

PHYSICAL ACTIVITY, EXERCISE, AND INFLAMMATION: EXAMINING THE
INDEPENDENT AND INTERACTIVE EFFECTS OF ORAL CONTRACEPTIVE USE
AND ADIPOSITY ON C-REACTIVE PROTEIN IN YOUNG WOMEN

by

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(Under the Direction of Ellen M. Evans)

ABSTRACT

Physical activity (PA) is inversely associated with C-reactive protein (CRP) levels in adults. However, exercise training interventions aimed at reducing CRP have yielded inconsistent results, with the cumulative body of evidence indicating exercise training in the absence of improvements in adiposity (%Fat) will not result a decrease in CRP. This study aimed to: 1) determine the independent and interactive associations of PA, %Fat, and oral contraceptive (OC) use on CRP in young women in a cross-sectional analysis (n=340), and 2) to determine the relative efficacy of 6-weeks of cycle exercise training, differing in intensity and matched on energy expenditure, on cardiometabolic risk factors, with a special emphasis on CRP in inactive, overweight/obese, young women (n=48). To assess primary aim 1, %Fat was measured via dual energy x-ray absorptiometry (DXA), PA was measured via accelerometer [steps·day⁻¹], OC-use was measured via self-report, and CRP was determined from fasting blood samples obtained via venipuncture following a 12-hour fast using standard clinical procedures. Using Poisson regression, a significant 3-way interaction (%Fat×OC-use×PA) was found, indicating that higher

levels of PA reduced the %Fat and OC-induced elevation in CRP (Wald $\chi^2=6.6$, $P=.011$). To assess primary aim 2, lipid, insulin, HOMA-IR, and CRP concentrations were measured using similar methods. Participants were randomly assigned to vigorous sprint-interval cycling (VIG-SIC) or continuous moderate-intensity cycling (MOD-C) for 6 weeks of exercise training. Participants in the VIG-SIC completed 5-7 repeated bouts of 30-second sprints, followed by 4 minutes of active recovery. MOD-C participants cycled at 60-70% of heart rate reserve for 20-30 minutes, with duration increasing biweekly, maintaining equal energy expenditure between groups. Total cholesterol, HDL-C, LDL-C, and triglyceride concentrations significantly improved after controlling for baseline values in both groups (all $P<.05$). A significant GROUP \times TIME interaction ($P=.031$) indicated exercise intensity modified the training response, with a reduction in CRP observed in the MOD-C group, but not in the VIG-SIC group after controlling for change in %Fat and baseline CRP. Young women, especially OC-users with higher %Fat, should engage in greater amounts of PA to reduce CRP. In addition, moderate intensity exercise may be more effective in reducing CRP in OW/OB young women.

INDEX WORDS: inflammation, physical activity, exercise training, obesity, oral contraceptives, young women

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2015

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August 2015

DEDICATION

I dedicate this document to my parents, Linda and Mike, who loved all three of their sons more than anything in the world, and who have supported me through my entire journey. My parents taught me to explore and discover. I also dedicate this work to Ben and Jake, for allowing sibling rivalry and brotherly competition to push the three of us beyond our own expectations. And, to my wife Megan, who inspires everything I do. Megan used her photographer's eye to show me that art and creativity can be seen in anything...including research.

ACKNOWLEDGEMENTS

The science sandbox is more confusing than I imagined. Thank you to my mentor, Dr. Ellen Evans, for explaining the rules of the game, teaching me to play nicely with other scientists, and helping me to develop a passion for mentoring, student development, and curriculum that fit nicely with my “research” tools. Sincere thanks go to my committee, Dr. Nathan Jenkins, Dr. Patrick O’Connor, and Dr. Michael Schmidt, whose expertise and guidance helped the development of this project from start to finish. Thank you to Dr. Rod Dishman, who set high standards for excellence, and refused to accept anything but my best. I would not have pursued a doctoral degree without the encouragement of Dr. Paul Visich, who showed me it was possible to raise a family and balance the “best job in the world,” all while coaching hockey, climbing mountains, and riding his bike around the country. All of these professors showed patience, as they trusted me with responsibility, and allowed me to fail, repeatedly, until I got it right.

Although the results of my dissertation will undoubtedly change the world, the most exciting results from SPINDawgs are yet to come. Thank you to Liz Hathaway and Simon Higgins for being a part of this study. Simon’s youthful enthusiasm on this project was unparalleled. Tackling a project of this size was ambitious, and forced us to realize how much of the little stuff we “ain’t got time for.” As Liz phrased it, “we are research homies for life.” To Christie, for her friendship and attentive ear through my time at UGA, Bhibha for her countless hours spent coordinating PowerDawgs,

and Chad for sharing an office, our teaching responsibilities, and our coursework for the first two years in Athens. Thank you to the rest the BCML Team, for their laughter and support. Let this project not be an example of the countless hours spent in our lab or at our desk, but a celebration of the amazing work that can be produced when many come together. Finally, thank you to James, who provided encouragement from our first morning on the stairs, and the perfect break from the academic stress when I needed it.

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CHAPTER 1

INTRODUCTION

1.1 Significance

Cardiovascular disease (CVD) is the leading cause of death among women in the United States, totaling nearly 300,000 deaths in 2009.[1] Metabolic syndrome (Mets) is a clustering of CVD risk factors that appear in constellation, identifying individuals with unhealthy cardiometabolic risk profiles before disease manifests.[2] Primordial prevention of metabolic syndrome (MetS) and CVD involves successfully adopting a number of health related behaviors aimed at slowing the progression and development of traditional disease risk factors (hypertension, dyslipidemia, obesity, etc.). Recent estimates indicate that 54% of young women in the U.S. have at least one risk factor for MetS, with 17% presenting 3 or more.[3, 4] It is well established that physical activity and exercise training reduces risks for both MetS and CVD and is also of primary importance for weight management. Although many types of activity programs have been investigated, data suggests that sprint interval cycling exercise (EX-SIC), similar to the popular training modality spinning, is a feasible, effective and time-efficient training modality for sedentary individuals to improve aerobic fitness.[5, 6] However, the efficacy of EX-SIC to reduce risk for MetS and CVD in young adult women, especially those using oral contraceptives (OCs), who may have increased risks for CVD, as described below, is not well established.

For young adult women who use OCs for birth control or to regulate their menstrual cycles, additional risks for MetS and CVD may be present. Notably, OCs are the most commonly used type of birth control among women aged 15-44 years; however, for all their popularity, it is established that OC-use negatively impacts c-reactive protein (CRP), a marker of systemic inflammation, theoretically increasing risks for MetS and CVD.

In addition to the need for more efficacy data for EX-SIC to reduce MetS risk factors in the young adult female population, more work is also needed characterizing the potential influence of OC-use on training adaptations to exercise training in general and in particular the contemporary EX-SIC. The goals of this project were to 1) to examine the independent and interactive effects of %Fat, PA, and OC-use on CRP in young, relatively healthy adult women using a cross-sectional design, and 2) to determine the relative effectiveness of exercise training differing in intensity, but matched in energy expenditure (EE) on cardiometabolic risk factors in overweight and obese young women. To accomplish this second aim, a parallel-arm trial was conducted with young adult females randomized to a Moderate Intensity Cycling (MOD-C) or Vigorous Sprint Interval Cycling (VIG-SIC) training protocol for 6 weeks, after blocked stratification on OC-use and weight status.

1.2 Primary Aims

Thus, the primary aims and hypotheses include:

Primary Aim 1: To examine the independent and interactive associations of %Fat, PA, and OC-use on CRP in young, relatively healthy adult women using a cross-sectional design.

Hypothesis: Higher %Fat, lower PA, and OC-use is independently associated with CRP, with the cumulative effect being greater than each independent factor. In addition, higher levels of PA will attenuate the increase in CRP caused by greater %Fat and OC-use.

Primary Aim 2: To determine the relative effectiveness of 6 weeks of cycle exercise training matched in EE but differing in intensity (MOD-C or VIG-SIC) on the primary outcome measures of fasting TRG, INS and CRP in overweight and obese young women.

Hypothesis: VIG-SIC will cause a greater reduction in TRG, INS and CRP when compared to MOD-C, even after controlling for potential differences in baseline levels or changes in adiposity.

1.3 Public Health Related Significance

Over half of young adult women have at least one risk factor for MetS.[3, 4] CRP levels are positively associated with the number of risk factors for MetS, potentially impacting future disease risk.[7, 8] It is well established that PA and EX training reduces risks for MetS and CVD; however, current data suggest that 55% of young adult women do not gain enough PA/EX to confer beneficial effects for the prevention of MetS and CVD, and may spend nearly 30 hours per week engaged in sedentary behavior.[9-11]

A large body of literature supports the role of EX training in lowering TRG, INS, and CRP levels. A novel training approach involving EX-SIC provides an opportunity for further research determining the efficacy of this program design in slowing the development and progression of MetS in young women, especially those using OCs. Previous research has examined the efficacy of VIG-SIC on aerobic capacity and cardiovascular function in young overweight and obese women, and concluded VIG-SIC can be utilized as a safe and effective mode of exercise training.[5] A paucity of original

research exists examining the moderating effect of OC-use on physical activity and cardiometabolic markers in young women, and further research is warranted given the adverse effect of OC-use on CRP. The proposed study is novel in that the efficacy of VIG-SIC on cardio-metabolic risk factors has been largely understudied in inactive overweight young women at risk for metabolic syndrome.

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CHAPTER 2

LITERATURE REVIEW

2.1 Incidence and Prevalence of OC-use among Women in the United States

Over 80% of US women have used OCs in their life, making them the most commonly used contraceptive method in the United States.[1] OCs are the primary contraceptive method in young women 15-24 yrs., with 48-54% reporting their use in 2010.[1] In addition, the prevalence of OC-use increases with education level, making their use increasingly popular among college-aged women.[2, 3]

2.2 OC-use and MetS Associated Risk Factors of TRG, INS and CRP

OCs typically combine a synthetic form of estrogen and a progestin component to regulate the menstrual cycle and reduce unfavorable side effects experienced during menstruation. Despite the benefit of preventing unwanted pregnancy, OC-use is associated with unfavorable changes in cardiometabolic risk factors. OC-users have higher triglyceride (TRG) concentrations when compared to non-users.[4] The increase in plasma TRG concentration is due to an increase in hepatic TRG secretion, and typically correlates strongly with the amount of synthetic estrogen.[5, 6] New OC-users were 3 times more likely to have elevated TRG following 3 years of continuous use, when compared to controls.[7] Accompanying the apparent increase in TRG levels, OC-use is also associated with a decrease in insulin sensitivity.[8] In addition, fasting insulin (INS) and insulin resistance (IR) increased in 38 obese OC-users following 3 months of treatment.[9, 10] Finally, OC-use is one of the strongest predictors of C-reactive protein

(CRP) level in young women.[11, 12] Cross-sectional data in young healthy women has indicated OC-users were 6-9 times more likely to have elevated CRP when compared to Non OC-users.[13-16] CRP levels increased 3- and 4-fold in new OC-users following two months of treatment in the absence of underlying disease pathology.[10, 17]

2.3 Physical Activity/Exercise and MetS Associated Factors of TRG, INS and CRP

It is well established that exercise reduces TRG, INS and CRP. The cumulative body of evidence indicates exercise training can favorably affect blood lipid and lipoprotein concentrations in women.[18] Exercise training significantly improves TRG levels between 5.0-9.0% in adults,[18-21] with the beneficial effects of exercise likely greatest in overweight or obese participants.[22] In addition, the largest improvements resulting from exercise training are observed in participants with higher TRG levels, and among those who experience larger reductions in adiposity.[19] Aerobic exercise significantly improved total cholesterol, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and TRG concentrations in 12 overweight young women (19-24yrs) following 12 weeks of training,[23] with similar improvements in HDL-C and TRG concentrations observed in 10 young women following sixteen weeks of training.[24]

Given the beneficial effect of exercise on TRG, coupled with the adverse side effect of OC-use on TRG, research has attempted to examine the possible interactive effect of exercise training and OC-use in women. Cross sectional studies found aerobic exercise is associated with reduced TRG concentrations in OC-users but not in non-users, and suggested an interactive effect between OC-use and exercise.[25] Sedentary OC-users have higher TRG when compared to non-users and nearly double the TRG level of active non-users.[26-28] Even among active women, OC-users have higher TRG when

compared to non-users.[27, 29] Exercise training interventions in OC-users have found no effect on TRG, indicating a possible moderating effect of OC-use.[30] Due to the excess hepatic TRG production associated with increased adiposity and OC-use, exercise training may improve TRG levels in overweight and obese OC-users by reducing hepatic production and increasing TRG clearance.

Similar to TRG, the cumulative body of evidence indicates exercise training improves fasting INS and INS resistance, especially in overweight and obese participants, even in the absence of significant weight loss or change in body composition.[31, 32] For Example, INS and HOMA-IR decreased 33% and 34% respectively following 12 weeks of exercise training in a cohort of young women when included as part of a lifestyle intervention.[33] INS and HOMA-IR also improved in obese young women following 12 weeks of moderate or high intensity aerobic exercise.[34] Despite the consistent results indicating exercise can lower insulin and HOMA-IR, the possible moderating effect of OC-use has not been examined.[35]

It is also well established that PA/exercise is inversely associated with CRP, with greater levels of PA/exercise associated with lower CRP.[36] CRP levels are higher among sedentary adults when compared to their more active peers.[37] In total, across all age groups physically active adults have CRP concentrations 19–35% lower than less active adults.[38] Recent research supports the beneficial effect of physical activity with a decrease in CRP following exercise training in healthy adults[39] and clinical populations.[40] A dose-response relationship was observed in a cohort of 14,000 US adults, with lower risk of elevated CRP found for adults engaging in light, moderate, and vigorous PA when compared to adults that reported no PA (OR=0.98, 0.85, and 0.53,

respectively).[41] In addition, cross-sectional analysis of old (60-80 yrs.) and young (18-30 yrs.) adults revealed that CRP levels were 60% lower in active individuals when compared to inactive adults.[42] The results of the INFLAME, DREW, HART-D, and Seattle studies concluded that exercise training did not improve CRP unless accompanied by a significant reduction in body weight or improvement in body composition.[43-46] However data from more recent intervention studies indicate that exercise training can reduce CRP levels in the absence of improvements in %Fat.[47, 48]

Vigorous leisure time PA is negatively associated with CRP; with OC-use positively associated with CRP.[49] However, no research to date has examined the moderating effect of OC-use on CRP following exercise training.

2.4 High Intensity Interval Training and MetS Associated Factors of TRG, INS and CRP

High intensity interval training is characterized by repeated short-duration exercise bouts of maximal effort followed by a period of active recovery.[50] This type of training approach allows for training at higher intensities that cannot be maintained for long durations, and may provide physiological adaptations equal to continuous MOD-C, when matched on energy expenditure, allowing for rapid training adaptations to occur with significantly lower time commitment by the participant.[51] High intensity interval exercise improved TRG in 8 sedentary participants (aged 20-40 yrs.) by 28% following 8 weeks of training, and 10% in 17 sedentary participants (53.0 ± 5.6 yrs.) over 8 months.[52, 53] Fasting INS improved 16% in 7 previously sedentary male and female participants (45.0 ± 5.0 yrs.) following 2 weeks of sprint interval cycling.[54] Similar

significant results were also observed in high intensity interval training studies of longer duration.[55]

Despite the recent rise in popularity, the effect of high-intensity interval training on CRP has not been thoroughly examined; however, recent research has shown promising results. High-intensity interval training effectively reduced CRP levels in 20 participants following 6 months of training.[56] Although CRP levels decreased 8% following 2 weeks of sprint interval cycling, the change was similar to that observed with moderate intensity exercise.[57] Despite the apparent effect of high intensity exercise training on TRG, INS, and CRP, some debate exists whether this is due to greater total energy expenditure, rather than due to exercise intensity, with the former not being well controlled.[32, 53]

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CHAPTER 3

**INDEPENDENT AND INTERACTIVE ASSOCIATIONS OF PHYSICAL
ACTIVITY, ADIPOSITY, AND ORAL CONTRACEPTIVE USE ON C-
REACTIVE PROTEIN LEVELS IN YOUNG WOMEN¹**

¹Fedewa MV, Hathaway ED, Higgins S, Das BM, Forehand RL, Schmidt MD, Evans EM. To be submitted to Am J Prev Med.

3.1 Abstract

Introduction:

Oral contraceptives (OCs) are the most commonly used type of birth control among young women. OC-users have higher systemic inflammation, as assessed by C-reactive protein (CRP) levels, than non-users. Relative adiposity (%Fat) and physical activity (PA) are also both associated with CRP. The study aimed to determine the independent and interactive associations of %Fat, OC-use, and PA on CRP in young women.

Methods:

Female participants (n=340, 18.8±1.3 yrs., 65.0% Caucasian) were assessed for objective PA via pedometer (NL-1000). %Fat was measured via dual x-ray absorptiometry. OC-use was categorized as yes or no, via self-report. CRP was obtained using conventional clinical methods. Poisson regression analysis was used to determine the independent and interactive associations of %Fat, OC-use, and PA on CRP in young women.

Results:

Participants were normal weight (body mass index=24.3±4.7 kg/m²) with a higher than expected average %Fat (35.0±6.9%), and 51.5% were classified as physically active, meeting PA guidelines averaging 10,208±3,766 steps/day. OC-use was reported by 35.0% of the sample (n=119) with 20.9% (n=71) having elevated CRP, defined as > 3.0mg/L. A significant 3-way interaction was found ($\chi^2=10.6$, P=.001), suggesting higher PA was associated with lower CRP in OC-users with higher %Fat.

Conclusion:

Higher PA attenuated the adverse effects of increased adiposity on CRP, specifically in OC-users who had higher CRP. Future research should consider OC-use when examining

the effect of exercise/weight management interventions on risk factors for cardiovascular disease, and encourage PA in OC-users to potentially lower future disease risk.

Grant Support: USDA 2008-55215-18825

3.2 Introduction

The paradigm of atherosclerosis has extended beyond the theory of lipid accumulation to include the role of inflammation in promoting plaque formation and its complications.[1] C-reactive protein (CRP) is one of the most commonly used circulating markers of chronic low-grade inflammation, and is one of the strongest predictors of a future cardiovascular event in young women when compared to other markers of inflammation.[2] The role of CRP in risk stratification in primary prevention has been clearly defined in the past decade,[3] with the cumulative body of evidence gathered from cross-sectional and prospective studies indicating individuals with higher CRP are 1.4-2.3 times more likely to develop cardiovascular disease (CVD).[4-8] Furthermore, including CRP in a battery of markers assessing CVD risk significantly increases the predictive value.[9]

There are numerous factors that influence CRP, including relative adiposity (%Fat), physical activity (PA), and oral contraceptive (OC) use. Data from cross-sectional studies consistently indicate greater %Fat and obesity, as assessed via body mass index (BMI), are associated with higher CRP in both children and adults, regardless of sex or race.[10-12] Levels of this inflammatory marker are ~2-fold higher in young women with excess adiposity compared to their lean peers due to the positive moderate association of %Fat and CRP.[13, 14] It is also well established that PA is inversely associated with CRP, with greater levels of PA associated with lower CRP.[15] In total, physically active adults have CRP concentrations 19–35% lower than less active adults.[16] Although the majority of cross-sectional studies have relied on self-reported physical activity and anthropometric measures of obesity, data from intervention studies

also indicate that increased PA as part of an exercise training program can reduce CRP levels in the absence of improvements in %Fat.[17, 18]

In addition to the independent associations of %Fat and PA on CRP, OC-use is also commonly associated with increased CRP levels in the absence of underlying disease pathology.[19] Cross-sectional data has indicated OC-users are 6-9 times more likely to have elevated CRP when compared to non OC-users,[20, 21] in young healthy women.[22-25] Although the mechanism is unclear, CRP levels increased 3- and 4-fold in new OC-users following two months of treatment.[19, 26]

Given the high prevalence of overweight/obesity and low levels of PA in young women,[27-30] the potential interactive effects of %Fat and PA coupled with the additional effect of OC-use on CRP is alarming. OC-use and %Fat have been consistently associated with CRP level in young women, however previous literature has not accounted for the effect of PA, and has not assessed the potential interactive associations of %Fat, OC-use, and PA on CRP level with %Fat and PA being assessed with objective methods.[14, 31] Thus, the primary aim of this study was to examine the independent and interactive associations of these variables on CRP in young, healthy adult women. It was hypothesized that lower PA, higher %Fat, and OC-use would be independently associated with CRP, with the cumulative effects on CRP being greater than each factor independently.

3.3 Methods

Study Design and Sample

This study utilized cross-sectional pooled data collected between 2012 and 2015 from female college students (n=340) recruited via email and print advertising at the

University of Georgia. E-mail messages were sent directly to the e-mail account created by the university for each student, through a listserve created by the Office of the Registrar. Participants were required to be full-time college students aged 18 to 24 years. Varsity athletes were excluded, as were participants who were pregnant, planning to become pregnant, or had given birth in the previous 12 months due to the known effects of pregnancy on body composition. This study protocol and informed consent document were approved by the university Institutional Review Board.

Measures

Demographic information and health history were collected via questionnaire, including items addressing current and past medical conditions, and current medication use. Participant menstrual history, OC-use, OC-brand, duration of OC-use (in months), and pill cycle (21 vs. 24 vs. 81 vs. 84 days), were included as part of the health history questionnaire.

Serum CRP was measured from fasting blood samples obtained via venipuncture following a 12-hour fast using standard clinical practice (Quest Diagnostics, Atlanta, GA, USA), with good test-retest reliability.[32] Standing height was measured by a stadiometer (Seca 242, SECA Corp, Hamburg, Germany) to the nearest 0.1 cm. Weight was measured with a digital scale (Tanita WB-110A class III, Tanita Corporation, Tokyo, Japan) to the nearest 0.1 kg. BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference (WC) was measured at the umbilical waist with a flexible, tension-sensitive, non-elastic vinyl tape measure (Gulick, Lafayette Instrument Co, Lafayette, IN). %Fat was measured via dual x-ray absorptiometry (DXA) (Lunar iDXA, v 11.30.062, GE Healthcare, Madison, Wisconsin).

Free-living PA was measured using the NL-1000 accelerometer (New Lifestyles Inc., Lee's Summit, Missouri) to provide an objective measure of total ambulatory PA measured in steps/day. Participants were asked to wear the accelerometer at the waist over the midline of the right thigh using a belt clip for 7 consecutive days, and were instructed to wear the activity monitor during all waking hours, except during activities that involved potential contact with water (bathing, swimming, etc.). A threshold of 500 steps per day was required for the inclusion of a given day in analyses. Participants with fewer than 4 valid days, defined as at least 10 hours of wear time, were excluded.[33, 34] The validity of the NL-1000 as a research pedometer has been established in laboratory and free-living environments at various walking speeds. The NL-1000 is suitable for use across all BMI groups.[35]

Statistical Analysis

Statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina). Bivariate associations between %Fat, OC-use, PA and CRP were calculated using Kendall's tau, and selected a priori. Poisson logistic regression was used to quantify the associations of the independent variables of %Fat, OC-use, and PA with CRP. %Fat, PA, and CRP were first included in the Poisson regression model as continuous variables, with OC-use categorized as YES or NO (coded as 1 and 0, respectively) for statistical analysis. A second model was used with %Fat categorized as "obese" and "non-obese" using an established threshold value of 35.0% (coded as 1 and 0, respectively),[36] as well as PA categorized as "inactive" and "active" (coded as 1 and 0, respectively) using a threshold of 10,000 steps/day.[37] All data are expressed as mean \pm standard deviation ($M \pm SD$). Continuous independent variables (%Fat & PA) were

centered around the respective mean for each parameter before being included in any statistical analysis. Parameter estimates for continuous variables used in the Poisson regression model are presented as unstandardized β coefficients, as well as odds ratios (ORs) when appropriate. The parameter estimate for PA was expressed as steps per day divided by 1,000. Cases with missing data or incomplete data (n=88) were excluded from the analysis. Statistical significance was indicated using an α level of 0.05.

3.4 Results

Flow of participants included in analysis is presented in Figure 3.1. As presented in Table 1, the sample of young women (n=340; 18.8 ± 1.3 y) varied in race (65.0% Caucasian, 12.9% African American, 6.5% Hispanic), and weight status (BMI= 24.3 ± 4.7 kg/m²) with 32.9% being classified as overweight/obese by BMI. Using DXA estimates of %Fat, 48.5% (n=165) of participants exhibited %Fat $\geq 35.0\%$ and were deemed obese (35.0 ± 6.9 %Fat). In addition, 51.5% (n=175) of participants accumulated $\geq 10,000$ steps/day ($10,207 \pm 3,766$ steps/day). Thirteen participants (3.8%) identified themselves as current or previous cigarette smokers. One hundred nineteen participants (35.0%) reported OC-use. The majority of OC-users reported the use of monophasic regimens, containing the same dose of estrogen and progestogen each day (n=86, 72.3%), and combined OC-pills, containing both synthetic estrogens and progestins (n=109, 91.6%). All OC-pills contained ethinyl estradiol in varying amounts (range 20-35 μ g ethinyl estradiol), and one of a number of different progestogens, with norethindrone acetate (n=41, 34.5%) and norgestimate (n=30, 25.6%) included as the most common progestin agents.

Kendall's tau correlation coefficient was calculated to assess the bivariate relationships between CRP, and the independent variables of %Fat, OC-use, and PA. A moderate positive association was found between %Fat and OC-use and CRP ($\tau=.30$ and $.39$ respectively, both $P<.0001$). OC-users and individuals with higher %Fat had higher CRP levels. In addition, a moderate inverse association was found between PA and CRP ($\tau=-.21$ $P<.001$); individuals who accumulated higher levels of PA had lower CRP levels. Additional bivariate correlations are presented in Table 2.

A significant 3-way interaction (%Fat \times OC-use \times PA) was found (see Table 3), indicating that higher levels of PA were inversely associated with CRP levels, especially in OC-users with higher %Fat (Wald $\chi^2=6.6$, $P=.011$). OC-users, as well as women with higher levels of %Fat and lower levels of PA had higher CRP levels. Controlling for race/ethnicity or smoking status did not alter the significance of the associations of %Fat, OC-use, and PA and CRP (data not shown).

3.5 Discussion

The primary aim of this study was to determine the independent and interactive associations of %Fat, OC-use, and PA, on CRP in young women, and to our knowledge this is the first reporting of a significant 3-way interaction indicating higher PA can attenuate the %Fat and OC-use induced elevation in CRP. Given the obesity and physical inactivity prevalence rates for young adult females and the fact that OC-use is the birth control method of choice for this cohort, these results are not only novel but also have major public health implications. The results of the current study are consistent with previous cross-sectional studies, which have found associations between %Fat, OC-use, and PA, and CRP. This study is novel in that it combined laboratory methods of

objectively measured PA and %Fat, whereas previous research relied predominantly on self-reported PA and anthropometric measurements to classify weight status. In addition, including OC-use as a novel factor showed the cumulative effect of lower PA, higher %Fat, and OC-use, which collectively produces a greater risk of elevated CRP than any factor individually.

Our study employed gold standard methodology and focused on an understudied population. Despite the large body of evidence supporting a relationship between excess adiposity and CRP, the majority of cross-sectional studies have been limited to anthropometric measures, such as BMI, waist circumference (WC), and waist-hip ratio (WHR).[12] Specific to this age group, BMI, WC, and skinfold thickness were positively associated with CRP levels in separate cohorts of young women aged 18-26 in New Zealand,[31] Brazil,[22] as well as in a combined sample of young women from the Philippines and United States.[38] Adding to the anthropometric measurements, %Fat has also been positively associated with CRP level in children, adolescents, and adults regardless of race or ethnicity,[10, 11] despite few studies examining this relationship specifically in college-aged young adult women. One such study found a moderate positive association between %Fat and CRP ($r=.41$), indicating that higher %Fat is associated with higher CRP in a sample of 171 women aged 18-24 yrs., but did not control for PA.[39]

Although the mechanism is unknown, OC-use is associated with increased CRP without signs of underlying pathology in apparently healthy women.[19, 31] OC-use was one of the strongest predictors of chronic low-grade inflammation in a cohort of over 7,000 premenopausal women, where OC-users were nearly 9 times more likely to have

elevated CRP levels.[21] More specific to the age group examined in the current study, OC-use was associated with higher CRP levels in a sample of 1,370 young women aged 21-24 yrs.,[22] and in a sample of 376 women aged 26 yrs.[31] CRP levels were nearly 4-fold higher in separate studies of 34 OC-users (26.8±3.8 yrs.), and 77 OC-users (23.5±3.8 yrs.), as well as 5-fold higher in a sample of 45 OC-users (25.2±2.3 yrs.).[23-25] Intervention studies found a 3-fold increase in CRP occurred following 2 months of OC-use in 24 young healthy women (26.0±4.9 yrs.), [26] and 3- and 4-fold increases in CRP in 35 new OC-users (18-33 yrs.) assigned to receive OC's containing either levonorgestrel or desogestrel, respectively, following 2 months of treatment.[19]

Consistent with the results of the current study, PA has been inversely associated with CRP. The cumulative body of evidence from cross-sectional studies indicates that physically active adults have CRP concentrations 19–35% lower than less active adults,[16] and that the inverse association of PA and CRP level in adults is consistent across age, sex, and racial/ethnic groups.[15] A dose-response relationship was observed in a cohort of 14,000 US adults, with lower risk of elevated CRP found for adults engaging in light, moderate, and vigorous PA when compared to adults that reported no PA (OR=0.98, 0.85, and 0.53, respectively).[40] In addition, cross-sectional analysis of old (60-80 yrs.) and young (18-30) adults reported that CRP levels were 60% lower in active individuals when compared to inactive adults.[41] Despite the consistent relationships between habitual PA and CRP levels observed in children, adolescents, and adults in the population as a whole, this relationship has not been specifically evaluated in young healthy women, especially using objective measures of PA and accounting for %Fat and OC-use. Although the associations of PA and CRP in previous research were

confounded by the influence of %Fat or underlying pathology,[42] data from intervention studies in young women indicate CRP can be lowered with a short-term increase in PA as part of an exercise training program, independent of improvements in %FAT.[17, 18]

The results of the current study are clinically important, as OC-use may be considered as a plausible candidate for atherogenesis. OCs are the most common form of contraception used by women in the US, and estimates suggest that 82% of women aged 15-44 years will use OCs during their lifetime,[43] with ~50% contraceptive users aged 15-24 using the OC pill.[44] In addition, inactive and overweight/obese young women are now in the majority,[27-30] and may be at greater risk of developing CVD given the known independent effects of physical inactivity and %Fat on cardiometabolic health and subsequent disease risk.

Strengths

This cross-sectional study adds to the literature due to a number of strengths. First, the study was conducted in a sample of young women enrolled at a major university. Because of their young age, the sample was relatively healthy and free of disease. This allowed for the exploration of the proposed hypotheses in the absence of a disease state, which may have confounded the results of the study, specifically CRP as a marker of systemic inflammation. Second, the majority of previous investigations relied primarily on self-reported PA.[16] In the present study, PA was measured objectively, reducing the possibility of inaccuracies introduced due to cognitive recall errors and social desirability bias. Third, %Fat was measured objectively using DXA, which allowed for greater accuracy characterizing body composition than with standard anthropometric measurements (BMI, WC, etc.).[12] Finally, CRP values correlate closely

with other markers of inflammation, some of which show similar, albeit less significant predictive associations with coronary events. The attention focused on CRP reflects the fact that it is a stable marker of inflammation, with well standardized, robust, and reproducible assays available from national laboratories.[45]

Limitations

Although these results are of interest, the study is not without limitations. First, the cross-sectional nature of this study provides only one measurement for each participant on a single occasion and does not measure change over time. The causal role of higher levels of PA attenuating the %Fat and OC-induced increase in CRP can only be hypothesized. In addition, although norethindrone acetate and norgestimate were the two most prevalent progestin types, examining the independent effect of each estradiol level and/or individual progestin component on CRP was beyond the scope of the current study. It is likely that differing estradiol levels and progestin agents have differing effects on CRP. Finally, future research should measure a battery of pro- and anti-inflammatory cytokines in addition to CRP to more completely characterize the associations of %Fat, OC-use, and PA and chronic, low-grade inflammation.

Conclusions

This study provides novel evidence that PA may play a role in regulating chronic low-grade inflammation, and is the first to measure the independent and interactive associations of %Fat, OC-use, and PA on CRP levels. In light of the apparent increase in chronic low-grade inflammation caused by OC-use, the results of the current study indicating PA may attenuate the %FAT and OC-induced elevation in CRP should be of interest to health care professionals concerned with issues related to women's health. If

PA provides a protective effect against elevated CRP in the presence of higher %Fat and OC-use in women, these results should provide an additional catalyst encouraging young women to become physically active, especially OC-users.

3.6 Acknowledgments

This study was supported by US Department of Agriculture grant 2008-55215-18825.

The authors have no potential, perceived, or real conflicts of interest to disclose.

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Table 3.1. Demographic characteristics of study participants.

	No OC-use (n=221)		OC-Users (n=119)		P Value
	M	SD	M	SD	
BMI (kg/m ²)	24.3	4.9	24.1	4.3	.708
%Fat	35.0	6.7	35.0	7.3	.945
Waist Circumference (cm)	74.5	10.8	74.9	9.4	.721
Physical Activity (steps/day)	10,478.1	3,717.0	9,705.5	3,819.5	.071
C-reactive Protein (mg/L)	1.4	3.3	3.7	4.3	<.001

Note: M, mean; SD, standard deviation. BMI=Body Mass. CRP=C-reactive Protein.
 %FAT=Relative adiposity measured via Dual X-ray Absorptiometry. Significance value
 for CRP calculated using independent-samples t-test with equal variances not assumed.
 Raw values presented for CRP, with P value presented for t-test following natural log
 transformation.

Table 3.2. Bivariate associations of CRP, %Fat, OC-use, and PA in young women (n=340).

	CRP	%Fat	PA	OC-use
C-reactive Protein (mg/L)	1.00			
%FAT	.30**	1.00		
Physical Activity (steps/day)	-.21**	-.25**	1.00	
Oral Contraceptive use	.39**	.00	-.08	1.00

Note: Bivariate associations measured using Kendall's tau. CRP=C-reactive Protein. %Fat=Relative adiposity measured via Dual X-ray Absorptiometry. PA=Physical Activity. OC-use=Self-reported Oral Contraceptive use.

** P<.001

Table 3.3. Poisson regression analysis for CRP in young women (n=340).

	β	SE	Wald χ^2	P Value
%Fat	0.0835	0.0111	56.89	<.001
PA (steps/day)	-0.0703	0.0216	10.60	.001
OC-use	1.7480	0.0902	199.61	<.001
%Fat×OC-use	-0.0305	.0144	4.51	.034
PA×OC	0.0601	0.0265	5.14	.023
PA×%Fat	-0.0050	0.0016	9.89	.002
PA×%Fat×OC-use	0.0065	0.0025	6.55	.011

Note: CRP=C-reactive Protein. %Fat=Relative adiposity measured via Dual X-ray Absorptiometry. PA=Physical Activity. OC-use=Self-reported Oral Contraceptive use. Parameter estimates presented as unstandardized β coefficients.

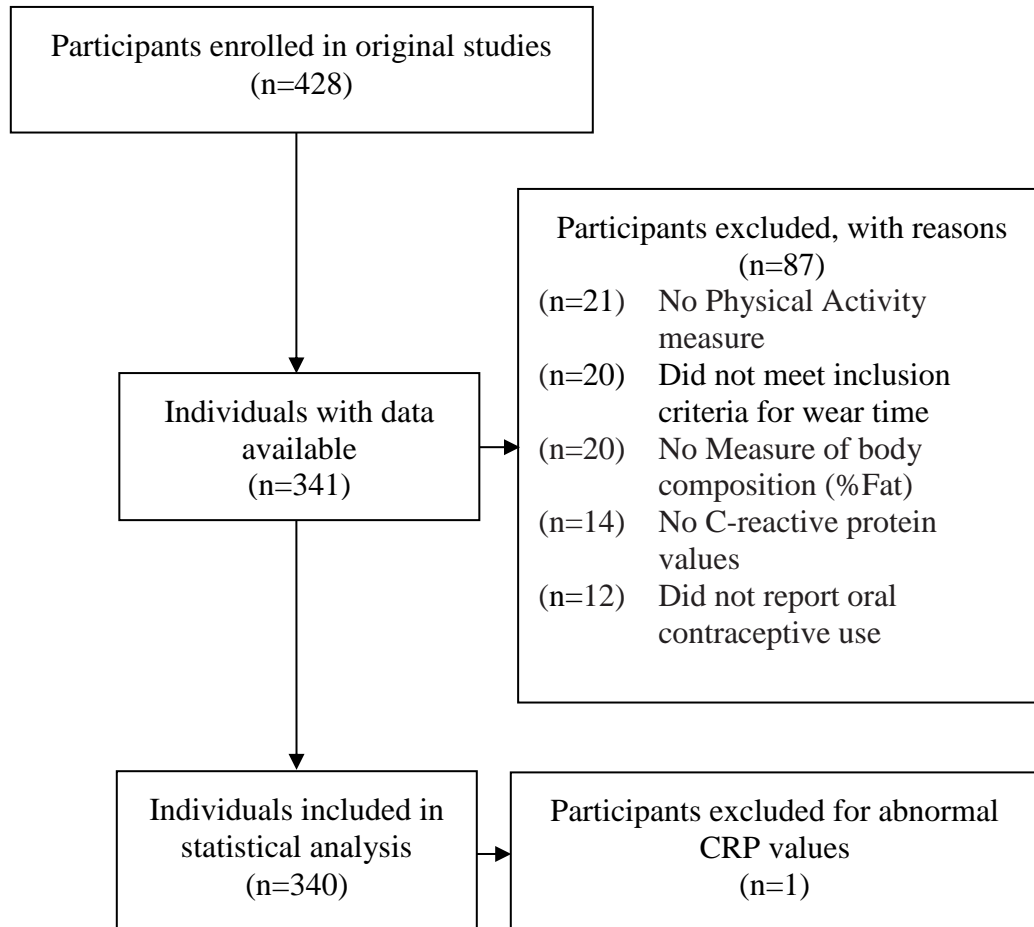


Figure 3.1. Diagram of participant flow through the study analysis.

CHAPTER 4

MODERATE, BUT NOT VIGOROUS, INTENSITY EXERCISE TRAINING

REDUCES C-REACTIVE PROTEIN¹

¹Fedewa MV, Hathaway ED, Higgins S, Forehand RL, Schmidt MD, Evans EM. To be submitted to Med Sci Sports Exerc.

4.1 Abstract

PURPOSE:

Sprint interval cycle training is a contemporary popular mode of training but its relative efficacy to reduce risk factors for cardiometabolic disease is incompletely characterized. The purpose of this investigation was to determine the relative efficacy of 6-weeks of moderate-intensity cycling (MOD-C) and vigorous sprint-interval cycling (VIG-SIC) on lipids, insulin (INS), and insulin resistance using the homeostatic model assessment (HOMA-IR), and C-reactive protein (CRP) in inactive, overweight/obese (OW/OB) young women.

METHODS:

OW/OB females (n=48, 20.3±1.5y, BMI≥ 25 kg/m², waist circumference ≥88cm,) were randomly assigned to 6 weeks of 3 days/week supervised MOD-C (20-30 min at 60-70% of heart rate reserve) or 3 days/week VIG-SIC (5-7 repeated bouts 30-second sprints at maximal effort, followed by 4 minutes of active recovery) training matched on energy expenditure. Outcomes of interest were measured using standard clinical procedures from fasting blood samples. Adiposity (%Fat) was measured using dual x-ray absorptiometry. Statistical significance was set at P < 0.05.

RESULTS:

Forty-eight participants with complete data (20.3±1.5 yrs., 64.6% Caucasian, 30.7±5.0 kg/m²) were included in the analysis. Overall, total cholesterol, HDL-C, LDL-C, and triglyceride levels significantly improved after controlling for baseline values in both groups (all P<.05); however, INS and HOMA-IR did not improve (P>.05). A significant GROUP×TIME interaction (P=.031) indicated exercise intensity modified the training

response, with a reduction in CRP observed in the MOD-C group, but not in the VIG-SIC group after controlling for baseline CRP and change in %Fat.

CONCLUSION:

These results indicate exercise training improves lipid concentrations, but not insulin or HOMA-IR in sedentary OW/OB young women. Also, MOD-C training may be more effective in reducing CRP than VIG-SIC.

Key Words: inflammation, lipids, insulin, moderate intensity, sprint-interval, young women

4.2 Introduction

Cardiovascular disease (CVD) is the leading cause of death among women in the United States, totaling nearly 300,000 deaths among women in 2009.[1] The development and progression of CVD has historically focused on a number of traditional risk factors (age, hypertension, dyslipidemia, impaired fasting glucose, obesity).[2] A vast amount of research substantiates that inactive overweight/obese (OW/OB) individuals, across the lifespan, are at greater risk of presenting an unfavorable metabolic health profile.[3-6] Over half of young women in the United States have at least one risk factor for CVD, with 17% presenting 3 or more.[7, 8] C-reactive protein (CRP) is a marker of chronic low-grade inflammation that can provide additional predictive value beyond traditional risk factors,[9] and may identify those at greater risk when elevated CRP is accompanied by an unfavorable CVD risk profile.[10, 11]

Increased focus on primordial prevention involves adopting a number of health related behaviors, including physical activity (PA) and exercise, aimed at reducing and controlling disease risk factors.[12] The cumulative body of evidence indicates exercise training can improve blood lipids and insulin (INS).[13, 14] However, despite consistent inverse associations of PA and CRP,[6] data from longitudinal exercise training studies has yielded inconsistent results.[15, 16] Identifying behavioral intervention strategies that improve the lipid profile, insulin, and CRP have important clinical implications because of the epidemiological evidence substantiating these factors for future CVD risk.

High-intensity interval training, typically performed on a cycle, has been used in numerous clinical studies to enhance cardiometabolic health,[17] and is a novel, feasible and effective mode of training in previously inactive individuals.[17-19] It is also an

increasingly popular training mode, especially as it considered time efficient.[20] Moreover, the effect of training intensity on cardiometabolic risk factors has been incompletely characterized in OW/OB young women. As such, the primary aim of this study was to determine the relative effectiveness of two 6-week cycle exercise training protocols differing in intensity but matched in energy expenditure (EE) on cardiometabolic risk factors in inactive, OW/OB, young women. Based on previous research examining the efficacy of moderate and vigorous exercise training on cardiometabolic outcomes,[14, 18, 21] it was hypothesized that changes in the lipid profile, INS, HOMA-IR, and CRP would be of similar magnitude in the moderate-intensity cycling (MOD-C) and vigorous sprint-interval cycling (VIG-SIC).

4.3 Methods

The university Institutional Review Board approved this study protocol and informed consent document, with written informed consent obtained by each participant prior to enrollment.

Participants. Female college students (n=72) were recruited via email and print advertising between 2014 and 2015 with e-mail messages sent directly to the e-mail account created by the university for each student upon enrollment, through a listserve created by the Office of the Registrar. Male students, current smokers, young women not enrolled at the University, varsity athletes, as well as women who were currently pregnant, or who had been pregnant within the previous 12 months were excluded from participation. Individuals with a neuromuscular, orthopedic, pulmonary, or cardiovascular condition that could have been exacerbated by moderate or vigorous exercise, or any health condition for which moderate or vigorous intensity exercise may be unsafe, were

not included in this study. Inclusion in this study was limited to female students between 18-24 years, body mass index (BMI) $>25 \text{ kg/m}^2$, waist circumference (WC) $>88\text{cm}$, and inactive (self-reported PA <30 minutes of PA, <2 days per week).

Health History and Body Composition. General health history was assessed via questionnaire, including items addressing current medical conditions, and past and current medical conditions. Anthropometric measures included waist circumference (WC), body weight, height, and body mass index (BMI). Standing height was measured by a stadiometer (Seca 242, SECA Corp, Hamburg, Germany) to the nearest 0.1 cm. Body weight was measured with a digital scale (Tanita WB-110A class III, Tanita Corporation, Tokyo, Japan) to the nearest 0.1 kg. BMI was calculated as weight divided by height squared (kg/m^2). WC was measured at the umbilical waist with a flexible, tension-sensitive, non-elastic vinyl tape measure (Gulick, Lafayette Instrument Co, Lafayette, IN). Whole body adiposity (%Fat) was measured via dual x-ray absorptiometry (DXA) (Lunar iDXA, v 11.30.062, GE Healthcare, Madison, Wisconsin).

Cardiometabolic Risk Factors. Fasting blood samples were obtained via venipuncture following a 12-hour fast using standard clinical procedures (Quest Diagnostics, Atlanta, GA, USA). Fasting lipid measures of total cholesterol (TC), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and triglyceride (TRG) levels, as well as fasting glucose (GLU), INS, and CRP were obtained. Following the fasting blood draw, participants were contacted within 7 days to assess signs and symptoms of illness (cold, fever, sore throat, etc.), which could cause an increase in CRP levels. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was used to quantify insulin resistance, and was calculated using the equation $HOMA - IR =$

$\frac{GLU \times INS}{405}$, where GLU was measured in mg/dL and INS was measured in IU/L.[22] The reliability and validity of HOMA-IR, as well as its use as a measure of steady-state glucose uptake and INS production, has been previously established.[23-25]

Exercise Interventions. The exercise training intervention was a parallel-arm design, with participants randomized to a conventional exercise treatment group (MOD-C) or to a novel treatment group receiving an experimental protocol (VIG-SIC) after being stratified on BMI. A friction-loaded stationary cycle ergometer was used for all exercise sessions for both the MOD-C and VIG-SIC groups (Keiser, Keiser M3 Indoor Cycle, Fresno, California). The Keiser 3m cycle has been previously used in research, primarily for its ability to estimate EE and power output, the drive mechanism which prevents the pedals from continuously rotating when pressure is no longer applied by the participant.[26-28]

Participants assigned to the VIG-SIC group began each exercise session with a brief warm-up, followed by repeated bouts of 30-second sprints interspersed with four minutes of active recovery pedaling against minimal resistance at a low pedal frequency. During weeks one and two, participants repeated this procedure, totaling 5 sprints, which equated to 2.5 minutes of near-maximal effort sprinting interspersed with a total of 16 minutes of recovery with a subsequent cool-down period following each session. Training progression increased the number of sprint repetitions from 5 during weeks 1 and 2 to 6 sprints during weeks 3 and 4, and to seven sprints during weeks five and six.

Participants assigned to the MOD-C group were instructed to cycle continuously at an intensity of 60-70% heart rate reserve (HRR) for 20-30 minutes, with the duration of each training session increased on a biweekly basis in order to maintain equal EE

between groups.

Maximal HR was measured in all participants with a maximal graded exercise test prior to randomization. During the test, all participants pedaled continuously on an electronically braked cycle ergometer (Lode Excalibur Sport 2000, Lode B.V., Groningen, Netherlands) while breathing through a mouthpiece. Oxygen uptake and gas exchange variables ($\dot{V}O_2$, CO_2 , ventilation, RER) were measured using a computerized indirect calorimetry system (ParvoMedics True Max 2400; ParvoMedics, Sandy, UT) with values assessed every 15s. Volume and gas calibrations were conducted prior to each test. Heart rate was measured continuously throughout the test (Polar FT1; Polar Electro, Kempele, Finland). Ratings of perceived exertion were assessed every 3 min using a standard 6-20 scale[29] prior to advancing to the next stage of the graded exercise test, after which resistance was increased gradually until reaching volitional fatigue. Maximal effort was determined by satisfying at least 2 of the following criteria to establish $\dot{V}O_{2peak}$: $RER \geq 1.05$, $RPE \geq 17$, $HR \geq 90\%$ of age predicted HR_{max} .

Participants performed their assigned training protocol three times weekly in a group-training format under the supervision of trained research staff. Training groups were matched on EE to isolate the effect of exercise intensity on the outcomes of interest. Heart rate during exercise, rating of perceived exertion, and estimated EE from the cycle ergometer were recorded during each exercise session to ensure compliance with the training protocol. The training protocol lasted 6 weeks, with participants required to attend a minimum of 13 sessions (70%) to be included in the final analysis.

Statistical Analysis. Statistical analyses were performed using SPSS for Windows (SPSS 20.0, Chicago, IL). Relations between variables of interest were estimated using

Pearson's r . Change (Δ) in the dependent variables of interest were analyzed using a 2-way ANCOVA (GROUP \times TIME) with GROUP included as the between training group factor, and TIME included as the within factor. Analysis was performed while statistically controlling for potential covariates associated with our primary outcomes of interest (e.g. %Fat, baseline values) when necessary. Effect sizes (ES) for unadjusted pre-post contrasts are reported as the standardized mean change, expressed relative to the pooled standard deviation of the change scores, using the equation $\frac{\bar{X}_{T1}-\bar{X}_{T2}}{s_g/\sqrt{2(1-r)}}$, where \bar{X}_{T1} is the mean at Time 1, \bar{X}_{T2} is the mean at Time 2, s_g is the standard deviation of the gain scores, and r is the correlation between the Time 1 and Time 2 scores.[30] Effect sizes (ES) for adjusted pre-post contrasts, controlling for covariates, are reported as the partial eta squared (η_p^2), expressed as using the equation $\eta_p^2 = \frac{SS_{Effect}}{SS_{Effect}+SS_{Error}}$, where SS_{Effect} is the sum of squares for the effect and SS_{Error} is the sum of squares for the error term. As in previous research, small, medium, and large ES were defined using 0.20, 0.50, and 0.80, respectively, as the threshold values.[31] Cases with missing or incomplete data, as well as participants who attended less than 70% of the prescribed sessions were excluded from the analysis. Data were screened for normal distribution with skewness or kurtosis >2 indicating non-normal distribution. Distributions of TC, HDL-C, and LDL-C were normal, however skewness or kurtosis was >2.0 for TRG, INS, HOMA-IR, and CRP. Non-normal data was transformed using a natural logarithmic transformation, with skewness and kurtosis in all variables being <2.0 prior to analysis. Statistical significance was indicated using an α level of $P<.05$. All data are expressed as mean \pm standard deviation ($M\pm SD$), unless otherwise indicated.

4.4 Results

Baseline characteristics for the study sample are presented in Table 1. A total of 72 participants were randomly assigned to one of the two training groups, of which 51 successfully completed the program (70.1% retention). Of these, participants were excluded from the final analysis due to $\Delta\text{CRP} > 3\text{SD}$ from the mean ΔCRP ($n=1$), and recent illnesses or antibiotic use at the time of blood draw ($n=2$) leaving 48 women included in the final analyses (20.3 ± 1.5 yrs., 64.6% Caucasian). Twenty one (43.8%) women were classified as obese according to BMI ($n=13$ and $n=8$ in the MOD-C and VIG-SIC groups, respectively), with an average BMI of $30.7 \pm 5.0 \text{ kg/m}^2$. Attendance to the 18 prescribed training sessions (15.9 ± 2.0 sessions vs. 15.0 ± 1.5 sessions, $P=.073$) and average activity EE per session ($131.0 \pm 19.9 \text{ kcal/session}$ vs. $127.7 \pm 14.7 \text{ kcal/session}$, $P=.569$) did not differ between the MOD-C and VIG-SIC groups, respectively.

Unadjusted pre- and post-intervention lipid, GLU, INS, and CRP values are presented in Table 2. Baseline %Fat was positively associated with baseline INS, HOMA-IR, and TRG, ($r=.52$, $.56$, and $.42$, respectively, all $P<.01$), but not TC, HDL-C, or LDL-C (all $P>.05$). After adjusting for baseline values, there were no GROUP \times TIME effects on any lipid outcome, INS, or HOMA-IR (all $P>.05$) suggesting the effects of the different exercise intensities were similar. After adjusting for baseline values, a significant TIME effect was found when examining the pooled data from both groups, with improvements in TC ($ES=.15$, $P=.006$), HDL-C ($ES=.12$, $P=.017$), LDL-C ($ES=.18$, $P=.003$), and TRG ($ES=.13$, $P=.014$) following exercise training. However, this same pooled analysis determined no improvements in INS ($ES=.05$, $P=.148$) or HOMA-IR ($ES=.06$, $P=.109$)

Following 6-weeks of exercise training, the Δ CRP level was positively associated with $\Delta\%$ Fat ($r=.44$, $P=.002$) indicating larger improvements in CRP were observed in young women with greater improvements in %Fat, and inversely associated with baseline CRP ($r=-.42$, $P=.003$) with greater reduction in CRP observed in participants with higher baseline levels. A GROUP \times TIME repeated measures ANCOVA yielded a significant interaction ($P=.031$) for CRP after controlling for baseline CRP and $\Delta\%$ Fat (see Figure 1). MOD-C was more effective in reducing CRP levels when compared to VIG-C. Elevated CRP levels ($>3\text{mg/L}$) were reduced to normal levels ($<3\text{mg/L}$) in 5 participants assigned to the MOD-C group, and in 2 participants assigned to the VIG-SIC group. Interestingly, 2 participants in the VIG-SIC with normal CRP levels at baseline had elevated CRP levels following the intervention.

4.5 Discussion

The primary aim of the study was to determine the relative efficacy of 6 weeks of MOD-C and VIG-SIC exercise training on traditional cardiometabolic risk factors, and C-reactive protein in young women. As hypothesized, the magnitude of improvement in fasting lipids was similar between moderate and vigorous exercise intensities with significant improvements following 6-weeks of exercise training. However, contrary to our hypothesis, INS and HOMA-IR did not improve following exercise training ($ES=.05$ and $.06$, respectively, both $P>.05$). Also contrary to our original hypothesis, the magnitude of the change in CRP levels was greater in the MOD-C group, with no significant change following 6-weeks of VIG-C, after controlling for $\Delta\%$ Fat.

This relatively short duration exercise training intervention suggests improvements in lipid concentration can occur in OW/OB young women with minimal

increase in activity EE. The beneficial effect of aerobic exercise observed in the current study is consistent with a previous meta-analysis, which found exercise training can favorably impact blood lipid and lipoprotein concentrations in women.[13] Similarly, it aligns with reports that aerobic exercise significantly improves TC, HDL-C, LDL-C, and TRG in overweight young women (19-24yrs.) following 12 weeks of training, [32] with similar improvements in HDL-C and TRG concentrations observed in young women following sixteen weeks of training.[33]

In the current study, no significant improvement occurred in INS or HOMA-IR following 6-weeks of exercise training, in contrast to the strong body of evidence supporting the effectiveness of exercise to reduce fasting INS and HOMA-IR in youth and adults.[14, 34] In contrast to the current results, INS and HOMA-IR decreased 33% and 34% respectively, following 12 weeks of exercise training in a cohort of young women when included as part of a lifestyle intervention.[35] INS and HOMA-IR also improved in obese young women following 12 weeks of moderate or high intensity aerobic exercise.[36] The lack of significant improvements observed for INS and HOMA-IR may be due to the shorter intervention period (6 weeks vs. 12 weeks), or due to a lower volume and exercise intensity prescribed to the MOD-C group in order to maintain equal activity EE. In addition, based on the average number of sessions attended and estimated EE per session, the overall training dose for the intervention was relatively small, totaling less than 2,000 kcal over the 6-week period. Improvements in INS and HOMA observed in previous studies were accompanied by significant weight loss, while participants in the current study remained weight stable throughout the intervention.

The results of the current study are also inconsistent with the results of the INFLAME, DREW, HART-D, and Seattle studies which concluded that exercise training did not improve CRP unless accompanied by a significant reduction in body weight or improvement in body composition.[37-40] However, our results are consistent with previous research in younger, non-clinical populations, that found CRP levels in healthy normal-weight women decreased 34.3% and 60.0% in participants assigned to aerobic training or combined aerobic and resistance training groups, respectively, compared to a 15.2% increase in the control group independent of $\Delta\%$ Fat.[41] Additional research in 319 inactive young women aged 18-30 yrs. found 16 weeks of aerobic exercise training effectively reduced CRP, especially in obese individuals, with no evidence that the effect was mediated by improvements in %Fat.[42] The lack of significant improvements in CRP following exercise training in previous studies, as well as the VIG-SIC group in the present study, may be due in part to the lack of significant changes in body weight, as the chronic elevation in CRP observed with obesity is stimulated primarily by IL-6 secreted from adipose tissue.[43, 44] Transient increase in IL-6 levels can also occur in response to an acute bout of exercise, and are reduced following chronic exercise training.[6, 44, 45] Although the exact mechanism by which exercise training reduces CRP levels is still unclear, examining the potential effect of exercise intensity on IL-6 levels, and subsequent changes in CRP following exercise training are warranted given that improvements in CRP were observed in the MOD-C, but not VIG-SIC, after controlling for $\Delta\%$ FAT.

The novelty of the current data is bolstered by several strengths within the study design and methods. First, the study was conducted in a sample of young women enrolled

at a major university. Because of their young age, the sample was relatively healthy and free of disease despite being OW/OB. This allowed for the exploration of the proposed hypotheses in the absence of a disease state (e.g. diabetes), which may have confounded the primary dependent measures of interest, especially CRP. Second, unlike many reports, %Fat was measured objectively using DXA, which allowed greater accuracy than with standard anthropometric measurements and allowed for controlling of $\Delta\%$ Fat when evaluating the changes in our outcomes of interest, especially CRP. Third, the study was designed to intentionally limit change in weight and %Fat to reduce the potential confounds for the change CRP. Thus, a) participants were stratified on BMI when randomized to the intervention group, b) a nutrition component was intentionally lacking, c) the training intervention was limited to 6 weeks, and d) the EE during each exercise session was matched. This allowed for the comparison of the relative efficacy of the two exercise intensities on our cardiometabolic variables, without the potential confounding influence of weight status, adiposity, or overall dose of exercise. Finally, aerobic exercise typically is not associated with an increase in CRP ≥ 24 hours following exercise, [46] and it should be noted that the amount of time between the final bout of exercise (2.40 ± 1.57 days) and time of blood sampling was not significantly associated with Δ CRP levels in either the MOD-C ($r = -.20$, $P = .350$) or VIG-SIC ($r = .23$, $P = .312$) after adjusting for baseline CRP.

Several limitations of our study warrant mentioning. First, although advertised as an exercise intervention study, it is important to note that 87.5% (N=42) of participants in the current study indicated their plans to exercise outside of the structured exercise training sessions to help “lose weight” during the study. In addition, 72.9% of

participants (N=35) indicated that they planned to diet to help lose weight during this study. Importantly, despite their intention to lose weight and lack of instructions for other PA and dietary intake, no significant change in weight occurred following the intervention in either group (see Table 1). Additionally, although we believe the potential confound to be small; it is possible that our results may have been influenced by change in diet (e.g. macronutrient composition of the diet) or PA outside of the structured exercise program not captured by our measures of these covariates. Finally, because of the group training protocol, blood samples may have been obtained during different phases of each participant's menstrual cycle potentially influencing cardiometabolic outcomes of interest due to known cyclical variations.[47-49]

The results of this study are inconsistent with previous research, indicating that exercise training cannot effectively lower CRP levels in the absence of a reduction in adiposity. Although these results may appear trivial, they are in fact crucial in terms of today's concern over CVD and possible interventions to slow the development and progression of disease. The conclusion that exercise training can elicit improvements in fasting lipid concentrations, regardless of exercise intensity, coupled with the significant improvement in CRP observed following MOD-C training align with a recent American Heart Association call for additional research related to physical activity and exercise performed at a lower intensity or dose, or different modes of exercise, and the relative potential to reduce CVD risk factors.[50] In addition, these results add to the body of evidence supporting the role of exercise in the prevention and treatment of health related complications of obesity in younger at-risk populations.

Assessing the potential independent and interactive effects of exercise intensity is innovative due to the fact that inactive and OW/OB young women are now in the majority of this cohort, and may be at greater risk of developing CVD given the known independent effects of physical inactivity and %Fat on metabolic health and subsequent disease risk.[51-56] Although the results of this study appear to affect only a small segment of the population, the results have tremendous impact for the population as a whole. In line with the 2008 Physical Activity Guidelines, it is important to advocate for exercise and disease prevention, as well as a “lifespan” approach toward physical activity and exercise in which healthy behaviors can be maintained for a lifetime.[57] Rather than advocating for all individuals to begin a demanding high-intensity exercise program, including VIG-SIC as a part of a physically active lifestyle may be more prudent than complete substitution of the conventional moderate intensity program.

4.6 Acknowledgments

The authors have no potential, perceived, or real conflicts of interest to disclose.

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Table 4.1. Baseline characteristics of study participants (n=48).

	M±SD	Median (25 th -75 th Percentile)
Age	20.3±1.5	20.0 (19.0-21.0)
BMI (kg/m ²)	30.7±5.0	28.7 (27.2-33.9)
%Fat	43.7±5.5	42.7 (40.4-47.1)
Waist Circumference (cm)	100.9±11.8	98.0 (90.3-111.3)
TC (mg/dL)	171.9±40.9	162.5 (141.3-202.5)
HDL (mg/dL)	57.9±15.3	54.5 (48.5-64.8)
LDL (mg/dL)	96.8±36.0	88.5 (70.3-120)
TRG (mg/dL)	86.6±59.8	70.0 (50.5-99.8)
HOMA-IR	2.2±1.5	1.9 (1.3-2.8)
Insulin (IU/L)	10.2±6.2	8.9 (6.4-12.1)
CRP (mg/L)	3.9±4.7	2.35 (0.9-5.8)

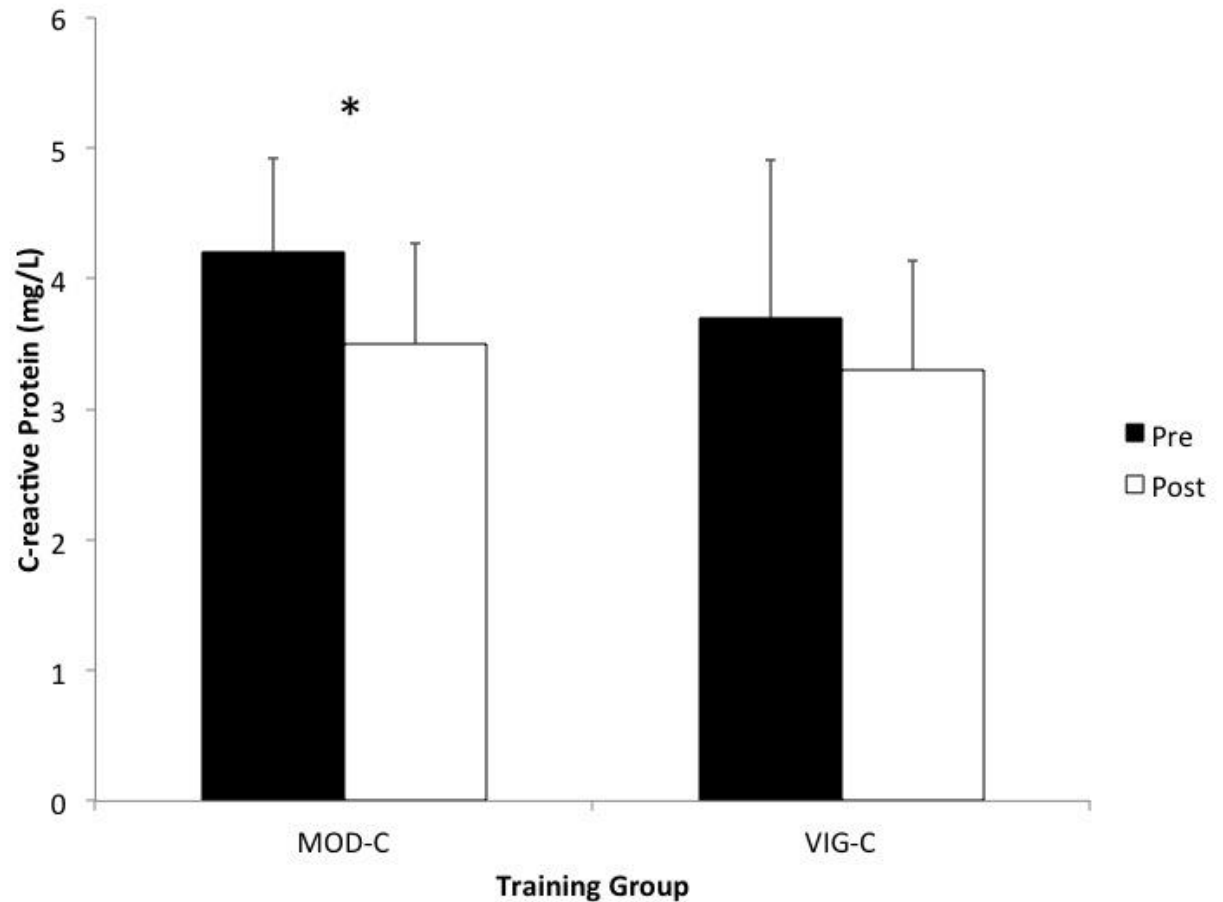
Note: BMI=Body Mass Index calculated as body weight in kilograms divided by height in meters squared. %Fat=Relative adiposity measured via Dual X-ray Absorptiometry. Waist circumference measured at the level of the umbilicus. TC=Total Cholesterol. HDL-C=High Density Lipoprotein Cholesterol. LDL-C=Low Density Lipoprotein Cholesterol. TRG=Triglyceride. HOMA-IR=Homeostatic Model Assessment of Insulin Resistance. CRP=C-reactive protein.

Table 4.2. Effect of moderate intensity cycle training and vigorous sprint-interval cycle training on metabolic outcomes in young women.

	MOD-C (n=25)		P value	VIG-SIC (n=23)		P value
	Baseline	Post		Baseline	Post	
Weight (kg)	84.2±15.1	84.4±15.4	.668	82.8±13.7	82.2±13.2	.200
TC (mg/dL)	175.0±37.0	170.3±36.5	.161	168.6±43.8	166.9±38.0	.587
HDL (mg/dL)	59.2±14.3	60.1±13.5	.485	56.4±16.6	59.4±15.9	.114
LDL (mg/dL)	98.6±32.3	92.2±30.3	.029	94.7±40.4	92.2±35.7	.393
TRG (mg/dL)	86.3±46.2	89.9±47.3	.356	87.0±72.9	76.8±44.5	.202
Insulin (IU/L)	11.1±5.2	11.1±5.2	.863	9.1±5.7	8.9±5.0	.932
HOMA-IR	2.5±1.5	2.5±1.1	.901	2.0±1.6	2.0±1.2	.922
CRP (mg/L)	4.2±3.6	3.5±3.9	.007*	3.7±5.8	3.3±4.0	.506

Note: Data presented as M±SD. Within group comparison pre- and post-intervention using paired t-test. MOD-C=Moderate-intensity cycling. VIG-SIC=Vigorous sprint-interval cycling. TC=Total Cholesterol. HDL-C=High Density Lipoprotein Cholesterol. LDL-C=Low Density Lipoprotein Cholesterol. TRG=Triglyceride. HOMA-IR=Homeostatic Model Assessment of Insulin Resistance. CRP=C-reactive protein. Significance values for CRP, HOMA-IR, INS, TRG values presented after natural logarithmic transformation. * P<.05 GROUP×TIME interaction (P=.031) after controlling for baseline CRP and change in %Fat

Figure 4.1— Effect of exercise training on unadjusted CRP levels in young women. Note: C-reactive protein (CRP) expressed in milligrams per liter, assessed pre and post 6-weeks of structured exercise training for participants assigned to continuous moderate intensity (MOD-C) or vigorous sprint-interval cycling (VIG-SIC). * $P < .05$ for mean difference between pre- and post-test. A significant GROUP \times TIME interaction ($P = .031$) after controlling for baseline CRP and change in %Fat.



CHAPTER 5

SUMMARY AND CONCLUSIONS

The results from the present study add to the growing body of literature examining the independent and interactive effects of %Fat, PA/exercise, and OC-use on cardiometabolic risk factors, including CRP, in young women. The cross-sectional analyses with a large sample size and criterion laboratory methodology for measurement of PA and adiposity provided excellent data to highlight the need for more research regarding the potentially important interactive effects of low PA, higher %Fat and OC-use on systemic inflammation in young women. In addition, the results from our 6-week exercise training intervention study align with previous data indicating exercise training favorably influences the fasting lipid profile, and provide new data supporting the role of moderate-intensity exercise to reduce CRP levels in OB/OW young women.

Our cross-sectional findings indicate that in healthy young women, %Fat, PA, and OC-use are independently associated with CRP levels. In addition, higher levels of PA may attenuate elevated CRP levels due to excess %Fat and OC-use. Inactive, obese, OC-users appear to have significantly higher CRP levels when compared to active, lean, non-users. Based on these data, our results suggest that young women, especially those with higher %Fat and OC-use, should engage in recommended amounts of PA to lower CRP a marker for chronic low-grade inflammation, potentially reducing future CVD risk.

With regard to the potential role of exercise training to favorably modulate risk for cardiometabolic disease, in inactive, overweight/obese young women, our results

indicate exercise training improves lipid concentrations, but not insulin or HOMA-IR. However, importantly, MOD-C training may be more effective in reducing CRP when compared to VIG-SIC. Despite the popularity of high-intensity interval training, our results indicate that more vigorous training intensities may not confer additional protective benefit for reducing risk factors for CVD, after controlling for energy expenditure.

Results from these cross-sectional and exercise training investigations will inform the development of future interventions for young women aimed at reducing the primordial risk of CVD. Our findings highlight yet another reason for young women to meet PA recommendations, as PA reduced the elevation in CRP seen in women with higher %Fat and OC-use. Moreover, moderate intensity exercise, but not vigorous, reduced CRP levels in addition to improvements in lipid concentrations observed in both participants following 6-weeks of exercise training. PA and exercise training may improve CVD risk profiles by attenuating %Fat, but our results strongly suggest that PA and exercise may reduce cardiometabolic risk independent of any major changes in body composition.