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C-REACTIVE PROTEIN LEVELS ACCORDING TO PHYSICAL ACTIVITY
AND BODY WEIGHT FOR PARTICIPANTS IN THE CORONARY
HEALTH IMPROVEMENT PROJECT

by

Michael T. Massey

A thesis submitted to the faculty of

Brigham Young University

in partial fulfillment of the requirements for the degree of

Master of Science

Department of Exercise Sciences

Brigham Young University

August 2007

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BRIGHAM YOUNG UNIVERSITY

GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Michael T. Massey

This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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As chair of the candidate's graduate committee, I have read the thesis of Michael T. Massey in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

C-REACTIVE PROTEIN LEVELS ACCORDING TO PHYSICAL ACTIVITY AND BODY WEIGHT FOR PARTICIPANTS IN THE CORONARY HEALTH IMPROVEMENT PROJECT

Michael T. Massey

Department of Exercise Sciences

Master of Science

Objectives. To evaluate C-reactive protein (CRP) levels according to weight and physical activity. The study explored how changes in CRP were associated with baseline CRP, weight, and physical activity and changes in these variables.

Methods. A randomized controlled study design assigned 348 individuals to the intervention or control group with measurements taken at baseline, 6 weeks, and 6 months of body weight, physical activity, and serum CRP levels. Participants attended an intensive 40-hour educational course delivered over a four-week period.

Results. At baseline, CRP was negatively associated with total steps/week, and positively associated with weight, BMI, percent fat, and saturated fat at baseline. CRP significantly decreased through 6 weeks and also through 6 months for only those with high CRP at baseline. For those with high CRP at baseline, the decrease was significant for normal,

overweight, and obese groups of people, albeit most pronounced for those with normal weight at baseline and least pronounced for those who were obese at baseline. Changes in weight or physical activity were not significantly associated with changes in CRP.

Conclusions. Over 6 week and 6 month follow-up periods, the intervention failed to discriminate changes in CRP. Changes in CRP were only associated with baseline levels of CRP and BMI and were not associated with changes in any of the selected variables considered.

ACKNOWLEDGMENTS

I would first like to acknowledge my thesis committee for all the hard work they have put into help me write this study. Gary Mack, my committee chair, for taking me on as a student and guiding me through the research process. Ray Merrill for the many hours he put into help get the results and tables finished. Larry Tucker for his consistent feedback and insight. Thank you all.

I would also like to thank Steven Aldana for introducing me to this topic and giving me confidence to complete my educational and career goals.

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**C-reactive Protein Levels According to Physical Activity and Body
Weight for Participants in the Coronary Health
Improvement Project**

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ABSTRACT

Objectives. Evaluate C-reactive protein (CRP) levels according to weight and physical activity. The study explored how changes in CRP were associated with baseline CRP, weight, and physical activity and changes in these variables.

Methods. A randomized controlled study design assigned 348 individuals to the intervention or control group with measurements taken at baseline, 6 weeks, and 6 months of body weight, physical activity, and serum CRP levels. Participants attended an intensive 40-hour educational course delivered over a four-week period.

Results. At baseline, CRP was negatively associated with total steps/week, and positively associated with weight, BMI, percent fat, and saturated fat at baseline. CRP significantly decreased through 6 weeks and also through 6 months for only those with high CRP at baseline. For those with high CRP at baseline, the decrease was significant for normal, overweight, and obese groups of people. Changes in weight or physical activity were not significantly associated with changes in CRP.

Conclusions. Over 6 week and 6 month follow-up periods, the intervention failed to discriminate changes in CRP. Changes in CRP were only associated with baseline levels of CRP and BMI and were not associated with changes in any of the selected variables considered.

Keywords: C-reactive protein (CRP); physical activity; body weight; percent body fat; saturated fat; body mass index (BMI); Coronary Health Improvement Project (CHIP)

INTRODUCTION

C-reactive protein (CRP) is a nonspecific acute-phase reactant that has traditionally been used to detect acute injury infection and inflammation (Backes, et al., 2004). Some of the typical causes of inflammation are cytomegalovirus, chlamydia pneumoniae, dyslipidemia, obesity and/or hormone replacement therapy (Sobal and Sinzinger, 2005). Recent studies have shown a positive independent association between CRP and atherosclerotic events (Backes, et al., 2004, Kasapis and Thompson, 2005, Ledue and Rifai, 2003). It has also been shown that high CRP is a significant and independent risk predictor of nontraumatic bone fractures (Schett, et al., 2006).

The level of serum CRP in the plasma indicates the severity of inflammation (Coppola, et al., 2006). Studies have determined that CRP levels above 3 mg/L are a good prognostic marker for future vascular events. People with and without cardiovascular arterial disease (CAD) with CRP levels above 3 mg/L are at significantly greater risk of ischemic episodes than those with CRP below 3 mg/L (Backes, et al., 2004, Coppola, et al., 2006). Serum CRP levels from 1-3 mg/L and below 1 mg/L have been shown to be at normal and low risk for CAD, respectively. Further research is needed to develop standard CRP prognostic markers for nontraumatic bone fractures.

CRP levels can be lowered through drug therapy (Sobal and Sinzinger, 2005). There is also evidence that a lifestyle intervention that improves a person's physical activity and/or eating habits may reduce CRP (Caulin-Glaser, et al., 2005, Blum, et al., 2006). Reduction of CRP levels through physical activity and weight loss is a practical

way of reducing the likelihood of a cardiac event. The Coronary Health Improvement Project (CHIP) is a specific lifestyle intervention program that may positively impact CRP levels. It is a 4-week health education intervention program that teaches participants how to improve their eating habits and physical activity in order to benefit their cardiac health. It is assumed that the program will reduce CAD risk factors, including CRP levels.

This study evaluated CRP levels according to weight and physical activity. It also explored how changes in CRP are associated with baseline CRP, weight, and physical activity and changes in these variables.

METHODS

Subject Recruitment

The SwedishAmerican Center for Complementary Medicine (SACCM) recruited participants for this study. Recruitment was from the Rockford, IL, metropolitan area with the requirement that participants be 18 years of age or older. All participants provided informed consent. Many enrolled with a spouse or significant other. For those who participated in the program with their partner (42%), the unit of randomization was *pairs*. For those who participated as individuals (58%), the unit of randomization was *individuals*. No significant differences were observed between pairs and individuals. Randomization was determined by a random number generator. The study coordinator conducted the participant sign-up process, randomization, and group assignments. Participants were excluded from the study if they had a significant condition or major illness that would prevent them from exercising. There were 403 individuals assessed for

eligibility. Three-hundred and seventy-seven were eligible, but 29 refused to participate, leaving 348 who were randomly assigned to start the program immediately (intervention group) or to wait for 6 months before beginning the program (control group). None of the participants were lost to follow-up throughout the 6 month period. The Institutional Review Board of the SwedishAmerican Health System approved the study on August 29, 2002.

Design

The study was a randomized study involving intervention and control groups. Measurements were taken at baseline, 6 weeks, and 6 months. The health education intervention was received during the first 4 weeks. The control group received no intervention during the 6 month follow-up period and was specifically told not to alter their lifestyle during the investigative period.

Intervention

The CHIP (Diehl, 1998) is an intensive community-based education intervention. Participants are required to attend a 4-week class. Each class is 2 hours long and there are 4 classes per week. Total class time is approximately 32 hours. Theory-based intervention planning was used to develop the curriculum, class design, alumni association, and each of the take-home assignments (Lupton, et al., 2003, Tudor-Locke, et al., 2004, Toobert, et al., 2003). The intervention incorporated learning theory (behaviorism) in which changes in physical and dietary behaviors were promoted using health education and positive reinforcement. In addition to encouragement and positive feedback from staff, the CHIP alumni program was designed to help participants maintain positive behavior changes.

The curriculum included the following topics: modern medicine and health myths, atherosclerosis, coronary risk factors, obesity, dietary fiber, dietary fat, diabetes, hypertension, cholesterol, physical activity, osteoporosis, cancer, lifestyle and health, the Optimal Diet, behavioral change, and self-worth.

The intervention group received a textbook and workbooks in conjunction with the CHIP lectures that closely followed the discussion topics and contained assignments with learning objectives that correlated with the discussion topics. These assignments were designed to assist the intervention group in understanding and integrating the concepts and information presented into their lives. Dietitians and medical professionals introduced the intervention group to current nutritional and medical information that related to the prevention of chronic diseases weekly.

The intervention group made dietary and physical activity goals, which they were encouraged to then follow. The dietary goal involved adopting a more plant-food based diet that emphasizes "as-grown," unrefined food. Prescribed foods included whole grains, legumes, vegetables, and fresh fruits. The diet was low in fat, animal protein, sugar, salt, very low in cholesterol, and high in fiber. The intervention group was also, accordingly with their new diet, encouraged to progressively work toward walking or exercising at a mild-to-moderate intensity for at least 30 minutes a day. A pedometer was given to the intervention group and they were encouraged to keep a physical activity log that recorded the miles they walked each day.

The primary objective of the health education intervention was to improve cognitive understanding of the importance of healthy lifestyles, nutrition and physical

activity behavior, risk factors associated with diabetes, hypertension, cardiovascular disease, and cancer.

Measures

A number of variables were considered to evaluate cardiovascular risk factors and individual health variation among participants. Measures included pedometer readings (step counts), CRP levels, body fat percentage, saturated fat levels, weight, height, BMI, chronic inflammatory conditions, age, and gender.

To determine the level of physical activity, a 7-day self-recorded pedometer log was maintained by each participant a week before measurements at baseline, 6 weeks, and 6 months. Participants from the intervention and control group wore the Walk4Life Model 2000 Life Stepper pedometer on a belt at the right hip directly above the right knee cap each day for 7 consecutive days. Immediately prior to going to bed the pedometer counts for the day were recorded and the number reset. Strike counts from pedometers are a valid and reliable method of monitoring and measuring free-living physical activity (Crouter, et al., 2003, Beets, et al., 2005, Tudor-Locke, et al., 2005). Crouter et al. (Crouter, et al., 2003) determined the validity of the Walk4Life Life Stepper pedometer by comparing pedometer readings with the number of actual steps of participants walking for 5 min on a treadmill at various speeds (54, 67, 80, 94, and 107 m/min). The correlation coefficient was ($r = 0.81, p < 0.05$) which Crouter indicates is acceptable to ascertain physical activity and “a good choice to use in research.” Beets et al. (Beets, et al., 2005) also assessed the Walk4Life pedometer by comparing it to actual steps from self-paced walking and treadmill walking (speeds: 40, 54, 67, 80, and 94

m/min). The intraclass correlation coefficient was ($R = 0.997$) for self-paced walking and ($R = 0.516, 0.745, 0.946, 0.988$ and 0.992) ($p < 0.05$) for treadmill speeds 40, 54, 67, 80, and 94 m/min respectively. Beets and Crouter both mention the Walk4Life pedometer to be more accurate the faster one walks.

Tudor-Locke et al. (Tudor-Locke, et al., 2005) conducted a study to determine how many days of pedometer readings are needed to determine the weekly physical activity of an adult. There were 90 participants enrolled (33 men and 57 female aged 49 +/-16.2 years and 44.8 +/-16.9 years respectively). BMI ranged from 21.1 to 33.9 kg/m². There was an R^2 of 0.94 and an intraclass correlation of 0.80 for wearing the pedometer for three days. The conclusion was that the time necessary to determine physical activity in a week with a pedometer was 3 days. Thus, the time taken from this study (7 days) to determine physical activity was sufficient.

Phlebotomists from the SwedishAmerican Health System's outpatient laboratory drew blood using a vacutainer (Becton-Dickinson Vacutainer Systems, Rutherford, NJ) after the participants had a 12-hour fast. Samples were allowed to clot and then centrifuged. Clinical analyses were completed at the SwedishAmerican Health System laboratory. CRP was determined using a microplate protocol based on a latex bead enhanced immunoturbidity assay from the same technician and has been demonstrated to be a valid and reliable method (Wu, et al., 2002, Otsuji, et al., 1982, Rifai, et al., 1999). Otsuji et al. (Otsuji, et al., 1982) conducted a study that demonstrates a turbidimetric immunoassay method for determining serum CRP that can be used with any spectrophotometer that can measure turbidimetrically at 340 nm. The measured

absorbances of a test sample (50 μL of serum + 2.0 mL of working antiserum solution), sample blank (50 μL of serum + 2.0 mL of sample blank buffer), reagent blank (50 μL of saline + 2.0 mL of working antiserum solution), and buffer blank (50 μL of saline + 2.0 mL of sample blank buffer) were prepared and incubated at 37 °C for 30 min.

Absorbances for the test sample (A_S), sample blank (A_{SB}), the reagent blank (A_{RB}), and the buffer blank (A_{BB}) were measured. The CRP absorbance (A_{CRP}) indicating serum CRP was calculated by the following equation: $A_{CRP} = A_S - A_{SB} - (A_{RB} - A_{BB})$. It was indicated that precision was good due to low CV% and SD at various CRP concentrations.

Rifai et al. (Rifai, et al., 1999) compared latex-enhanced immunoassay for hs-CRP with Enzyme-Linked ImmunoSorbent Assay (ELISA), a previously validated method for measuring hs-CRP, (Wu, et al., 2002) in a case controlled study using patients with peripheral arterial disease. There were 288 subjects (144 case subjects and 144 control subjects) enrolled. The Latex assay used monoclonal anti-CRP antibodies coated to polystyrene particles that formed a complex with hs-CRP present in the measured study sample. The amount of scattered light was directly proportional to the size of the antigen-antibody complex and reflected the hs-CRP concentration present in the study sample. The run-to-run CVs, at hs-CRP concentrations of 0.47, 10.5, and 54.9 mg/L were 6.4%, 3.7%, and 2.9%, respectively. The correlation between the Latex and ELISA methods based on original and log-normalized data were ($r = 0.95$, $p < 0.001$) and ($r = 0.93$, $p < 0.001$) respectively for both case and control groups. Thus, Rifai concluded

that the Latex method was comparable to ELISA and can be used in research to determine CRP levels.

Percent body fat was estimated using the Tanita TBF - 300A electrical impedance scale, which has been shown to be a valid and reliable method (Kyle, et al., 2001, Rubiano, et al., 2000). Kyle et al. (Kyle, et al., 2001) developed a study to validate a single bioelectrical impedance analysis (BIA) equation for 343 healthy white subjects (202 men and 141 women) aged 22-94 with a BMI between 17.0 and 33.8 kg/m. Fat-free mass (FFM) was measured by dual-energy x-ray absorptiometry (DEXA) (Hologic QDR-4500) and BIA (Xitron 4000B). Significant correlations were high ($r = 0.986-0.987$, $p < 0.05$), the standard error for the estimates was low ($SEE = 1.72$ kg), and technical error was 1.74 kg. A valid single prediction for FFM was developed: $FFM = -4.104 + (0.518 \times \text{height(m)}^2 / \text{resistance (kg)}) + (0.231 \times \text{weight (kg)}) + (0.130 \times \text{reactance (ohm)}) + (4.229 \times \text{sex: men} = 1, \text{ women} = 0)$. He concluded that the single prediction equation was valid for prediction of FFM in populations with large variations of age and body composition.

Rubiano et al. (Rubiano, et al., 2000) investigated the validity of the leg-to-leg BIA (Tanita TBF 310) to measure body composition using DEXA as a reference. He enrolled 39 subjects (8 male and 31 female) aged from 37 to 73 years of age with a BMI from 24.6 to 32.6. A significantly high correlation was observed ($r = 0.94$, $p < 0.001$, $SEE = 3.6$ percent body fat) for measuring body fat percentage. Rubiano concludes that leg-to-leg BIA systems may be useful in assessing total body composition in a population varying in BMI.

Saturated fat was estimated by the participants completing a self administered food survey based on the 98 item Block questionnaire (Block, et al., 1990, Hartman, et al., 1996). Block et al. (Block, et al., 1990) determined this type of questionnaire to be a valid method for determining saturated fat intake. Hartman et al. (Hartman, et al., 1996) determined the reliability of this questionnaire to be adequate for research purposes. They reported intraclass correlations between three administrations of the same survey during three different seasons between 0.56 and 0.82.

Weight and height were measured using standard medical weight and height scales calibrated by the Biometrics Department of the SwedishAmerican Health System. Body mass index (BMI) was determined using the formula weight (kg)/height (m)².

Presence of chronic inflammatory conditions were determined by health history questionnaire and documented by medical staff. Age and gender were also ascertained.

Statistical Analyses

Frequency distributions were computed and cross-tabulations were used to perform bivariate analyses between intervention status (intervention vs. control) and selected demographic variables and medication use, with statistical significance based on the Chi-square test (χ^2) (Fienberg, 1977). The Pearson correlation coefficient (r) was used to assess associations between selected variables at baseline. Comparisons between means were made and multiple regression analysis was used to assess the simultaneous influence of selected variables on CRP. The F- and t-statistics were used to evaluate regression models and measures of association. Analyses were performed using SAS

version 9.1 (SAS Institute Inc., Cary, NC, USA, 2003). Statistical significance and confidence intervals were based on the 0.05 level.

RESULTS

Of the 348 participants, ages ranged from 24 to 81 years ($M = 50.4$, $SD = 11.0$). The mean age for the intervention group was 50.1 years compared with 50.8 years in the control group [$t(346) = -0.57$, $p = 0.57$]. Characteristics of the study participants are presented for selected demographic variables in Table 1. Distributions across the levels of each variable were not significantly different between the intervention and control groups. The most common characteristic observed for each variable was: female; white; married; an annual family income of at least \$60,000; and at least some college education.

At baseline, there were significant correlations between CRP and total steps/week ($r = -0.23$, $p < 0.0001$), weight ($r = 0.46$, $p < 0.0001$), BMI ($r = 0.54$, $p < 0.0001$), percent fat ($r = 0.12$, $p = 0.0258$), and saturated fat ($r = 0.13$, $p = 0.0158$). Improvements in total steps/week, weight, BMI, percent fat, and saturated fat were observed, and were significantly greater for those in the intervention group compared with the control group (Table 2). There was not a significant difference in change in C-reactive protein (CRP) between intervention and control groups.

C-reactive protein was categorized into low, normal, and high groups (Table 3). C-reactive protein tended to increase for those with low or normal CRP at baseline, albeit only significantly so through 6 weeks for those with low CRP at baseline. On the other hand, CRP significantly decreased through 6 weeks and 6 months for those with high

CRP at baseline. The level of CRP did not significantly differ between the intervention and control groups across the baseline levels of CRP.

C-reactive protein at 6 weeks was regressed on those variables shown in Table 2. Using backward elimination, baseline BMI ($t = 5.83$, $p < 0.0001$) and baseline CRP ($t = 11.21$, $p < 0.0001$) were the only variables that remained significant in the model. The same was true for the model involving CRP at 6 months (i.e., $t = 6.61$, $p < 0.0001$ for baseline BMI and $t = 10.31$, $p < 0.0001$ for baseline CRP).

Mean change in CRP through 6 weeks and 6 months is presented according to categories of CRP and BMI at baseline in Table 4. We collapsed over intervention and control groups because no difference was observed in changes in CRP between these groups. For low CRP at baseline, changes in CRP were greater with higher BMI at baseline. The increase observed earlier for those with low CRP at baseline was greatest among individuals who were obese. On the other hand, the decrease observed for those with high CRP at baseline was greatest among those who are of normal weight.

DISCUSSION

At baseline, serum CRP levels were significantly associated with total steps/week, weight, BMI, percent fat, and saturated fat. However, although the intervention resulted in significant improvements in total steps/week, body weight, BMI, percent fat, and saturated fat among participants in the intervention group, there was no significant improvement observed in CRP levels. However, by stratifying according to baseline CRP, those with high CRP at baseline showed a significant decrease over the study period. CRP levels at 6 weeks and again at 6 months were significantly associated with

baseline BMI and baseline CRP. For those with low CRP at baseline, a significant positive change was observed through 6 weeks and 6 months. For those with high CRP at baseline, a significant decrease was observed in CRP through 6 weeks and 6 months. The decrease was most pronounced for those of normal weight and least pronounced for those who are obese. The intervention did not discriminate these patterns.

Excess adipose increases inflammation and expression of CRP (Anty, et al., 2006). The lifestyle changes of exercise and nutrition, as implemented by the CHIP, should have the greatest affect on individuals that are sedentary and obese. Typically these attributes, particularly obesity, are altered significantly through positive lifestyle changes of physical activity and nutrition. BMI, in a normal population, often has a positive association with individual CRP levels due to its association with adiposity. The majority of individuals who were obese, as indicated by their BMI, had high levels of CRP at baseline. These individuals significantly lowered their baseline CRP levels as the trial progressed. This occurred, most likely, in response to improvements in dietary intake. It should be noted that the overweight and normal weight individuals with high CRP levels experienced a greater reduction of CRP, normal weight achieving the greatest reduction, than the obese individuals as the trial progressed. A possible explanation for this occurrence is that obese individuals are not as likely to maintain improvements in their exercise and eating habits as are people with lower weight.

Individuals with low CRP levels, according to BMI at baseline, increased their mean CRP levels as the study progressed. There is nothing in the current literature that would indicate that the CHIP intervention would raise CRP levels for individuals who

have low CRP at baseline. Obese individuals with low CRP levels at baseline increased their CRP levels more so than those with low CRP at baseline who were of normal weight or overweight. The reason for this result is unclear, and deserves further study.

The intervention group's physical activity increased during the six month trial, from 40,579 steps per week (5,797 steps per day) to 47,290 steps per week (6,756 steps per day), after controlling for extra steps from the control group. Tudor-Locke et al. (Tudor-Locke and Bassett, 2004) indicate that these pedometer readings would rank the intervention group in a "low active" category. This was not enough physical activity to determine a significant independent correlation of physical activity with CRP. However, despite the low physical activity reported by the intervention group, it should be noted that they increased their physical activity significantly, by 30.5%. Hence, overall the efficacy of the CHIP intervention was demonstrated for improving physical activity and lowering weight, but not for CRP.

The participants in this study were mostly white and sufficiently self-motivated to volunteer to participate in the study. On average, participants were slightly more educated than the community average. Participants had lifestyles that permitted them to attend most, if not all, of the classes. This is evident in the high rate of attendance to this time-intensive program. Hence, generalization of these findings should be done with caution. Because the participants were self-selected, the results from this intervention may represent a best-case scenario.

The study is limited by the fact that physical activity, weight, and nutrition data were self-reported. For some variables, the control group also experienced significant

improvement, as seen with decreases in CRP among those with high CRP at baseline. Interviews with control group participants revealed that some participants were anxious to get started on improving their health behaviors, and may have begun prior to starting CHIP six months after the intervention group.

CONCLUSION

CRP was negatively associated with total steps/week, and positively associated with weight, BMI, percent fat, and saturated fat at baseline. However, CRP at 6 weeks and again at 6 months was only associated with baseline BMI and CRP. Participants with high levels of CRP at baseline who were obese showed a significant reduction of CRP over the course of the study. This is consistent with the literature, as a reduction in adiposity would reduce inflammation and CRP levels. A longer trial and improvements in the methods for measuring CRP data should be included in further such studies for better understanding the relationships between changes in CRP levels and changes in physical activity and changes in body weight.

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Table 1: Comparison of Demographic Characteristics of Intervention and Control Groups in a Therapeutic Lifestyle-Modification Program, Rockford, Illinois

Demographic Characteristic	Intervention		Control		$\chi^2_{df} (P)$
	No.	%	No.	%	
Sex					
Male	47	27.0	51	29.3	0.23, 1 (0.63)
Female	127	73.0	123	70.7	
Race					
White	167	96.0	160	93.0	2.91, 2 (0.23)
Black	4	2.3	10	5.8	
Other	3	1.7	2	1.2	
Marital Status					
Never married	12	6.9	20	11.6	2.99, 3 (0.39)
Married	138	79.8	127	73.4	
Divorced	16	9.2	16	9.2	
Widowed	7	4.1	10	5.8	
Annual family income, \$					
\$0– <\$20,000	14	8.2	12	7.1	1.03, 3 (0.79)
\$20,000– <\$40,000	34	20.0	28	16.5	
\$40,000– <\$60,000	37	21.8	41	24.1	
\$60,000 +	85	50.0	89	52.3	
Education					
<High school	4	2.3	7	4.0	6.58, 4 (0.16)
High school	37	21.5	46	26.6	
Some college	58	33.7	39	22.5	
Bachelor degree	39	22.7	38	22.0	
Post-bachelor degree	34	19.8	43	24.9	

Table 2: Physical activity and other selected variables at baseline and 6-month follow-up among participants in a therapeutic lifestyle-modification program, Rockford, Illinois

Variable	No.	Baseline Mean	6 Week Mean	Difference In Mean between Baseline and 6 Weeks	95% CI	6 Month Mean	Difference In Mean between Baseline and 6 Months	95% CI
Total steps/week								
Intervention	173	40,579	52,429	11,656	9,122, 14,190	52,951	12,476	9,773, 15,179
Control	173	43,869	45,825	2,105*	-277, 4,487	49,530	5,315*	2,769, 7,862
Body mass index								
Intervention	174	33.3	32.1	-1.2	-1.3, -1.1	31.7	-1.6	-1.9, -1.3
Control	174	31.4	31.3	-0.1*	-0.2, -0.0	31.1	-0.3*	-0.5, -0.1
Weight, kg								
Intervention	174	93.3	90.0	-3.3	-3.6, -3.0	88.8	-4.5	-5.3, -3.8
Control	174	87.7	87.6	-0.1*	-0.7, 0.4	87.1	-0.6*	-1.5, 0.1
Body fat, %								
Intervention	173	36.7	28.0	-8.7	-10.3, -7.4	28.5	-8.2	-9.3, -7.0
Control	174	34.6	34.1	-0.5*	-1.4, 0.2	35.5	0.9*	-0.0, 1.9
Saturated fat, g								
Intervention	173	26.3	15.0	-11.3	-13.7, -9.2	13.3	-13.0	-14.9, -11.0
Control	174	21.8	19.2	-2.6*	-3.7, -1.4	20.5	-1.3*	-2.6, -0.1
C-reactive protein, mg/L								
Intervention	168	4.2	3.9	-0.3	-0.7, 0.2	3.8	-0.4	-0.9, 0.1
Control	167	3.8	3.7	0.1	-0.5, 0.5	3.6	-0.1	-0.6, 0.4

*Significant difference between intervention and control group's mean difference scores from baseline through follow-up, $p < 0.05$.

Table 3: C-reactive protein, mg/L, at baseline, 6 weeks and 6-month follow-up among participants in a therapeutic lifestyle-modification program, Rockford, Illinois

CRP Group	No.	Baseline Mean	6 Week Mean	Difference In Mean between Baseline and 6 Weeks	95% CI	6 Month Mean	Difference In Mean between Baseline and 6 Months	95% CI
Low < 1								
Intervention	28	0.6	1.5	0.9	0.1, 1.7	1.4	0.8	-0.0, 1.7
Control	38	0.6	1.5	0.9	0.2, 1.7	1.2	0.6	-0.1, 1.3
Normal 1 - 3								
Intervention	60	1.8	2.4	0.6	-0.1, 1.1	2.2	0.4	-0.2, 1.0
Control	55	1.8	2.5	0.7	-0.1, 1.5	2.3	0.5	-0.2, 1.2
High > 3								
Intervention	80	7.1	5.8	-1.3	-2.0, -0.6	5.7	-1.4	-2.2, -0.6
Control	74	6.8	5.8	-1.0	-1.8, -0.2	5.8	-1.0	-1.8, -0.1

Note: there was no difference between intervention and control groups at 6 weeks or 6 months after adjusting for baseline differences in CRP.

Table 4: Change in C-reactive protein, mg/L, through 6 weeks and 6 months according to CRP and BMI at baseline, among participants in a therapeutic lifestyle-modification program, Rockford, Illinois

CRP		C-Reactive protein, mg/L through 6 weeks		C-Reactive protein, mg/L through 6 months	
BMI	No.	Mean Change	95% CI	Mean Change	95% CI
Low < 1					
Normal (BMI < 25)	32	0.47	-0.03, 0.97	-0.77	-0.25, 0.10
Overweight (25 ≤ BMI < 30)	22	0.41	0.08, 0.76	0.60	-0.21, 1.35
Obese (BMI ≥ 30)	12	3.08*	0.49, 5.68	2.98*	0.71, 5.25
Medium 1 – 3					
Normal (BMI < 25)	19	0.76	-0.63, 2.15	0.90	-0.61, 2.40
Overweight (25 ≤ BMI < 30)	43	0.07	-0.67, 0.82	-0.25	-0.76, 0.26
Obese (BMI ≥ 30)	53	0.95	0.22, 1.69	0.79	0.03, 1.56
High > 3					
Normal (BMI < 25)	13	-3.48	-5.86, -1.10	-3.08	-5.90, -0.26
Overweight (25 ≤ BMI < 30)	30	-1.64	-2.92, -0.37	-1.80	-3.12, -0.47
Obese (BMI ≥ 30)	111	-0.71*	-1.28, -0.13	-0.78*	-1.42, -0.15

*Significant difference in changes in mean CRP levels across categories of BMI, $p < 0.05$.

Appendix A

Prospectus

Chapter 1

Introduction

Atherosclerosis is the primary cause of coronary artery disease (CAD). It is an inflammatory vascular disease that hardens the walls of medium and large arteries due to the deposits of fatty-fibrous tissue (plaque) that develop from subendothelial accumulation and is caused by lifestyle and genetic factors.^[1] As plaque develops, blood flow is restricted and ischemia results. Atherosclerosis occurs throughout the body and takes decades to develop before a cardiac event (i.e. myocardial infarction, sudden death, angina pectoris). Many risk factors are associated with the development of atherosclerosis. Some of the traditional risk factors that can be manipulated through lifestyle intervention include dyslipidemia, hypertension, smoking, diabetes, body mass index (BMI) and high homocysteine levels.^[2]

C-reactive protein (CRP) is a nonspecific acute-phase reactant that has traditionally been used to detect acute injury infection and inflammation.^[3] Some of the typical causes of inflammation are cytomegalovirus, chlamydia pneumoniae, dyslipidemia, obesity and/or hormone replacement therapy.^[1] Several recent studies have shown a strong independent association between CRP and atherosclerotic events.^[3-5]

The level of CRP in the plasma indicates the severity of inflammation.^[6] Studies have determined that CRP levels above 3 mg/L are a good prognostic marker for future vascular events. That is, people, with and without CAD, with levels above 3 mg/L will likely have more ischemic episodes than those below 3 mg/L.^[3, 6]

CRP Levels can be lowered through drug therapy^[1] and/or a lifestyle intervention that improves a person's exercise and/or eating habits.^[7, 8] Reduction of CRP levels through exercise and weight loss is thought to be a practical way of ensuring a higher quality of life by reducing the likelihood of a cardiac event. The Coronary Health Improvement Project (CHIP) is a specific lifestyle intervention program that may positively impact CRP levels. It is a 4-week lecture program with a curriculum that teaches participants how to change their lifestyle habits to benefit their cardiac health through proper eating habits and exercise. It is assumed that the program will reduce CAD risk factors, including CRP levels.

Statement of the Problem

The literature on CRP describes different methods to lower its levels through drugs and different lifestyle intervention programs. Currently there is not a program that has been established to be the most efficacious in lowering CRP levels. The purpose of this study is to determine the independent and interactive correlation of changes in physical activity and weight with CRP levels. Participants in a CHIP group and control group have been assessed at baseline, six weeks, and six months to determine the changes in CRP levels that are associated with changes in body weight and/or exercise habits.

Research Questions

1. What is the independent correlation of changes in physical activity with CRP levels for CHIP participants?
2. What is the independent correlation of changes in weight with CRP levels for CHIP participants?

3. What is the interactive correlation of changes in physical activity and weight with CRP levels for CHIP participants?
4. What impact do medications, gender, body composition, chronic diseases, and confounding variables have on the correlation of changes in physical activity and weight with CRP levels for CHIP participants?

Assumptions

All participants self-reported answers to questions regarding nutrition and exercise were reported truthfully.

Delimitations

Participants were only selected from the greater Rockford, IL, metropolitan area and have very little minority representation.

Limitations

Participants are primarily from one area and are mostly white, which may affect the generalization of the study.

Significance

The CHIP program was designed to improve a person's lifestyle habits related to exercise and nutrition to reduce the likelihood of a cardiac event. Reducing CRP levels is less understood than the reduction of other CAD risk factors. Currently there is not a standard protocol to follow when a patient presents with high levels of CRP and other risk factors that contribute to CAD. Lifestyle intervention (i.e. exercise and nutrition) may be an efficient and practical way of lowering CRP levels to reduce the likelihood of CAD.

Chapter 2

Review of the Literature

C-reactive protein (CRP) is a nonspecific acute-phase reactant that has traditionally been used to detect acute injury infection and inflammation.^[3] It is synthesized by hepatocytes and has an overall molecular weight of 11,800 Da. CRP production is stimulated by cytokines upon inflammation and rapidly declines upon inflammatory resolution. Despite these transient fluctuations, CRP levels remain relatively constant in an individual.^[9] Some of the possible causes of inflammation are cytomegalovirus, chlamydia pneumoniae, dyslipidemia, obesity and/or hormone replacement therapy. However, recent evidence suggests that CRP levels are a good indicator of predicting the presence and production of atherosclerosis, an inflammatory CAD.^[1, 3, 6]

Kuller and associates^[10] conducted a study that measured the relation between CRP and subsequent risk of myocardial infarction (MI) and coronary heart disease (CHD) with subjects who participated in the Multiple Risk Factor Intervention Trial (MRFIT). They followed up with the participants up to 17 years for deaths and 6-7 years for MIs. Upon follow-up, there were 98 MI cases and 148 CHD deaths. They found a significant association between high CRP levels and subsequent CHD mortality. This is the first prospective study to document the correlation between CRP and CHD mortality in individuals with high CAD risk factors that were otherwise healthy.

Jãnoskuti and associates^[11] conducted a study where they determined the risk of mortality for an individual with high total cholesterol and low CRP levels compared to an

individual with low cholesterol and high CRP levels. The study found that patients with high CRP and low total cholesterol have a higher risk for mortality than patients with low CRP and high total cholesterol. This is a significant finding considering that for years it has been emphasized that cholesterol is a primary risk factor determinant of CAD.

There have been many recent studies that have investigated the mechanisms of how CRP contributes to the atherosclerotic process independently and through interaction with other CAD risk factors. Aggregated CRP binds to LDL and very low density lipoprotein (VLDL) which stimulates tissue factor production by macrophages and starts coagulation. CRP has also been shown to mediate uptake of LDL into the macrophages in the subendothelium. Smooth muscle cells and CRP are found to be in close apposition with each other in the atherosclerotic process.^[9] These mechanisms contribute to the production of foam cells, which is a primary contributor to the formation of atherosclerotic plaque. The MRFIT study found that only CRP and total cholesterol were independent predictors of future cardiovascular risk.^[10] However, they have also been found to interact together in the formation of arterial plaque.^[9] The oxidation of low density lipoprotein (LDL) to foam cells is a continuous positive feedback inflammatory response. As a result, production of CRP is mildly increased indicating that atherosclerosis is taking place. CRP and cholesterol compliment each other in increasing the risk of CAD.^[12]

A meta-analysis of CRP concluded that it was the single most powerful predictor of future CAD of all the inflammatory and lipid markers.^[9] A study conducted with 437 patients that had unstable angina and non-Q-wave MI, showed that significantly

increased CRP levels at the presentation of their cardiac episodes was a good predictor of 14-day mortality in that population.^[13] Albert and associates^[14] conducted a prospective case-control study to determine the importance of CRP, homocysteine, and lipids as long-term predictors of sudden cardiac death. There were 97 cases of sudden cardiac death among apparently healthy men examined. Baseline measures of CRP, homocysteine and lipids were taken prior to their sudden cardiac death episode. Follow-up was carried out over the next 17 years and determined that only baseline CRP levels were associated with the risk of sudden cardiac death. The study's conclusion suggests that CRP levels may be useful in identifying apparently healthy men who are at an increased long-term risk of sudden cardiac death. Thus, studying CRP levels as a determinant of CAD risk is a focus in much of the literature.

Currently the clinical interventions to treat high CRP levels vary. Therapy consists of one or a combination of the following interventions: medication, smoking cessation, exercise, and weight loss. There is an abundance of literature that describes how these factors affect CRP levels, but further studies are still needed.^[9] Despite the abundance of therapy interventions, it is unclear which are most effective in lowering CRP levels.

Physical Activity

Physical activity has been studied as a potential treatment to reduce CRP levels. Different results and conclusions have been made concerning the effects of exercise on CRP levels. The discrepancies in the literature may be attributed to the type of exercise intervention and its effects that are not directly related to CRP levels.

Kelley and associates^[15] examined the effects of aerobic exercise on CRP levels in subjects that were older than 18 years of age. Their meta-analysis assessed randomized controlled trials for 16 years. Measurements of CRP, body weight, percentage of body fat, and maximum oxygen consumption were taken. The exercise interventions lasted 4 weeks or more. Their methods included in the study were limited to randomized controlled trials with aerobic exercise as the only intervention. Their conclusion was that aerobic exercise does not reduce CRP levels in adults.

Marcell and associates^[16] conducted a study to determine the effects of exercise training on CRP levels. They also wanted to assess whether exercise-induced changes in insulin resistance could be explained by changes in inflammatory markers, including CRP. There were 51 participants who were middle-aged and overweight. Body fat and CRP were measured before and after 16 weeks of moderate, intense or no exercise training. They concluded that participation in moderate to intense exercise was not found to be associated with improved CRP levels. Furthermore, they found exercise-induced improvements in insulin sensitivity not related to CRP.

Other studies dispute the assertion that exercise itself does not reduce CRP levels. Goldhammer and associates^[17] examined the exercise effects on cytokines, a primary stimulus for CRP production. They enrolled 28 CAD patients to participate in a 12-week aerobic exercise program typical for cardiac rehabilitation programs. Participants were required to train at 70-80% of their individual maximal heart rate. Training produced a significant reduction of pro-inflammatory cytokines, including CRP levels. They

concluded that aerobic exercise should be an effective means in reducing CRP levels since pro-inflammatory cytokines are reduced with training.

Ford and associates^[18] examined the association of physical activity and CRP levels in a large national sample of the United States population. There were 13,748 participants who were 20 years of age or older enrolled in the study. The participants were given the Third National Health and Nutrition Examination Survey between 1988 and 1994 and were asked questions regarding their physical activity in the past month before taking the survey. Through the participants' frequency and type of activity, four levels of physical activity were defined: vigorously active, moderately active, lightly active and sedentary. Blood was drawn after 10-16 hrs of fasting from each participant at the session they attended and CRP levels were measured. After adjusting for confounding variables, it was found that participants who engaged in light, moderate and vigorous physical activity had lower CRP levels than participants who did not engage in any physical activity. They concluded that physical activity reduces inflammation and effectively lowers one's CRP levels.

Williams and associates^[19] examined the relationship between cardiorespiratory fitness and CRP levels in early adulthood. The participants consisted of 400 men and 315 women all aged 26 years old. Measurements of their CRP levels, cardiorespiratory fitness, anthropometric variables, and blood pressure were taken. The analysis controlled for BMI and indicated that the participants' CRP levels are inversely associated with cardiorespiratory fitness levels. This finding was independent of obesity, blood pressure,

and smoking. The conclusion is that a reduction of inflammation (and CRP levels) due to exercise may reduce the risk of CAD.

Plaisance and associates^[20] reviewed the literature on the affects of weight loss and exercise on CRP levels. Various cross-sectional and longitudinal studies indicate that weight loss independently lowers CRP levels. However, the methods used in the studies that concluded exercise does not independently lower CRP were insufficient protocols for answering the question. Based upon studies with appropriate design and methods, they conclude “exercise independently lowers CRP as much or more than statins” (pg. 458). Thus, lifestyle intervention could be a very effective way of reducing CRP levels. However, this conclusion is still controversial given the literature indicating that exercise does not independently lower CRP.

Weight Loss

The literature supports the hypothesis that a reduction in adiposity is the primary factor in exercise's efficiency in reducing CRP levels.^[21-23] Florez and associates^[21] examined the correlation of CRP levels with waist circumference and BMI in people with the metabolic syndrome (MS). They screened 190 participants for glucose intolerance and BMI over 25. They did not include anyone with cardiac disease or that were taking drugs that lower CRP. They then examined the relationship between CRP levels and MS components and found abdominal obesity to be the most important component of MS that was associated with high CRP levels.

Diaz and associates^[22] evaluated the effect of weight and fitness on CRP levels. They enrolled 2,112 adults from 20 to 49 years of age with a BMI greater that 18.5

kg/m². Fitness was determined by calculating the estimated maximal oxygen consumption for each participant from submaximal graded exercise testing. Their analyses indicated weight loss to be significantly and independently associated with lower CRP levels regardless of their fitness level.

Reinehr and associates^[23] compared the differences between the serum concentrations of 14 non-obese children and 31 obese children. The obese children were found to have significantly higher CRP levels than the non-obese children. They further examined the changes in CRP levels in the 31 obese children. They observed that 16 of the obese children lost weight and 15 obese children did not lose weight for one year. The 15 obese children who did not lose weight had significantly higher serum CRP levels than the 16 obese children who lost weight. They postulated that weight loss was associated with a significant decrease in CRP levels.

Adipose tissue can add to the body's expression of CRP. Anty and associates^[24] conducted a study with 46 severely obese individuals where 21 had MS and 25 did not. Liver and adipose tissue biopsies were collected and used to determine the CRP gene expression by real-time polymerase chain reaction. They found CRP levels to be elevated in the severely obese individuals independent of the presence of MS. One unique observation was that CRP gene expression was found in adipose tissue. Thus, liver and adipose tissue could both contribute to high CRP levels in obese individuals. This study leaves reason to assert that a lifestyle intervention to reduce adiposity could contribute to lowering one's CRP levels.

Medications

Statins are drugs that lower the levels of LDL cholesterol and CRP.^[25] A study evaluated the relationships between the LDL cholesterol and CRP levels. There were 3745 patients with acute coronary syndromes that received statin therapy, which consisted of 80 mg of atorvastatin or 40 mg of pravastatin per day, and were evaluated for risk of recurrent MI or death from CAD. One of the key findings of the study was that patients with lower CRP levels before receiving statin therapy had better clinical outcomes than those with higher levels.^[25] Statin therapy undoubtedly contributes to a better clinical outcome for patients at risk for CAD, but additional research is necessary to understand the independent effect of reducing CRP levels on CAD risk.

Ridker and associates^[26] studied whether inflammation increases or decreases the risk of thrombosis with aspirin therapy. They randomly assigned 543 apparently healthy men who had, developed within the eight years of the trial, myocardial infarction, stroke, or development of venous thrombosis and 543 other apparently healthy men, who did not develop CAD within the eight years, and treated each with aspirin or placebo therapy. Serum CRP levels were measured after the participants received their treatment. Their conclusion states that aspirin could prevent MI and ischemic stroke due to its anti-inflammatory effects and reduction of CRP. However, the effects of aspirin therapy are relative to each individual. Therefore, CRP may serve as a suitable marker in identifying the extent aspirin reduces one's inflammation.

Confounding Variables

The literature on the effects of smoking on CRP levels is very interesting. The research on smoking has delved into the frequency of smoking, the effects of tar, the duration of being a smoker and other factors that may contribute to CRP levels.

CRP levels were measured in 400 men and women older than 65 years and free of clinical CAD at baseline as part of the Cardiovascular Health Study. The association between CRP levels and pack-years was found to be independent of the length of time since cessation of smoking. That is, levels of CRP in these participants were elevated and reflected their lifetime exposure to smoking independent of cessation.^[27] CRP levels were not reduced one year following smoking cessation.^[28] Smoking did have inflammatory effects on the body.^[29] However, the literature supports the hypothesis that elevated levels of CRP "are not directly attributable to tobacco use and are more likely to be elevated due to a secondary process that is yet to be established" (pg. 865).^[28]

The research on alcohol consumption indicates that it has a negative correlation with CRP due to its anti-inflammatory effect. Stewart and associates^[30] conducted a study with 11,572 participants aged 17 and older to evaluate the association between alcohol consumption and CRP levels. Using a model that adjusted for confounding variables, they found that alcohol drinkers were less likely to have elevated CRP levels than nondrinkers. They suggested moderate alcohol consumption might protect against cardiovascular death.

Diet

There are many studies that have examined the effects of dieting on CRP levels. One diet in particular that has been studied is the Mediterranean diet. Michalsen and associates^[31] conducted a study where they examined the effects of the Mediterranean diet on markers on inflammation, including CRP, in patients with CAD. A one-year intervention of increased fish, fruits/vegetables and moderate canola/olive oil, the main constituents of the Mediterranean diet, was consumed by 101 patients. The results showed that adherence to the Mediterranean diet had no affect on CRP levels, indicating that the diet had no affect on the metabolic and inflammation risks.

Chrysohoou and associates^[32] explored the relationship between the Mediterranean diet and CAD risk. They randomly enrolled 1514 men (18 to 87 years old) and 1528 women (18 to 89 years old) from the Attica area of Greece, adherent to Mediterranean diet. Contrary to Michalsen,^[31] Chrysohoou found that adherence to the traditional Mediterranean diet was associated with lower concentrations of biomarkers of inflammation and coagulation, including CRP levels. They suggested that this may partly explain the beneficial actions of this diet on the cardiovascular system.

Blum and associates^[8] also determined explanations for reduced CAD risk when one is adhering to the Mediterranean diet. They enrolled 80 healthy males and compared levels of CRP when one consumes a Mediterranean-like meal compared to western-like meal (high in saturated fat). They found that a postprandial Mediterranean meal decreases CRP levels and concluded that these results provide a "potential explanation to the protective properties of a Mediterranean diet against atherogenesis" (pg. 24). Thus,

the literature is not consistent on the effects of adherence to the Mediterranean diet on CRP levels.

The effects of other diets on CRP levels have also been studied. Bergesio and associates^[33] investigated the influence of the vegan diet supplemented with essential amino acids (EAA) and ketoanalogues (VSD) on CRP levels. The Vegan diet is known for its better lipoprotein profile and antioxidant vitamins content, which could protect individuals against CAD. Bergesio enrolled 29 patients with advanced chronic renal failure to be on a very low protein vegetarian diet and 31 patients with similar renal function were enrolled on a conventional low-protein diet. The results indicated that the patients on the vegan diet had lower CRP and LDL levels. They concluded that their findings suggest that low-protein diets, and vegan in particular, may have a beneficial effect reducing CAD risk for patients with renal disease. Although this study was conducted in a population with renal disease, the findings may support research to determine the most efficient diet in lowering CRP levels.

Jenkins and associates^[34] compared the efficacy of cholesterol lowering diets with statins in lowering CRP levels. They enrolled 34 hyperlipidemic subjects who randomly completed three one-month treatments of a very low-saturated fat diet (control), the same diet with 20 mg lovastatin (statin), and a diet high in plant sterols, soy protein, viscous fibers, and almonds. Fasting blood samples and measurements of total cholesterol and CRP were obtained at weeks 0, 2, and 4. The results and conclusions indicated that a combination of cholesterol-lowering foods reduced CRP levels similar to the extent of a

statin drug. These findings indicate that dietary interventions can be just as effective as drug therapy.

Huffman and associates^[35] conducted a study with the objective of determining if exercise training imposes change on CRP levels for men and women who are at risk for cardiovascular disease. They took baseline and postintervention values of CRP for 193 sedentary individuals that were randomized to 1 of 3 groups that exercise with varying intensities or a group assigned to be inactive for 6 months. They found cardiorespiratory fitness to be inversely related to CRP levels independent of gender. However, they also determined that the lack of a significant change in diet to accompany the exercise will not significantly change CRP levels. Thus, diet may impact the effect exercise may have on lowering CRP levels.

The combination of diet and exercise to reduce CRP is an area of research that may help in developing a practical method of reducing the risk of cardiac events. The CHIP program focuses on a diet and exercise intervention designed to reduce cardiac risk factors. The data from these types of programs should contribute to our understanding of the role of weight loss and exercise in reducing CRP levels.

Summary

CRP is an independent risk factor for CAD and can be used to predict future CAD in an individual.^[4,5] The CHIP program is a lifestyle intervention program that uses diet and exercise as a means to reduce CAD risk factors, thereby effectively reducing cardiac episodes. Weight loss and exercise regimens adhered to by CHIP participants will be examined and correlated with CRP levels. The results of this study will contribute to the

literature in determining the independent and collective effects of exercise and weight loss on CRP levels and help to design an efficient and practical intervention in reducing CRP levels.

Chapter 3

Methods

Subject Recruitment

The SwedishAmerican Center for Complementary Medicine (SACCM) recruited the subjects for this study. Recruitment was from the Rockford, IL, metropolitan area with the guidelines that all subjects be 18 years of age or older. All subjects provided informed consent. Many subjects participated with a spouse or significant other. These subjects were randomized as paired units while the other subjects were randomized as individual units. Randomization of subject units was determined by a random number generator. A study coordinator conducted the sign-up process for the program, subject randomization, and subject group assignments. Subjects were excluded from the study if they had a significant condition or major illness that would prevent them from exercising. Total enrollment for the study was 366 randomly selected individuals with 180 in the intervention group and 186 in the control group. Within the intervention group, 13 were excluded due to illness unrelated to the study, unwillingness to commit to the CHIP program and/or did not provide measurements after baseline, leaving 167 included in the analysis. Within the control group, 16 were excluded due to illness unrelated to the study, unwilling to commit to the CHIP program, lost interest and/or failed to complete follow up measurements, leaving 170 to include in the analysis. The Institutional Review Board of the SwedishAmerican Health System approved the study on August 29, 2002.

Design

The study is a randomized control trial with a treatment group and a control group that had measurements taken at baseline, six weeks, and six months. The treatment group received their intervention in the first four weeks after baseline measurements. The control group received no intervention and was specifically told not to alter their lifestyle during the investigative period. They were also told that after six months they would be able to participate in the CHIP program.

Intervention

The Coronary Health Improvement Project (CHIP)^[36] served as the intervention for this study. The program required the intervention group to meet and receive instruction for two hours at each meeting. Instruction took place four times a week for four weeks at the beginning of the study. The curriculum included the following topics: modern medicine and health myths, atherosclerosis, coronary risk factors, obesity, dietary fiber, dietary fat, diabetes, hypertension, cholesterol, exercise, osteoporosis, cancer, lifestyle and health, the Optimal Diet, behavioral change, and self-worth.

The intervention group received a textbook and workbooks in conjunction with the CHIP lectures that closely followed the discussion topics and contained assignments with learning objectives that correlated with the discussion topics. These assignments were designed to assist the intervention group in understanding and integrating the concepts and information presented into their lives. Dieticians and medical professionals introduced the intervention group to current nutritional and medical information that related to the prevention of chronic diseases weekly.

The intervention group was encouraged to follow pre-set dietary and exercise goals. The dietary goal involved adopting the more plant-food based diet that emphasizes "as-grown," unrefined food. The foods encouraged to eat were whole grains, legumes, vegetables, and fresh fruits. The diet was low in fat, animal protein, sugar, salt, very low in cholesterol, and high in fiber. The intervention group was also, accordingly with their new diet, encouraged to progressively work toward walking or exercising at a mild-to-moderate intensity for at least 30 minutes a day. A pedometer was given to the intervention group and they were encouraged to keep an exercise log that recorded the miles they walked each day.

The primary objectives of this therapeutic lifestyle change program were to improve the intervention group's cognitive understanding of the importance of healthy lifestyles, nutrition and physical activity behavior, risk factors associated with diabetes, hypertension, cardiovascular disease, and cancer.

Measures

The variables gathered from the CHIP program to evaluate cardiovascular risk factors and individual health variation were extensive. The CHIP variables used in the proposed study are pedometer readings (strike counts), body weight, BMI, body fat percentage, chronic inflammatory conditions, age, gender, and CRP levels. Baseline values were taken for all participants and additional measurements were taken six weeks and six months after the intervention group began the program.

Physical Activity

To determine the level of physical activity, a 7-day self-recorded pedometer log was maintained by each participant a week before measurements at baseline, six weeks, and six months. Participants from the intervention and control group wore the Walk4Life Model 2000 Life Stepper pedometer on a belt at the right hip directly above the right knee cap each day for 7 consecutive days. Immediately prior to going to bed the pedometer counts for the day were recorded and the number reset. Strike counts from pedometers are a valid and reliable method of monitoring and measuring free-living physical activity.^[37-39] Crouter^[37] determined the validity of the Walk4Life Life Stepper pedometer by comparing pedometer readings with the number of actual steps of participants walking for 5 min on a treadmill at various speeds (54, 67, 80, 94, and 107 m/min). The correlation coefficient was ($r = 0.81$, $P < 0.05$) which Crouter indicates is acceptable to ascertain physical activity and “a good choice to use in research.” Beets^[38] also assessed the Walk4Life pedometer by comparing it to actual steps from self-paced walking and treadmill walking (speeds: 40, 54, 67, 80, and 94 m/min). The intraclass correlation coefficient was ($R = 0.997$) for self-paced walking and ($R = 0.516, 0.745, 0.946, 0.988$ and 0.992) for treadmill speeds 40, 54, 67, 80, and 94 m/min respectively at 0.05 level of significance. Beets and Crouter both mention the Walk4Life pedometer to be more accurate the faster one walks.

Tudor-Locke^[39] conducted a study to determine how many days of pedometer readings are needed to determine the weekly physical activity of an adult. There were 90 participants enrolled (33 men and 57 female aged 49 +/- 16.2 years and 44.8 +/- 16.9

years respectively). BMI ranged from 21.1 to 33.9 kg/m². There was an R² of 0.94 and an intraclass correlation of 0.80 for wearing the pedometer for three days. The conclusion was that the time necessary to determine physical activity in a week with a pedometer was 3 days. Thus, the time taken from this study (7 days) to determine physical activity is sufficient.

CRP levels

Phlebotomists from the SwedishAmerican Health System's outpatient laboratory drew blood using a vacutainer (Becton-Dickinson Vacutainer Systems, Rutherford, NJ) after the participants had a 12-hour fast. Samples were allowed to clot and then centrifuged. Clinical analyses were completed at the SwedishAmerican Health System laboratory. CRP was determined using a microplate protocol based on a latex bead enhanced immunoturbidity assay from the same technician and has been demonstrated to be a valid and reliable method.^[40-42] Otsuji^[41] conducted a study that demonstrates a turbidimetric immunoassay method for determining serum CRP that can be used with any spectrophotometer that can measure turbidimetrically at 340 nm. The measured absorbances of a test sample (50 μ L of serum + 2.0 mL of working antiserum solution), sample blank (50 μ L of serum + 2.0 mL of sample blank buffer), reagent blank (50 μ L of saline + 2.0 mL of working antiserum solution), and buffer blank (50 μ L of saline + 2.0 mL of sample blank buffer) were prepared and incubated at 37 °C for 30 min. Absorbances for the test sample (A_S), sample blank (A_{SB}), the reagent blank (A_{RB}), and the buffer blank (A_{BB}) were measured. The CRP absorbance (A_{CRP}) indicating serum CRP was calculated by the following equation: $A_{CRP} = A_S - A_{SB} - (A_{RB} - A_{BB})$. It was

indicated that precision was good due to low CV% and SD at various CRP concentrations.

Rifai^[42] compared latex-enhanced immunoassay for hs-CRP with Enzyme-Linked ImmunoSorbent Assay (ELISA), a previously validated method for measuring hs-CRP,^[40] in a case controlled study using patients with peripheral arterial disease. There were 288 subjects (144 case subjects and 144 control subjects) enrolled. The Latex assay used monoclonal anti-CRP antibodies coated to polystyrene particles that formed a complex with hs-CRP present in the measured study sample. The amount of scattered light was directly proportional to the size of the antigen-antibody complex and reflected the hs-CRP concentration present in the study sample. The run-to-run CVs, at hs-CRP concentrations of 0.47, 10.5, and 54.9 mg/L were 6.4%, 3.7%, and 2.9%, respectively. The correlation between the Latex and ELISA methods based on original and log-normalized data were ($r = 0.95$, $P < 0.001$) and ($r = 0.93$, $P < 0.001$) respectively for both case and control groups. Thus, Rifai concluded that the Latex method was comparable to ELISA and can be used in research to determine CRP levels.

Percent body fat

Percent body fat was estimated using the Tanita TBF - 300A electrical impedance scale, which has been shown to be a valid and reliable method.^[43, 44] Kyle^[43] developed a study to validate a single bioelectrical impedance analysis (BIA) equation for 343 healthy white subjects (202 men and 141 women) aged 22-94 with a BMI between 17.0 and 33.8 kg/m. Fat-free mass (FFM) was measured by dual-energy x-ray absorptiometry (DEXA) (Hologic QDR-4500) and BIA (Xitron 4000B). Significant correlations were high ($r =$

0.986—0.987, $P < 0.05$), the standard error for the estimates was low (SEE = 1.72 kg), and technical error was 1.74 kg. A valid single prediction for FFM was developed:

$$\text{FFM} = -4.104 + (0.518 \times \text{height(m)}^2 / \text{resistance (kg)}) + (0.231 \times \text{weight (kg)}) + (0.130 \times \text{reactance (ohm)}) + (4.229 \times \text{sex: men} = 1, \text{women} = 0).$$
 He concluded that the single prediction equation was valid for prediction of FFM in populations with large variations of age and body composition.

Rubiano^[44] investigated the validity of the leg-to-leg BIA (Tanita TBF 310) to measure body composition using DEXA as a reference. He enrolled 39 subjects (8 male and 31 female) aged from 37 to 73 years of age with a BMI from 24.6 to 32.6. A significantly high correlation was observed ($r = 0.94$, $P < 0.001$, SEE = 3.58). Rubiano concludes that leg-to-leg BIA systems may be useful in assessing total body composition in a population varying in BMI.

Weight and height were measured using standard medical weight and height scales calibrated by the Biometrics Department of the SwedishAmerican Health System. Body mass index (BMI) was determined using the formula $\text{weight (kg)} / \text{height (m)}^2$.

Presence of chronic conditions were determined by health history questionnaire and documented by medical staff. Age and gender were also ascertained.

Statistical Analyses

Cross-tabulations will be used to perform bivariate analyses between intervention status (intervention vs. control) and selected demographic variables and medication use, with statistical significance based on the chi-square test for equal proportions (χ^2).^[45] Repeated measures analysis of variance will be used to assess time and time by group

effects on CRP level. Analyses were performed using SAS version 9.0 (SAS Institute Inc., Cary, NC, USA, 2003). The polynomial option in the repeated statement will be used with the SAS procedure statement GLM such that the transformation used to implement the repeated measures analysis was an orthogonal polynomial transformation. The spacing of the orthogonal polynomial used will reflect the time from baseline to six weeks to six months. Wilks' Lambda will be used to evaluate time and time by group effects.^[46] If the time effect is significantly different between groups, then the role of physical activity, weight loss, smoking status, and alcohol consumption on explaining the difference will be explored. The SAS procedure PROC FREQ will also be employed. Statistical significance will be based on the 0.05 level.

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