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Physical Activity and C-reactive Protein Levels: The Confounding Role of Body Fat Percentage

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PHYSICAL ACTIVITY AND C-REACTIVE PROTEIN LEVELS:

THE CONFOUNDING ROLE OF BODY FAT PERCENTAGE

by

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GRADUATE COMMITTEE APPROVAL

of a thesis submitted by

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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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BRIGHAM YOUNG UNIVERSITY

As chair of the candidate's graduate committee, I have read the thesis of Kenric L. Russell 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

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The purpose of the present study was to examine the cross-sectional relationship between physical activity and C-reactive protein (CRP) in 211 middle-aged women (43.1 $\pm$ 3.0 years). A secondary objective was to determine the extent to which body fat percentage operated as a confounder in the association between physical activity and CRP. Physical activity was objectively measured using MTI accelerometers, which the subjects wore for seven continuous days. Fasting blood samples were taken, from which CRP was measured using a solid phase ELISA. Body fat percentage was assessed using the Bod Pod. Results showed that physical activity was significantly and inversely related to CRP concentrations (F = 4.20, p = 0.042). Specifically, regression analysis showed that for each 100,000 count increase in physical activity (about 25 minutes of moderate exercise), there was a decrease of 0.026 mg/L of CRP. However, after

adjusting for differences in body fat percentage, measured physical activity was no longer

a significant predictor of CRP (F = 0.01, p = 0.927). These findings suggest that

although higher physical activity levels are related to lower CRP levels, this relationship

is almost entirely a function of differences in body fat.

ACKNOWLEDGMENTS

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PHYSICAL ACTIVITY AND C-REACTIVE PROTEIN LEVELS:

THE CONFOUNDING ROLE OF BODY FAT PERCENTAGE

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Abstract

The purpose of the present study was to examine the cross-sectional relationship between physical activity and C-reactive protein (CRP) in 211 middle-aged women (43.1 $\pm$ 3.0 years). A secondary objective was to determine the extent to which body fat percentage operated as a confounder in the association between physical activity and CRP. Physical activity was objectively measured using MTI accelerometers, which the subjects wore for seven continuous days. Fasting blood samples were taken, from which CRP was measured using a solid phase ELISA. Body fat percentage was assessed using the Bod Pod. Results showed that physical activity was significantly and inversely related to CRP concentrations (F = 4.20, p = 0.042). Specifically, regression analysis showed that for each 100,000 count increase in physical activity (about 25 minutes of moderate exercise), there was a decrease of 0.026 mg/L of CRP. However, after adjusting for differences in body fat percentage, measured physical activity was no longer a significant predictor of CRP (F = 0.01, p = 0.927). These findings suggest that although higher physical activity levels are related to lower CRP levels, this relationship is almost entirely a function of differences in body fat.

Key Words: C-reactive protein; physical activity; body fat

INTRODUCTION

In America, 40% of all deaths are a result of cardiovascular disease, making this disease the nation's leading killer of men and women of all races and ethnicities (1). In 1998, the National Hospital Discharge Survey reported cardiovascular disease (CVD) as the principal diagnosis in five million patients, and according to the American Heart Association's 2003 statistical report, CVD led to the death of 1.4 million American adults (1).

Coronary artery disease (CAD) causes approximately one-half of all CVD cases (1). CAD is a narrowing of the coronary arteries to the point that sufficient blood cannot be delivered to the myocardium. The resulting decrease in myocardial oxygen levels leads to tissue damage, myocardial infarction, and possible death. Five major risk factors of CAD include smoking, obesity, hypertension, hypercholesterolemia, and physical inactivity (1).

Atherosclerosis is the leading cause of CAD, characterized by cholesterol-lipid-calcium deposits in arterial linings (2). These deposits accumulate until they severely obstruct blood flow, destroy the artery, or become an atherothrombosis (3). The body responds to atherosclerosis with inflammation of the injured area, the severity of which is an important indicator of plaque stability (3). This inflammation can be estimated, and risk of future complications approximated, by a number of currently available tests.

One screening test that is rapidly gaining attention among CVD researchers is C-reactive protein (CRP). CRP is a nonglycosylated polymeric hepatic protein produced by

most vertebrate species in response to foreign invasion of antigens (4, 5). It was recently shown that CRP binds specifically to phosphocholine in cell membranes (5). CRP binds to artery walls, causing phagocytosis in leukocytes, macrophages, and other cells (5).

Until recently, CRP has only been used to diagnose tissue injury or inflammation. The role of inflammation in cardiovascular disease is now better understood, which has sparked a renewed interest in this protein. A number of recent studies have shown CRP to more strongly predict CVD than homocystine (6, 7), cardiac troponin (8), ESR (erythrocyte sedimentation rate) screening tests (9), and the blood lipid profile (7).

Many studies have shown low levels of physical activity to increase risk of heart disease, accounting for 35% of coronary heart disease (CHD) deaths (10). Studies also show that higher levels of physical activity reflect low risk of coronary heart disease (11). This correlation between increased physical activity and decreased CHD risk may be due partially to a decrease in endothelial inflammation (11).

Recently, a handful of studies have examined the effects of both acute and chronic exercise on CRP levels. Meyer et al. studied the acute effects of short exercise bouts on the acute phase reactants: interleukin 6 and 8, neutrophils, premacrophages, cortisol, and CRP (12). They found CRP to be the only elevated parameter 24 hours after exercise (12). Another study found untrained individuals to have higher CRP levels than trained individuals 24 hours after one bout of extreme exercise (13). A group of Dutch scientists noted a 20-fold increase of CRP 16 hours after a triathlon (14). In another study of triathletes, CRP levels were three times higher than baseline after a triathlon (15). Increases of CRP have also been found in athletes 16 hours after a marathon and 24 hours

after a 90-kilometer race (16, 17). Apparently, acute physical activity raises plasma CRP levels.

Although the acute effect of vigorous exercise seems to be a short-term increase in CRP, chronic endurance training has been found to cause a systemic anti-inflammatory effect, indicated by lowered plasma CRP (18-20). Dufaux et al. demonstrated the suppressive effect of regular training on CRP by finding lower CRP levels in swimmers and rowers, compared to a control group (19). Additionally, in older individuals, increased fitness (faster maximum walking speed and greater handgrip strength) correlated with lower CRP levels (20).

In order to better understand the relationship between physical activity and CHD, a closer look at the interaction of physical activity and C-reactive protein is needed. Very few investigations have examined the association between chronic physical activity and CRP levels. Moreover, CRP has not been studied as it relates to physical activity measured objectively. Additionally, few investigations have controlled for potential confounders, such as body composition, injury, illness, infection, or acute exercise, and sample sizes have been relatively small. However, the current study included an objective measure of physical activity and controlled for body composition, acute illness, injury, infection, and acute exercise. The study also employed a large sample.

The purpose of the present study was to determine the extent to which objectively measured physical activity is associated with resting C-reactive protein levels in middle-age females. A secondary purpose was to measure the extent to which potentially

confounding factors, such as total body fat and age, affect the CRP and physical activity association.

MATERIALS AND METHODS

Subjects

A total of 211 women participated in the present study. Average age was $43 \pm$ 3.03 years. Approximately 90% of the participants were white, 80% were married, and 35% were college graduates. All subjects were required to sign a university-approved informed consent before participating in the study. Subjects had no apparent health problems and did not smoke.

Instruments and measurement methods

C-reactive protein. C-reactive protein (CRP) was measured using the solid phase ELISA (cat no. 1000; Alpha Diagnostics International (ADI), Inc., San Antonio, TX). This kit was chosen because of its sensitivity, precision, availability, and cost (21). The ADI assay is most sensitive within the normal range of 0-5 mg/L (21). The intra-assay variation in the normal range is low (C.V. = 3.0%; sd = 0.3), and the inter-assay variation is acceptable (C.V. = 7.0%; sd = 0.4) (21).

Blood serum was collected by a certified hospital laboratory. Subjects were asked not to exercise for at least 48 hours prior to being tested. If a subject was sick, recovering from an illness or infection, bruised, or injured, she was asked to wait until all symptoms were gone before having her blood drawn. If she was experiencing hay-fever symptoms, she was asked to rate the severity of the symptoms. Blood samples were drawn, centrifuged, and stored in aliquots at -20ºC.

After subjects had their blood drawn, the serum was transported to the biochemistry laboratory at the university where an ELISA for C-reactive protein was performed for each subject after an adequate number of samples were collected. Assays were performed in duplicate and the mean of the two analyses was used to index CRP.

Physical activity. Accelerometers (Manufacturing Technology, Inc. (MTI), Fort Walton Beach, FL) were used to objectively measure physical activity. Originally, the company name was Computer Science and Application ((CSA), Shalimar, FL). These devices have been shown to give valid and reliable activity counts, and can precisely monitor everyday activities (22-26).

In a study performed by Bassett et al., strong correlations were found between physical activity measured using various accelerometers and energy expenditure (25). Specifically, four accelerometers were used to measure physical activity and a portable metabolic system was used to measure energy expenditure during 28 different everyday activities, such as vacuuming, cooking, cleaning, child care, and walking (25). In comparing the four different accelerometers, the MTI monitor was the only one that did not differ significantly from energy expenditure measured with a portable metabolic system ($p = 0.473$) (25). Additionally, in studying 24 adults, Nelson et al. showed a strong correlation ($r^2 = 0.85$) between energy expenditure during a treadmill test and MTI accelerometer measurements (26).

MTI accelerometers were worn over the left hip, in line with the outer seam of the pants and the umbilicus, and were secured by a small pouch fastened to a nylon belt. They were worn at all times during a seven day period except while bathing and during

water activities. The monitors summed the motion counts of each subject into one-minute intervals, which were later collapsed into 10-minute intervals. Therefore, the motion of each subject was recorded in 144 time segments (epochs) each day, each with a length of 10 minutes. Over the course of the seven days, each subject had a total of 1008 epochs which were summed to index total physical activity.

Body fat percentage. Body fat percentage was measured using the Bod Pod (Life Measurement Instruments, Concord, CA), which uses air displacement plethysmography. We evaluated the test-retest reliability of the Bod Pod using a subset of 100 women from our sample and found a strong intra-class correlation (r = 0.999) (27). Body composition for the 100 subjects was also measured using Duel Energy X-ray Absorptiometery (DXA) (Hologic 4500W, Bedford, MA). When the DXA results were compared to the Bod Pod findings, the Pearson correlation was 0.94 ($p < 0.001$), and the intra-class correlation was 0.97 ($p < 0.001$) (28). Others have also shown that the Bod Pod produces reliable and valid measurements of body fat (29).

Subjects fasted for 4 hours before being tested, and wore a standard university-issued swimsuit and a swim cap. Before each test, the Bod Pod was calibrated with a known-volume cylinder according to the manufacturer's instructions. At least two tests were performed, and the average taken. If the two tests were not within one percentage point of each other, subsequent tests were performed until two were within one percentage point. Approximately 25% of subjects required a third measurement. The average of the two results within one percentage point was used.

Procedure

Subjects reported to the Human Performance Research Center at the University for two appointments. During the first appointment, subjects read and signed an informed consent, which contained all procedures of the study. Benefits and possible risks were described, and subjects were assured that all information acquired through the study would be confidential. A medical screening questionnaire was also given in order to assess any health problems.

Subjects were asked to eliminate any bodily waste and to change into a standard nylon swimsuit. Weight was measured using an electronic scale (Tanita Corporation, Japan) and body fat percentage was assessed using the Bod Pod.

After changing back into street clothes, the proper technique for wearing the accelerometer was shown to and discussed with the subject. An accelerometer was properly fitted, located over the left hip. A second appointment was made for eight days later. Subjects wore the monitor at all times (except bathing) for the next seven days. During the next seven days, subjects received three reminder calls to encourage them to follow proper procedure.

At the second appointment, the activity monitor was returned and the data were collected. Each subject was then given a blood requisition form for a nearby regional hospital blood laboratory, and was directed to have her blood drawn within the next week. Subjects were instructed to fast for twelve hours, to be free of any illness, injury, or infection, and to refrain from moderate and intense physical activity 48 hours prior to the draw. At the time of the blood draw, subjects completed a short questionnaire to

confirm that they had fasted, free from illness, injury and infection, and that they had not

exercised for at least 48 hours. Subjects who did not meet these conditions were asked to

return to the lab to have their blood drawn at another time.

Data analysis

The magnitude and direction of the bivariate relationship between objectively

measured total physical activity and resting CRP was indexed using regression analysis.

Additionally, the association between physical activity and CRP was measured with

subjects categorized according to their CRP levels. Specifically, subjects were divided

into quartiles based on their CRP levels, and then the middle two quartiles were

collapsed, forming three groups. Mean differences in physical activity across the

different categories of CRP were calculated using regression analysis. The affect of

potentially confounding factors, such as age and body fat percentage, on the physical

activity and CRP association, was analyzed using partial correlation. Alpha was set at the

0.05 level and all calculations were performed using SAS software, version 9.0.

RESULTS

In this investigation, 211 women were studied. Table 1 shows that mean age was

43.1 $\pm$ 3.0 yrs, average weight was 67.1 $\pm$ 11.0 kg, and mean body fat percent was 32.5 $\pm$

7.4%. Average BMI was 24.1 $\pm$ 3.5. Mean CRP concentration was 1.28 $\pm$ 1.70 mg/L,

and the mean weekly activity count was 2.6 million $\pm$ 0.9 million counts.

With both of the key variables treated as continuous measures, physical activity

was significantly and inversely related to CRP concentrations (F = 4.20, $p = 0.042$).

Specifically, regression analysis showed that for each 100,000 count increase in physical

activity, there was a decrease of 0.026 mg/L of CRP. In the present study, subjects in the lowest quartile of CRP differed from participants in the highest quartile of CRP by approximately 400,000 activity counts (Table 2).

The association between physical activity and CRP was not affected by differences in age ($F = 4.15$, $p = 0.043$). However, after adjusting for differences in body fat percentage, objectively measured physical activity was no longer a significant predictor of CRP ($F = 0.01$, $p = 0.927$). Controlling for age and body fat percentage simultaneously resulted in no relationship between physical activity and CRP ($F = 0.00$, $p = 0.948$).

As shown in Table 2, with CRP divided into quartiles and the two middle quartiles combined, there were significant differences in objectively measured physical activity counts across the CRP levels ($F = 3.26$, $p = 0.040$). When differences in age were controlled, the relationship between activity counts and CRP remained significant ($F = 3.22$, $p = 0.042$). However, after adjusting for body fat percentage, differences in activity counts across CRP levels were no longer significant ($F = 0.92$, $p = 0.399$). Likewise, after controlling for age and body fat percentage simultaneously, with CRP divided into three categories, there was no relationship between activity counts and CRP ($F = 0.92$, $p = 0.402$).

DISCUSSION

Results of the present study indicate that there is a significant relationship between objectively measured physical activity and CRP in middle-age women. In short, subjects with higher physical activity levels also tend to have lower CRP levels.

However, when body fat percentage was controlled statistically, the relationship between physical activity and CRP was eliminated. It appears that the association between objectively measured physical activity and CRP is almost entirely a result of differences in body fat percentage—physically active women tend to have lower levels of CRP, not because they are physically active, but because physically active women tend to be leaner.

Before adjusting for differences in body fat, the relationship between physical activity and CRP appears significant. In the present study, regression analysis results showed that for each increase of 100,000 activity counts there was a corresponding decrease of 0.026 mg/L in CRP. Given walking briskly for 10 minutes generates approximately 40,000 activity counts in the typical women (30), if a causal relationship were assumed, walking briskly 30 minutes per day, five days per week (i.e., 600,000 activity counts), on average, would result in a reduction of more than 0.15 mg/L in CRP.

Other studies have also revealed significant associations between physical activity or exercise and CRP. None of these investigations controlled for differences in body fat, however. Mattusch et al. found that after eight months of training for a marathon, 8 subjects experienced a decrease in CRP levels (18). In another investigation, Taafe et al. studied the relationship between physical performance and CRP in 1189 subjects and found that total annual physical exercise was related to higher CRP levels in less active individuals (19). Likewise, increased physical activity contributed to lower CRP levels in 120 women in a study conducted by Esposito et al. (31). Another study suggested that

the intense, regular exercise of 63 ultramarathon runners was associated with low CRP levels when compared to 63 sedentary control subjects of similar age and BMI (32).

Few investigations that have studied the association between physical activity and CRP have noted the possible role of changes in adiposity (33, 34). Hammett et al. found that four months of regular physical exercise lowered CRP levels in 61 elderly men and women (33). These researchers speculated that the favorable changes in CRP were likely caused by reductions in adiposity during training, although this hypothesis was not studied empirically (33).

In a study by Okita et al., a reduction in CRP was found in 199 healthy middle-aged women who completed a 2-month aerobic exercise weight reduction program (34). They found that CRP was related to BMI ($r = 0.41$, $p < 0.001$) and to several liver function profiles, suggesting that a fatty liver caused by obesity may be the link between CRP and adiposity (34). According to Okita, adipose tissue is a likely modulator for CRP (34). Cytokines released by adipocytes, especially adipose cells located within the liver, may trigger increased production of CRP by the liver (34).

Although CRP levels tended to decrease as a result of participation in the aerobic exercise weight reduction program in Okita's study, the extent to which CRP levels changed as a result of regular exercise or weight loss was not measured directly (34). Hence, the researchers could only speculate about the roles of exercise and weight loss in the observed decrease in CRP levels.

As shown in Table 2, in the present study, the influence of body fat on the relationship between physical activity and CRP was measured empirically. There was a

significant linear relationship between CRP and body fat percentage (r = 0.37, p = 0.0001), suggesting that high levels of CRP are partly a function of high levels of body fat.

Excess body fat may be an important determinant of CRP, and in turn heart disease. Forouhi et al. suggested that visceral adiposity is a main contributor to arterial inflammation, marked by elevated CRP (35). A study by Lemieux et al. suggests that obesity and abdominal fat are the most significant contributors to elevated plasma CRP in men (36). Another study by Tanne et al. found that body fat percentage and distribution predicted long-term mortality from heart disease (37).

The present study was limited because it used a cross-sectional design. Without the use of a true experimental design, cause-and-effect conclusions are not warranted. Although physical activity may have a direct effect on CRP levels, given the design of this study, other factors could influence the relationship between activity and CRP. However, because potentially confounding factors were controlled statistically in this study, the influence of body fat percentage on the physical activity and CRP association was identified.

Numerous studies have shown physical activity to decrease the risk of coronary heart disease, atherosclerosis, diabetes and many types of cancer (10, 11, 38, 39). Osteoporosis, arthritis, and other joint diseases are also reduced with regular physical activity (40, 41). Increased physical activity also reduces stress, and has been shown to relieve symptoms of depression (42, 43). Exercise has been shown to elicit a decrease in blood pressure and an increase in stroke volume, which enables the heart to work more

efficiently (44). Another benefit of exercise is increased HDL cholesterol, which aids in maintaining the integrity of arterial walls (45).

Regular exercise is a critical factor in maintaining health and preventing disease. However, being lean is of utmost importance. When comparing lean and overweight subjects with the same physical activity level, those with higher body fat levels are more prone to disease (46). Specifically, Hu et al. found that a higher mortality rate existed in individuals with greater adiposity regardless of their physical activity levels (46).

Given the apparent role of body fat in the association between physical activity and C-reactive protein revealed in the present study, additional research to evaluate the intricacies of this relationship is warranted. Specifically, randomized, controlled trials would be of particular value. Moreover, experiments using only lean or only obese subjects would help to ascertain the extent to which physical activity and exercise influence CRP levels directly.

In conclusion, the present study suggests that high physical activity levels are significantly related to lower C-reactive protein levels in middle-age women. This relationship, however, is almost entirely a function of differences in body fat. As many other studies have indicated, high levels of physical activity contribute to lower levels of body fat. Decreased body fat seems to ultimately be a critical factor in reducing the risk of heart disease possibly by lowering CRP levels.

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Table 1. Descriptive information for all subjects

Variables	Mean (N=211)	Standard Deviation	25th Percentile	50th Percentile	75th Percentile	Low	High
Age (years)	43.1	3.0	41.0	43.0	46.0	37.0	49.0
Weight (kg)	67.1	11.0	58.9	65.9	74.3	42.4	97.0
Body Mass Index (kg/m2)	24.1	3.5	21.3	23.8	26.6	16.2	34.1
Body Fat %	32.5	7.4	26.9	32.9	38.2	12.4	49.9
Physical Activity*	2601.0	931.8	1983.9	2430.4	6064.2	912.9	6824.6
CRP (mg/L)	1.28	1.70	0.24	0.67	1.66	0.01	12.00

*Average weekly activity counts measured objectively with MTI accelerometers, divided by 1,000

Table 2. Differences in physical activity levels by C-reactive protein categories
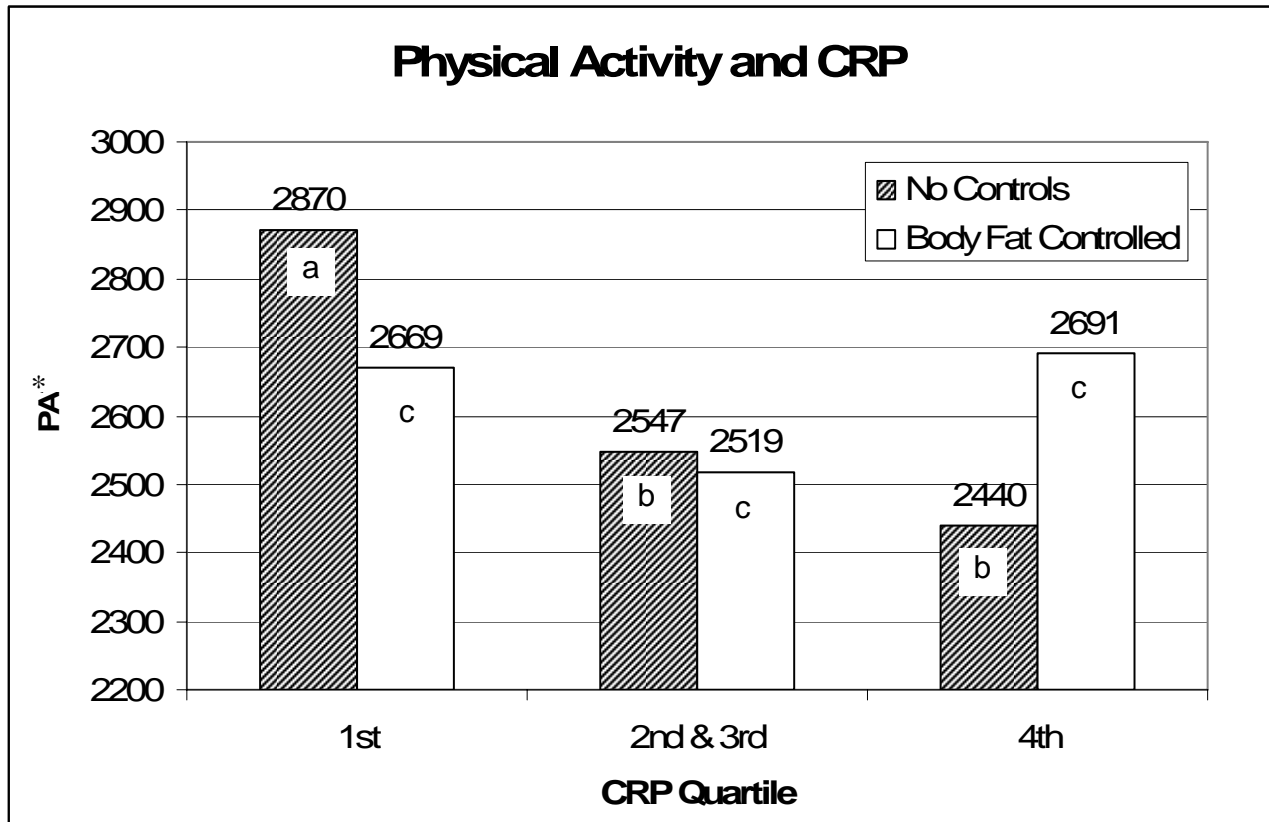
| Variable | C-reactive Protein Category | | | F | p |
| | Low | Moderate | High | | |
Variable Controlled	(n = 53)	(n = 104)	(n = 54)		
	Mean (SD)	Mean (SD)	Mean (SD)		
Physical activity*					
None	2,870.4^a (1,099.5)	2,547.1^b (883.9)	2,440.3^b (794.7)	3.26	0.040
Age (years)	2,870.4^a	2,547.1^b	2,440.3^b	3.22	0.042
Body fat (%)	2,669.2^a	2,519.2^a	2,691.5^a	0.92	0.399
Age, body fat	2,671.8^a	2,519.2^a	2,688.9^a	0.92	0.402

*Average daily activity counts divided by 1,000

Means on the same row with different superscript letters are significantly different ($p<0.05$). On rows showing that a variable was controlled, means are adjusted means.

C-reactive protein categories represent quartiles, with the two middle quartiles combined. Table 1 shows the quartile cut-points associated with each CRP category.

Figure 1. Physical activity and CRP, with and without controlling for body fat.

Physical Activity and CRP

Legend:
- ▨ No Controls
- ☐ Body Fat Controlled

1st quartile: No Controls 2870 (a), Body Fat Controlled 2669 (c)
2nd & 3rd quartile: No Controls 2547 (b), Body Fat Controlled 2519 (c)
4th quartile: No Controls 2440 (b), Body Fat Controlled 2691 (c)

Y-axis: PA* (2200 to 3000)
X-axis: CRP Quartile (1st, 2nd & 3rd, 4th)

*Average weekly activity counts divided by 1,000

No Controls (F = 3.26, p = 0.040), Body Fat Controlled (F = 0.92, p = 0.399)

Bars of the same color with different superscript letters are significantly different

Appendix A

Prospectus

Chapter 1

Introduction

In America, 40% of all deaths are a result of cardiovascular disease, making this disease the nation's leading killer of men and women of all races and ethnicities (1). In 1998, the National Hospital Discharge Survey reported cardiovascular disease (CVD) as the principal diagnosis in 5 million patients, and according to the American Heart Association's 2000 statistical report, cardiovascular disease (CVD) led to the death of 1,400,000 American adults (1).

Coronary artery disease (CAD) causes approximately one-half of all CVD cases (1). CAD is a narrowing of the coronary arteries to the point that sufficient blood cannot be delivered to the myocardium. The resulting decrease in myocardial oxygen levels leads to tissue damage, myocardial infarction, and possible death. Five major risk factors of CAD include smoking, obesity, hypertension, hypercholesterolemia, and physical inactivity (1).

Atherosclerosis is the leading cause of CAD, characterized by cholesterol-lipid-calcium deposits in arterial linings (2). These deposits accumulate until they severely obstruct blood flow, destroy the artery, or become an atherothrombosis (3). The body responds to atherosclerosis with an inflammation of the injured area, the severity of which is an important indicator of plaque stability (3). This inflammation can be estimated, and risk of future complications approximated, by a number of currently available tests (3).

One screening test that is rapidly gaining attention among CVD researchers is C-reactive Protein (CRP). CRP is a nonglycosylated polymeric hepatic protein produced by most vertebrate species in response to foreign invasion of antigens (4,5). It was recently shown that CRP binds specifically to phosphocholine in cell membranes (5). CRP binds to artery walls, causing phagocytosis in leukocytes, macrophages, and other cells (5).

Until recently, CRP has only been used to diagnose tissue injury or inflammation (5). The role of inflammation in cardiovascular disease is now better understood, which has sparked a renewed interest in this protein (5). A number of recent studies have shown CRP to more strongly predict CVD than homocystine (6,7), cardiac troponin (8), ESR (erythrocyte sedimentation rate) screening tests (9), and the blood lipid profile (7).

Many studies have shown low levels of physical activity to increase risk of heart disease, accounting for 35% of coronary heart disease (CHD) deaths in 1993 (10). Studies also show that higher levels of physical activity reflect low risk of coronary heart disease (11). This correlation between increased physical activity and decreased CHD risk may be due partially to a decrease in endothelial inflammation (11).

Recently, a handful of studies have examined the effects of both acute and chronic exercise on CRP levels. Meyer et al. studied the acute effects of short exercise bouts on the acute phase reactants: interleukin 6 and 8, neutrophils, premacrophages, cortisol, and CRP (12). They found CRP to be the only elevated parameter 24 hours after exercise (12). Another study found untrained individuals to have higher CRP levels than trained individuals 24 hours after one bout of extreme exercise (13). A group of Dutch scientists noted a 20-fold increase of CRP 16 hours after a triathlon (14). In another study of

triathletes, CRP levels were three times higher than baseline after a 160-kilometer race (15). Increases of CRP have also been found in athletes 16 hours after a marathon and 24 hours after a 90-kilometer race (16,17). Apparently, acute physical activity raises plasma CRP levels.

Although the acute effect of vigorous exercise seems to be a short-term increase in CRP, chronic endurance training has been found to cause a systemic anti-inflammatory effect, indicated by lowered plasma CRP (18,19,20). Dufaux et al. demonstrated the suppressive effect of regular training on CRP by finding lower CRP levels in swimmers and rowers, compared to a control group (20). Additionally, in older individuals, increased fitness (faster maximum walking speed and greater handgrip strength) correlated with lower CRP levels (19).

In order to better understand the relationship between physical activity and CHD, a closer look at the interaction of physical activity and C-reactive protein is needed. To date, very few investigations have examined the association between chronic physical activity and CRP levels. Moreover, CRP has not been studied as it relates to physical activity measured objectively. Additionally, few investigations have controlled for potential confounders, such as body composition, injury, illness, infection, or acute exercise, and sample sizes have been relatively small. The current study will objectively measure physical activity, will control for body composition and for acute illness, injury, infection, and acute exercise. The study will also have a large sample size.

Statement of the Problem

The purpose of this study is to determine the extent to which objectively measured physical activity is associated with resting C-reactive protein levels in middle-age females. A secondary purpose will be to measure the extent to which potentially confounding factors, such as total body fat and age, affect the CRP and physical activity association.

Research Questions

1. To what extent is total physical activity level associated with resting C-reactive protein level?

2. To what extent is the relationship between physical activity and C-reactive protein influenced by age and body fat percent?

Assumptions

1. Subjects will wear the accelerometer over the left hip continuously for seven full days.

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

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

4. Subjects will not have their blood drawn if they have an acute illness, injury, or infection.

Delimitations

The purpose of this study will be to determine the extent to which objectively measured physical activity is associated with resting C-reactive protein levels in middle-age females. A sample of approximately 200 women will be used as participants. All subjects will live in Utah Valley, and will be between the ages of 37-49 years. Data collection will begin in January 2002. C-reactive protein will be measured using Alpha Diagnostic International's ELISA (enzyme-linked immunosorbent assay) kit for CRP. Accelerometers will be used to measure physical activity, and body composition will be assessed using the Bod Pod.

Limitations

Subjects may wear the accelerometer incorrectly, or may not wear it continuously. Additionally, subjects may not properly fast or refrain from exercise before having their blood tested. Moreover, sub-clinical illness or injury could result in elevated CRP levels that could confound the association between physical activity and CRP.

Definitions

C-reactive Protein (CRP): An acute-phase protein that is synthesized in the liver. It consists of five identical, nonglycosylated polypeptide subunits that are noncovalently linked, forming a disk-shaped pantamer. The molecular weight of this molecule is ~125,000.

Accelerometer: A small electronic device that measures and records the duration and intensity of movement of the wearer.

Physical Activity: Body movement measured by the accelerometer.

Cardiovascular Disease (CVD): Disease in the heart and/or arteries.

Coronary Artery Disease (CAD): A narrowing of the coronary arteries, causing

insufficient oxygen in the myocardium. CAD is usually the result of atherosclerosis, and

often leads to myocardial infarction and death.

Atherosclerosis: A hardening and eventual thickening of artery walls due to an

accumulation of plaque.

Chapter 2

Review of the Literature

Cardiovascular disease (CVD) is the leading killer of men and women of all races in America, resulting in 40% of all deaths (1). According to the American Heart Association, CVD led to the death of 1,400,000 Americans in the year 2000 (1).

About one-half of all CVD cases are caused by coronary artery disease (CAD), a narrowing of the coronary arteries resulting in insufficient oxygen transportation to the myocardium (1). The leading cause of CAD is a build-up of plaque deposits in the lining of artery walls, known as atherosclerosis (1). Atherosclerotic deposits can restrict blood flow, deteriorate the artery, or become atherothrombosis (3). The body responds to atherosclerosis with an inflammation of the injured area, the severity of which is an important indicator of plaque stability. A number of tests are currently available to estimate this inflammation (3). Recently, C-reactive Protein (CRP) has been gaining attention among CVD researchers as a method of screening for arterial inflammation.

CRP and Cardiovascular Disease

A number of prospective, large-scale investigations have shown CRP to be a very strong independent predictor of myocardial infarction and stroke among men and women who appear to be healthy (7). Research suggests that if CRP were to be added to the standard lipid screening, global risk prediction among individuals with high and low cholesterol may be improved (7). CRP may also be useful in targeting proven therapies for primary prevention of CVD, such as aspirin and statins (7).

Because recent evidence has shown atherosclerosis to be partly the result of inflammation, Nader et al. compared severity of atherosclerosis to CRP levels (31). In this study, 100 men who had angiographically-confirmed CHD were compared to 100 men with no history of CHD (31). Age, gender, and smoking were controlled, and severity of disease was indicated by > 0% obstruction in one vessel (n = 30), two vessels (n = 39), or three vessels (n = 31) (31). They found significantly higher CRP levels in the CHD group when compared to controls (median for cases vs. controls, 3.4 vs. 1.5 mg/L; $p < 0.0001$) (31). Although CRP concentration was increased in CHD patients, it did not correlate with severity of the disease (31). Nader suggests that rather than reflecting the degree of obstruction in arteries, CRP may be an indicator of the rate of atherosclerotic development (31).

Ridker et al. studied whether inflammation (measured by CRP) predicted stroke in 543 apparently healthy subjects in whom myocardial infarction (MI), stroke, or venous thrombosis later developed (32). These subjects were compared to a control group of 543 who did not report any disease during the 8-year follow-up period (32). Higher plasma CRP levels were found in men who, during the 8-year period, had an MI (1.51 vs. 1.13 mg/L, $p < 0.001$) or stroke (1.38 vs. 1.13 mg/L, $p = 0.02$) (32). Patients in the highest CRP quartile had three times the risk of having an MI (relative risk, 2.9; $p < 0.001$) and two times the risk of stroke (relative risk, 1.9; $p = 0.02$) (32). It was concluded that baseline CRP concentrations predict the risk of future MI and stroke (32).

In western Turkey, Onat et al. studied the relationship between CRP and CHD among 532 men and 523 women (33). The mean CRP level for this population was 1.9

mg/L, with an inter-quartile range of 0.8 to 4.3 (33). After adjusting for age, the odds

ratio for CHD in the highest quartile compared to the lowest quartile was 4.48 (p < 0.001)

(33). This finding was particularly interesting because of the relatively low cholesterol

levels of the population (33).

In Osaka, Japan, Hiroyuki et al. performed a longitudinal study comparing CRP

levels to atherosclerosis development (34). A total of 179 outpatients between the ages

of 40 and 79, who did not show signs of advanced atherosclerosis, were studied (34).

These patients had previously been treated at the Osaka University Graduate School of

Medicine for traditional risk factors for CVD (hypertension, hypercholesterolemia,

diabetes mellitus, smoking, ischemic heart disease, cerebrovascular disease, aortic

aneurysm, or peripheral vascular disease) (34). Each patient received repeated

ultrasonographic evaluations of the carotid arteries and blood tests for CRP over a period

of 35 ± 10 months (34). A significant correlation was found between the change in

number of carotid plaques over time and level of baseline CRP (r = 0.223, p = 0.003)

(34). A relationship between baseline CRP and change in carotid plaque score over time

was also found (r = 0.268, p < 0.001) (34). It was concluded that although CRP was not

related to the number of plaques or plaque score at baseline, CRP was related to the

change in number of plaques per year and the change in plaque score per year (p < 0.001

for both) independent of the effect of traditional risk factors (34).

CRP is supported as a main cardiovascular risk assessment tool (6). This opinion

is based on the fact that because CRP is an acute phase reactant, it directly measures

inflammation (6). It is also recommended that CRP testing be used in conjunction with

total cholesterol/HDL ratios and homocysteine levels in order to improve CHD risk

assessment (6).

Acute Physical Activity and CRP

A number of studies have examined the acute effect of physical activity on CRP

levels. Specifically, Meyer et al. measured CRP levels in twelve non-specifically trained

males after short bouts of aerobic exercise (12). The exercise protocol consisted of one

60-second all-out test and eight 10-second all-out tests (12). These tests were assigned in

a random order to each subject (12). Higher levels of CRP were found in individuals

after repeated 10-second tests than after a single 60-second test (12). This suggests that

short aerobic exercise bouts trigger an acute inflammatory response, which is more

pronounced following repeated short bouts than one longer bout of similar duration (12).

Acute phase protein responses to sustained strenuous exercise were studied by

Taylor et al. (15). Eighteen athletes competed in a 160-kilometer triathlon, consisting of

canoeing, cycling, and running (15). A number of plasma components were measured

before and 30 minutes, 24 hours, and 48 hours after the triathlon, including CRP (15).

They found CRP to be elevated by nearly 300% at 24 hours after exercise (15). Castell et

al. also found elevated CRP levels in marathoners 16 hours after a race (16).

Smith el al. compared trained and untrained subjects who performed one hour of

cycling at 60% of VO_{2max} (13). CRP was measured by immunoassay before, one hour

after, and 24 hours after cycling (13). Their results showed no significant difference in

CRP levels between trained and untrained individuals one hour post-exercise (13).

However, only untrained individuals had higher CRP levels 24 hours after exercise (13).

The effect of vitamin C supplementation and exercise on the acute CRP response was studied by Peters et al. (17). Sixteen ultramarathoners participated in a 90-kilometer race, having their blood drawn 16 hours prior to the race and 30 minutes, 24 hours, and 48 hours after finishing (17). Elevated CRP levels were observed at each of the post-race points (17). Similarly, Jeukendrup et al. noted a 20-fold increase in CRP levels in 29 athletes 16 hours after a long-distance triathlon (14).

A cross-sectional study performed by Drent et al. investigated the association between fatigue and acute CRP response among sarcoidosis patients (35). High levels of CRP were strongly correlated with increased fatigue ($p < 0.0001$) (35). CRP was also significantly associated with resting energy expenditure/fat free mass ($r = 0.54$, $p = 0.001$) (35).

Payne et al. studied the inflammatory effects of uphill, downhill, and near-level running on eight extremely well-trained males (36). On three different days, each subject performed eight 5-minute bouts of treadmill running (36). They were randomly assigned to run for 40 minutes at one of the three grades at 90% (uphill), 50% (downhill), and 50% (near-level) of their VO_{2max} (36). There were no significant differences in plasma CRP between the three days in any of the subjects 24 hours after running (36).

Apparently, acute physical activity, especially intense or very long duration bouts, triggers an acute inflammatory response in the body. Drastic elevations in inflammation (measured by CRP levels) are seen immediately after the onset of exercise, and can last for as long as 24 hours. Therefore, it can be concluded that acute physical activity raises plasma CRP levels.

Chronic Physical Activity and CRP

The American Heart Association 2000 Heart and Stroke Statistical Update

reported that as many as 250,000 deaths can be attributed to a lack of regular physical

activity, accounting for more than 11% of total deaths (12). Less physically active

individuals have reduced longevity and increased risk of CHD, CVD, stroke, and colon

cancer (30). However, very few studies comparing chronic physical activity and CRP

have been conducted.

In an experiment conducted by Mattusch et al., the effects of training on CRP

levels were studied in eight subjects preparing for a marathon (18). After eight months of

training, an unexpected decrease in CRP resulted, despite a general increase in training

intensity (18). Their study suggests that regular physical exercise has a chronic systemic

anti-inflammatory effect (18).

As part of the MacArthur study of successful aging, Taafe et al. studied the

relationship of physical performance with CRP (19). A total of 1189 subjects age 70 to

79 participated (19). Total annual physical exercise was related to higher CRP levels ($p <$

0.001) in less active individuals (19). A relationship also existed ($p = 0.038$) for lower

CRP levels in subjects with faster 6-minute walking speeds (19). Also, at the conclusion

of the 7-year study, those who died or were not able to undergo testing had much higher

baseline CRP levels ($p < 0.01$) and slower 6-minute walking speeds ($p < 0.05$) (19).

In a study conducted by Dufaux et al., basal CRP levels in athletes from a variety

of genres were measured (20). The aim of the study was to evaluate the effect of physical

training on plasma CRP levels (20). A total of 356 male and 103 female athletes were

recruited for the study (20). When compared to untrained controls, male swimmers had significantly lower CRP levels ($p < 0.001$) (20). Rowers also exhibited significantly lower CRP than controls ($p < 0.01$) (20). CRP levels of long distance runners, racing cyclists, and soccer players did not differ significantly from controls (20). In female athletes, lowest CRP levels were also shown in swimmers, which were significantly lower than the female controls ($p < 0.001$) (20).

Although an immediate escalation of CRP levels seems to occur at the onset of successive training sessions, these studies suggest that physical training over a period of time may lower basal CRP levels in adults. Also suggested is the idea that prolonged training suppresses the body's inflammatory response, demonstrated by lowered CRP.

Conclusion

There appears to be a clear connection between CRP and CHD. As resting CRP levels increase, risk for developing CHD also increases. Apparently, exercise, particularly intense exercise, has an unfavorable acute effect on the body, especially the circulatory system. However, recent studies suggest that chronic exercise can actually lessen the body's inflammatory response over time. This chronic reduction of inflammation, indicated by lowered CRP levels, also suggests a decreased CHD risk.

Unfortunately, very little research has been conducted to examine the connection between chronic physical activity and CRP. Current studies have not controlled for such confounding factors such as body fat, smoking, acute illness, and infection. Some studies have also failed to use adequate sample sizes and most have only subjectively measured physical activity. The proposed investigation will control for potential confounders, will

study 200 subjects, and will objectively measure physical activity, which should help to

elucidate the association between chronic physical activity and CRP.

Chapter 3

Methods

Subjects

Subjects for this study will come from an existing cohort of approximately 250

women. These women participated in baseline testing 36 months prior to this study. The

women were recruited during 1998 and 1999 using several advertising methods. At

baseline, all subjects were between the ages of 35-45 years old, had no apparent health

problems, did not smoke, had a BMI (body mass index) less than 30, were

premenopausal, and were not planning on having children during the next six years.

These criteria were established in order that confounding factors be avoided, such as

obesity, smoking, childbirth, menopause, and serious disease.

A total of 200 women from the original cohort will be used as subjects in the

present study. All subjects will be required to sign an informed consent document before

participating in the study.

Instruments and Measurement Methods

C-reactive Protein

C-reactive Protein (CRP) will be measured using the solid phase ELISA (cat no.

1000) developed by Alpha Diagnostics International (ADI), Inc. (San Antonio, TX).

Many similar high-sensitivity assay kits to measure CRP have become available in the

past five years. This kit was chosen because of its sensitivity, precision, availability, and

cost. The ADI assay is most sensitive within the normal range of 0-5 µg/ml. The intra-

assay variation in the normal range is low (C.V. = 3.0%; sd = 0.3), and the inter-assay

variation is reasonable (C.V. = 7.0%; sd = 0.4). Other CRP kits report higher levels of intra- and inter-assay variation. Moreover, other CRP assay kits are manufactured by small companies and distributed from foreign countries, such as Mexico, Ireland, Germany, and Korea. ADI is a well-known, well-established American company located in San Antonio, Texas.

Blood serum will be collected by a certified laboratory at Timpanogas Regional Hospital in Orem, UT. Subjects will be asked not to exercise for at least 48 hours prior to being tested. If a subject is sick or recovering from an illness, she will be asked to wait until all symptoms are gone before having her blood drawn. Blood samples will be drawn, centrifuged, and stored in aliquots at -20ºC.

After subjects have had their blood drawn, the serum will be collected periodically and transported to the biochemistry laboratory at BYU. Samples will be transported in a cooler at approximately 4ºC. After an adequate number of samples have been collected, an ELISA for C-reactive protein will be performed for each subject. The Alpha Diagnostic International, Inc. (San Antonio, TX), C-reactive Protein ELISA Kit Cat No. 1000 will be used. Assays will be performed in duplicate and the mean of the two analyses will be used to index CRP. If an assay pair differs by more than 8%, a third assay will be performed and the closest two results will be averaged. A third assay will also be performed if any test reads higher than 30µg/ml.

Physical Activity

Computer Science and Application (CSA) accelerometers (Shalimar, FL) will be used to objectively measure physical activity. These devices have been shown to give

valid and reliable activity counts, and can precisely monitor everyday activities (22,23,24,25,26).

In a study performed by Bassett et al., strong correlations were found between energy expenditure and physical activity (26). Specifically, four accelerometers were used to measure physical activity and a portable metabolic system was used to measure energy expenditure during 28 different everyday activities, such as vacuuming, cooking, cleaning, child care, and walking (26). In comparing the four different accelerometers, the CSA monitor was the only one that did not differ significantly from energy expenditure measured with a portable metabolic system (p=0.473) (26). Additionally, in studying 24 adults, Nelson et al. showed a strong correlation ($r^2 = 0.85$) between energy expenditure during a treadmill test and CSA accelerometer measurements (25).

CSA accelerometers will be worn over the left hip, in line with the outer seam of the pants and the umbilicus. They will be worn at all times during a seven day period except while bathing and during water activities. The monitors will sum the motion counts of each subject into one-minute intervals, which will later be collapsed into 10-minute intervals. Therefore, the motion of each subject will be recorded in 144 time segments (epochs) each day, each with a length of 10 minutes. Over the course of the seven days, each subject will have a total of 1008 activity counts which will be summed to index total physical activity.

Body Fat Percentage

Body fat percentage will be measured using the Bod Pod (Life Measurement Instruments, Concord, CA), which implements the technique of air displacement

plethysmography. It has been shown that the Bod Pod produces a reliable and valid measurement of body fat (28). When measuring test-retest reliability in 100 subjects during a pilot study, a strong intra-class correlation (r = 0.999) was observed (27). Body composition for the 100 subjects was then measured using Duel Energy X-ray Absorptiometery (DEXA) (Hologic 4500W, Bedford, MA). When the DEXA results were compared to the Bod Pod findings, the Pearson correlation was 0.94 (p < 0.001), and the intra-class correlation was 0.97 (p < 0.001) (29).

Subjects will have fasted for 12 hours before being tested, and will wear a standard BYU-issued swimsuit and a swim cap. Each subject will eliminate any waste, and will then be weighed. Before each test, the Bod Pod will be calibrated with a known-volume cylinder, according to the manufacturer's instructions. A minimum of two tests will be performed, and the average taken. If the two tests are not within one percentage point of each other, subsequent tests will be performed until two are within one percentage point. The average of the two nearest measurements will be calculated.

<div align="center">Procedure</div>

Subjects will report to the Human Performance Research Center at Brigham Young University in Provo, UT for two appointments. During the first appointment, subjects will read and sign an informed consent, which will contain all procedures of the study. The document will be reviewed with the subjects, and any questions will be answered. Benefits and possible risks will be described, and the subjects will be assured that all information acquired through the study will be confidential. A medical screening questionnaire will also be given in order to assess any health problems.

Subjects will be asked to eliminate any bodily waste, and to change into a standard BYU swimsuit. Weight will be measured using an electronic scale (Tanita Corporation, Japan). The procedure for the Bod Pod will be described to the subjects, and two tests will be administered. If the two tests are not within one percentage point, additional tests will be performed until two tests are within one percentage point. The average of the two tests will be calculated.

After changing back into street clothes, the proper techniques for wearing the accelerometer will be shown to, and discussed with, the subjects. An accelerometer for each subject will be properly fitted, located over the left hip. A second appointment will be made for eight days later. Subjects will wear the monitor at all times (except bathing) for the next seven days. During the next week, subjects will receive three reminder calls to encourage them to follow proper procedure.

At the second appointment, the activity monitor will be collected and downloaded. Any abnormalities in the data will be discussed with the subject. Each subject will then be given a blood profile requisition form for Timpanogos Regional Hospital blood laboratory, and will be directed to have her blood drawn within the next week. Subjects will be instructed to fast for twelve hours, to be free of any illness or infection, and to refrain from moderate and intense physical activity 48 hours prior to the draw. After completing the blood draw, each subject will be thanked for her participation and receive a $25 gift certificate.

Data Analysis

The magnitude and direction of the bivariate relationship between objectively measured total physical activity and resting CRP will be indexed using the Pearson product-movement correlation coefficient. Additionally, the association between physical activity and CRP will be measured with subjects categorized according to their physical activity levels. Specifically, subjects will be divided into quartiles based on their total activity counts, then the middle two quartiles will be collapsed, forming three groups. Mean differences in CRP across the different categories of physical activity will be calculated using regression analysis. The affect of potentially confounding factors, such as age and body fat percentage, on the physical activity and CRP association, will be studied using partial correlation. Alpha will be set at the 0.05 level and all calculations will be determined using SAS software, version 8.01

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