile by Reducing Stress

Mariam Kashani MS, CRNP, Arn Eliasson MD, Marina Vernalis DO

Integrative Cardiac Health Project  
Henry M. Jackson Foundation for the Advancement of Military Medicine  
Walter Reed Army Medical Center, Washington, DC

**Background:** High stress levels trigger a negative cascade of hormones mediated in part by cortropin-releasing factor, CRF. The effect of CRF on cortisol is well known but recent science has demonstrated its pivotal role on insulin levels as well. Currently, pharmacologic agents are being developed to assist patients with this proposed hyperinsulinemia.

**Objective:** We sought to examine the effect of stress along with its hormonal mediators on a prediabetic population.

**Methods:** Subjects entering a 6-month cardiovascular (CV) risk reduction program (to improve sleep, exercise, nutrition and stress) completed questionnaires including the Perceived Stress Scale (PSS), anthropometric measures and a cardiac-relevant lab panel. Differences between subjects with high stress (PSS $\geq$ 23) and those with low stress (PSS $<$ 23) were analyzed by t-test.

**Results:** Of 24 prediabetic subjects, 12 (50%) scored high on the PSS (mean 29.5 $\pm$ 4.9 vs 18.4 $\pm$ 3.3). These high-stress subjects demonstrated higher mean insulin (20.6 $\pm$ 11.7 vs 10.8 $\pm$ 4.2, p $<$ 0.01), higher insulin resistance, as demonstrated by HOMA, (5.3 $\pm$ 2.9 vs 2.8 $\pm$ 1.2, p $<$ 0.01) and greater percent body fat (38.8 $\pm$ 7.6 vs 30.7 $\pm$ 8.5, p $<$ 0.02), than their low-stress counterparts. There were no differences in glucose or weight between the two groups at baseline.

| Change at 6 mo  | Low stress n=12 | High stress n=12 | p value  |
|-----------------|-----------------|------------------|----------|
| PSS             | 1.4 $\pm$ 8.3   | -14.4 $\pm$ 9.3  | $<$ 0.01 |
| Insulin (ug/dL) | 3.4 $\pm$ 7.7   | -11.8 $\pm$ 11.8 | $<$ 0.01 |
| CRP (mg/dL)     | 0.3 $\pm$ 0.41  | -0.2 $\pm$ 0.7   | $<$ 0.03 |

**Conclusion:** High stress correlates with numerous unhealthy metabolic states which place patients at higher risk for CV disease. Prediabetic patients can significantly improve their CV risk profile by reducing stress. We hypothesize that an integrative lifestyle change program may interrupt the negative sequence of events caused by CRF and potentially provide prediabetic patients an adjunct to their CV risk reduction action plan.

**Citation:** Kashani M, Eliasson A, Vernalis M. Prediabetics improve CV risk profile by reducing stress. American Heart Association—Quality of Care and Outcomes Research, Washington DC, 19 May 2010, poster presentation

## Racial Differences in Perceived Stress, Sleep Habits, and Daytime Symptoms

Arn Eliasson MD, Mariam Kashani CRNP, Jacqueline Hoffman MA, Marina Vernalis DO

**Introduction:** Racial disparities are important to understand in order to design effective programs for evaluation and intervention. We hypothesized that important racial differences exist in subjects enrolling in a heart health program.

**Methods:** The Integrative Cardiac Health Project (IChP) is a heart health program that includes goals of improving sleep and stress management. At program entry, participants complete validated questionnaires, specifically the Berlin Questionnaire for sleep apnea, Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), fatigue visual analog scale (FVAS) and the Perceived Stress Scale. Subjects also submit to anthropomorphic measures and a cardiac-relevant lab panel. Differences between whites and blacks were compared using unpaired t-test and Wilcoxon rank sum test (2-tailed) as appropriate.

**Results:** Of 350 consecutive subjects (mean age 55.1 yrs, 28% men), there were 133 white (38%), 105 black (30%), 90 mixed race/undeclared, 14 Latino, and 8 others. For this analysis, only white and black subjects were considered. White subjects were somewhat older ( $57.4 \pm 12.6$  yr vs  $52.1 \pm 12.4$ ,  $p=0.001$ ) and included more men (47% v 34%,  $p=0.04$ ). BMI was similar between groups ( $29.5 \pm 5.1$  kg/m<sup>2</sup> vs  $30.6 \pm 6.6$ ,  $p=0.18$ ). White subjects had lower perceived stress (PSS= $19.4 \pm 9.6$  vs  $23.6 \pm 6.8$ ,  $p<0.001$ ), better sleep quality (PSQI= $6.1 \pm 4.1$  vs  $7.1 \pm 3.9$ ,  $p=0.05$ ), and less daytime sleepiness (ESS= $8.0 \pm 4.9$  vs  $9.8 \pm 5.0$ ,  $p=0.01$ ). White subjects tended to have less fatigue (FVAS= $3.9 \pm 2.5$  vs  $4.5 \pm 2.4$ ,  $p=0.08$ ) and longer sleep duration (20 min longer per night,  $p=0.07$ ). However, there was no difference in sleep latency (24.4 min vs 23.0,  $p=0.85$ ) or likelihood for sleep apnea (Berlin positive 44% vs 51%,  $p=0.40$ ).

**Conclusions:** There are important differences in levels of perceived stress, sleep quality and daytime sleepiness between white and black subjects in our program. These differences deserve explanation and may be valuable in designing interventions tailored for specific groups.

Citation: Eliasson A, Kashani M, Hoffman J, Vernalis M. Racial differences in perceived stress, sleep habits, and daytime symptoms. *Sleep* 2011; 34:A262.

## The Berlin Questionnaire Identifies a Population with Traits Inhibiting Adherence

Arn Eliasson MD, Mariam Kashani MS, CRNP, Karla Bailey BS, RDMS,  
Marina Vernalis DO

Walter Reed Army Medical Center and Jackson Foundation for the Advancement of  
Military Medicine, Washington, DC

**Rationale:** As part of a cardiovascular (CV) risk assessment, we screen for sleep apnea using the Berlin Questionnaire. To enhance outcomes of therapies aimed at reducing CV risk it is important to assess for traits that affect adherence to those therapies. We hypothesize that while the Berlin Questionnaire successfully screens for patients with sleep apnea and detects a population at higher risk for CV disease, the survey tool may also identify a population at risk for traits that compromise adherence to therapies for CV risk improvement.

**Methods:** Consecutive subjects entering our CV disease prevention program completed validated surveys including Berlin Questionnaire for Sleep Apnea and Perceived Stress Scale (PSS). Data collection also included a carotid intima-medial thickness (CIMT) and a CV-relevant lab panel. Differences between subjects scoring positive and those scoring negative for sleep apnea by Berlin Questionnaire were analyzed by t-test and correlations were sought using the Pearson product-moment correlation coefficient.

**Results:** Of 126 consecutive subjects, demographic data showed a mean age of  $51.2 \pm 13.9$  years, 55 men (44%), and racial diversity (64 Caucasian, 49 African-American, 7 Latino, 2 Asian, and 4 undeclared). Fiftysix subjects (44%) scored a high likelihood of sleep apnea on the Berlin Questionnaire and their CV health assessments demonstrated a higher risk profile:

|                    | Mean CIMT mm      | HDL mg/dL       | TG mg/dL         | HgbA1C %      | HOMA          | Dx Dep/Anx | PSS            |
|--------------------|-------------------|-----------------|------------------|---------------|---------------|------------|----------------|
| Berlin Neg<br>n=56 | 0.718 $\pm$ 0.144 | 60.6 $\pm$ 18.2 | 95.0 $\pm$ 47.6  | 5.7 $\pm$ 0.4 | 1.8 $\pm$ 0.9 | 11%        | 19.3 $\pm$ 6.9 |
| Berlin Pos<br>n=70 | 0.794 $\pm$ 0.153 | 50.5 $\pm$ 14.1 | 127.0 $\pm$ 71.6 | 6.2 $\pm$ 1.4 | 3.4 $\pm$ 3.3 | 28%        | 23.6 $\pm$ 8.6 |
| t-test p           | 0.005             | 0.001           | 0.005            | 0.01          | 0.002         | 0.02       | 0.003          |
| Pearson r          | 0.25              | -0.30           | 0.25             | 0.22          | 0.31          | 0.21       | 0.27           |

There were no differences between groups for other traditional risk factors such as total cholesterol (p=0.43), LDL cholesterol (p=0.08), lipoprotein (a) (p=0.63), and CRP (p=0.12).

**Conclusions:** The Berlin Questionnaire does help identify a population of subjects at

greater risk for CV disease as well as traits of anxiety and perceived stress that may diminish the patients' ability to comply with CV risk reduction therapy. An important implication is that sleep apnea therapy should be implemented not only to improve CV risk but to aid the management of anxiety trait and perceived stress trait with the goal of enhancing adherence to behavior change and improving overall quality of life.

**Journal Citation:** Eliasson A, Kashani M, Bailey K, Vernalis M. The Berlin questionnaire identifies a population with traits inhibiting adherence. *Am J Respir Crit Care Med* 2011; 183:A1444

## Reducing Perceived Stress Improves Sleep Quality—A Longitudinal Outcomes Study

Arn Eliasson MD, Mariam Kashani CRNP, Maren Mayhew CRNP, Assumpta Ude CRNP, Jacqueline Hoffman MS, Marina Vernalis DO  
Walter Reed Army Medical Center and Jackson Foundation for the Advancement of Military Medicine, Washington, DC

**Introduction:** Anecdotal experience suggests that stress is a major impediment to sleep, eroding overall sleep quality. Clinical programs universally endorse interventions for stress reduction to improve sleep, but there are few reports validating this therapeutic approach. To examine the relationship between stress reduction and sleep improvement, we measured changes in perceived stress and its correlation with sleep quality in a longitudinal outcomes study.

**Methods:** The Integrative Cardiac Health Project (IHP) is a heart health program with goals of improving diet, exercise, sleep and stress. At program entry and at graduation, participants were assessed with the Perceived Stress Scale (PSS14) and the Pittsburgh Sleep Quality Index (PSQI) which includes sleep duration along with sleep latency, sleep fragmentation, perceived restfulness, daytime functioning, nocturnal behaviors, and use of sleep aids. Subjects were divided into groups that improved PSS score and those that did not. Differences between groups were compared using unpaired t-test.

**Results:** 66 consecutive graduates (mean age 59.6±11.6, 28 men) reduced their PSS 3.1±5.8 points and improved their PSQI 1.2±2.9 points. Fifty subjects were able to reduce their PSS by a mean of 5.5±4.5 points accompanied by improvements in PSQI (1.9±3.0 points), Lp-PLA2 (41.6±53.8 mg/dL), glucose (2.0±9.1 mg/dL), insulin (2.2±7.0 ug/dL) and HOMA (0.04±1.69). The other 16 subjects showed increases in PSS of 4.3±2.0,  $p < 0.001$  accompanied by worsening PSQI (0.27±2.49,  $p = 0.02$ ), Lp-PLA2 (21.7±65.5,  $p = 0.02$ ), glucose (2.8±11.0,  $p = 0.08$ ), insulin (1.4±6.1,  $p = 0.07$ ) and HOMA (0.49±1.51,  $p = 0.04$ ).

**Conclusions:** Reductions in perceived stress correlate significantly with improvements in sleep quality. Improvements in perceived stress and sleep quality are accompanied by improvements in cardiovascular risk markers including glucose metabolism and lipids. Our findings underscore the importance and value of utilizing stress management techniques as a teachable sleep improvement intervention.

**Journal Citation:** Eliasson A, Kashani M, Mayhew M, Ude A, Hoffman J, Vernalis M. Reducing Perceived Stress Improves Sleep Quality—A Longitudinal Outcomes Study. CHEST 2010; 137:913A

## Improving Sleep Quality Correlates with Lower Weight —A Longitudinal Outcomes Study

Arn Eliasson MD, Mariam Kashani CRNP, Maren Mayhew CRNP, Marina Vernalis DO

**Introduction:** Numerous cross-sectional studies have shown an association between shorter total sleep time (TST) and increased weight. However, longitudinal studies examining weight as a function of TST have shown mixed results. In order to examine the relationship between sleep and weight loss, we measured sleep quality rather than TST alone in a longitudinal outcomes study.

**Methods:** The Integrative Cardiac Health Project (IChP) is a heart health program with goals of improving diet, exercise, sleep and stress. At program entry and at graduation, participants were weighed and completed the Pittsburgh Sleep Quality Index (PSQI) which includes sleep duration along with sleep latency, sleep fragmentation, perceived restfulness, daytime functioning, nocturnal behaviors, and use of sleep aids. Subjects were divided into groups that improved PSQI score and those that did not. Differences between groups were compared using unpaired t-test.

**Results:** 78 consecutive graduates completed IChP at a mean of 9.4±2.7 mo. Nine subjects had a body mass index (BMI) <25 kg/m<sup>2</sup> at enrollment and were excluded from analysis. The other 69 graduates were overweight (mean BMI=31.1±5.0 kg/m<sup>2</sup>), had a mean age of 59.0±12.7 yrs, included 31 men (45%), and were racially diverse (34 Caucasian, 30 African-American, 4 Hispanic, and 1 Asian). Of these 69 participants, 43 (age 58.2±13.4 yrs, 17 or 40% men) showed mean improvement in PSQI of 3.5±3.1 points along with mean decrease in BMI=0.74±1.3 kg/m<sup>2</sup>. In contrast, 26 subjects (age 60.5±11.6 yrs, 14 men or 54%) showed worsening PSQI score of 1.2±1.4 points and a limited decrease in BMI=0.09±1.01, p=0.04.

**Conclusions:** In overweight subjects, improvements in sleep quality correlated with greater weight loss. Global assessment of sleep quality, rather than a focus on TST alone, may clarify the mechanism between sleep and weight loss. Identifying these components of sleep quality also provides targets for therapeutic intervention.

**Citation:** Eliasson AH, Kashani M, Mayhew M, Vernalis M. Improving sleep quality correlates with lower weight—A longitudinal outcomes study. *Sleep* 2010; 33:A378

## Longer Sleep Time Confers Cardiovascular Health Benefit

Arn Eliasson MD, Mariam Kashani CRNP, Marina Vernalis DO  
Walter Reed Army Medical Center and Jackson Foundation for the Advancement of Military Medicine, Washington, DC

**Background:** Prior research using actigraphy as an objective measure of sleep time showed correlation between short sleep duration and increased BMI. This work was limited by a small cohort and a restricted number of measured parameters. We sought to further examine the relationship between sleep, maintenance of healthy weight, and cardiovascular (CV) health by utilizing validated sleep questionnaires and self-reported sleep time in a larger cohort.

**Methods:** Consecutive subjects entering a 6-month integrative healthy lifestyle program completed questionnaires including the Berlin Questionnaire for sleep apnea risk, Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), Fatigue Scale and Perceived Stress Scale (PSS). Data collection also included anthropometrics and cardiac-relevant lab panel. Differences between subjects with short sleep (total sleep time or TST ≤ 5 hrs/night) and long sleep (TST ≥ 7 hrs/night) were analyzed by t-test.

**Results:** In 478 participants (age 54.1±12.4y, 36% men, 169 Caucasian, 121 African-American, 22 Hispanic, 3 Asian, 12 other, 151 undeclared), Berlin Questionnaire indicated high risk for sleep apnea in 53%. Group TST=6.3±1.3h; Sleep Latency (SL)=23.6±38.4 min; PSQI=7.0±4.3; ESS=8.9±5.0 and Fatigue=4.3±2.5; mean BMI=29.8±5.8; PSS=22.4±8.1. For 108 short sleepers (age 50.0±13.0y), Berlin Questionnaire indicated high risk in 66% of subjects; TST=4.5±0.7h; SL=45.9±69.0 min; PSQI=10.9±3.9; ESS=10.7±5.3 and Fatigue=5.7±2.2; mean BMI=31.3±6.5; PSS=24.9±8.7; and hsCRP=0.44±0.55 mg/L. By contrast, the 175 long sleepers, were older (57.2±11.7y, p<0.001); had lower % of subjects at risk for sleep apnea (42%); slept longer (TST=7.6±0.8h, p<0.001); fell asleep more quickly (SL=15.2±14.3 min, p<0.001); had better sleep quality (PSQI=4.6±3.6, p<0.001); had less daytime sleepiness (ESS=7.2±4.8, p<0.001); and less fatigue (Fatigue=3.3±2.6, p<0.001). Long sleepers also weighed less (BMI=29.1±6.0, p=0.004); experienced lower stress levels (PSS=20.4±7.3, p<0.001); and had lower levels of the inflammatory marker hsCRP (0.32±0.47 mg/L, p=0.05). Importantly, there were no differences in lipids, glucose or HgbA1C between short and long sleepers.

**Conclusions:** Participants who slept longer showed a better CV risk profile and enjoyed higher quality of life by a number of indicators. Despite a lack of difference in the more traditional risk factors, total sleep time is strongly associated with lower stress, healthier body weight, and lower inflammation. These findings underscore the importance of addressing adequate sleep time as a modifiable risk factor in an integrative program for CV risk reduction.

**Journal Citation:** Eliasson A, Kashani M, Vernalis M. Longer sleep confers cardiovascular health benefit. *Am J Respir Crit Care Med* 2010; 181:A6524

## Assessing Perceived Stress Provides Targets for Stroke Prevention

Mariam Kashani MS, CRNP, Arn Eliasson MD, Jacqueline Hoffman MS,  
Marina Vernalis DO  
Walter Reed Army Medical Center and Jackson Foundation for the Advancement of Military Medicine, Washington, DC

**Background:** Stroke prevention traditionally targets cholesterol and blood pressure control. While these measures are valuable, this limited focus may overlook other variables that increase risk for stroke.

**Objective:** We sought to examine a broader approach to stroke prevention in an integrative cardiovascular prevention program (CPP) by identifying multiple behavioral factors associated with stroke risk. Our integrative program targets cardiovascular (CV) risk reduction through behavioral interventions to improve nutrition, exercise, sleep and stress.

**Methods:** Subjects entering the CPP completed questionnaires including the Perceived Stress Scale (PSS), Epworth Sleepiness Scale (ESS), Fatigue Scale, Pittsburgh Sleep Quality Index (PSQI) and Berlin Questionnaire for Sleep Apnea. Data collection also included anthropometrics and a CV-relevant lab panel. Differences between subjects with high stress (PSS≥23) and those with low stress (PSS<23) were analyzed by t-test.

**Results:** Of 351 consecutively enrolled subjects: 166 (47%) scored above the median PSS. These high-stress subjects displayed an increased cardiovascular risk profile including elevated BMI (31.1±5.9 vs 29.0±5.9, p=0.001), increased Waist Circumference (101.5±17.4 cm vs 98.2±13.8, p=0.04), glucose (98.1±28.2 mg/dL vs 92.8±14.6, p=0.03) and Lp-PLA2 (strongly associated with stroke risk, 220.6±104.7 ng/mL vs 195.6±67.1, p=0.02). High-stress subjects also demonstrated greater daytime sleepiness (ESS=10.4±5.1 vs 7.8±4.8, p<0.001), greater fatigue (5.4±2.2 vs 3.4±2.4, p<0.001), lower sleep quality (PSQI 8.5±4.4 vs 5.9±4.0, p<0.001) and shorter sleep duration (19 min less/24 hr, p=0.04) with a higher risk for sleep apnea (60% at high risk vs 41%, p=0.003) than their low-stress counterparts.

**Conclusions:** Assessing stress levels in patients can provide targets for intervention in stroke prevention. High stress is associated with numerous behavioral, biochemical and anthropometric factors that increase stroke risk. Comprehensive stroke risk prevention could benefit from an integrative approach that includes lifestyle behavioral assessment to identify as well as to reduce stroke risk and improve quality of life indicators.

**Journal Citation:** Kashani M, Eliasson A, Hoffman J, Vernalis M. Assessing perceived stress provides targets for stroke prevention. *Stroke* 2010; 41:e292

## **Stress Therapy Empowers Prevention (STEP): A Healthy-Lifestyle Program for Breast Cancer Patients**

Amy Burke\*, Jane Haberkorn, Fran Lechak, Judith Sullivan, James Vizza, Marina N. Vernalis, Darrell L. Ellsworth

Integrative Cardiac Health Program, Windber Research Institute, Windber, PA, and Walter Reed Army Medical Center, Washington, DC.

\*primary author

**Purpose:** Breast cancer has a significant emotional, psychological, and social impact, often associated with high levels of stress and sleep deprivation, which promotes unhealthy behaviors causing weight gain, decreased physical fitness, and an increased risk for cardiovascular disease (CVD). Because women with breast cancer often exhibit emotional distress for years following diagnosis, it is important to develop programs that effectively manage stress and promote mental and physical health in breast cancer patients.

**Methods:** This program is designed to help breast cancer patients lose weight, improve strength and endurance, sleep better, and reduce disease risk factors. Women 18+ years of age with breast disease (or at high risk) are offered a program of lifestyle change, consisting of a Healthy Lifestyle intervention for 3 months with instruction and demonstrations on exercise, nutrition, stress reduction, and mind/body health, followed by a five-year follow-up with additional reinforcement to help integrate healthy behaviors into daily life. Testing is conducted at baseline and after the Healthy Lifestyle intervention, with follow-up examinations every 6 months for 5 years. Information collected includes perceived stress/anxiety, sleep disturbances and psychosocial measurements, carotid ultrasound, traditional CVD risk factors (weight, blood pressure, and body composition), and biochemical assays.

**Results:** Recruitment has been conducted primarily through newspaper ads and newly designed brochures. Of 43 women who expressed interest in the program, 20 have enrolled. Demographic characteristics of participants are: average age 65, 6% with diagnosed CVD, and 61% with breast cancer. Obstacles to recruitment include out-of-pocket costs to patients, lack of local physician referrals, limited time to devote to participation, and lack of knowledge among patients about the benefits of lifestyle change on quality of life or clinical outcome. Once women make the commitment to participate, satisfaction surveys indicate a high degree of satisfaction with the program.

**Conclusions:** Proven strategies to reduce risk of recurrence and improve quality of life in breast cancer patients are best implemented as a comprehensive program for lifestyle change, empowering the individual patient to make healthy lifestyle choices. Improving the health and well-being of women with breast disease may have a positive impact on breast disease and cardiovascular outcomes.

Citation: Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Vernalis MN, Ellsworth DL. Stress Therapy Empowers Prevention (STEP): A healthy-lifestyle program for breast cancer patients. *Am J Clin Oncol* 2011;34(5):551.

## Improvement in Cardiovascular Risk Factors in Breast Cancer Patients Participating in the Stress Therapy Empowers Prevention (STEP) Program

Darrell L. Ellsworth\*, Heather L. Patney, Amy Burke, Jane Haberkorn, Fran Lechak, Judith Sullivan, James Vizza, David M. Neatrou, Marina N. Vernalis

Integrative Cardiac Health Program, Windber Research Institute, Windber, PA, and Walter Reed Army Medical Center, Washington, DC.

\*primary author

**Introduction:** Breast cancer is the most frequently occurring cancer and leading cause of death in women between 20 and 59 years of age in the US. Significant emotional and psychological sequelae, including stress and sleep disturbance, often degrade the quality of life in breast cancer patients. Similar to cardiovascular disease, breast cancer susceptibility is influenced in part by modifiable risk factors, suggesting that a healthy lifestyle program may lead to significant improvements in mental health and quality of life as well as reductions in cancer recurrence and cardiovascular risk.

**Methods:** Eighteen women with breast disease are participating in a lifestyle program designed to improve quality of life and reduce disease risk factors. The intervention provides instruction and demonstrations on exercise, nutrition, stress reduction, and mind/body health, and includes a five-year follow-up with reinforcement to help integrate healthy behaviors into daily life. Examinations at baseline, after the healthy lifestyle intervention, and at one year, collected information on age, ethnicity, health history, medication use, and diet. Psychosocial surveys assessed depression, perceived stress, general well-being, and sleep quality. Physical exams measured heart rate, blood pressure, weight, and percent body fat. Blood was collected to measure lipids, glucose, glycosylated hemoglobin, and a panel of biochemical variables.

**Results:** Over the initial 12 week period, participants showed change in the desired direction for many risk factors: body weight (-1.8%;  $p < 0.01$ ), diastolic blood pressure (-8.5%;  $p < 0.05$ ), and perceived stress (-24.2%;  $p < 0.05$ ) decreased significantly, while triglycerides (-14.4%;  $p = 0.09$ ), systolic blood pressure (-7.6%;  $p = 0.08$ ), depression (-25.3%;  $p = 0.09$ ), hostility (-25.6%;  $p = 0.07$ ), and sleep quality (+20%;  $p = 0.05$ ) showed near significant changes. In patients who have reached the one year time-point ( $n = 9$ ), perceived stress (-32.0%;  $p < 0.05$ ) and sleep quality (+30.8%;  $p < 0.01$ ) improved significantly, and glycosylated hemoglobin levels decreased (-4.5%;  $p = 0.09$ ).

**Conclusions:** A comprehensive program for lifestyle change that empowers patients to make healthy lifestyle choices can successfully improve quality of life and overall health in as little as three months. As participants continue in the program, we will evaluate the health and morale of these women to determine if lifestyle changes result in improved clinical outcomes over the long-term.

Citation: Ellsworth DL, Patney HL, Burke A, Haberkorn J, Lechak F, Sullivan J, Vizza J, Neatrou DM, Vernalis MN. Improvement in cardiovascular risk factors in breast cancer patients participating in the Stress Therapy Empowers Prevention (STEP) Program. *Am J Clin Oncol* 2011;34(5):552.

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Instrumentation. Total walk-away time associated with each run time, including all wait time where the operator is available to perform other tasks and not be present at the instrumentation, was also captured. Equipment required for both methods was also compared including equipment needed for extraction. **Results:** For 92 reportable results the Hologic Invader® Factor V Leiden Test had 20 minutes hands on time, 90 minutes cycle time, and 10 minutes for data analysis. This allowed for 90-minute operator walk-away time that was 75% of the total run time. For 90 samples the Roche Factor V Leiden Detection Kit for the LightCycler 1.2 had 45 minutes hands-on time, 114 minutes cycle time, and 45 minutes for data analysis. This allowed for 114 minute operator walk-away time that was 59% of the total run time. **Conclusions:** Both systems provide opportunities for the operator to walk-away during the testing process with the Hologic Invader® Factor V test having the highest percentage versus total run time. The Hologic Invader® test also demonstrated the least total hands-on time and had the shortest run time to result for approximately 92 samples compared to Roche LightCycler 1.2 for 90 samples. Both systems have a similar amount of equipment required post extraction. The difference between the methods occurs with the extraction. The Roche method requires the Magna Pure LC Instrument be used for reproducible DNA preparation and PCR set-up. The Hologic method does not require a specific extraction method be followed, only that commercially available extraction kits or laboratory validated methods should provide sufficient concentration at sufficient purity per the Invader® Factor V package insert.

**TT44. Performance of Whole-Genome Amplified DNA Isolated from Serum and Plasma for Estimating Copy Number Variation with High Density Single Nucleotide Polymorphism Arrays**  
D.T. Croft\*, L.M. Voegtly\*, H.L. Patney\*, C.D. Shriver\*, M.N. Vernalis\*, D.L. Ellsworth\*  
\*Windber Research Institute, Windber, PA, †Walter Reed Army Medical Center, Washington, DC.

**Introduction:** Defining genetic variation associated with complex human diseases requires high-quality DNA from well-characterized patients. With the development of robust technologies for whole-genome amplification, sample repositories such as serum banks now represent a potentially valuable source of DNA for genomic studies and clinical diagnostics. We assessed the performance of whole-genome amplified (wga) DNA derived from stored serum/plasma for estimating chromosome copy number (CN) variation on high-density single nucleotide polymorphism (SNP) arrays. **Methods:** Fresh serum and plasma samples were obtained from subjects who voluntarily agreed to participate in this study and gave written informed consent. DNA was extracted from 200  $\mu$ l of serum or plasma using the QIAamp® DNA Blood Mini Kit. Genomic (g) DNA was isolated from peripheral blood mononuclear cells with the Puregene® DNA Purification Kit according to the manufacturer's protocol. Whole-genome amplification was then performed on 2.5  $\mu$ l of serum/plasma DNA using the REPLI-g® whole-genome amplification kit. Genotypes were determined using Affymetrix GeneChip® Genotyping Analysis Software and CN variation was assessed with Genotyping Console™. **Results:** Storage time and usage history did not affect DNA extraction or whole-genome amplification yields; however, samples that had been thawed and refrozen showed significantly lower call rates (73.9 + 7.8%) compared to samples that had never been thawed (92.0 + 3.3%) ( $P < 0.001$ ). Genotype call rates did not differ significantly ( $P = 0.13$ ) between wgaDNA from never-thawed serum/plasma (92.9 + 2.6%) and gDNA (97.5 + 0.3%) isolated from whole blood. Approximately 400,000+ genotypes were consistent between wgaDNA and gDNA; however, patterns of CN variation were highly discordant between serum/plasma wgaDNA and gDNA from the same patients. The CNV in the wgaDNA samples showed sporadic regions of amplifications and deletions compared to the unamplified gDNA. These regions showed much larger areas of amplification and deletions across all the chromosomes compared to the unamplified gDNA CNV. **Conclusions:** While use of stringent quality control requirements can facilitate the collection of quality SNP genotype data from wgaDNA, our data suggest that more advanced analyses, such as CN and loss of heterozygosity assessments, may be compromised due to spurious amplification during the whole-genome amplification process.

**TT45. Rapid High Throughput TaqMan SNP Genotyping Assay for FVL, PT, and MTHFR**  
E.I. Reader, H.B. Steinmetz, C.L. Leferts, B. Wood, G.J. Tsongalis, L.J. Tafe  
Dartmouth Hitchcock Medical Center, Lebanon, NH.  
**Introduction:** Testing for Factor V Leiden (FVL) 1691G>A, Prothrombin (PT) 20210G>A, and 5, 10- methylene tetrahydrofolate reductase (MTHFR) 677C>T polymorphisms is commonly performed in clinical molecular laboratories as part of a work up for thrombophilia. A variety of testing methods are commercially available that utilize different platforms and chemistries. In this study, we compare the performance of the Hologic Invader assay for these three polymorphisms with a TaqMan laboratory developed assay. **Methods:** We utilized DNA samples from 18 patients who had been

previously tested for FVL, PT, and/or MTHFR polymorphisms by the Hologic Invader technology. DNA was isolated from whole blood using the EZ1 DNA Blood Kits (Qiagen, Valencia, CA). All cases were analyzed using custom TaqMan SNP Genotyping Assays for FVL (rs6025), PT (rs1799963) and MTHFR (rs1801133) polymorphisms (Life Technologies, Carlsbad, CA). Real-time PCR was performed on the AB 7500 Instrument using AB 2x fast universal master mix, 10-20 ng of genomic DNA in a total reaction volume of 10  $\mu$ l with the default fast cycling conditions. A post amplification plate read was used for allelic discrimination. Known wild-type and mutant samples were tested as controls in each assay run. **Results:** Of 18 patient samples, the results were as follows: FVL - 10 wild type (WT), 5 heterozygous and 3 homozygous; PT - 13 WT, 3 heterozygous and 1 homozygous; MTHFR - 6 WT, 7 heterozygous and 5 homozygous. There was 100% concordance between the two assays. All control samples gave the expected results. Hands-on time for this assay was approximately 45 minutes and the time to result was approximately 3 hours versus 5 hours for the Hologic Invader assay. **Conclusions:** Our study shows that the 7500 FAST TaqMan SNP Genotyping Assay is comparable to the Hologic Invader assay in detecting and characterizing FVL, PT, and MTHFR polymorphisms. In addition, the TaqMan assay is easy to perform, requires less technologist time, shorter incubation and an improved time to result as compared to the Hologic Invader assay.

**TT46. A Workflow Integrating High-Throughput Automated RNA and DNA Extraction from FFPE Samples and Second Generation Sequencing**  
T. Guettouche, D. Hedges, J. Rantus, K. Slosak, I. Konidari, W. Hulme, A. Andersen, A. Diaz, R. Gentry, Y. Pasco, M. Pericak-Vance, J. Gilbert  
University of Miami, School of Medicine, Miami, FL

**Introduction:** Biorepositories around the world store vast numbers of formalin-fixed, paraffin-embedded (FFPE) samples. These samples contain a large collection of phenotypic, histological and pathological data. For genomics studies, such as biomarker discovery, targeted resequencing or gene expression profiling, this resource has been largely unutilized because sample extraction can be challenging and the quality of the extracted nucleic acids is often poor. Currently, RNA and DNA extractions from FFPE samples are commonly performed manually and involve laborious protocols that are not amenable to high throughput processing. In addition there are no standard quality control methods for downstream applications such as second generation sequencing. **Methods:** In this study, we present a fully automated RNA and DNA extraction method (Tissue Preparation System, Siemens Healthcare Diagnostics, Tarrytown, NY) for IVD and research use that allows simultaneous or separate extraction of DNA and RNA from up to 48 samples in 4h with minimum operator interaction. In contrast to most other FFPE extraction protocols this method uses an innovative deparaffinization step on the instrument that does not require incubation with Xylene or other solvents. We have successfully extracted DNA and RNA from breast and prostate cancer tissue ( $n = 100$ ) and the study is being extended to an additional 5 different tumor tissues including breast, prostate, pancreas, bladder and cervix ( $n = 350$ ). Qualitative and quantitative analysis was carried out by Bioanalyzer, real-time PCR and Qubit. RNA and DNA were sequenced on a HiSeq2000 second generation sequencing instrument. **Results:** Extraction of both DNA and RNA was successful in FFPE samples from breast and prostate cancer tissue. From adjacent 10  $\mu$ m paraffin sections the automated system, on average, extracts longer nucleic acid fragments as judged by Bioanalyzer traces and similar amounts of RNA and DNA measured by the Qubit method, compared to a standard commercially available manual protocol. Matched fresh frozen and FFPE samples are being analyzed by second generation sequencing on a HiSeq2000. **Conclusions:** We present an integrated FFPE analysis workflow that includes standardized and fully automated nucleic acid extraction and quality controls for high-quality, high-throughput preparation of FFPE samples (96 samples/8 hr work day) for downstream analysis such as resequencing or transcriptome analysis by second generation sequencing. Our integrated workflow should significantly simplify utilization of FFPE samples for downstream genomic analyses such as second generation sequencing and as a consequence unlock a largely untapped source of information.

**TT47. RNA Is Isolated in High Yield and Purity from PAXgene® Stabilized Blood and from Buffy Coat Using the Maxwell® 16 SimplyRNA Method**  
J. English, M. Mandrekar, A.K. Brekke, B.A. Hook, T.L. Shagat, T. Lubben  
Promega, Madison, WI.

**Introduction:** The analysis of gene expression data in research has needed a critical evaluation of easy, reproducible methods for RNA extraction. The reliability of a novel chemistry on the Maxwell 16 instrument provided a middle-throughput automated procedure to isolate RNA from whole blood or its leukocyte compartment (buffy coat) to evaluate this. **Methods:** Evaluation included reproducibility, yield, and purity using the Nanodrop, Agilent Bioanalyzer and denaturing gels. A combination of quantitative RT-PCR and quantitative PCR was used to evaluate DNA contamination of the RNA. Buffy

Citation: Croft DT Jr, Voegtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. *J Mol Diag* 2011;13(6):781.

with the Genfind® DNA Extraction Kit and the set up of the Cervista reaction. This report describes a new Investigational Use Only automated platform for performing the test, the Cervista HPV HR High Throughput Automation (HTA). Methods: All system testing was in accordance with FDA guidance document "Assay Migration Studies for In Vitro Diagnostic Devices" (<http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm092752.pdf>). Results: The Cervista HTA fully automates DNA extraction, reaction setup, and results processing on a single platform. The system has a compact footprint of 15.6 square feet and provides flexible processing capabilities to perform multiple sample batch sizes. A batch size of 192 samples can be processed in an eight hour shift with fully automated results generated before the next day. For a 192 sample run, hands-on time was less than one hour including deck set up and daily maintenance. More than 90% of processing time was completely walk-away. To determine system reproducibility, a 9-member sample panel including high negatives, low positives, and moderate positives was tested across three different study sites. A comparative sample panel consisting of 288 residual clinical samples with various levels of HPV was tested at three external sites. The percent negative and positive agreement were both greater than 96% for the HTA versus the FDA-approved test. The potential for cross-contamination on the HTA system was also evaluated and no cross-contamination was observed. Conclusions: The Investigational Use Only HTA system, for use with the Cervista HPV HR test, is a fully automated, flexible system, designed to enable high throughput screening of cervical specimens for the presence of HPV DNA with limited hands-on time.

**TT39. Analysis of JAK2 V617F Mutational Burden in Myeloproliferative Neoplasia by Deep Sequencing**  
Z. Wang, B. Evans, M. Weiss, J. Gong, S.C. Peiper  
Thomas Jefferson University, Philadelphia, PA  
Introduction: The spectrum of Philadelphia chromosome negative myeloproliferative neoplasms (MPN) includes polycythemia vera (PV), essential thrombocythemia (ET), and acute myelofibrosis (MF). The G>T somatic mutation in codon 617 of the JAK2 gene resulting in a substitution of phenylalanine for the naturally occurring valine residue (V617F) has emerged as a diagnostic criterion for PV, ET, and MF. Since the natural progression of MPN involves expansion of the clonal progenitor population containing the JAK2V617F mutation, analysis of the mutational burden can provide important clues to prognosis. Current methods for analysis of this mutation include qualitative and quantitative PCR with allele specific probes and pyrosequencing. We describe the use of next generation sequencing for quantification of mutation burden. Methods: With approval by the IRB for analysis of de-identified specimens, 23 genomic DNA samples were selected. All cases were previously analyzed using Ipsogen Mutascreen qualitative/semi-quantitative assay (assay sensitivity 2%). Among them, 5 were positive at various levels, 17 were borderline positives, and one was negative. These gDNA were amplified with primers flanking exon 14 of the JAK2 gene by PCR and subjected to NextGen sequencing using a 454 Junior and the percentage of fragments carrying the V617F mutation was determined. Up to 12 specimens were performed in the same sequencing run. Positive cases were analyzed in parallel by Sanger sequencing. Results: The 5 positive specimens had a V617F mutation burden from 2.3% to 78%. Of 17 cases with low level positivity with the Ipsogen test, only 1 contained this mutation, which was quantified at 0.4% by deep sequencing. Average Junior read number per sample was 8697 combining forward and reverse reads. Positive cases were confirmed by Sanger Sequencing but mutation loads below 10% by deep sequencing were below the limits of detection. Conclusions: Deep sequencing of exon 14 of the JAK2 gene is a sensitive and specific approach for the quantification of the V617F mutation in patients with MPN. The sensitivity of Sanger sequencing was insufficient for the detection of low levels of mutational load and the Ipsogen Mutascreen assay gave false positive values in the borderline positive range. Deep sequencing is superior in comparison to other widely used mutation detection assays when sensitivity and accuracy are required. Simplifying procedure for deep sequencing with automation is needed for future diagnostic applications of this technology.

**TT41. Automated Total Nucleic Acid Purification from FFPE Tissues Using Maxwell® 16**  
D.J. Waczorek, C. Cowan, N. Nassif, D.R. Storts, T.L. Schagat  
Promega, Madison, WI  
Introduction: Traditional methods for the purification of nucleic acids from formalin-fixed, paraffin-embedded (FFPE) tissue samples are often labor intensive and include the use of hazardous organic reagents. In addition, carryover of formalin throughout the purification process can inhibit amplification. Thus, a consistent method using appropriate extraction and purification techniques is essential for the success of purified nucleic acids from FFPE samples in downstream applications. Here, we describe an automated method for the purification of total nucleic acid from FFPE tissue sections using the Maxwell® 16 instrument that eliminates the need for toxic reagents and with

minimal hands on time. Methods: Pre-processing of 10 micron mouse FFPE tissue sections involved a simplified protocol with no xylene or phenol extraction required. Following pre-processing, samples were placed directly into the Maxwell® 16 cartridges, and purified total nucleic acid was ready in approximately 45 minutes. All samples were eluted in 50µl of nuclease-free water. Yield and purity of the purified total nucleic acid was analyzed by the NanoDrop 1000. RNA recovery was analyzed by quantitative RT-PCR using primers specific to mouse actin. Results: Total nucleic acid from a variety of mouse FFPE tissue sections was successfully purified, including liver, brain, heart, kidney, and spleen. Automated RNA purification with Maxwell® 16 was compared to manual purification kits from Qiagen and Invitrogen. RNA recovery was equivalent to these commonly used manual methods as determined by quantitative RT-PCR analysis. Conclusions: Total nucleic acid from FFPE tissue samples was successfully purified using the Maxwell® 16 instrument. Automated nucleic acid purification decreases hands on time spent manually extracting, provides more consistent results from difficult to purify sample types, and reduces the risk of RNase contamination. The low elution volume format provides for highly concentrated nucleic acid ready for downstream applications. The method does not require the use of hazardous organic solvents, providing a safer method of extraction and purification.

**TT42. Utility of Whole Genome Amplification for Assessing Copy Number Variation with High Density SNP Arrays from Formalin-Fixed, Paraffin-Embedded Tissue**  
L.M. Voeghtly<sup>1</sup>, D.T. Croft<sup>1</sup>, B. Deyarmin<sup>1</sup>, M.N. Vernalis<sup>2</sup>, C.D. Shriver<sup>2</sup>, D.L. Ellsworth<sup>1</sup>  
<sup>1</sup>Windber Research Institute, Windber, PA; <sup>2</sup>Walter Reed Army Medical Center, Washington, DC  
Introduction: The ability to obtain sufficient high quality DNA from archival formalin-fixed, paraffin-embedded (FFPE) tissue often limits genomic analysis for researchers and clinicians alike. Of numerous methods developed to optimize the quantity of DNA extracted from FFPE tissues, whole genome amplification (WGA) has become a robust and reliable technique for obtaining sufficient genomic material for a variety of molecular applications. Previous studies suggest that DNA obtained from FFPE samples may be used on high-density single nucleotide polymorphism (SNP) arrays to provide information on SNP genotypes, chromosome copy number (CN), and loss of heterozygosity, but spurious results occur with insufficient DNA template. Methods: We examined the feasibility of assessing chromosome CN variation using whole-genome amplification on DNA extracted from FFPE tissue, as well as fresh frozen (FF) tissue in OCT, and high-density Affymetrix GeneChip® 500K SNP Mapping Arrays. Genomic DNA was extracted from microdissected regions (approx 2.9 mm<sup>2</sup>) of human tissue preserved in paraffin using the GenomePlex® Tissue Whole Genome Amplification Kit (Sigma®) and from human FF tissue using QiaAmp® DNA Mini Kit (Qiagen®). Whole-genome amplification was then performed on 1.5 µl of FF or FFPE DNA using the REPLI-g® whole-genome amplification kit (Qiagen®). Genotypes were determined using the Dynamic Model Mapping Algorithm in the Affymetrix GeneChip® Genotyping Analysis Software (GTTYPE 4.0) package and CN variation was assessed with Genotyping Console™ (Affymetrix). Results: Acceptable genotyping call rates were obtained for all unamplified DNA samples (96.3 ± 1.5%) and wgaDNA samples (93.3 ± 1.6%) from FF tissue. Call rates were significantly lower, however, for wgaDNA samples from FFPE (67.5 ± 5.1%) (p<0.001). Assessment of CN variation was highly consistent between unamplified and whole-genome amplified FF samples, but was clearly discordant between amplified FF and amplified FFPE samples. Conclusions: These results indicate that FF tissue, even if whole-genome amplified, is useful for genome-wide SNP genotyping and determining chromosome CN variation, but large discrepancies are likely to occur when using whole-genome amplification on DNA isolated from FFPE. CN variation may be affected by uneven amplification of the genome with small quantities of suboptimal DNA template extracted from FFPE samples.

**TT43. Workflow Comparison of Two FDA-Cleared Factor V Leiden Detection Systems**  
A. Sanders, M. Doleshal, S. Day  
Hologic Molecular Diagnostics, Madison, WI  
Introduction: To provide an operational review of the Roche Factor V Leiden Mutation Detection Kit for use on the LightCycler Instrument 1.2 and the Hologic Invader® Factor V using Invader® Plus Chemistry. Extraction methods, equipment, and total time requirements were evaluated for a similar number of samples. Both manufacturers also have Prothrombin (Factor II) Detection Kits that have equivalent workflows to the Factor V Leiden tests. Methods: Manufacturer's package inserts and peer reviewed publications were used to gather information to compare the methods. Multiple factors outlining the workflow of the procedure and ease of use were evaluated. Comparison data was expressed as total run time post extraction to final result for a similar number of samples. Hands-on-time included all interaction with the samples and

Citation: Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. J Mol Diag 2011;13(6):780.

## SNPs associated with plasma triglyceride levels in the general population influence response during intensive cardiovascular risk reduction

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL

Integrative Cardiac Health Program, Windber Research Institute, Windber, PA; Windber Medical Center, Windber, PA, USA; Integrative Cardiac Health Program, Walter Reed National Military Medical Center, Bethesda, MD

**Background:** Triglycerides are lipid fractions that represent an important risk factor for cardiovascular disease (CVD) because they play a fundamental role in development and progression of atherosclerosis. Although current guidelines advocate lifestyle change involving diet, physical activity, and weight control for management of hypertriglyceridemic patients, plasma triglyceride levels may be influenced by genetic composition in addition to lifestyle behaviors. Recent genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) associated with plasma triglyceride levels in the general population.

**Methods:** We examined the influence of genetic variation on variability in triglyceride response in 178 participants who completed a prospective, non-randomized intervention designed to stabilize or reverse progression of CVD through dietary changes, exercise, and stress reduction. Cardiovascular risk factors were assessed at baseline, 12 weeks, and 52 weeks by standard methods. SNPs (n=19) associated with plasma triglycerides were genotyped by TaqMan® allelic discrimination assays.

**Results:** Patients experienced significant improvement ( $P < 0.05$ ) in most risk factors, including weight (-9%), blood pressure (-6%), total cholesterol (-7%), and triglycerides (-9%). Triglyceride response during the program differed significantly ( $P < 0.05$ ) between genotypes for three SNPs (rs442177, rs3846662, and rs17145738) located close to the following genes: transcriptional activator AF4/FMR2 family member 1 (AFF1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), which catalyzes the rate-limiting step in cholesterol synthesis, and MLX interacting protein-like (MLXIPL), which controls transcription of genes involved in glycolysis.

**Discussion:** Lifestyle modification for cardiovascular risk reduction may be more beneficial to certain individuals based on genetic composition. Genetic variation associated with CVD risk may provide a basis for personalized treatments to optimize cardiovascular health.

Accepted for poster presentation at the ASHG Meeting, San Francisco, CA, 6-10 Nov 12.

## The Importance of Weight Loss for Effecting Molecular Change during Intensive Cardiovascular Risk Reduction

Ellsworth DL, Croft DT Jr, Burke A, Haberkorn MJ, Patney HL, Mamula KA, Vernalis MN. Windber Research Institute, Windber, PA; Windber Medical Center, Windber, PA; Concurrent Technologies Corporation, Johnstown, PA; Walter Reed National Military Medical Center, Bethesda, MD

Obesity is a major risk factor for cardiovascular (CV) disease. Behavioral lifestyle change is the cornerstone of therapy for weight management. Currently little is known about molecular responses accompanying weight loss that may be important in weight control and CV risk reduction.

Patients (n=89) participated in a prospective, nonrandomized, lifestyle change program designed to stabilize or reverse progression of CV disease through dietary changes, exercise, and stress reduction. Nonintervention controls (n=63) were matched to patients based on age, gender, and disease status. CV risk factors (BMI, blood pressure, lipids) and peripheral blood gene expression profiles were assessed at three time points over one year.

Most patients were obese (63%;  $BMI \geq 30$ ) or overweight (25%;  $25 \leq BMI < 30$  kg/m<sup>2</sup>) at baseline, but showed significant improvement in CV risk factors compared to controls during the program. Following stratification based on weight loss, we observed significant expression changes (FDR  $P < 0.05$ ) for 41 genes in participants who lost the most weight (mean weight loss=11%) from baseline to three months and for 3223 genes in those who lost the most weight (mean weight loss=15%) from baseline to one year. No significant expression changes were observed in patients who lost the least weight (mean weight loss<4%) or in controls. Functional ontologies of genes showing the most significant changes in expression included immune/defense response and symbiosis at three months and metabolism/biosynthesis at one year.

Intensive lifestyle modification can effectively alter CV risk factors, but successful weight loss may accentuate molecular change. Defining the role of weight loss in molecular response to lifestyle modification provides another dimension to understanding complex biological processes involved in CV health.

Presented as poster for Obesity 2012: 30<sup>th</sup> Annual Scientific Meeting, San Antonio, TX, 20-24 Sep 12.

**FEASIBILITY OF INCLUDING LIMITED MINDFULNESS TRAINING IN AN EXISTING THERAPEUTIC LIFESTYLE CHANGE (TLC) PROGRAM**

Nancy S. Saum, MS, AHN-BC, Elaine Walizer, MSN, Marina Vernalis, DO, Henry M. Jackson Foundation, Rockville, MD

**Background/problem being addressed:** Since mindfulness (the simple act of paying attention to what is happening in any moment--without judgment or criticism), encourages us to take greater responsibility for our choices, a potential role for mindfulness in improving diet and exercise lifestyle habits and behaviors has been suggested. However, most often, mindfulness training occurs as a referral to a separate, time-intensive program.

**Description:** This presentation describes the novel integration of brief mindfulness training into an existing TLC curriculum within a cardiovascular risk reduction program. Abbreviated (10-minute) mindfulness practices (mindful eating, body scan, awareness of breath, mindful movement, walking meditation) were successfully incorporated into 12 weekly 1-hour support groups. Over a 2.5 year period, 142 participants met as 30 cohorts, for a total of 360 support group sessions.

**Evaluation:** All 142 group members participated in the mindfulness practices. On the post-curriculum evaluations (n=123), the mean participant rating for "meeting personal objectives and expectations" (weight loss and increased physical activity) was 4.6, on a scale of 1(low) to 5(high). Stated benefits of the mindfulness practices included: relaxation, self-compassion, body awareness, increased patience, improved sleep, greater health consciousness, and better management of time and stress. 32% of the respondents also reported incorporating mindfulness practices into their daily lives during the 12 weeks of the TLC program.

**Conclusions:** While some participants were indeed skeptical, all were willing to learn about mindfulness and participate in the practices. Many participants reported benefit from even brief exposure to mindfulness principles and practice. A formative evaluation of the TLC program is underway to explore the extent of the positive impact of mindfulness and its influence on participant success.

**Implications for practice:** Abbreviated mindfulness training has the potential to augment the benefits of a TLC program. Further studies are needed to demonstrate its efficacy in health promotion.

**Disclaimer:** "The views expressed in this abstract are those of the author and do not reflect the official policy of the Department of the Army, Department of Defense, or U.S. Government."

## **INTEGRATIVE CARDIAC HEALTH PROJECT RISK SCORE IMPROVES CARDIOVASCULAR RISK ASSESSMENT IN WOMEN WITH SUBCLINICAL ATHEROSCLEROSIS**

Randolph Modlin, MD, Elaine Walizer, MSN, Mariam Kashani, CRNP,  
Arn Eliasson, MD, Marina Vernalis, DO

Walter Reed Army Medical Center, Washington, DC  
Henry M. Jackson Foundation, Rockville, MD

**Background:** The Framingham Risk Score (FRS) substantially underestimates lifetime risk of cardiovascular (CV) disease, especially in women, when only a 10-year risk model is used. The Integrative Cardiac Health Project (ICHP) CV Risk Score, which incorporates family history and novel risk factors such as BMI, waist circumference, diastolic BP, LDL-cholesterol, triglycerides, and hsCRP, has shown enhanced predictive performance in middle-aged men.

**Objective:** To examine our hypothesis that the ICHP Risk Score may improve CV disease risk identification in women, we compared risk prediction using FRS and ICHP Risk Score in a cohort of women with abnormal carotid intima-media thickness (CIMT).

**Methods:** 128 women underwent clinical and serologic risk factor screening for entry into a lifestyle change intervention study. All had at least 2 CV disease risk factors and subclinical atherosclerosis by CIMT (>75<sup>th</sup> percentile by age/gender). For this analysis 15 women with diabetes were excluded. FRS and ICHP Risk Score were calculated and compared.

**Results:** Of 113 non-diabetic (mean age=54, range 26 to 81), predominately black (50%) women, 4% smoked, 47% were hypertensive and 81% were dyslipidemic including 27% with low HDL and 33% with LDL>130 mg/dL. Family history of CV disease was positive in 65%. Subjects were obese (mean BMI=32; mean waist circumference=100 cm). Triglycerides were not elevated (mean=109 mg/dL); 50% had hsCRP  $\geq$  0.3 mg/dL. All subjects were identified as having a low 10-year risk by FRS. When the ICHP Risk Score was applied, 60% shifted from low to medium risk ( $p<0.0001$ ).

**Conclusions:** The ICHP Risk Score dramatically improves CV disease risk prediction in this cohort of women with subclinical atherosclerosis. These findings emphasize the need for improved CV disease risk identification in women. Family history and other novel risk factors add predictive value to current risk models and identify potential therapeutic targets.

**Citation:** Modlin R, Walizer E, Kashani M, Vernalis M, Eliasson A. Integrative Cardiac Health Project risk score improves cardiovascular risk assessment in women with subclinical atherosclerosis. Conference of the American College of Physicians—Army Chapter, Bethesda, MD, 19 Nov 2010, podium presentation

## **Cardiac Rehabilitation Involving Lifestyle Modification Alters Comprehensive Plasma Metabolomic Profiles Defined by LC-FTMS**

Ellsworth DL, Soltow QA, Kolli K, Patney HL, Jones DP, and Vernalis MN. Windber Research Institute, Windber, PA; Emory University, Atlanta, GA; Walter Reed Army Medical Center, Washington, DC.

**Background:** Noninvasive coronary artery disease (CAD) management involves risk factor modification through comprehensive lifestyle change. Although lifestyle change is effective in improving traditional CAD risk profiles, little is known about other physiologic responses that influence disease progression. We assessed the hypothesis that metabolomic profiling, which integrates information on dietary, behavioral, and lifestyle factors, can provide important information on CAD risk reduction.

**Methods:** Patients (n=17) participated in a prospective, nonrandomized, cardiac rehabilitation program designed to stabilize or reverse CAD progression through dietary changes, exercise, stress management, and group support. Nonintervention controls (n=17) were matched to patients based on age, gender, and disease status. Plasma metabolic profiles run in duplicate were generated by liquid chromatography-Fourier transform mass spectrometry (LC-FTMS) and evaluated over 1 year.

**Results:** Participants showed significant improvement in traditional CAD risk factors at three months and one year. Metabolomic profiling identified 12,859 metabolite features in plasma; 4,432 features were present in more than 90% of the 102 samples analyzed. False discovery rate (FDR, 10%) analysis detected changes in 19 metabolites after 3 months and 7 metabolites after 1 year in participants, but changes in only 1 metabolite at 3 months in controls. At the 1-year examination, 87 differences in metabolite profiles distinguished participants from controls. Metabolites changing significantly in abundance were matched to primarily plant-derived compounds associated with inflammation and platelet aggregation in metabolomics databases (METLIN and Madison Metabolomics Consortium Database). Principal component analysis (PCA) showed clear differences in metabolite abundance during the program and distinct profiles of metabolite change in participants with diagnosed heart disease compared to those with only elevated risk factors.

**Discussion:** Cardiac rehabilitation involving lifestyle change can effectively alter traditional risk factors and plasma metabolomic profiles, thus reducing risk for cardiac events. In conclusion, metabolomic profiling detects a vast array of diverse metabolites and provides another dimension to understanding complex biological processes involved in cardiac rehabilitation.

## Sleep Parameters Associated With Hyperinsulinemia Increase CVD Risk

Henry M. Jackson Foundation for the Advancement of Military Medicine  
Walter Reed Army Medical Center, Washington, DC

Mariam Kashani MS, CRNP, Arn Eliasson MD, Marina Vernalis DO

**Introduction:** Obstructive Sleep Apnea Syndrome (OSAS) is a well-established risk factor for cardiovascular disease (CVD). Multiple mechanisms have been proposed to explain the association between OSAS and CVD. Hyperinsulinemia may play a role in this association.

**Objective:** We sought to examine the relationship between insulin levels and parameters of sleep.

**Methods:** Consecutive participants entering a 6-month integrative CVD risk reduction program to improve nutrition, exercise, stress and sleep completed questionnaires including the Epworth Sleepiness Scale (ESS), Fatigue Scale, Pittsburgh Sleep Quality Index (PSQI) and the Berlin Questionnaire for sleep apnea risk. Data collection also included a cardiac-relevant lab panel. Differences between subjects with normal insulin (<20 ug/dL) and abnormal insulin (insulin  $\geq$  20 ug/dL) were analyzed by t-test.

**Results:** Of 127 consecutive participants entering the program, mean age  $51.3 \pm 13.9$  years there were 54 men (43%), 65 Caucasian, 49 African-American, 7 Hispanic, 2 Asian and 4 other. Insulin levels in 104 (82%) participants were normal and abnormal in 23(18%). There were no differences in age, gender or race between groups.

|                            | Epworth  | Fatigue | PSQI    | Berlin | % HgbA1C | CVD Risk Score |
|----------------------------|----------|---------|---------|--------|----------|----------------|
| Insulin (<20 ug/dL)        | 9.2+5.5  | 4.6+2.3 | 7.3+3.8 | 49%    | 5.9+0.9  | 16.7           |
| Insulin ( $\geq$ 20 ug/dL) | 13.5+5.8 | 5.9+1.9 | 9.0+4.4 | 86%    | 6.8+1.6  | 23.7           |
| p                          | 0.001    | 0.02    | 0.07    | 0.001  | 0.001    | 0.03           |

**Conclusion:** Abnormal insulin levels that are associated with unhealthy metabolic and behavioral states place patients at higher risk for CVD. Higher insulin levels associated with increased CVD risk include states of glucose dysregulation, sleepiness, fatigue and high risk for OSAS. We hypothesize that an integrative lifestyle change program can help patients to avoid the resulting adverse consequences of hyperinsulinemia by reducing their insulin levels and potentially providing targets for intervention to reduce CVD risk and to improve parameters of sleep.

## COMPREHENSIVE, EARLY ASSESSMENT IS CRITICAL FOR CARDIOVASCULAR PREVENTION

Randolph Modlin MD, Mariam Kashani MS, CRNP,  
Arn Eliasson MD, Marina Vernalis DO

Walter Reed Army Medical Center, Washington, DC;  
Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, MD

**Background:** During the Korean and Viet Nam conflicts, autopsy findings demonstrated the early development of atherosclerosis in Active Duty (AD) soldiers. Given that cardiovascular (CV) risk factors are on the rise in the US military population, there is a clear need to identify and change reversible risk factors and health behaviors in soldiers long before clinical symptoms present. Prevalence patterns for co-existing risk factors may inform efforts for prevention of CV disease.

**Objective:** We assessed multiple CV risk factors and health behaviors in a group of young soldiers with hypertension utilizing laboratory studies, anthropometrics, validated questionnaires and sophisticated actigraphic devices to measure activity levels and sleep.

**Methods:** 12 consecutive AD soldiers entering the Integrative Cardiac Health Project at Walter Reed had lipid and glucose metabolism studies done along with measurement of body mass index (BMI), waist circumference (WC) and % body fat. The soldiers also completed Berlin Questionnaire for sleep apnea, and wore Sensewear actigraphic armbands continuously for up to 5 days. Soldiers with high systolic ( $\geq$ 120 mm Hg) or diastolic ( $\geq$ 80 mm Hg) blood pressure were compared with soldiers who had normal blood pressure using the Student's t-test or chi-square test as appropriate.

**Results:** Of 12 participants (7 men, average age 27.8 years, 8 Caucasian, 3 African-American, 1 other), 7 had systolic hypertension or diastolic hypertension (6 had both). These hypertensive soldiers were older (32 vs 22 years,  $p=0.02$ ) and had multiple risk factors for metabolic syndrome: Dyslipidemia (total cholesterol 198 vs 153 mg/dL,  $p=0.02$ ; LDL cholesterol 122 vs 83 mg/dL,  $p=0.03$ ); Obesity (BMI 31.9 vs 27.8 kg/m<sup>2</sup>,  $p=0.19$ , WC 105 vs 85 cm,  $p=0.01$ , and % body fat 37 vs 29%,  $p=0.26$ ); and Glucose Dysregulation (insulin 26 vs 9 uIU/ml,  $p=0.26$ ; HOMA 5.8 vs 1.7,  $p=0.27$ ). The hypertensive soldiers also had higher prevalence of sleep apnea (43 vs 0%,  $p=0.11$ ), and were at markedly higher risk for CV disease (IChP CV Risk Score 10.3=moderate risk vs 4.8 points=low risk,  $p=0.02$ ). There were no significant differences in sleep time but soldiers with normal blood pressure exercised  $\frac{1}{2}$  hour more each day compared to hypertensive soldiers.

**Conclusion:** The findings demonstrate clustering of multiple risk factors for CV disease in young soldiers emphasizing the need for comprehensive early assessment for CV disease prevention. In view of the multiple co-morbid risk factors, the use of an integrative intervention strategy may be highly effective.

**Citation:** Modlin R, Kashani M, Eliasson A, Vernalis M. Comprehensive early assessment is critical for cardiovascular prevention. Conference of the American College of Physicians—Army Chapter, Bethesda, MD, 18 Nov 2010, podium presentation

## **The Need for Cardiovascular Prevention in Young Military Service Members**

Randolph Modlin MD, Mariam Kashani MS, CRNP,  
Arn Eliasson MD, Karla Bailey BS, RDMS, Marina Vernalis DO

Walter Reed Army Medical Center and Jackson Foundation for the Advancement of Military  
Medicine, Washington, DC

**Background:** Recent data suggest worrisome trends in the prevalence of risk factors for atherosclerosis in Active Duty (AD) soldiers.

**Objective:** We sought to examine CV risk in a group of young AD members.

**Methods:** 14 consecutive AD soldiers completed Carotid Intima-Medial Thickness measurement (CIMT--a measure of atherosclerosis), IPAQ (international physical activity questionnaire), BMI, and labs. Differences between subjects with normal and abnormal CIMT (>75% for gender and age) were analyzed by t-test.

**Results:** Of 14 participants (9 men), average age 27.7 years, 5 had abnormal CIMT. These five soldiers exercised less ( $524 \pm 183$  MET-min/week versus  $1577 \pm 1253$ ,  $p=0.10$ ), showed more snoring/OSA (60% versus 11%,  $p=0.05$ ), weighed more ( $BMI=32.4 \pm 5.6$  kg/m<sup>2</sup> versus  $28.8 \pm 4.0$ ,  $p=0.18$ ), had dyslipidemia (100% versus 33%,  $p=0.01$ ), lower HDL ( $43.2 \pm 11.8$  mg/dL versus  $55.7 \pm 11.4$ ,  $p=0.08$ ), and lower vitamin D ( $12.5 \pm 4.7$  pg/mL versus  $20.0 \pm 7.9$ ,  $p=0.08$ ).

**Conclusion:** In this cohort of young soldiers, subclinical atherosclerosis was prevalent. Reversible risk factors were identified with easily obtained and inexpensive assessment tools. Our experience supports earlier assessment and prevention to conserve the Fighting Force.

**Citation:** Modlin R, Kashani M, Eliasson A, Bailey K, Vernalis M. The need for cardiovascular prevention in young military service members. Conference of the American College of Physicians—Army Chapter, Bethesda, MD, 18 Nov 2010, poster presentation

## Should Subclinical Hypothyroidism Be Treated to Lower Cardiovascular Risk?

Maren Mayhew MS, CRNP, Arn Eliasson MD, Mariam Kashani MS, CRNP, Marina Vernalis DO

**Background:** Subclinical hypothyroidism (SCH) is diagnosed when TSH is mildly elevated but thyroid hormone levels are normal. Treatment guidelines endorse individualized therapy, offering thyroid replacement for SCH only when symptoms of hypothyroidism are clinically convincing. However, SCH has been associated with increased risk of coronary heart disease (CHD) and while controversial, research has shown that replacement therapy may improve CHD risk factors. In order to inform therapeutic decisions in our cardiovascular disease prevention program (CPP), a program managed by Nurse Practitioners, we sought to evaluate the important health associations of SCH in subjects entering our CPP.

**Methods:** Patients entering our CPP through self-referral or referral by a provider are evaluated by a Nurse Practitioner with history and physical examination, anthropometrics, a panel of laboratory tests, and validated questionnaires assessing sleep behaviors and stress levels. Consecutive patients over a two year period were considered in this analysis. Patients with the diagnosis of overt thyroid disease and patients on thyroid replacement therapy were excluded. Using a TSH cutoff of  $>4.2$  uIU/dL, subjects with SCH were compared with patients whose thyroid panel was normal, using unpaired t-tests. Relationships between TSH and other continuous clinical variables were assessed with the Spearman's rank-order correlation.

**Results:** Of 340 consecutive patients, 51 (15%) were excluded for diagnosed thyroid disease or thyroid replacement medication. The remaining 289 patients (165 women) comprised the study set with 111 Caucasian, 89 African-American, 12 Hispanic, 2 Asian and 75 undeclared. There were 10 patients (3.5%) with SCH (6 women, mean TSH  $4.74 \pm 0.41$ ) and 279 patients with normal thyroid studies (158 women, mean TSH  $1.78 \pm 0.82$ ). For patients with and without SCH, two sample t-tests showed no differences in BMI, waist circumference, perceived stress levels, or C-reactive protein. Indices of glucose metabolism between groups were not statistically different, including fasting glucose, HbA1c, and HOMA. Compared to normal subjects, patients with SCH showed no differences in sleep habits and symptoms, including sleep latency, sleep duration, habitual snoring, risk for sleep apnea, daytime sleepiness and fatigue. Lipid studies showed no statistical differences in total cholesterol ( $p=0.55$ ), LDL ( $p=0.71$ ), HDL ( $p=0.16$ ), TG ( $p=0.77$ ), PLA2 ( $p=0.18$ ) or LPa ( $p=0.68$ ). Spearman's rank-order correlation showed a statistically significant inverse correlation between TSH level and LPa ( $\rho = -0.146$ ,  $p=0.012$ ) and identified a correlation between TSH level and HDL ( $\rho = 0.146$ ,  $p=0.013$ ). Framingham risk index was not statistically different between patients with SCH and normals ( $p=0.33$ ).

**Conclusion:** SCH was not associated with an extensive array of CHD risk factors in our population. Our findings support following the current endocrinology guidelines, offering thyroid replacement for SCH only when symptoms of hypothyroidism are clinically compelling. In our Nurse Practitioner managed CPP, the diagnosis of SCH does not appear to warrant thyroid replacement therapy for cardiovascular benefit but should be carefully considered for each patient's circumstances.

**Citation:** Mayhew M, Eliasson A, Kashani M, Vernalis M. Should subclinical hypothyroidism be treated to lower cardiovascular risk? Conference of the American College of Nurse Practitioners, Tampa, FL, 20-24 Oct 2010, poster presentation

## **The Need for Cardiovascular Prevention in Young Military Service Members**

Mariam Kashani MS, CRNP, Arn Eliasson MD, Marina Vernalis DO

Walter Reed Army Medical Center and Jackson Foundation for the Advancement  
of Military Medicine, Washington, DC

**Background:** Recent data suggest worrisome trends in the prevalence of risk factors for atherosclerosis in Active Duty (AD) soldiers.

**Objective:** We sought to examine CV risk in a group of young AD members.

**Methods:** 14 AD soldiers completed Carotid Intima-Medial Thickness measurement (CIMT--a measure of atherosclerosis), IPAQ (international physical activity questionnaire), BMI, and labs. Differences between subjects with normal and abnormal CIMT (>75% for gender and age) were analyzed by t-test.

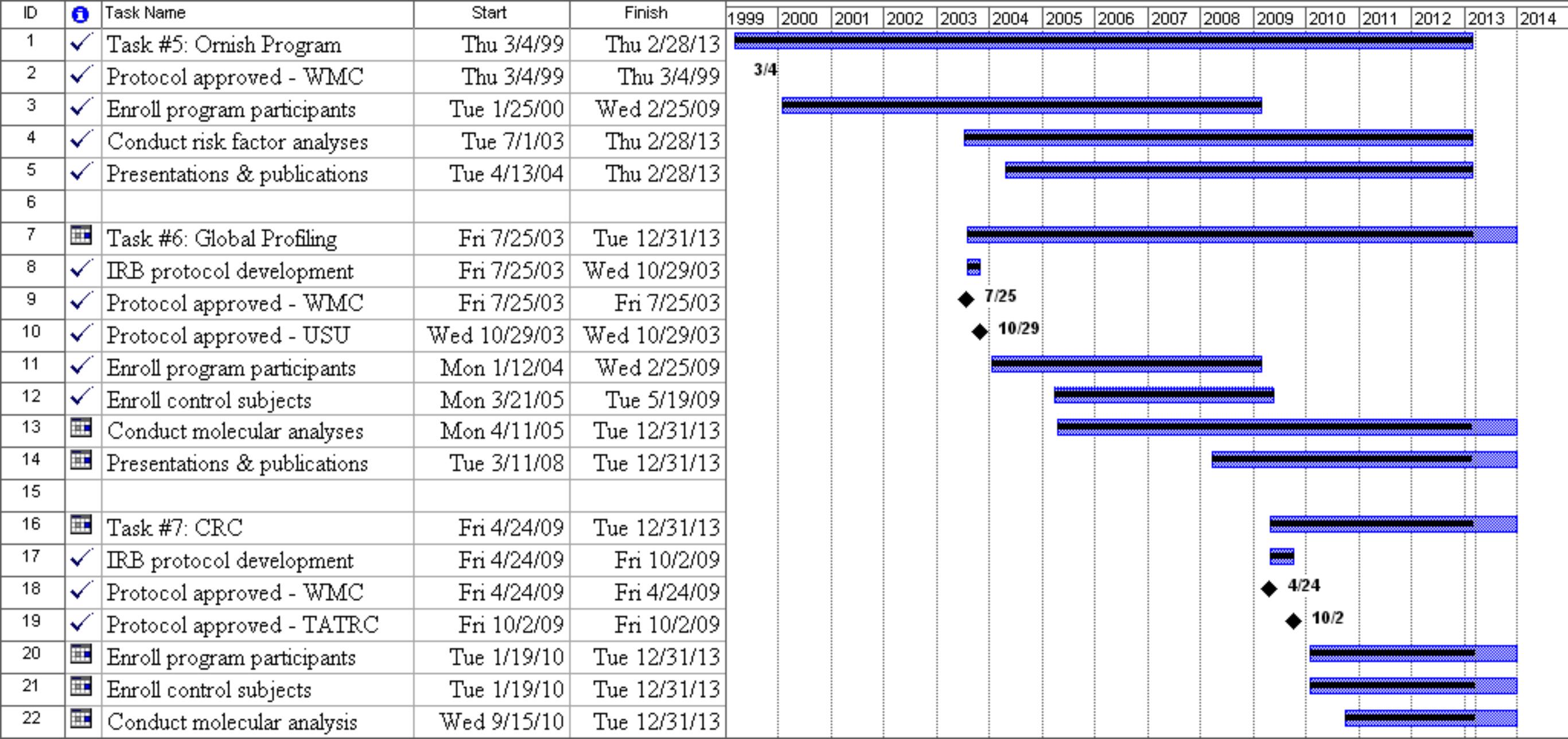
**Results:** Of 14 participants (9 men), average age 27.7 years, 5 had abnormal CIMT. These five soldiers exercised less ( $524 \pm 183$  MET-min/week versus  $1577 \pm 1253$ ,  $p=0.10$ ), showed more snoring/OSA (60% versus 11%,  $p=0.05$ ), weighed more (BMI= $32.4 \pm 5.6$  kg/m<sup>2</sup> versus  $28.8 \pm 4.0$ ,  $p=0.18$ ), had dyslipidemia (100% versus 33%,  $p=0.01$ ), lower HDL ( $43.2 \pm 11.8$  mg/dL versus  $55.7 \pm 11.4$ ,  $p=0.08$ ), and lower vitamin D ( $12.5 \pm 4.7$  pg/mL versus  $20.0 \pm 7.9$ ,  $p=0.08$ ).

**Conclusion:** In this cohort of young soldiers, subclinical atherosclerosis was prevalent. Reversible risk factors were identified with easily obtained and inexpensive assessment tools. Our experience supports earlier assessment and prevention to conserve the Fighting Force.

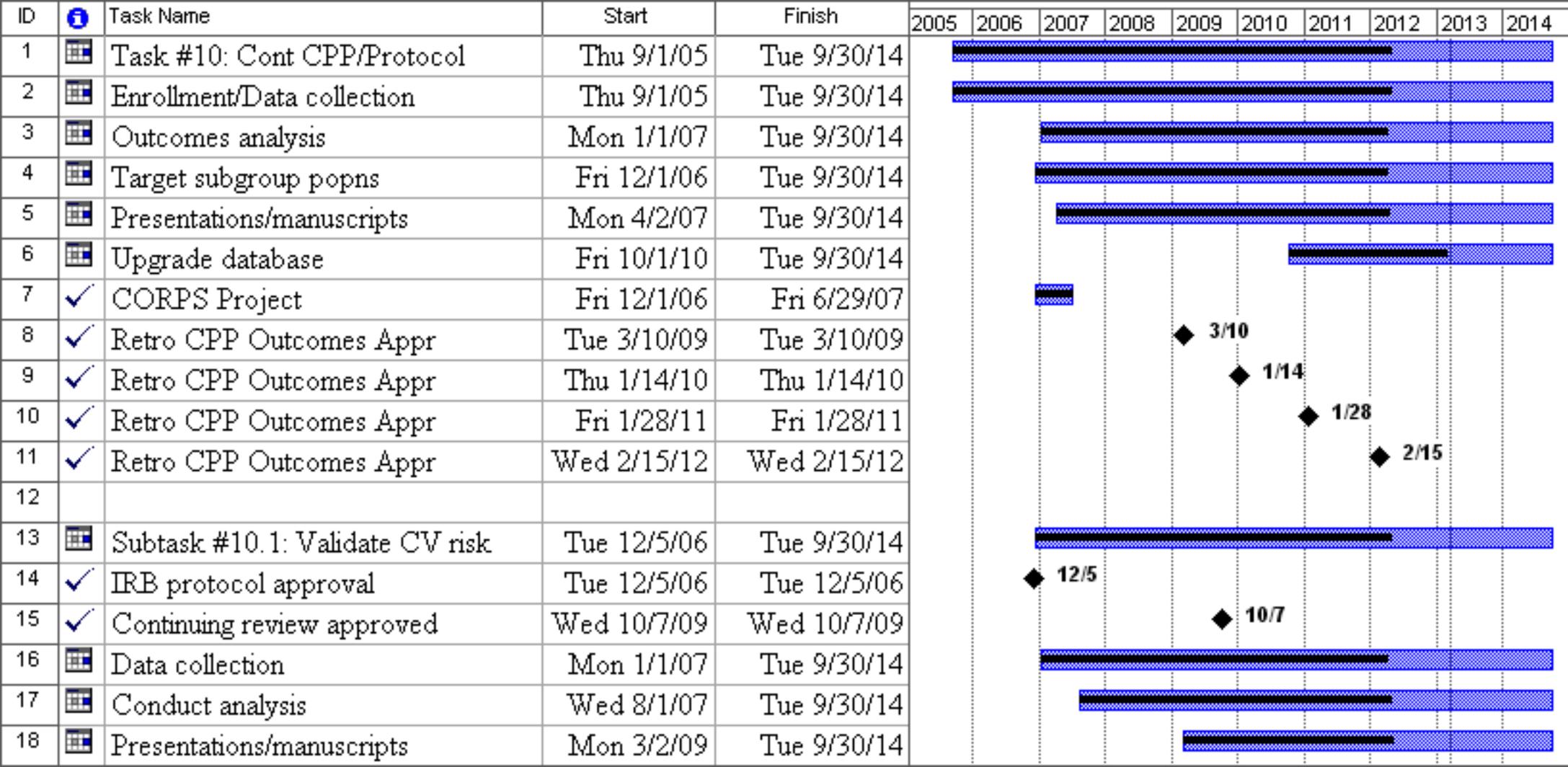
**Citation:** Kashani M, Eliasson A, Vernalis M. The need for cardiovascular prevention in young military service members. Force Health Protection Conference, Phoenix, AZ, 10 Aug 2010, poster presentation

**Appendix B**  
Gantt Charts

| ID | Task Name                              | Start        | Finish       | 2005 | 2006   | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 |
|----|--|--------------|--------------|------|--------|------|------|------|------|------|------|------|
| 1  | Task #3: CADRe 5-Year Follow-up        | Wed 3/1/06   | Tue 4/30/13  |      |        |      |      |      |      |      |      |      |
| 2  | IRB protocol approval                  | Tue 5/23/06  | Tue 5/23/06  |      | ◆ 5/23 |      |      |      |      |      |      |      |
| 3  | Participant enrollment/data collection | Fri 2/2/07   | Wed 6/30/10  |      |        |      |      |      |      |      |      |      |
| 4  | Data reconciliation                    | Fri 10/1/10  | Fri 9/30/11  |      |        |      |      |      |      |      |      |      |
| 5  | Conduct analysis                       | Wed 12/1/10  | Thu 1/31/13  |      |        |      |      |      |      |      |      |      |
| 6  | Publication plan                       | Wed 12/1/10  | Fri 2/15/13  |      |        |      |      |      |      |      |      |      |
| 7  | Presentations and manuscripts          | Tue 2/1/11   | Tue 4/30/13  |      |        |      |      |      |      |      |      |      |
| 8  |  |              |              |      |        |      |      |      |      |      |      |      |
| 9  | Task #4: BATTLE trial                  | Thu 9/1/05   | Fri 10/29/10 |      |        |      |      |      |      |      |      |      |
| 10 | IRB protocol approval                  | Tue 4/25/06  | Tue 4/25/06  |      | ◆ 4/25 |      |      |      |      |      |      |      |
| 11 | Intervention preparation               | Tue 4/25/06  | Fri 11/30/07 |      |        |      |      |      |      |      |      |      |
| 12 | Recruitment/Enrollment/Data            | Thu 11/15/07 | Wed 3/10/10  |      |        |      |      |      |      |      |      |      |
| 13 | Addendum submission/approval           | Thu 7/1/10   | Fri 1/14/11  |      |        |      |      |      |      |      |      |      |
| 14 | Data collection (Main study)           | Tue 1/1/08   | Thu 7/15/10  |      |        |      |      |      |      |      |      |      |
| 15 | Data collection (Addendum)             | Tue 1/18/11  | Wed 5/18/11  |      |        |      |      |      |      |      |      |      |
| 16 | Database reconciliation (Main study)   | Thu 7/15/10  | Wed 6/15/11  |      |        |      |      |      |      |      |      |      |
| 17 | Data analysis (Main study)             | Mon 1/3/11   | Fri 7/29/11  |      |        |      |      |      |      |      |      |      |
| 18 | Quantitative analysis (Addendum)       | Fri 4/1/11   | Fri 9/30/11  |      |        |      |      |      |      |      |      |      |
| 19 | Qualitative analysis (Addendum)        | Fri 4/1/11   | Tue 1/31/12  |      |        |      |      |      |      |      |      |      |
| 20 | Publication plan                       | Fri 4/1/11   | Fri 9/30/11  |      |        |      |      |      |      |      |      |      |
| 21 | Presentations and manuscripts          | Wed 9/1/10   | Wed 10/31/12 |      |        |      |      |      |      |      |      |      |



| ID | Task Name                     | Start        | Finish       | 2008   | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
|----|-------------------------------|--------------|--------------|--------|------|------|------|------|------|------|------|------|
| 1  | Subtask #7a: STEP             | Fri 8/29/08  | Tue 12/31/13 |        |      |      |      |      |      |      |      |      |
| 2  | ✓ IRB protocol development    | Fri 8/29/08  | Mon 5/11/09  |        |      |      |      |      |      |      |      |      |
| 3  | ✓ Protocol approved at WMC    | Fri 8/29/08  | Fri 8/29/08  | ◆ 8/29 |      |      |      |      |      |      |      |      |
| 4  | ✓ Protocol approved at TATRC  | Mon 5/11/09  | Mon 5/11/09  | ◆ 5/11 |      |      |      |      |      |      |      |      |
| 5  | ✓ Enroll program participants | Tue 9/15/09  | Mon 4/30/12  |        |      |      |      |      |      |      |      |      |
| 6  | Conduct molecular analysis    | Wed 9/15/10  | Tue 12/31/13 |        |      |      |      |      |      |      |      |      |
| 7  |                               |              |              |        |      |      |      |      |      |      |      |      |
| 8  | Task #9: MI in Young Military | Fri 6/27/08  | Tue 12/31/13 |        |      |      |      |      |      |      |      |      |
| 9  | ✓ IRB protocol development    | Fri 6/27/08  | Thu 9/30/10  |        |      |      |      |      |      |      |      |      |
| 10 | ✓ Protocol approved at WMC    | Fri 6/27/08  | Fri 6/27/08  | ◆ 6/27 |      |      |      |      |      |      |      |      |
| 11 | ✓ WRNMMC protocol development | Wed 8/10/11  | Fri 10/14/11 |        |      |      |      |      |      |      |      |      |
| 12 | ✓ WRNMMC protocol submitted   | Fri 10/14/11 | Fri 10/14/11 |        |      |      |      |      |      |      |      |      |
| 13 | Protocol approved at WRNMMC   | Mon 10/31/11 | Fri 2/17/12  |        |      |      |      |      |      |      |      |      |
| 14 | Protocol approved at TATRC    | Wed 11/30/11 | Mon 4/30/12  |        |      |      |      |      |      |      |      |      |
| 15 | Conduct molecular analysis    | Thu 3/1/12   | Tue 12/31/13 |        |      |      |      |      |      |      |      |      |



| ID | Task Name                              | Start       | Finish       | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
|----|--|-------------|--------------|------|------|------|------|------|------|------|------|
|    |  |             |              |      |      |      |      |      |      |      |      |
| 1  | Subtask #10.5: Initiate ZENTH trial    | Fri 1/1/10  | Fri 10/30/15 |      |      |      |      |      |      |      |      |
| 2  | Protocol development                   | Fri 1/1/10  | Wed 5/9/12   |      |      |      |      |      |      |      |      |
| 3  | Protocol approval                      | Wed 5/9/12  | Mon 4/1/13   |      |      |      |      |      |      |      |      |
| 4  | Recruitment/enrollment/data collection | Mon 4/1/13  | Tue 9/30/14  |      |      |      |      |      |      |      |      |
| 5  | Conduct analysis                       | Thu 10/1/15 | Thu 6/30/16  |      |      |      |      |      |      |      |      |
| 6  | Publication plan                       | Wed 4/1/15  | Fri 9/30/16  |      |      |      |      |      |      |      |      |
| 7  | Presentations and manuscripts          | Tue 10/1/13 | Fri 12/30/16 |      |      |      |      |      |      |      |      |

**Attachement 1****ICHP Personnel List**

| <b>Employee ID</b> | <b>Name</b>               | <b>Role on project</b>         |
|--------------------|---------------------------|--------------------------------|
| 100263             | Curry,Troy T              | Graphic Artist                 |
| 100884             | Ford,Deborah A            | Graphic Artist                 |
| 101469             | Nixon,Audra H             | Program Manager                |
| 101525             | Walizer,Elaine M          | Dir., Clinical Research Coord  |
| 101640             | Turner,Ellen              | Health Coach/Coordinator       |
| 102286             | Kashani,Mariam            | Director, Clinical Programs    |
| 107563             | Vernalis,Marina N.        | Medical Director, ICHP         |
| 107698             | Hill,Lydia                | Clinical/Admin Project Officer |
| 107807             | Tschiltz,Nancy            | Dietician                      |
| 107878             | Chrosniak,Linda Doss      | Psychologist                   |
| 107883             | Hoffman,Jacqueline Anne   | Stress Management Instructor   |
| 108069             | Saum,Nancy Seaby          | Senior Nurse                   |
| 108070             | Eliasson,Arn H.           | Clin/Res. Physician Consultant |
| 108424             | Phillips,Jill S           | Nurse Practitioner             |
| 108487             | Caporiccio,Christa        | Administrative Assistant       |
| 108596             | Halsey,Joy F              | Dietician                      |
| 108660             | Mayhew,Maren S            | Nurse Practitioner             |
| 108704             | Buenafior,Graeme          | Health Fitness Instructor      |
| 108748             | Henderson,Josephine       | Administrative Assistant       |
| 109318             | Lampkin,Bettina Corinne   | Senior Financial Analyst       |
| 109754             | Ude,Assumpta Onyinye      | Nurse Practitioner             |
| 109918             | Bailey,Karla Christian    | Data Outcomes Specialist       |
| 110252             | Connally,Brooke D.        | Graphic Artist                 |
| 111205             | Bishop,Adina Oakes        | Nurse                          |
| 112272             | Jones,Phyllis Ann         | Nurse Practitioner             |
| 112693             | Lalicato,Amanda Rose      | Nurse Practitioner             |
| 112843             | Edinger RN,Rosemarie Anne | Chief Nurse Officer            |

## Attachement 2

### WRI Personnel List

| <b>WRI ICHP FY09 Personnel</b>     | <b>Title</b>              |
|------------------------------------|---------------------------|
| Chen, Yaqin                        | Statistical Analyst       |
| Croft, Daniel                      | Research Associate III    |
| Decewicz, Alisha                   | Research Associate II     |
| Decewicz, David                    | Research Physician        |
| Ellsworth, Darrell                 | Senior Director           |
| Elston, Ed                         | IT Manager                |
| Furmanchik, Lydia                  | Finance Assistant         |
| Greenawalt, Amber                  | Research Assistant        |
| Hicks, Marissa                     | Intern                    |
| Jordan, Rick                       | Casual Employee           |
| Kolli, Kumar                       | Senior Director           |
| Mamula, Kimberly                   | Sr. Statistical Analyst   |
| Masiello, Matthew (funds returned) | Director, CHPDP           |
| Melley, Jen                        | Research Associate II     |
| Mural, Richard                     | Chief Scientific Officer  |
| O'Donnell, Amy                     | Research Associate II     |
| Patney, Heather                    | Research Associate II     |
| Rigby, Sean                        | Research Assistant        |
| Seitz, Brianne                     | Intern                    |
| Slavik, Julianna                   | Research Associate II     |
| Trostle, Lynn                      | Grant Program Coordinator |
| Voegtly, Laura                     | Postdoctoral Fellow       |
| Weise, Jonathan                    | IT Support Specialist     |
| Woznick, Kristen                   | Intern                    |

| <b>WMC ICHP FY09 Personnel</b> | <b>Title</b>                         |
|--------------------------------|--------------------------------------|
| Adams, Bernice                 | Licensed Clinical Social Worker      |
| Burke, Amy                     | Clinical Research Manager            |
| Gray, Patrick                  | Medical Director CRC & STEP          |
| Haberkorn, Mary Jane           | Research Nurse                       |
| Klucik, Cathy                  | US Tech                              |
| Lechak, Fran                   | Dietician                            |
| Mislanovich, Judy              | US Tech                              |
| Mladjan, Mary                  | Research Nurse                       |
| Oakes, Amy                     | US Tech                              |
| Prazich, Kathy                 | Data Entry Clerk                     |
| Rokita, Angie                  | Exercise Physiologist                |
| Sullivan, Judith               | Stress Management                    |
| Vizza, James                   | Behavioral Health Specialist for CRC |
| Walker, Kathy                  | Stress Management                    |
| Warshel, Kelly                 | Medical Director CRC & STEP          |