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Inflammation and Exercise (INFLAME): study rationale, design, and methods

Angela Thompson, MSPH[1], **Catherine Mikus, MS**[1], **Ruben Q. Rodarte, MS**[1], **Brandy Distefano, BS**[2], **Elisa L. Priest, MPH**[3], **Erin Sinclair, BS**[4], **Conrad P. Earnest, PhD**[1], **Steven N. Blair, PED**[5], and **Timothy S. Church, MD, MPH, PhD**[1]

1 *Pennington Biomedical Research Center, Baton Rouge, LA, USA*

2 *The Cooper Aerobics Center, Dallas, TX, USA*

3 *Institute for Health Care Research and Improvement, Baylor Health Care System, Dallas, TX, USA*

4 *Department of Preventive Medicine, Northwestern University, Chicago, IL, USA*

5 *Department of Exercise Science, University of South Carolina, Columbia, SC, USA*

Abstract

Purpose—The INFLAME study is designed to determine the effect of exercise training on elevated high-sensitivity C-Reactive Protein (CRP) concentrations in initially sedentary women and men.

Methods—INFLAME will recruit 170 healthy, sedentary women and men with elevated CRP (≥2.0 mg/L) to be randomized to either an exercise group or non-exercise control group. Exercising individuals will participate in four months of supervised aerobic exercise with a total energy expenditure of 16 kcal • kg^{-1} • $week^{-1}$ (KKW). Exercise intensity will be 60–80% of maximal oxygen consumption (VO_2 max).

Outcome—The primary outcome will be change in plasma CRP concentration. Secondary outcomes include visceral adiposity, the cytokines IL-6 and TNF-α, and heart rate variability (HRV) in order to examine potential biological mechanisms whereby exercise might affect CRP concentrations.

Summary—INFLAME will help us understand the effects of moderate to vigorous exercise on CRP concentrations in sedentary individuals. To our knowledge this will be the largest training study specifically designed to examine the effect of exercise on CRP concentrations. This study has the potential to influence therapeutic applications since CRP measurement is becoming an important clinical measurement in Coronary Heart Disease risk assessment. This study will also contribute to the limited body of literature examining the effect of exercise on the variables of visceral adiposity, cytokines, and heart rate variability.

Keywords

CRP; fitness; exercise; adiposity; cytokines; HRV

Address for correspondence and reprints: Tim Church MD, MPH, PhD, Pennington Biomedical Research Center, 6400 Perkins Rd., Baton Rouge, LA 70808, Telephone: 225-763-2632, Telefax: 225-763-3014, Email: Tim.Church@PBRC.edu.

Conflict of Interest
The authors have no conflict of interests.

INTRODUCTION

C-reactive protein (CRP), a marker of systemic inflammation, is an independent predictor of cardiovascular disease (CVD) in both women and men.[1;2] There is evidence that CRP is an important clinical measure to consider and interventions to improve it may have significant therapeutic applications. Numerous cross sectional studies have observed an inverse association between CRP and regular physical activity and/or exercise.[3–8] In contrast few studies have prospectively examined the effect of exercise training alone on resting levels of CRP, particularly in individuals with elevated levels of CRP. [9;10]

The Inflammation and Exercise study (INFLAME) is designed to investigate the effect of exercise training on elevated CRP concentrations (≥2.0 mg/L), visceral adiposity, the cytokines IL-6 and TNF-α, and heart rate variability (HRV) in men and women aged 30–75 yr. As an inflammatory marker CRP can increase exponentially in response to injury, stress or illness. Further, CRP can be affected positively and negatively by various medications. Thus designing a clinical trial that examines the role of a lifestyle intervention such as exercise in reducing CRP presents many complicating issues. The purpose of this report is to describe the design and methods for INFLAME as well as to provide some insight into the protocol decisions made in studying this methodologically challenging outcome variable.

Specific Objectives

Sedentary individuals with elevated CRP concentration will be randomly assigned to either an exercise group or to a no exercise control group. Individuals in the exercise group will train for four months at a total energy expenditure of 16 kcal • kg^{-1} • $week^{-1}$ (KKW) and an intensity of 60–80% of maximal oxygen consumption (VO_2 max). The primary study outcome will be change in plasma CRP concentration. Secondary outcomes will include evaluating biological mechanisms whereby exercise might affect CRP concentrations such as visceral adiposity, the cytokines IL-6 and TNF-α, and HRV as a measure of autonomic balance.

Methods

Description and selection criteria of participants—INFLAME will recruit 170 sedentary, healthy women and men aged 30 to 75 years who had an elevated plasma CRP concentration (≥2.0 mg/L but <10.0 mg/L) at the initial screening. CRP values ≥2.0 mg/L are common within sedentary populations but less common in higher fit populations and associated health risks appear to increase starting at this level.[1] In our recent publication, the prevalence of CRP ≥2.0 mg/L was approximately 50% in lowest fit group compared to about 25% in the moderate fit group and 17% in the highest fit group.[6] In the DREW study, which is composed exclusively of sedentary post-menopausal women, approximately 78% of the participants have a CRP ≥2.0 mg/L at baseline. It has been reported that less than 5% of the general population has CRP values greater than 10.0 mg/L and such values are often the result of acute infection or other inflammatory conditions.[11] Utilizing a minimum CRP concentration that is both associated with elevated CVD risk and is prevalent in sedentary populations will increase the clinical significance and applicability of the research question and will allow for realistic recruitment goals. In the ongoing JUPITER study in which 15,000 individuals with elevated CRP but normal LDL will be randomized to statin therapy or placebo, the same CRP cut-off value (≥2.0 mg/L) that we have proposed is also being used.[12]

The recruitment goal is to have at least 25% of study participants to come from minority groups, primarily African American and Mexican American women and men. We have enhanced our ability to recruit individuals from minority groups because of our Community Advisory Board. This diverse group of women and men represent various minority interests, has played an important role in previous studies and continue to play an integral role in this study. Our off

site intervention site is located in the Oak Cliff neighborhood of Dallas, an area with a high concentration of African-Americans and Hispanics. For the convenience of participants, orientations, blood draws, run-in sessions, and supervised exercise sessions are conducted at the off site facility. All baseline and 4-month follow-up medical assessments are conducted at the Cooper Institute main campus in North Dallas. The INFLAME study has been approved by the Institutional Review Board (IRB) of the Cooper Institute and reviewed quarterly by the IRB and a Data Safety and Monitoring Board (DSMB).

Inclusion and exclusion criteria for INFLAME are shown in Table 1. The study steering committee had numerous discussions related to medication related inclusion/exclusion criteria. The final consensus was that any medication that has been documented to substantially increase CRP was an exclusion criterion. Our reasoning was that medications represent an artificial means of raising CRP, which may not be affected by changes in exercise. Based on our review of the literature and on the medications our study population used, this criterion applied only to hormone replacement therapy.[13] With regard to medications that have been demonstrated to reduce CRP, the committee ruled that use of these medications was not cause for exclusion as long as the minimum CRP level was met and the participant was on a stable dose for a minimum of two consecutive months prior to screening. Examples of these types of medications are statins, ace-inhibitors and multi-vitamins.[14–16] However, if the medication has been shown to reduce CRP but was used in the treatment of chronic inflammatory conditions, then the medication was cause of exclusion. Systemic steroids are an example of such a medication and the exclusion was based more on the underlying medical condition and less on the use of the medicine. One medication that gave the committee particular difficulty was oral contraceptives. While hormone replacement therapy has definitively been shown to increase CRP, oral contraceptives have not.[13] We concluded that female participants who had been on a stable dose for a minimum of two consecutive months were eligible for the study.

Individuals who use tobacco products were excluded because smoking is associated with increased CRP levels and changes in tobacco use could have large effects on changes in CRP. Most of the non-medication inclusion/exclusion criteria listed in Table 1 are either standard screening criteria related to blood pressure (BP) and lipids and/or related to being able to safely participate in an exercise training program.

Since CRP is an inflammatory molecule that can be influenced by numerous acute factors including changes in medication, injury, infection, or hormonal variations, we felt it was important to allow for temporary exclusion of people who initially were ineligible due to what could be an acute condition (table 2). Individuals who were temporarily ineligible due to CRP levels could be retested after 2 weeks to allow acute inflammation to subside.[11] If his/her CRP is still ≥10 mg/L, the participant may become ineligible or be temporarily excluded for up to 6 months depending on the cause of the elevated CRP levels. After the period of temporary exclusion, s/he could be screened again. Individuals with repeat CRP measurements >10.0 mg/L are ineligible for further study participation.

Timeline and rationale—Participant timeline and flow from screening through randomization is shown in Table 3. Initial eligibility will be determined by a brief screening interview. Eligible participants will be invited to an orientation session where the study will be explained in detail and questions will be answered. Individuals who remain interested in assessing eligibility for the study will review and sign a written informed consent form. Height and weight will be measured to calculate BMI and a blood sample will be drawn for screening CRP concentration.

Following the orientation session, eligible participants will attend three run-in sessions over the course of one to two weeks where they will complete study questionnaires, BP

measurements, watch a presentation about living a healthy lifestyle and will be asked to sign a behavioral contract to affirm their commitment to study adherence. Any effect of the educational presentations will be distributed across groups and should not affect internal validity since they will be completed prior to randomization. Since many participants will be at moderate to high risk for CVD and other health problems, the primary goal of the educational presentation will be to provide information on risk reduction, which is the current minimum standard medical practice.

Baseline evaluations will be scheduled after the participant successfully completes the run-in sessions. During baseline evaluations, participants will be tested for final inclusion/exclusion criteria such as cholesterol, triglycerides, glucose, and ECG abnormalities described in table 1. Baseline and the four-month follow-up evaluations may require up to three visits. At baseline evaluation, participants will be randomized after completion of the maximal exercise test, physician review of the exercise ECG and clinical labs. Final eligibility will be determined and randomization conducted after the run-in for several reasons: 1) the baseline examinations may reveal that some participants meet exclusion criteria not discovered at the preliminary screening visits, such as cardiac arrhythmia and/or abnormal blood pressure or blood chemistries and 2) the baseline evaluations will be used as part of the run-in to improve adherence rates by testing the ability of the participant to come to the intervention site multiple times in one week.

Eligible participants will then be enrolled as directed by the next assignment letter contained in a series of sequentially numbered, opaque, sealed envelopes.[17] The randomization sequence of 170 treatment assignments will be determined using Efron's biased coin method, in order to keep the treatment assignments closely aligned to enrollment targets over time, yet unpredictable. The randomization sequence will be generated in advance using a pseudo-random number generator. The statistician will fill envelopes with treatment assignment sheets according to the sequence, then number, seal, and deliver the envelopes to the project manager. The project biostatistician will keep a secure record of the entire assignment sequence, which when combined with participant tracking records will provide an audit trail.[17] At randomization, the envelopes will be opened in order by a health educator independent of the statistical staff.

Participant incentives—Individuals will receive up to $200 as an incentive for participation in the study, with $50 for baseline and follow-up evaluations, and $100 for adherence to exercise assignment. The incentive will be disbursed in the following ways: for the exercise group, the $100 payment will be reduced by $20 for each week of missed sessions beyond the 90% adherence target. The payment will be reduced by $2.50 for any missing study forms (8 total). The maximum deduction for failing to exercise and missing study forms will be $100. Exercise and control group participants will receive $50 for completing the four-month follow-up medical evaluations. This incentive payment schedule is designed to promote adherence to both the exercise program and returning for follow-up examinations. Participants in the control group will be able to earn the $100 incentive if they turned in all study forms (8 total). The $100 payment will be reduced by $10 for each missing form (8 total). The remaining $20 will be added to the amount for all forms being turned in and adherence to their group. Since the control group will not have to come in for exercise sessions, their forms will have a higher value.

While this incentive is a substantial amount, we feel that it is appropriate because our objective is to evaluate the effects of exercise on CRP. Excellent adherence to both intervention and measurement is necessary. We will not be testing whether or not financial incentives encourage individuals to exercise, but will be evaluating specific responses to a dose of exercise. Upon completion of the follow-up evaluation, participants randomized to the control group will be offered a 2-month voluntary exercise program essentially identical to that undertaken by

participants in the exercise group. Accepting or declining the voluntary exercise program will not effect eligibility for INFLAME.

Outcome Measures and Methods

C-Reactive Protein Concentrations and Blood lipids—Prior to visits for blood draws and HRV measurements, participants will be asked to fast for 10–12 hours, refrain from consuming alcohol or exercising for 24 hours, and to refrain from acutely using aspirin or anti-inflammatory medications for 48 hours since these types of medications may modify CRP, IL-6 and TNF-α concentrations. Participants who report using these medications at the time of the orientation visit will not be asked to discontinue the medication if they have been taking them regularly and/or take them on the recommendation of a physician.

Blood will be drawn at three separate visits throughout the trial: 3 ml at the orientation session, 20 ml at baseline approximately two weeks later and 20ml at the 4-month follow-up assessment. Baseline and follow-up plasma, serum and RBC with buffy coat samples will be stored in a −80°c freezer. CRP and cytokines assays will be performed only after both baseline and follow-up are available in order for each individual's set of samples to be measured using the same assay kit. Serum CRP will be measured by a solid-phase, chemiluminescent immunometric assay (Immulite 2000 High-Sensitivity CRP, Diagnostic Products Corporation, Los Angeles, CA). Serum IL-6 and TNF-α will be measured with a high sensitivity multiplex assay kit (High Sensitivity Human Cytokine LINCO*plex* Kit, LINCO Research Inc, St. Charles, MO). Changes in ovarian hormones affect both IL-6 and HRV, therefore blood will be drawn from premenopausal women within eight days of menstrual cessation.[18]

Anthropometry—Height and weight will be measured using a traditional standiometer and balance-beam scale and recorded to the nearest centimeter and 0.1 kg, respectively. Body composition will be measured using dual X-ray absorptiometry (DXA) scans using a Hologic Bone Densitometer (Hologic Inc., Bedford, MA) according to the Hologic user's manual. Additionally, waist and hip circumference measurements will be made using standard techniques. Visceral adiposity and subcutaneous fat will be measured as described by Ross et al. [19]

Heart Rate Variability and Blood Pressure—All HRV measurements will be made between 0630 and 1000 in the morning and participants will report to the lab at the same time of day for both measures in order to control for diurnal variations.[20] HRV assessment will be performed and analyzed using well established, highly reproducible techniques. HRV will be quantified using both time-domain and frequency-domain methods.[21] Resting BP measurements will be made by means of a Colin STBP-780 automated BP unit (Colin Medical Instruments Corp., San Antonio, TX) using standard techniques following 20 min of supine rest during the HRV assessment.[22]

Food Frequency Analysis—In order to account for dietary intake, we will use the FIAS system (version 3.9, 2000) developed at the Human Nutrition Center, University of Texas Health Science Center School of Public Health. One reason we selected the FIAS is that it is linked with the Pyramid Serving Database (PSDB). The USDA food codes generated after the analysis of the dietary recalls in FIAS are linked to the PSDB to determine the number of servings of the major food groups consumed. This database was developed to analyze the number of servings of each of the Food Guide Pyramid's major food groups and the amounts of discretionary fat and sugars consumed.[23]

Medical History—At baseline and follow-up evaluations, detailed medical history questionnaires will be administered and participants questioned about acute medical problems

including colds, sinusitis and orthopedic injury. In addition, monthly health questionnaires will be administered to monitor participant health status and visits to physicians, dentists and other healthcare providers.

Fitness Assessments—For the baseline and follow-up fitness measurements, participants will complete a graded exercise test to exhaustion on a Lode Excalibur Sport Cycle Ergometer (Lode, BV, Groningen, Netherlands). Prior to testing, participants will be fitted with a 12-lead ECG and BP cuff. Heart rate (HR) and BP measurements will be made in the supine and standing position and then after 2 min after waiting quietly on the cycle ergometer. Women will initiate the testing protocol at 25 W and will progress 25 W per stage. Men will initiate the testing protocol at 35 W and will progress 35 W per stage. For both men and women, each stage will last 2 minutes. Each testing protocol will be terminated when the participant reaches fatigue, exhaustion or if an abnormal ECG or BP response to testing appears. During the final 30 s of each stage, BP, HR and rating of perceived exertion (RPE) will be recorded. Throughout the test, pulmonary ventilation, oxygen consumption (VO_2), carbon dioxide production, and respiratory exchange ratio will be measured and recorded every 15 s using a metabolic cart (Parvomedics True Max 2400 Metabolic Measurement Cart, Salt Lake City, UT). This information will be used to screen for abnormal BP and ECG responses to exercise and to create individual exercise prescriptions. Criteria for $V0_2$max will be: RER >1.1, a plateau in VO_2 (<100 mL/min change during the final 3 consecutive time increments of testing), and HR within 10 bpm of age-predicted max. All other tests will be considered VO_2peak.

Weekly Exercise Sessions and Daily Activity—For the weekly planned exercise sessions, exercise intensity will be quantified using data from the Polar XL HR monitor worn by participants. The appropriate heart rate range (HRR) for the prescribed intensity (60–80% VO_2 max) will be calculated following completion of the maximal exercise test by the study's exercise physiologist. If a participant's HR falls or rises out of range, the speed and/or grade of the treadmill or Watts on the cycle ergometer will be adjusted to maintain the prescribed intensity. All data including the participant's study number, group assignment, VO_2max, resting HR, maximal HR, desired HRR during exercise and self-selected exercise frequency, will be entered into a touch screen electronic data capture system (Clinaero Inc., Bellevue, WA; previously called Vital Link.net). Data from previous exercise sessions, including mode of exercise, HR, RPE, and speed/grade (treadmill) or Watts (cycle ergometer) may be accessed at any time and will provide real-time feedback on the duration of exercise needed to reach caloric expenditure goals given a participants' weight, power output and self-selected frequency.

Daily activity outside the assigned exercise session will be monitored via step counters (Accusplit Eagle, Japan). Each participant will be given a step counter at baseline and instructed to wear the step counter at all times during waking hours. Participants will be asked to remove the step counter only during planned exercise sessions, while swimming, or for purposes of bathing, sleeping, or dressing. At the end of each day, participants will record the number of steps taken and reset the step counter for the following day. Participants will also be asked to make note of any events resulting in significant changes in activity, for example, illness or injury.

Exercise program rationale and selection of doses—In order to maintain the clinical relevance of the intervention, we selected the exercise dose to be within the capability of sedentary but otherwise healthy adults and to be one that professionals could prescribe to patients with reasonable expectations of adherence. Therefore we selected an exercise dose of 16 KKW divided into three to five sessions per week in the controlled exercise laboratory. This dose falls within the consensus public health recommendation for moderate to vigorous intensity physical activity of 30 minutes or more on most days of the week, or approximately

150 to 210 minutes of moderate intensity activity. A 70 kg participant exercising at 16 KKW would expend 1120 kilocalories per week. This exercise prescription would require 373 kcal of energy expenditure per exercise session over three sessions or 224 kcal per exercise session over five sessions. A two-week ramping phase where exercise prescriptions require 10 KKW then 13KKW will be used to ease participants into the exercise intervention and to minimize injury, soreness, and dropout. Energy expenditure (EE) requirements will be elevated to 16 KKW by the third week and will remain constant throughout the remainder of the intervention. Participants will alternate between exercising on the treadmill and cycle ergometer in an effort to reduce injury, provide variety, and produce a well-rounded training response.[24]

Current public health recommendations advise moderate intensity exercise for sedentary persons initiating an exercise program. For the purposes of this study, moderate-to-vigorous-intensity exercise is defined as 60 to 80% VO_2max. Exercise at 60% VO_2max will be sufficient to elicit clinically significant physiological changes associated with exercise training; 80% VO_2max will allow those participants who wish to work at a higher intensity but not above most individuals' anaerobic threshold.[25–28]

Current data suggest that most physiological adaptations occur within the first four months of exercise training when exercise prescription remains unchanged.[29;30] Therefore, in view of the expectation of little further adaptation after four months and considering the increased logistical and participant burdens and costs of running a highly controlled, laboratory-based study for a longer period, the intervention will be limited to four months.

Each week, a tracking report will be generated to monitor adherence to exercise prescription. In order to maintain high adherence rates, flexibility will be required. If a participant fails to meet EE requirements one week, the EE requirement for the following week could be increased up to 1.5 KKW to compensate. Participants will be allowed to complete up to 10 exercise sessions outside of the laboratory by providing the participant with a HR monitor and an exercise prescription based on the exercise completed in the laboratory. Participants will record the duration and intensity of exercise completed outside the laboratory as well as their beginning and ending HR. This information will be used to calculate kcals expended during the session.

Statistical Analysis and Power—Statistical analysis of primary and secondary outcomes will be based on the intention-to-treat principle. These analyses will take into account pre-specified baseline covariates, including age, BMI, use of medications that might affect CRP, and baseline values of outcome measures. Analyses of continuous measures will be based on ANCOVA models of four-month change-scores since baseline, and will be summarized as least-squares adjusted means across control and exercise groups. Associations between changes in CRP and changes in the secondary outcomes will be studied by regressing change in each secondary outcome, one by one, against change in CRP, with adjustment for treatment group and baseline levels. Analyses of binary outcomes will be based on logistic regression. The potential effects of missing data will be explored under selection models for non-ignorable missing data mechanisms, and through multiple imputation models under ignorable missing data assumptions.[31] Out of concern for Type I error rates in performing repeated statistical tests, only one primary goal with one primary outcome measure has been pre-specified. Other outcomes are categorized as "secondary" or "exploratory," and associated results will be interpreted with appropriate caution.

For all power calculations the comparison of the exercise group versus the control was based on the two-sample t-test for change-score differences, with equal group size and 5% significance (2-sided). For CRP, average change values and variability were based on observations from published reports.[32;33] The correlation between pre- and post-trial CRP

measures was estimated from a previous trial. Power was calculated for 30 and 40 percent changes in CRP (compared to no change in control group), which were derived from previous reports of CRP being reduced 31 and 35 percent in response to exercise training in uncontrolled studies.[32;33] Assuming 10 percent drop out and 77 participants completing the study in each group, for CRP we calculated power to be 0.94, and 0.99 to detect differences between the experimental and control groups of 30, and 40 percent, respectively. In comparing the INFLAME study with the other two available CRP and exercise training studies, the INFLAME population will be considerably less fit at baseline, have much greater baseline CRP values, and will follow a much more tightly controlled exercise regimen.[32;33] Thus it is reasonable to expect a decrease of CRP of at least 30% in the exercise group of the INFLAME study.

Among the secondary variables, we calculated power to be 0.99 to detect significant changes in the exercise group compared to the control group for visceral adiposity, TNF-α, IL-6 and rMSSD-HRV and 0.93 for HRV based on literature available when the study was planned. The selection of a target of 77 participants per group finishing the study is only based on power calculations for the primary outcome measure, CRP. CRP is a highly variable measure and it is not surprising that a large sample size is needed to assure adequate power for an exercise training study. We calculated power for the secondary variables only to assure that there was an appropriate level of power to detect change during the study period.

There are few reports examining the effect of regular exercise on both the primary and secondary outcomes and we recognize that we have made numerous statistical assumptions. However, these assumptions are based on the literature at the time of study planning and the sample size should be large enough to compensate for the less than optimal estimates used in the power calculations.

As an acute phase reactant that is greatly elevated during acute illness or inflammatory conditions, CRP can temporarily be elevated 100 fold or more creating potential difficulties in interpretation. In order to address this issue conservatively, we will use all follow-up CRP values without any attempt to re-measure or remove CRP values >10 mg/L. The number of individuals with an acutely elevated CRP at follow-up should be equally distributed between the exercise and no exercise group. Nonparametric methods or robust regression methods might be utilized if there is an unequal distribution of acutely elevated CRP between treatment groups. Further, our power calculations were based on mean and standard deviations from existing data sets, which included individuals with elevated CRP at baseline or at follow-up. Thus the study should be powered to protect for the occurrence of the occasional elevated CRP at follow-up.

Discussion

INFLAME will evaluate the effects of chronic aerobic exercise on plasma CRP concentrations in sedentary women and men who had elevated CRP levels at the initial assessment. The study is adequately powered to evaluate CRP as the primary outcome and secondary outcomes that include visceral adiposity, cytokines and heart rate variability. The focus of this study is to evaluate the efficacy of moderately to vigorous intensity exercise on CRP levels. The moderately intense exercise dose is based on consensus public health recommendations and is considered practical for sedentary adults to undertake. To our knowledge this will be the largest training study specifically designed to examine the effect of exercise on CRP. This intervention will have increased clinical importance if it is found to improve CRP.

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Reference List

1. Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 2001 April 3;103(13):1813–8. [PubMed: 11282915]
2. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 2002 November 14;(347)(1557):65.
3. Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between Physical Activity and Markers of Inflammation in a Healthy Elderly Population. Am J Epidemiol 2001 February 1;153(3):242–50. [PubMed: 11157411]
4. Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology 2002 September;13(5):561–8. [PubMed: 12192226]
5. LaMonte MJ, Durstine JL, Yanowitz FG, Lim T, DuBose KD, Davis P, Ainsworth BE. Cardiorespiratory fitness and C-reactive protein among a tri-ethnic sample of women. Circulation 2002 July 23;106(4):403–6. [PubMed: 12135936]
6. Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. Arteriosclerosis Thrombosis Vascular Biology 2002;22:1869–76.
7. Festa A, D'Agostino JR, Williams K, Karter AJ, Mayer-Davis EJ, Tracy RP, Haffner SM. The relation of body fat mass and distribution to markers of chronic inflammation. Int J Obes Relat Metab Disord 2001 October;25(10):1407–15. [PubMed: 11673759]
8. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999 December 8;282(22):2131–5. [PubMed: 10591334]
9. Okita K, Nishijima H, Murakami T, Nagai T, Morita N, Yonezawa K, Iizuka K, Kawaguchi H, Kitabatake A. Can exercise training with weight loss lower serum C-reactive protein levels? Arterioscler Thromb Vasc Biol 2004 October;24(10):1868–73. [PubMed: 15284086]
10. Lakka TA, Lakka HM, Rankinen T, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. Effect of exercise training on plasma levels of C-reactive protein in healthy adults: the HERITAGE Family Study. Eur Heart J 2005 June 29;26(19):2018–25. [PubMed: 15987707]
11. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the american heart association. Circulation 2003 January 28;107(3):499–511. [PubMed: 12551878]
12. Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. Circulation 2003 November 11;108(19):2292–7. [PubMed: 14609996]
13. Davison S, Davis SR. New Markers for Cardiovascular Disease Risk in Women: Impact of Endogenous Estrogen Status and Exogenous Postmenopausal Hormone Therapy. Journal of Clinical Endocrinology Metabolism 2003 June 1;88(6):2470–8. [PubMed: 12788842]
14. Prasad K. C-reactive protein (CRP)-lowering agents. Cardiovasc Drug Rev 2006;24(1):33–50. [PubMed: 16939632]
15. Wilson AM, Ryan MC, Boyle AJ. The novel role of C-reactive protein in cardiovascular disease: risk marker or pathogen. Int J Cardiol 2006 January 26;106(3):291–7. [PubMed: 16337036]
16. Church TS, Earnest CP, Wood KA, Kampert JB. Reduction of C-reactive protein levels through use of a multivitamin. Am J Med 2003 December 15;115(9):702–7. [PubMed: 14693322]
17. Schulz KF, Grimes DA. Allocation concealment in randomised trials: defending against deciphering. Lancet JID - 2985213R 2002 February 16;359(9306):614–8.
18. Sato N, Miyake S, Akatsu J, Kumashiro M. Power spectral analysis of heart rate variability in healthy young women during the normal menstrual cycle. Psychosom Med 1995 July;57(4):331–5. [PubMed: 7480562]

19. Ross R, Leger L, Morris D, de Guise J, Guardo R. Quantification of adipose tissue by MRI: relationship with anthropometric variables. J Appl Physiol 1992 February 1;72(2):787–95. [PubMed: 1559959]
20. Molgaard H, Sorensen KE, Bjerregaard P. Circadian variation and influence of risk factors on heart rate variability in healthy subjects. Am J Cardiol 1991 September 15;68(8):777–84. [PubMed: 1892086]
21. Heslegrave RJ, Ogilvie JC, Furedy JJ. Measuring baseline-treatment differences in heart rate variability: variance versus successive difference mean square and beats per minute versus interbeat intervals. Psychophysiology 1979;16(2):151–7. [PubMed: 424498]
22. Perloff D, Grim C, Flack J, Frohlich ED, Hill M, McDonald M, Morgenstern BZ. Human blood pressure determination by sphygmomanometry. Circulation 1993 November;88(5 Pt 1):2460–70. [PubMed: 8222141]
23. Cleveland LE, Cook DA, Krebs-Smith SM, Friday J. Method for assessing food intakes in terms of servings based on food guidance. Am J Clin Nutr 1997;65(suppl):1254S–63S. [PubMed: 9094930]
24. American College of Sports Medicine. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness in healthy adults. Med Sci Sports Exerc 1990;22:265–74. [PubMed: 2355825]
25. NIH Consensus Development Panel on Physical Activity and Cardiovascular Health. Physical activity and cardiovascular health. JAMA 1996;276(3):241–6. [PubMed: 8667571]
26. Snyder KA, Donnelly JE, Jacobsen DJ, Hertner G, Jakicic JM. The effects of long-term, moderate intensity, intermittent exercise on aerobic capacity, body composition, blood lipids, insulin and glucose in overweight females. Int J Obes Relat Metab Disord 1997;21:1180–9. [PubMed: 9426387]
27. Perri MG, Anton SD, Durning PE, Ketterson TU, Sydeman SJ, Berlant NE, Kanasky WF Jr, Newton RL Jr, Limacher MC, Martin AD. Adherence to exercise prescriptions: effects of prescribing moderate versus higher levels of intensity and frequency. Health Psychol 2002 September;21(5): 452–8. [PubMed: 12211512]
28. U.S.Department of Health and Human Services. Physical activity and health: A report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion; 1996.
29. Badenhop DT, Cleary PA, Schaal SF, Fox EL, Bartels RL. Physiological adjustments to higher- or lower-intensity exercise in elders. Med Sci Sports Exerc 1983;15(6):496–502. [PubMed: 6656559]
30. Kohrt WM, Malley MT, Coggan AR, Spina RJ, Ogawa T, Ehsani AA, Bourey RE, Martin WH, Holloszy JO. Effects of gender, age, and fitness level on response of VO_2max to training in 60–71 yr olds. J Appl Physiol 1991 November;71(5):2004–11. [PubMed: 1761503]
31. Little, RJA.; Rubin, DB. Statistical analysis with missing data. New York, NY: John Wiley & Sons; 1987.
32. Mattusch F, Dufaux B, Heine O, Mertens I, Rost R. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. Int J Sports Med 2000 January;21(1): 21–4. [PubMed: 10683094]
33. Smith JK, Dykes R, Douglas JE, Krishnaswamy G, Berk S. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. JAMA 1999 May 12;281(18):1722–7. [PubMed: 10328073]

Table 1

Inclusion and Exclusion Criteria for the INFLAME study

Inclusion Criteria	
Age	30 to 75 years
Physically inactive	Exercising ≤3 d…wk^{-1} for 20 min each time.
C-Reactive Protein	≥2.0 mg/L but <10.0 mg/L, at the time of initial screening
BMI	18.5 kg/m^2 < BMI < 40.0 kg/m^2
Fasting Glucose	<126 mg/Dl
Blood Pressure and lipids	Resting blood pressure <140 mmHg systolic and/or 90 diastolic. Individuals on blood pressure or lipid lowering medications bu
Non-Smoker	Has not smoked or used chewing tobacco or tobacco products in the past 6 months
Capable of exercise	There is no minimal or maximal value for VO_2 max as long as participants are physically able to exercise safely at the required
Medications	Not currently taking the following medications or has been on a stable dose for at least 2 consecutive months: Statins, Ace Inhibitors, Oral Contraceptives, Multi-vitamins, Aspirin, Ibuprofen and other anti-inflammatory medications
Informed consent	Capability and willingness to give written informed consent, to understand exclusion criteria, and to accept the randomized gro
Permanent Exclusion Criteria: A past history and/or physical examination or laboratory findings related to the following medical conditions	
Medications	Hormone Replacement Therapy, beta blocker, allergy shots, or systemic corticosteroids (except inhalers)
Significant cardiovascular disease or disorders	Including but not limited to serious arrhythmias, cardiomyopathy, congestive heart failure, stroke or transient ischemic cerebra
Blood lipids	Total cholesterol ≥240 mg/dL with LDL-C ≥190 mg/dL or Triglyceride levels >300 mg/dL.
Other exclusions	Plans to be out of the city more than 4 weeks over the next 4 months For women, plans to become pregnant in the next year
Other significant medical conditions	Including but not limited to: chronic or recurrent respiratory, gastrointestinal, neuromuscular, neurological, or psychiatric cond

Table 2

Temporary Ineligibility Criteria for the INFLAME study

Reason	Minimum Duration of Exclusion
CRP >10 mg/L, at the time of initial screening	2 Weeks -6 months
Blood donation	6 Weeks
Out of range: blood pressure or cholesterol	2 Months
Colonoscopy, sprain or strain, any type of dental work including removal of wisdom teeth, cold, flu or other infection, taking antibiotics	3 Months
Broken bone, anaphylactic shock, Surgery	6 Months
Cardiac dysrhythmia	Discretion of medical staff

Table 3

Participant flow and timeline from screening to randomization

Timeline	Action	Participant Data Collected		Purpose
Week 1	Telephone Screen	• Demographic information • Self-reported medical information to screen		To determine initial illegibility
Week 2	Orientation & CRP Screening	• Informed consent • Measurements: height, weight • Blood chemistry screening: CRP		To ensure that the participant understands the expectations of the study and meets inclusion criteria
Week 3–4	3 Educational Run-In Sessions	• Questionnaires: FFQ, medication inventory, detailed medical history • Measurements: BP • Behavioral Contract		To ensure participant meets inclusion criteria and can visit the intervention site multiple times per week
Week 5	Baseline Evaluations	• Blood draw • Measurements: ECG, CT scan, DXA, height, weight, waist and hip circumference • Fitness Testing		Collect baseline data and ensure participant meet remaining study entry criteria
Week 6	**Randomization**			
Week 7–23	Intervention	Exercise Group: • Exercise 3–5x per wk • Self-reported data returned monthly[a]	Control Group: • Self-reported data returned monthly[a]	Collect intervention data as well as data on potential confounding variables such as health, diet and non-study related activity
Week 24	Follow-Up Evaluations	• Blood draw • Measurements: EBT, DXA, height, weight, waist and hip circumference, BMI, BP • Fitness Testing • Questionnaires: FFQ, MSQ, Exit Interview		Collect follow-up data

[a]Self Report Data Returned Monthly includes: Medical Symptoms Questionnaire, Medication Inventory and Daily Step Log