The Association Between Changes in Body Fat, Body Weight and Serum C-Reactive Protein: A Prospective Study

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THE ASSOCIATION BETWEEN CHANGES IN BODY FAT,
BODY WEIGHT AND SERUM C-REACTIVE PROTEIN:
A PROSPECTIVE STUDY

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

Benjamin T. Bikman

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

Date ___________________________  Larry Tucker, Chair

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As chair of the candidate’s graduate committee, I have read the thesis of Benjamin T. Bikman 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

THE ASSOCIATION BETWEEN CHANGES IN BODY FAT, BODY WEIGHT AND SERUM C-REACTIVE PROTEIN: A PROSPECTIVE STUDY

Benjamin T. Bikman
Department of Exercise Sciences
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Objective- To investigate the extent to which changes in body fat percentage (BF%) and weight (BW) relate to changes in C-reactive protein (CRP) in women, while statistically controlling for possible confounders, such as age, body weight, and menopause status.

Methods and Results- A prospective cohort of 150 free-living subjects was followed over a 2½-year period. BF% was measured using dual energy X-ray absorptiometry (DEXA), while BW was determined with a calibrated, electronic scale. There was no significant relationship between changes in BF% and CRP, regardless of age, BW, and menopause status. However, changes in BW were predictive of changes in CRP ($F=7.75, p=0.006, R^2=5.0\%$). The association remained significant after adjusting for differences in baseline BW, age, and menopause status ($F=9.17, p=0.003, R^2=7.9\%$).
Conclusions- Changes in BF% are not predictive of changes in CRP. However, in agreement with other studies, variations in BW are predictive of changes in CRP. Evidently, changes in CRP are more a function of changes in BW than changes in BF% in middle-aged women. If a causal relationship is assumed, then weight gain over time is likely to increase risk of elevated CRP levels and possibly cardiovascular disease.

Keywords: C-Reactive Protein · Body Fat Percent · Body Weight
ACKNOWLEDGEMENTS

Dr. Larry Tucker has been a wonderful mentor and friend. He is a fantastic example of one who is devoted to helping students excel. My Father has always been an advocate of mastering subjects and I would be remiss to not mention the role he has played in helping me appreciate hard work and knowledge. Finally, I acknowledge the role my sweet wife Cheryl has played in helping complete this degree. She is my greatest motivation.
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THE ASSOCIATION BETWEEN CHANGES IN BODY FAT, 
BODY WEIGHT AND SERUM C-REACTIVE PROTEIN: 
A PROSPECTIVE STUDY

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Running Title: Body Fat, Weight, and CRP
Abstract

Objective- To investigate the extent to which changes in body fat percentage (BF%) and weight (BW) relate to changes in C-reactive protein (CRP) in women, while statistically controlling for possible confounders, such as age, initial body weight, and menopause status.

Methods and Results- A cohort of 150 free-living subjects was followed prospectively over a 2½-year period. BF% was measured using dual energy X-ray absorptiometry (DEXA), while BW was determined with a calibrated, electronic scale. There was no significant relationship between changes in BF% and CRP, regardless of age, initial BW, and menopause status. However, changes in BW were predictive of changes in CRP (F=7.75, p=0.006, R²=0.05). The association remained significant after adjusting for differences in baseline age, initial BW, and menopause status (F=9.17, p=0.003, R²=0.08).

Conclusions- Changes in BF% are not predictive of changes in CRP. However, in agreement with other studies, variations in BW are predictive of changes in CRP. Evidently, changes in CRP are more a function of changes in BW than changes in BF% in middle-aged women. If a causal relationship is assumed, then weight gain over time is likely to increase risk of elevated CRP levels and possibly cardiovascular disease.

Keywords: C-Reactive Protein · Body Fat Percent · Body Weight
Cardiovascular disease (CVD) is the leading cause of death in the industrialized world and accounts for more lost years of potential life before the age of 75 than any other human condition.\textsuperscript{1} In fact, CVD has been responsible for more deaths in the United States than any other disease every year except one since 1900.\textsuperscript{2} In the absence of preventative measures, it is estimated that by the year 2020, CVD will be responsible for 36\% of all deaths and the leading cause of death in the world.\textsuperscript{3} Moreover, with the aging population of the United States, there will undoubtedly be an increase in the incidence of coronary artery disease, stroke, and heart failure.\textsuperscript{4} In an effort to curb these startling statistics, additional research is being conducted in the area of CVD in an effort to establish and improve methods of CVD prevention and prediction.

Nearly half of all CVD cases are caused by atherosclerosis, a narrowing of the arteries due to deposits of lipids and cholesterol in the intimal layer.\textsuperscript{5,6} This process often results in obstruction of blood flow or thrombogenesis, both of which may cause severe damage to vessels. This development is particularly damaging when it occurs in the coronary arteries; the consequence of which is insufficient blood flow to the heart.

The body’s response to atherosclerosis results in inflammation of the immediate area, which induces a change in the intimal layer that increases leukocyte, low-density lipoprotein (LDL), and platelet adhesion to the endothelium. As LDL is oxidized, it promotes a mounting inflammatory response and increasing macrophage concentration by a process which involves monocytes-turned-macrophages attempting to remove the oxidized LDL by binding to it, engulfing the LDL, and consequently turning into a foam cell.\textsuperscript{7-9} Inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor-alpha
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(TNF-α), and interleukin-6 (IL-6) increase the affinity of LDL to the endothelium to further up-regulate the inflammatory response.\textsuperscript{10}

Studies which have examined factors influencing the development of CVD have established the importance of vascular inflammation resulting in atherosclerosis.\textsuperscript{11,12} C-reactive protein (CRP) is an indicator of vascular inflammation and has been shown to be an accurate predictor of cardiovascular events in subjects despite the absence of diagnosed CVD and known risk factors.\textsuperscript{1,13-22} In fact, CRP is a better predictor of CVD than homocysteine,\textsuperscript{3,19} erythrocyte sedimentation rate screening tests,\textsuperscript{23} and more importantly, the blood lipid profile.\textsuperscript{19} Hence, the measurement of serum CRP concentrations is an increasingly used, relatively inexpensive method of determining risk of CVD. Other established approaches, such as imaging techniques, can be costly and less effective at determining risk. Moreover, studies demonstrate they have limited clinical utility.\textsuperscript{24}

Certain cardiovascular risk factors have strong associations with CRP levels, such as age, smoking, hypertension, exercise, plasma lipids, homocysteine, and body mass index.\textsuperscript{25} Perhaps most important is the direct association between obesity and CRP levels.\textsuperscript{26,27} Obesity, particularly in the abdominal region, is known to be a prominent risk factor in the development of CVD. This is due, in large part, to obesity's role as a primary participant in the development of atherosclerosis.\textsuperscript{1,28-34}

Synthesis of CRP is dictated predominantly by two cytokines: Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α),\textsuperscript{35,36} though IL-6 is the main stimulator. Interestingly, both of these cytokines are secreted by adipose tissue.\textsuperscript{37-39} In fact, mRNA
for both IL-6 and TNF-α have been found in human adipose tissue. Hence, not only do injury and infection result in vascular inflammation, but obesity as well.

While there is considerable evidence that obesity is highly correlated with elevated CRP levels, few studies have determined the relationship between changes in body fat percentage and CRP. Studies investigating the association between body composition and CRP have been both cross sectional and longitudinal. Of the longitudinal studies, each has had at least one significant shortcoming. Two well-conducted studies found a positive correlation between weight loss and CRP concentration, though neither study was longer than six months. Of the four studies that were longer than one year, each involved only obese women. Lastly, each longitudinal study involved an intervention designed to decrease body weight within the sample.

To date, no prospective research has studied the effects of body weight/fat changes on CRP in middle-aged, non-obese women. Because this study followed free-living individuals, some participants gained body weight/fat over time, whereas others lost body weight/fat.

Inasmuch as CRP is an accurate and early predictor of cardiovascular events, its importance in determining risk should not be underestimated. Moreover, because prospective research designed to study the relationship between changes in body fat and weight and changes in CRP is sparse, the objective of this study was to determine the
Body Fat, Weight, and CRP relationship between changes in body fat percent and body weight and CRP over a 2½ -year period.

Methods

Subjects

The present study was conducted in cooperation with the Brigham Young University Lifestyle Project, a prospective cohort study. The Lifestyle Project began in 1998-1999. Follow-up assessments occurred in 2000-2001, 2002-2003, and 2004-2005. The 2002-2003 data collection was used as the baseline phase for the current study. The 2004-2005 data collection was used as the follow-up phase. For the current study, there were 199 participants at baseline and 150 women participated in the 2004-2005 follow-up phase. At baseline, the average age was 42.9±2.99 years and at follow-up the mean age was 45.6±3.01 years. At follow up, 32 participants were premenopausal (21.3%), 29 perimenopausal (19.3%), and 89 were postmenopausal (59.3%). A vast majority of these women were Caucasian, educated, and married.

Measurements

C-reactive protein, body fat percentage, weight, age, menopause status, and changes in these variables across 2½ years were measured in this study. Methodology and testing standards remained the same for the baseline and follow-up assessments. Change in each variable was measured by subtracting baseline values from the follow-up values.

Body Fat Percent. Body fat percent was determined using Dual Energy X-ray Absorptiometry (DEXA) (Hologic QDR 4500 W, Waltham, MA). DEXA has been shown to be an accurate and precise method of measuring body fat. When compared to
Bod Pod findings, data from DEXA resulted in a Pearson correlation of 0.94 ($P<0.001$) and an intra-class correlation of 0.97 ($P<0.001$). A state licensed radiology technician performed all of the measurements with DEXA. Further, to reduce variation in analysis, the same investigator analyzed all of the DEXA scans.

**C-Reactive Protein.** CRP was measured using the solid phase ELISA (cat no. 1000) manufactured by Alpha Diagnostics International (ADI), Inc. (San Antonio, TX). The ADI assay was chosen because of its precision, availability, cost, and sensitivity.

Certified phlebotomists from a local regional hospital collected blood serum samples. Blood was not drawn from a participant if she was recovering from an illness or injury until all symptoms were gone. Further, participants were asked not to exercise for at least 48 hours prior to having blood drawn. Samples were centrifuged and stored in aliquots at -20°C.

Blood samples were collected frequently and transported to the biochemistry laboratory in a cooler at approximately 4°C. Once collected, each sample underwent an ELISA for CRP. The mean of two analyses per sample was used to index CRP, if the analyses differed by less than 8%. If the difference was more than 8%, a third assay was performed and the closest two results were averaged. In the current study, assay reliability, indexed by the coefficient of variations (CV), was $8.3\% \pm 6.5$.

**Body Weight.** All subjects were weighed using a computerized electronic scale manufactured by Tanita Corporation (Tokyo, Japan). Prior to use each day, the scale was calibrated with known weights to maintain its accuracy to within 10 grams. Subjects were weighed wearing a standard lab-issued swimsuit. Subjects were instructed not to
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eat for four hours prior to the appointment and to void any bodily waste before being weighed.

Menopause. Menopause status was determined by a series of questions that focused on regularity of the menstrual cycle. Subjects were considered premenopausal if they indicated that their menstrual periods were fairly to very regular, whereas subjects were considered postmenopausal if they indicated a cessation of menses. Subjects who indicated an irregular to very irregular menstrual cycle were considered perimenopausal.

Statistical Analyses

DEXA scans from both assessments were analyzed for body fat percentage and changes in these values over 2½ years. CRP change was calculated by subtracting each subject’s baseline from her follow-up data. Regression analysis using the general linear model (GLM) procedure was used to determine the extent to which changes in body fat percentage and changes in body weight were associated with changes in CRP over the study period. Each relationship was evaluated using two statistical strategies. First, the key variables, such as changes in body weight and changes in CRP, were analyzed with both measures treated as continuous variables. Then to aid interpretation, three categories were created for both changes in body fat percentage and body weight based on whether participants lost weight/fat, experienced moderate gains in weight/fat, or experienced high gains in weight/fat. The extent to which participants in these categories differed in mean CRP changes was then evaluated. Potential confounding variables, including age, initial weight, and menopause status, were controlled using partial
correlation. Alpha was set at the 0.05 level. All statistical analyses were computed using the SAS® system (Cary, NC).

Results

Descriptive data of key and potentially confounding variables in the present study, including differences between baseline and follow-up, are reported in Table 1. Generally, subjects experienced increases in both percent body fat and serum CRP (F=31.8, p=0.001 and F=46.24, p=0.001, respectively) over the 2½-year study. Body weight did not increase significantly from baseline to follow-up (F=2.46, p=0.1177), although variation was substantial. Over the course of the study, almost 60% of the participants gained weight and nearly 75% increased in body fat percentage.

Cross-Sectional Results

Cross-sectional data collected at baseline revealed that the association between body fat percentage and CRP was significant (F=13.44, p=0.0003, R²=0.08). Specifically, for each 1% difference in body fat, baseline CRP differed by 0.008 mg/dL. Significant results were also seen with body weight and CRP at baseline (F=5.80, p=0.017, R²=0.04). For each 1 kg difference in body weight, baseline CRP differed by 0.003 mg/dL. When measured at follow-up, the cross-sectional data of both relationships remained significant (F=15.08, p=0.0002, R²=0.09 and F=13.61, p=0.0003, R²=0.08 for body fat/CRP and body weight/CRP, respectively). Further, for each 1% difference in body fat, CRP levels differed by 0.015 mg/dL, and for each 1 kg difference in weight, CRP differed by 0.008 mg/dL.
Changes in Body Fat Percent and CRP

The relationship between changes in body fat percent and serum CRP concentration lacked significance, whether controlling for potentially confounding variables or not. With the key variables treated as continuous measures, CRP increased 0.006 mg/dL for each increase of one percentage point in body fat (F=1.09, p=0.299, R²=0.01). The potential confounders, age, initial body weight, and menopause status, each known to have a relationship with CRP concentration, had little meaningful effect on the association between changes in body fat percent and changes in CRP.

With participants grouped into three categories of body fat percentage loss, moderate gain, and high gain over the 2½-year period, there were no significant differences in CRP among the groups (Table 2). After adjusting for potential confounders, such as age, initial weight, and menopause status, the relationship between body fat percentage change and CRP change remained non-significant (Table 2).

Post hoc analysis revealed that the relationship between changes in fat mass and changes in CRP approached significance (F=2.85, p=0.093, R²=0.02). On the other hand, the association between changes in lean body mass and changes in CRP was less significant (F=2.19, p=0.141, R²=0.02).

Changes in Body Weight and CRP

Unlike body fat percentage, changes in body weight were significantly and positively related to changes in serum CRP. Specifically, with both measures treated as continuous variables, changes in body weight were predictive of changes in CRP (F=7.75, p=0.006, R²=0.05, see Figure 1). In fact, each kilogram change in weight was associated with a
0.013 mg/dL change in CRP concentration. Moreover, the relationship was strengthened after controlling for baseline weight (F=8.98, p=0.003, R²=0.06) and age (F=8.08, p=0.005, R²=0.05), but weakened slightly with menopause status (F=6.72, p=0.011, R²=0.04). Further, controlling for all three confounders simultaneously resulted in a strong and significant association between changes in body weight and changes in CRP (F=9.17, p=0.003, R²=0.08).

Finally, when divided into categories based on body weight change, there were significant differences in CRP changes (F=4.30, p=0.04, R²=0.03). As shown in Table 3, adjusting for differences in the potential confounding variables had little influence on the association between weight change and CRP change over the 2½-year study.

**Discussion**

The results of this study revealed no apparent relationship between changes in body fat percentage and changes in serum CRP concentration over 2½ years in middle-aged women. The lack of a significant relationship was somewhat unexpected given previously reported evidence from a treatment-based study involving obese women, which noted a significant relationship between decreases in body fat and CRP over a 1-year period. Moreover, increases from baseline to follow-up in body fat percentage and CRP within this study were both statistically significant (F=31.8, p=0.001 and F=46.24, p=0.001, respectively).

A noteworthy finding of the present study was the significant relationship between changes in body weight and changes in CRP. Although changes in body weight from baseline to follow-up were not significant (F=2.47, p=0.118), the association
between changes in body weight and CRP was significant (F=7.75, p=0.006). As mentioned previously, each kilogram change in weight was associated with a change of 0.013 mg/dL in CRP concentration. An increase of 0.013 mg/dL may appear to be slight, but it represents a meaningful change in CRP concentration relative to CVD risk. For example, if a subject weighed 67 kg at baseline (sample mean) with a CRP concentration of 0.08 mg/dL, she would be classified as having a mild risk of CVD. Assuming she gained 4 kg and weighed 72 kg at follow-up, her CRP level would be expected to increase to 0.132 mg/dL, which would place her at moderate risk of CVD. Moreover, similar to blood pressure and cholesterol, any significant increase in CRP, whether or not a change in risk category occurs, represents a greater risk of CVD.

Although CRP levels tended to increase as body weight increased over the 2½ year study, the relationship accounted for only 8% shared variance, after adjusting for the major covariates. Figure 1 shows that change in body weight did not explain all of the variance in CRP change. As revealed in Figure 1, some participants were located in quadrants 1 and 4, indicating that they either lost weight and increased in CRP, or they gained weight and experienced a decrease in CRP. This is because many factors contribute to CRP levels other than body weight. For instance, diet seems to contribute significantly to CRP, and systolic blood pressure is also a significant correlate. Additionally, differences in physical activity level also help to explain differences in CRP. Moreover, as mentioned previously, abdominal fat is a significant determinant of CRP levels. In short, body weight is but one of many important factors that contribute
to CRP levels, indicating that favorable changes in CRP will likely require a multivariate approach.

According to the results, change in body weight was a significant predictor of change in CRP, but change in body fat percentage was not. One possible explanation for this could be the greater variation that accompanied body weight changes compared to changes in body fat percentage. The standard deviation associated with weight change was 7.8 times the mean change, whereas the standard deviation associated with change in body percentage was 2.2 times the mean change. Moreover, there is less error associated with the measurement of body weight compared to body fat, which would tend to make body weight a better predictor than body fat, since error variance does not predict well. Additionally, given change scores were used as predictors, the error-variance associated with body weight change and body fat change was essentially doubled, which would tend to make weight change a better predictor than body fat percent change.

Another possible explanation for the lack of significance between changes in body fat percent and CRP could be that body fat percent, as measured by DEXA, provides an accurate assessment of whole body fat, whereas the relationship between adiposity and CRP may be more a function of a specific site of fat deposition (e.g., abdominal fat). Adipose tissue has been shown to be responsible for synthesizing CRP, albeit indirectly—mRNA for both IL-6 and TNF-α have been found in human adipose tissue. However, what remains to be determined is the extent to which adipose tissue at various sites throughout the body differ in the degree of synthesis of cytokines. If location of adipose tissue affects CRP synthesis, this would help to explain why change
in total body fat percentage was not a good predictor of change in CRP in the present study.

Of the six longitudinal studies that have observed changes in body fat, body weight and CRP, only one\textsuperscript{52} measured variations in body fat percent, while the other five\textsuperscript{27,42,45,51,53} focused on changes in body weight to highlight the relationship with CRP. The single longitudinal study that observed changes in body fat was conducted by Tchernof et al.\textsuperscript{52} Participants in the Tchernof study were not only exclusively obese, but they were also enrolled in a supervised weight loss program, during which body fat percentage dropped by an average of 25\% for the sample ($P<0.0001$). No gains in percent body fat among participants were indicated.

All five longitudinal studies that involved weight change and CRP change consisted entirely of obese participants who were either given treatments for weight loss or who had recently undergone surgery to induce weight loss. Similarly, no gains in body weight within the samples were mentioned.

A notable strength of the current study was the ability to determine the association between both increases and decreases in body fat percentage and body weight and serum CRP. Previous studies have imposed treatments resulting only in weight/fat loss. Because the current study involved no treatment, some participants gained body weight/fat over time, whereas others lost body weight/fat. Given almost 60\% of the participants gained weight over the duration of the current study, and nearly 75\% gained in body fat percentage, the focus of the present investigation was more on weight/fat gain and CRP than weight/fat loss and CRP.
In summary, results from the current prospective study were unable to establish a significant relationship between changes in total body fat percentage and serum CRP over 2½ years in a cohort of healthy, middle-aged women. Further, controlling the effects of age, initial weight, and menopause status did not produce significance in the body fat percentage and CRP relationship. However, changes in body weight proved to be significantly related to changes in CRP, regardless of age, initial weight, or menopause status. Further research concerning the relationship between changes in body fat percentage and CRP, possibly focusing on specific sites of fat gain or loss, is warranted.

In conclusion, it appears that variations in body weight over a 2½-year period are positively related to changes in CRP, indicating that as body weight increases, the risk of developing higher levels of CRP increases. Further, with higher CRP values comes a greater risk of CVD. Therefore, increases in body weight may exacerbate the risk of CVD through subsequent CRP gains.
References


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Table 1. Descriptive Data of Key and Controlled Variables at Baseline and Follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Baseline</th>
<th>Change</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Reactive Protein (mg/dL)</td>
<td>0.14±0.18</td>
<td>0.30±0.32</td>
<td>0.15±0.27</td>
<td>46.24</td>
<td>0.0001</td>
</tr>
<tr>
<td>Body Fat Percent</td>
<td>32.60±6.67</td>
<td>34.24±6.83</td>
<td>1.64±3.56</td>
<td>31.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.88±10.73</td>
<td>67.49±11.30</td>
<td>0.61±4.72</td>
<td>2.47</td>
<td>0.1177</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>42.99±2.99</td>
<td>45.62±3.01</td>
<td>2.64±0.55</td>
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**Table 2.** Changes in CRP by Percent Body Fat Change Category

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controlled Variable</th>
<th>BF%* Loss</th>
<th>Mod BF% Gain</th>
<th>High BF% Gain</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12±0.26</td>
<td>0.16±0.28</td>
<td>0.17±0.27</td>
<td>0.67</td>
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<td>0.12</td>
<td>0.16</td>
<td>0.17</td>
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<td>0.12</td>
<td>0.15</td>
<td>0.17</td>
<td>0.92</td>
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<td>0.13</td>
<td>0.15</td>
<td>0.16</td>
<td>0.22</td>
<td>0.643</td>
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<td></td>
</tr>
</tbody>
</table>

*Body Fat Percent*

Values are Mean±SD

The Body Fat Percent Change categories were defined as follows: BF% Loss (subjects who had a decrease in body fat percent), Moderate BF% Gain (subjects who gained between 0.1 and 2.5% body fat), and High BF% Gain (subjects who gained more than 2.5% body fat).
### Table 3. Changes in CRP by Body Weight Change Category

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wt Loss (n=64)</th>
<th>Mod Wt Gain (n=57)</th>
<th>High Wt Gain (n=29)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>△CRP</td>
<td>0.11±0.24\textsuperscript{a}</td>
<td>0.16±0.30\textsuperscript{a,b}</td>
<td>0.24±0.25\textsuperscript{b}</td>
<td>4.30</td>
<td>0.040</td>
</tr>
<tr>
<td>Age</td>
<td>0.10\textsuperscript{a}</td>
<td>0.16\textsuperscript{a,b}</td>
<td>0.24\textsuperscript{b}</td>
<td>4.44</td>
<td>0.037</td>
</tr>
<tr>
<td>Baseline Weight</td>
<td>0.10\textsuperscript{a}</td>
<td>0.16\textsuperscript{a,b}</td>
<td>0.24\textsuperscript{b}</td>
<td>4.74</td>
<td>0.031</td>
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<tr>
<td>Menopause</td>
<td>0.10\textsuperscript{a}</td>
<td>0.15\textsuperscript{a,b}</td>
<td>0.24\textsuperscript{b}</td>
<td>4.28</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Values are Mean±SD

The Weight Loss category includes all subjects who lost weight. The Moderate Weight Gain category includes those with weight gain up to 4 kg. The High Weight Gain category includes those who gained more than 4 kg over the 2½ years of the study.
Figure 1. The Relationship Between Change in Body Weight and Change in CRP

\[ y = 0.0128x + 0.1434 \]

\[ R^2 = 0.0498 \]
Appendix A

Prospectus
Chapter 1

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the industrialized world and accounts for more lost years of potential life before the age of 75 than any other human condition.\(^1\) In fact, CVD has been the number one killer in the United States every year since 1900, with the exception of 1918.\(^2\) CVD claimed the lives of over 1.4 million adults in 2001 alone—nearly 2,600 deaths each day.\(^2\) In the absence of preventative measures, it is estimated that by the year 2020 CVD will be responsible for 36% of all deaths and the leading cause of death in the world.\(^3\) Moreover, with the aging population of the United States, there will undoubtedly be an increase in the incidence of coronary artery disease, stroke, and heart failure.\(^4\) In an effort to curb these startling statistics, additional research is being conducted in the area of CVD in an effort to establish and improve methods of CVD prevention and prediction.

Nearly half of all CVD cases are caused by atherosclerosis, a narrowing of the arteries due to deposits of lipids and cholesterol in the intimal layer.\(^5,6\) This process often results in obstruction of blood flow or thrombogenesis, both of which may cause severe damage to vessels. This development is particularly damaging when it occurs in the coronary arteries; the consequence of which is insufficient blood flow to the heart.

The body’s response to atherosclerosis results in inflammation of the immediate area, which induces a change in the intimal layer that increases leukocyte, low-density lipoprotein (LDL), and platelet adhesion to the endothelium. As LDL is oxidized, it promotes a mounting inflammatory response and increasing macrophage concentration by a process which involves monocytes-turned-macrophages attempting to remove the
oxidized LDL by binding to it, engulfing the LDL, and consequently turning into a foam cell.\textsuperscript{7-9} Inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-\(\alpha\)), and interleukin-6 (IL-6) increase the affinity of LDL to the endothelium to further up-regulate the inflammatory response.\textsuperscript{10}

Studies which have examined factors influencing the development of CVD have established the importance of vascular inflammation resulting in atherosclerosis.\textsuperscript{11,12} C-reactive protein (CRP) is an indicator of vascular inflammation and has proved to be an accurate predictor of cardiovascular events in subjects despite the absence of diagnosed CVD and known risk factors.\textsuperscript{1,13-22} In fact, CRP is a better predictor of CVD than homocysteine,\textsuperscript{3,19} ESR (erythrocyte sedimentation rate) screening tests,\textsuperscript{23} and more importantly, the blood lipid profile.\textsuperscript{19} Hence, the measurement of serum CRP concentrations is an increasingly used, relatively inexpensive method of determining risk of CVD. Other established approaches, such as imaging techniques, can be costly and less effective at determining risk. Moreover, studies demonstrate they have limited clinical utility.\textsuperscript{24}

Certain cardiovascular risk factors have strong associations with CRP levels, such as age, smoking, hypertension, exercise, plasma lipids, homocysteine, and body mass index.\textsuperscript{25} Perhaps most important is the direct association between obesity and CRP levels.\textsuperscript{26,27} Obesity, particularly in the abdominal region, is known to be a prominent risk factor in the development of CVD. This is due, in large part, to obesity's role as a primary participant in the development of atherosclerosis.\textsuperscript{1,28-34}

Synthesis of CRP is dictated predominantly by two cytokines: Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-\(\alpha\)),\textsuperscript{35,36} though IL-6 is the main stimulator.
Interestingly, both of these cytokines are secreted by adipose tissue.\textsuperscript{37-39} In fact, mRNA for both IL-6 and TNF-\(\alpha\) have been found in human adipose tissue. Hence, not only do injury and infection result in vascular inflammation, but obesity as well.

While there is considerable evidence that obesity is highly correlated with elevated CRP levels, few studies have determined the relationship between changes in adiposity and CRP.\textsuperscript{40-45} Studies investigating the association between adiposity and CRP have been both cross sectional\textsuperscript{40,41,43,44,46-50} and longitudinal.\textsuperscript{27,42,51-54}

Of the few longitudinal studies, each has had at least one significant shortcoming. Two well-conducted studies found a positive correlation between weight loss and CRP concentration, though neither study was longer than six months.\textsuperscript{27,42} Of the four studies that were longer than one year, each involved only obese women.\textsuperscript{51-54} Lastly, each study involved an intervention to decrease adiposity within the population.

A notable strength of the proposed study is the opportunity to determine an association between both an increase and decrease in adiposity and serum CRP due to the unique conditions of the study. Because the proposed study does not impose an intervention, unlike previous studies, and the subjects are free-living, middle-aged women at various states of menopause, it is likely the population will display both an increase and decrease in adiposity. This has yet to be done.

Inasmuch as CRP is an accurate and early predictor of cardiovascular events, its importance in determining risk should not be underestimated. Moreover, because prospective research designed to study the relationship between adiposity and CRP is sparse, the objective of this proposed study will be to determine the relationship between changes in adiposity and CRP over an 18-month period.
Problem Statement

The purpose of this study will be to determine the relationship between changes in adiposity and serum CRP concentration. An additional purpose will be to determine the association between adiposity and CRP independent of age, menopause status, and weight.

Research Questions

1. To what degree is a change in adiposity associated with a change in serum C-reactive protein?

2. To what degree is the relationship between changes in adiposity and serum C-reactive protein independent of age, menopause status, and weight?

Assumptions

1. Subjects will fast for at least 12 hours prior to having blood drawn, with the exception of water.

2. Subjects will not engage in moderate or intense physical activity for at least 48 hours prior to having blood drawn.

3. Subjects will not have blood drawn if they are suffering from an acute illness, injury, or infection.

Delimitations

The purpose of this study will be to determine the relationship between changes in adiposity and serum C-reactive protein concentration. All subjects will be past participants of the BYU Lifestyle Study—a cohort of approximately 250 women. As many subjects as possible from the initial cohort that participated in the third phase will be recruited as subjects for this proposed study. Baseline data for this proposed study will
be third phase data from the Lifestyle Study, and the 18-month data for this proposed study will be collected as part of this master's thesis. All subjects will live in Utah Valley, and will be between the ages of 40-55. Data collection will begin September, 2004. C-reactive protein will be measured using Alpha Diagnostic International's ELISA (enzyme-linked immunosorbent assay) kit for C-reactive protein. Menopause status will be assessed using a series of questions that focus on signs and symptoms of menopause. Body composition will be assessed using the Bod Pod and dual-energy x-ray absorptiometry (DEXA).

Limitations

Subjects may not fast properly or refrain from exercise before having blood drawn. Further, subjects may unknowingly suffer from illness or injury at the time of the blood draw, which could result in elevated C-reactive protein levels.

Operational Definitions

Atherosclerosis - A form of arteriosclerosis characterized by the deposition of atheromatous plaques containing cholesterol and lipids on the innermost layer of the walls of large and medium-sized arteries.

C-reactive protein - A globulin that appears in the blood in certain acute inflammatory conditions, such as rheumatic fever, bacterial infections, and neoplastic diseases.

Cardiovascular Disease (CVD) - A disease of the heart or blood vessels.

Cytokine - Any of several regulatory proteins that are released by cells of the immune system and act as intercellular mediators in the generation of an immune response.
Chapter 2

Review of Literature

Since the discovery of an atherosclerotic aspect in the onset of cardiovascular disease (CVD) and other cardiovascular events, many studies have sought not only to better understand the etiology of vascular inflammation, but also to study the various inflammatory serum markers in hopes of finding a meaningful correlation. Of these, C-reactive protein has received the most attention, due to the standardized and reproducible assays that have been established.\textsuperscript{55-56}

The Inflammatory Process

An integral component of the pathogenesis of cardiovascular disease is inflammation. Vascular inflammation is believed to precede the development and the continual process of atherosclerosis.\textsuperscript{6,57} Further, it is believed that inflammation stems from the acute phase response, which is part of the body’s reaction to injury or infection.\textsuperscript{58}

Injury or infection induces a change in the intimal layer that increases leukocyte, low-density lipoprotein (LDL), and platelet adhesion to the endothelium. Steinberg outlined the following as possible causes of dysfunctional endothelium: free radical damage from environmental exposure; hypertension; direct toxic effects of homocysteine; infections with \textit{Chlamydia pneumonia} and herpes virus; and advanced glycosylated end-products.\textsuperscript{59}

LDL can damage the endothelium when it is oxidized, becomes immunogenic, aggregates, or undergoes glycation.\textsuperscript{7-9} The presence of oxidized LDL promotes the expression of growth factors and chemotactic proteins, which in turn cause a mounting
inflammatory response and increasing macrophage concentration. Inflammatory mediators interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6) increase the affinity of LDL to the endothelium to further up-regulate the inflammatory response.\textsuperscript{10}

The first identifiable characteristic of advanced atherosclerosis is the fatty streak, which is formed by an aggregation of foam cells.\textsuperscript{60} A foam cell is formed by a monocyte-turned-macrophage responding in an attempt to remove oxidized LDL by binding to it, engulfing the LDL, and turning it into a foam cell. Simultaneously, the permeability of the endothelial wall is altered such that macrophages and LDL can migrate into the wall, resulting in inflammation. The inflammation causes increasing numbers of macrophages and lymphocytes to multiply inside the lesion, resulting in the production of enzymes, cytokines, and growth factors. As this process continues, smooth muscle cells migrate into the fatty streak, resulting in a lesion of necrotic cellular debris inside the artery wall, which is eventually covered by a fibrous cap that protects the area. Initially, the artery dilates to accommodate the lesion and then the lumen begins to narrow.\textsuperscript{59}

The Acute Phase Response and C-Reactive Protein

The acute phase response occurs in reaction to a disturbance brought on by injury and trauma or disordered immunological activity. A local reaction at the site of injury or infection leads to activation of cytokines IL-1, TNF-α, and IL-6 that trigger systemic responses, among which is an increase in plasma levels of acute phase proteins fibrinogen, serum amyloid A, and CRP.\textsuperscript{61}
Ballou et al\textsuperscript{62} determined that CRP is manufactured in the liver and deposited in damaged tissue. CRP is also found in high levels in both the intimal layer of the atherosclerotic aortic artery and the foam cells within the lesions of atherosclerotic plaque. Research has found CRP to be a key in many of the inflammatory sequences that promote the progression of atherosclerosis.\textsuperscript{63}

While concentrations of various cytokines increase with the acute phase response and are positively correlated with the risk of primary and recurrent myocardial infarction and death, they cannot be used to accurately predict cardiovascular events. The risk associated with these elevated levels remains constant even when the data are adjusted for other major risk factors.\textsuperscript{21}

The most convincing data concerning the inflammatory response and the ability to predict risk of CVD is the relationship between CRP assays and cardiovascular events.\textsuperscript{57} Serum concentration of CRP has been shown to be elevated 100 times within 24-48 hours after an acute inflammatory stimulus as well as be significantly stable over long periods of time in the absence of trauma or acute infection.\textsuperscript{64}

At a conference convened by the American Heart Association in 1998, methods to measure the markers of inflammation were discussed.\textsuperscript{2} The conference concluded that “many of these markers (including inflammatory markers) are not yet considered applicable for routine assessment because of: 1) lack of measurement standardization, 2) lack of consistency in epidemiological findings from prospective studies with endpoints, and 3) lack of evidence that the novel marker adds to risk prediction over and above that already achievable through the use of established risk factors.”\textsuperscript{65,66} Currently, only fibrinogen and CRP have widely available assays. However, the existence of excellent
anti-CRP antibodies and a well-established World Health Organization (WHO) international reference standard for CRP makes the CRP assay the only acceptable candidate at this time.\textsuperscript{67}

C-reactive Protein and Cardiovascular Disease

\textit{Longitudinal Studies}

Several longitudinal studies have determined that CRP is a very strong indicator of CVD risk in both men and women, despite the absence of known risk factors. Ridker at Harvard Medical School spearheaded many of these studies. One study suggested that CVD risk prediction could be improved by the addition of CRP to the standard lipid screening and, furthermore, that CRP was a stronger predictor of relative risk of future cardiovascular events than the standard lipid profile (RR=2.3 and 4.4 for total cholesterol and CRP, respectively).\textsuperscript{19}

Another study evaluated interrelationships between CRP, the metabolic syndrome, and cardiovascular events among 14,719 women who were followed for an 8-year period for myocardial infarction, stroke, coronary revascularization, or cardiovascular death.\textsuperscript{18} It was found that among those suffering from the metabolic syndrome at baseline, age-adjusted incidence rates of future cardiovascular events were 3.4 and 5.9 per 1000 person-years of exposure for those with baseline CRP levels less than or greater than 3.0 mg/L, respectively. Also, at baseline, median CRP levels for those with 0, 1, 2, 3, 4, or 5 characteristics of the metabolic syndrome were 0.68, 1.09, 1.93, 3.04, 3.88, and 5.75 mg/L, respectively ($P=0.0001$).

Ridker et al\textsuperscript{11} also studied the predictive capabilities of CRP with future cardiovascular events in 122 apparently healthy participants who later suffered a first
cardiovascular event and from 244 age- and smoking-matched control subjects who remained free of CVD during a three-year follow-up period. Women who suffered from cardiovascular events had higher baseline CRP levels than control subjects ($P=0.0001$). Those with the highest levels at baseline had a five-fold increase in risk of any vascular event ($RR=4.8; 2.3$ to $10.1; P=0.0001$) and a seven-fold increase in risk of myocardial infarction (MI) or stroke ($RR=7.3; 2.7$ to $19.9; P=0.0001$).

Another study conducted by Ridker et al$^{20}$ measured CRP and LDL cholesterol in 27,939 apparently healthy women who were followed for a mean of eight years for the occurrence of cardiovascular events or death from cardiovascular causes. After adjustment for age, smoking status, and components of the metabolic syndrome, the relative risks of first cardiovascular events according to increasing quintiles of CRP, compared with women in the lowest quintile, were 1.4, 1.6, 2.0, and 2.3, respectively ($P<0.001$), whereas corresponding measurements with LDL cholesterol were 0.9, 1.1, 1.3, and 1.5, respectively ($P<0.001$).

Ridker et al$^{22}$ conducted a case-control study among 28,263 apparently healthy postmenopausal women over a mean follow-up period of three years. Of the 12 serum markers measured, CRP was the strongest univariate predictor of the risk of cardiovascular events. The relative risk of events for women in the highest compared to those in the lowest quartiles for CRP was 4.4 (2.2 to 8.9; $P<0.001$).

A final study by Ridker et al$^{15}$ determined whether inflammation (measured by CRP) predicted stroke in 543 apparently healthy participants in whom MI, stroke, or venous thrombosis subsequently developed compared to 543 participants who did not report CVD during an eight-year follow-up. Higher CRP levels were found in men who
had an MI (1.51 to 1.13 mg/L; \( P < 0.001 \)) or stroke (1.38 to 1.13 mg/L; \( P = 0.02 \)) than among men without vascular events. The men in the highest CRP quartile had three times the risk of MI (RR=2.9; \( P < 0.001 \)) and two times the risk of stroke (RR=1.9; \( P = 0.02 \)) of the men in the lowest quartile.

The authors of the MRFIT nested case-control study measured the relation between CRP and subsequent risk of death due to CVD. The participants were followed for up to 17 years. There was a significant association between CRP and subsequent CVD mortality. The risk of CVD deaths in the highest quartile of CRP as compared to the lowest was 4.3 (1.74 to 10.8; \( P < 0.001 \)).

The final longitudinal study concerning CRP and risk of CVD is one conducted by Koenig et al that examined the association of CRP with the incidence of CVD in 936 men 45 to 64 years of age. The participants were followed for eight years. The hazard rate ratio (HRR) of CVD with a 1-SD increase in CRP level was 1.67 (95% CI; 1.29 to 2.17). After adjusting for age and smoking, the HRR was 1.60 and 1.50, respectively.

**Cross-sectional Studies**

Only one cross-sectional study specific to the CRP and CVD relationship could be found. Mendall et al sought to test the hypothesis that minor chronic insults such as smoking, bronchitis, and bacterial infections may be associated with increases in CRP and that increased CRP in turn may be associated with increased risk of CVD. A random sample of 388 men aged 50-69 years were used as participants in this study. CVD was evaluated by the Rose angina questionnaire and Minnesota coded electrocardiograms. All the aforementioned minor insults were found to be associated with raised CRP concentrations. As expected, CRP was also strongly associated with CVD; the odds ratio
per doubling of CRP concentration was 1.36 (1.08 to 1.72; \( P<0.001 \)) for electrocardiographic abnormalities, 1.42 (1.13 to 1.78; \( P<0.001 \)) for those with a positive Rose angina questionnaire result or history of MI, 1.55 (1.25 to 1.92; \( P<0.001 \)) for those with either, and 1.83 (1.29 to 2.58; \( P<0.001 \)) for those with claudication.

C-Reactive Protein and Adiposity

Longitudinal Studies

Unlike the studies conducted on the association between CRP and CVD, studies that have examined the relationship between CRP and adiposity have been predominantly cross sectional, with only a few longitudinal studies.

Ziccardi et al\(^{54}\) age matched 56 healthy obese women and 40 normal weight women. Compared with nonobese women, obese women had increased basal concentrations of TNF-\( \alpha \) and IL-6, cytokines that stimulate CRP synthesis. After one year of a multidisciplinary program of weight reduction (diet, exercise, behavioral counseling) all obese women lost at least 10 percent of their original weight (9.8±1.5 kg). The weight loss was associated with a reduction of cytokine concentration (\( P<0.01 \)).

Sixty-one obese, postmenopausal women were recruited as participants in a study by Tchernof et al\(^{52}\) that found that plasma CRP levels were positively associated with dual x-ray absorptiometry-measured total body fatness (\( r=0.36, \ P<0.005 \)) and CT-measured intra-abdominal fat area (\( r=0.30, \ P<0.02 \)). Twenty-five of the 61 women completed a weight loss protocol, with an average weight loss of 14.5±6.2 kg (-15.6%, \( P<0.0001 \)) and a drop in CRP levels by 32.3 percent, from 3.06 to 1.63 g/mL (\( P<0.0001 \)).

Another study followed 20 morbidly obese women who experienced weight loss by surgical measures.\(^{53}\) The participants were measured for fat mass, CRP, IL-6 and
TNF-α before and one year after Swedish adjustable gastric banding. Along with a significant loss of fat mass from 53.9±10.3 to 29.8±12.1 kg, CRP levels decreased from 1.33±1.21 mg/dL to 0.40±0.61 mg/dL, respectively.

Valsamakis et al\textsuperscript{42} allocated 41 women into two groups according to the treatment prescribed. The first group, with a mean BMI of 46±8.6 kg/m\textsuperscript{2}, was treated with Sibutramine for weight loss. The second group, with a mean BMI of 45.2±5.2 kg/m\textsuperscript{2}, was treated with Orlistat after one month on a low-fat (≤ 30%) diet. After six months, groups one and two had a modest mean weight loss of 5.4% (\textit{P}=0.0001) and 2.5% (\textit{P}=0.002), respectively. Group one experienced a 9.7% (\textit{P}=0.03) drop in CRP levels, while group two, despite significant weight loss, had no change in CRP levels.

Esposito et al\textsuperscript{51} conducted a study in 2003 to determine the effect of a program of changes in lifestyle designed to cause a sustained reduction of body weight on markers of vascular inflammation and insulin resistance. To this end, 60 obese women were randomly assigned to an intervention group and received detailed advice on how to achieve a 10% reduction in body weight through a low-energy diet and increased physical activity. Another group of 60 women were used as controls. After 2 years, BMI of the women in the intervention group decreased more than the BMI of those in the control group (-4.2 kg/m\textsuperscript{2}; \textit{P}<0.01), as did mean CRP (-1.6 mg/L; \textit{P}=0.008).

Heilbronn et al\textsuperscript{45} conducted a final longitudinal study in this area. The investigation included 83 healthy, obese women (mean BMI: 33.8±04 kg/m\textsuperscript{2}). Subjects were placed on very-low-fat (15%) diets for 12 weeks. After 12 weeks, weight loss was 7.9±0.3 kg and CRP was significantly decreased by 26% (\textit{P}<0.001).

\textit{Cross-sectional Studies}
Eleven cross-sectional studies on the relationship between adiposity and CRP were found.\textsuperscript{26,40,41,43,44,46-50,68} Though the data from these studies are valuable, because they were cross-sectional, they are limited in that they cannot determine changes over time.

Among these studies, five used BMI and anthropometry (WHR, WC) as measures of adiposity.\textsuperscript{26,40,44,47,68} Nevertheless, the results of these studies displayed relationships between BMI, WHR, and WC and CRP levels similar to those found using more accurate measurements of adiposity, such as Bod Pod, DEXA, etc. In all but one of these five studies,\textsuperscript{26} BMI was measured along with WC and WHR. Each of these studies determined that WHR and WC had a stronger relationship to CRP levels than BMI alone. Hence, the definite conclusion drawn from these studies was that while BMI has a significant correlation with CRP levels, abdominal fat measured by WHR and WC is a stronger indicator of elevated CRP than BMI alone.

The remaining studies employed established, reliable, and valid means of measuring body fat, such as bioelectrical impedance, hydrostatic weighing, computed tomography (CT), and DEXA. Three studies used only bioelectrical impedance and found conflicting results. Two of these studies\textsuperscript{48,49} determined that fat mass measured by bioelectrical impedance had a stronger relationship to CRP levels and vascular inflammation than BMI. The third study found the opposite.\textsuperscript{43}

Measures more accurate than bioelectrical impedance were used to assess adiposity in the final three studies. Lemieux et al\textsuperscript{41} examined the contribution of body composition measured by hydrostatic weighing and of abdominal tissue accumulation assessed by CT to the variation in CRP levels in a sample of 159 men. The researchers
found that fat mass had the strongest correlation to CRP levels ($r=0.41; P<0.0001$), over WC and BMI ($r=0.37; P<0.0001$ and $r=0.36; P<0.0001$, respectively).

Kim et al\textsuperscript{46} investigated whether visceral fat (VF) accumulation measured by ultrasonography can adequately predict CVD measured by CRP in 346 subjects. As expected, VF had a significant correlation with CRP levels ($r=0.34; P<0.05$).

The final cross-sectional study concerning CRP and adiposity was one conducted by Manns et al\textsuperscript{50} to determine whether higher physical activity levels are associated with lower CRP levels, independent of body fatness measured by DEXA in 133 postmenopausal women. Higher physical activity energy expenditures were significantly associated with lower CRP levels, independent of a list of variables. However, body fat was one of the sole independent predictors of higher CRP levels ($r=0.61; P<0.001$). The researchers' conclusion was that the association between higher physical activity and lower CRP levels was dependent on adiposity.

Summary

Clearly, a statistically significant relationship exists between serum concentrations of CRP and risk of CVD. Equally impressive is the association between adiposity and serum CRP, making adiposity a risk factor of CVD. If it is found that changes in adiposity are predictive of changes in serum CRP concentration, the conclusion will be that a rise in adiposity increases the risk of elevated serum CRP levels, thus heightening the risk of atherosclerosis and CVD.
Chapter 3

Methods

Subjects

An existing cohort of 250 women, recruited during 1998 and 1999, will participate in this study. At baseline, approximately 54 months prior to the proposed study, all subjects were 35-45 years old. To avoid confounding factors like obesity, smoking, childbirth, menopause, and serious illness, at baseline all the women had no apparent health problems, had a BMI less than 30, were premenopausal, did not smoke, and were not planning to have children during the next six years.

As many subjects as possible from the initial cohort that participated in the third phase will be recruited as subjects for this proposed study. All subjects will sign an informed consent form before participation in this study.

Instrumentation and Measurement Methods

C-reactive Protein

Despite the existence of other known inflammatory markers, such as fibrinogen, C-reactive protein (CRP) will be the marker measured in this study due to the consistent and reproducible assays and reference standards that have been established with regards to measuring serum CRP concentration.\(^{67,69,70}\) CRP will be measured using the solid phase ELISA (cat no. 1000) manufactured by Alpha Diagnostics International (ADI), Inc. (San Antonio, TX). The ADI assay was chosen because of its precision, availability, cost, and sensitivity. Intra-assay variation in the normal range is low (CV=3.0%; sd=0.03), and the inter-assay variation is acceptable (CV=7.0%; sd=0.4). ADI is a well-known, established company located in San Antonio, Texas.
Certified phlebotomists at Timpanogos Regional Hospital in Orem, UT will collect blood serum samples. Blood will not be drawn from a participant if she is recovering from an illness until all symptoms are gone. Further, participants will be asked not to exercise for at least 48 hours prior to having blood drawn. Samples will be centrifuged and stored in aliquots at -20°C.

Blood samples will be collected frequently and transported to the biochemistry laboratory at BYU in a cooler at approximately 4°C. Once collected, each sample will undergo an ELISA for CRP. As mentioned previously, the Alpha Diagnostic International, Inc. (San Antonio, TX) C-reactive protein ELISA Kit Cat No. 1000 will be used. The mean of two analyses per sample will be used to index CRP if the analyses differ by less than eight percent. If the difference is more than eight percent, a third assay will be performed and the closest two results will be averaged.

Adiposity

Adiposity will be measured using two well-established methods; the Bod Pod (Life Measurement Instruments, Concord, CA) and Dual Energy X-ray Absorptiometry (DEXA) (Hologic QDR 4500 W, Waltham, MA). All measurements with DEXA will be performed by a state licensed radiologist. The Bod Pod has been shown to produce reliable and valid measurements with a strong intra-class correlation ($r=0.999$) when measuring test-retest reliability.71,72 When compared to Bod Pod findings, results from DEXA displayed a Pearson correlation of 0.94 ($P<0.001$), and an intra-class correlation of 0.97 ($P<0.001$).73

Body Weight
All subjects will be weighed using a computerized electronic scale manufactured by Tanita Corporation (Japan). Prior to use, the scale will be calibrated with known weights to maintain its accuracy to within ten grams. Subjects will be weighed wearing a standard BYU-issued swimsuit and cap. Subjects will be instructed not to eat for four hours prior to the appointment and will be asked to void any bodily waste before being weighed.

*Menopause Status*

The same menopause status instrument used in the third phase will be used in this study—a series of questions that focus on signs and symptoms of menopause. Subjects will be considered premenopausal if they have experienced at least 10 menstrual cycles during the past 12 months and are free from symptoms of menopause. Women will be considered postmenopausal if their menses have ceased naturally for at least 12 months. Women who have experienced fewer than 10 cycles during the past 12 months, will be considered perimenopausal.

**Procedures**

Subjects will report to the Human Performance Research Center at Brigham Young University in Provo, UT for one appointment. Subjects will read and sign an informed consent form, which will list all procedures of the study. A medical screening questionnaire will be given to determine health problems. Menopause status will be determined by a series of questions that focus on signs and symptoms of menopause. In addition to being assured that all information acquired through the study will be confidential, benefits and possible risks will be described.
Subjects will arrive having fasted for four hours and will be given a standard BYU-issued swimsuit and cap to change into. After eliminating any bodily waste, weight will be measured using an electronic scale (Tanita Corporation, Japan). Before each measurement, the Bod Pod will be calibrated according to the manufacturer’s specifications. The procedures for both the Bod Pod and DEXA will be explained. Testing on the Bod Pod and DEXA will be administered in a random order. A minimum of two tests will be performed on the Bod Pod. Subsequent tests will be performed as necessary until two measurements are within one percentage point. The average of the two nearest measurements will be taken. Tests performed on DEXA will be performed once.

Subjects will receive a blood profile requisition form for Timpanogos Regional Hospital blood laboratory to have blood drawn within the next week. Subjects will be instructed to arrive having fasted for 12 hours, be free from illness or infection, and refrain from moderate to intense physical activity for 48 hours prior to the draw. Each subject will receive a copy of her results.

Data Analysis

The relationship between adiposity and serum CRP concentration will be indexed using the Pearson product-movement correlation coefficient. Additionally, participants will be divided into quartiles based on change in adiposity. Mean differences in CRP across the different categories of adiposity change will be calculated using regression analysis. Potentially confounding variables, such as age, menopausal status, and weight, will be studied using partial correlation. Changes in adiposity and serum CRP will be determined by subtracting third phase data from the data collected as a result of this
study. Alpha will be set at the 0.05 level and all calculations will be determined using SAS, version 8.01.
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