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ASSOCIATION OF CARDIORESPIRATORY FITNESS AND ADIPOSITY WITH INFLAMMATORY BIOMARKERS IN YOUNG ADULTS

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ASSOCIATION OF CARDIORESPIRATORY FITNESS AND ADIPOSITY WITH
INFLAMMATORY BIOMARKERS IN YOUNG ADULTS

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN NURSING

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER AT HOUSTON
SCHOOL OF NURSING

BY
EUNDUCK PARK, MSN, RN

AUGUST, 2014

The University of Texas Health Science Center at Houston
School of Nursing
Houston, Texas

May 27, 2014

Date

To the Dean for the School of Nursing:

I am submitting a dissertation written by Eunduck Park and entitled "Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Young Adults." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Nursing.

Janet C. Meininger, Committee Chair

We have read this dissertation
and recommend its acceptance:

Accepted

Dean for the School of Nursing

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Association of Cardiorespiratory Fitness and Adiposity with Inflammatory
Biomarkers in Young Adults

August, 2014

Abstract

Background

Low grade systemic inflammation plays a key role in atherosclerosis, and C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), markers of systemic inflammation, are associated with cardiovascular events and adiposity. Cardiorespiratory fitness has shown health benefits by reducing circulating levels of inflammatory markers. However, it remains uncertain whether the negative association between cardiorespiratory fitness and inflammatory biomarkers is due to cardiorespiratory fitness itself or results from lower levels of adiposity. Moreover, the evidence examining the interaction between cardiorespiratory fitness and adiposity in inflammation in young adults is lacking.

Purpose

The aims of this study were to (1) determine the strength of the associations of cardiorespiratory fitness and adiposity (body mass index [BMI], waist circumference [WC]) with circulating levels of plasma hs-CRP, IL-6, and TNF- α ; and (2) test the moderating effect of adiposity on the strength of the association between cardiorespiratory fitness and circulating levels of plasma hs-CRP, IL-6, and TNF- α .

Methods

A cross-sectional study was conducted with 88 young adults aged 20-34 years without diagnosed diseases. A submaximal treadmill walking test was used to assess

cardiorespiratory fitness. BMI and WC were measured to assess adiposity. The hs-CRP, IL-6 and TNF- α were assayed and were log₁₀-transformed. For aim one, a separate multiple regression analysis was conducted with each of hs-CRP, IL-6, and TNF- α as dependent variables and adjusted for confounders. Analysis of covariance (ANCOVA) was used and adjusted for confounders for aim two. Confounding variables tested were sex, ethnicity, oral contraceptive use, and education level.

Results

Aim1: Cardiorespiratory fitness was not significantly associated with log₁₀hs-CRP after adjustment for BMI or WC and confounders. Cardiorespiratory fitness was not significantly associated with log₁₀IL-6 after adjustment for BMI and confounders. However, cardiorespiratory fitness was significantly and negatively associated with log₁₀IL-6 after adjustment for WC and confounders (Model adjusted $R^2 = .273$, $p < .0001$; $\beta = -.341$, $t = -1.995$, $p = .049$). Aim 2: Cardiorespiratory fitness $\times$ BMI or WC interaction was not significantly associated with log₁₀hs-CRP after adjustment for confounders. Similarly, cardiorespiratory fitness $\times$ BMI interaction was not significantly associated with log₁₀IL-6 after adjustment for confounders. However, cardiorespiratory fitness $\times$ WC interaction was significantly associated with log₁₀IL-6 after adjustment for confounders (Model adjusted $R^2 = .258$, $p < .0001$; partial $\eta^2 = .056$, $F = 4.730$, $p = .033$). There were no associations of cardiorespiratory fitness, adiposity, and log₁₀TNF- α .

Conclusions

In young adults, higher cardiorespiratory fitness is significantly associated with lower levels of IL-6, particularly in young adults with central adiposity. Further studies are warranted to determine if experimentally induced increases in cardiorespiratory fitness

reduce inflammatory markers in young adults. Longitudinal studies are needed to understand the underlying inflammatory mechanisms related to interaction between cardiorespiratory fitness and adiposity, and its influence on cardiovascular disease risk in young adults.

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Summary of Study

The dissertation consists of the proposal, two manuscripts reporting study results, and appendixes. The proposal consists of specific aims, research strategy, facilities/ resources/ consultants, and human subject concerns. Two manuscripts are included: manuscript 1. Anthropometric Adiposity Measures and Inflammatory Biomarkers in Adolescents and Young Adults: A Systematic Review and manuscript 2. Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Young Adults: dissertation.

Manuscript 1 is the systematic review on the associations between anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF%) and inflammatory biomarkers (CRP, IL-6, and TNF- α) in adolescents and young adults. This review found that anthropometric adiposity measures are positively associated with CRP in adolescents and young adults. However, anthropometric adiposity measures and IL-6 and TNF- α in adolescents and young adults is inconclusive. Additional studies are needed to identify the strength and patterns of association of anthropometric adiposity measures with CRP, IL-6 and TNF- α .

Manuscript 2 is a dissertation study. This cross-sectional study is to (1) determine the strength of the associations of cardiorespiratory fitness and adiposity (body mass index [BMI], waist circumference [WC]) with circulating levels of plasma hs-CRP, IL-6, and TNF- α ; and (2) test the moderating effect of adiposity on the strength of the association between cardiorespiratory fitness and circulating levels of plasma hs-CRP, IL-6, and TNF- α . This study found that in young adults, higher cardiorespiratory fitness is significantly associated with lower levels of IL-6, particularly in young adults with

central adiposity. Further studies are warranted to determine if experimentally induced increases in cardiorespiratory fitness reduce inflammatory markers in young adults.

Appendices include study protocols, IRB approval letters and human subject concerns. Curriculum vitae constitute the final section of the dissertation.

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EUNDUCK PARK, MSN, RN

July, 2012

Abstract

Purpose: The proposed study is to investigate the association of cardiorespiratory fitness (CRF) and adiposity with inflammatory biomarkers in healthy young adults. Specific aims are (1) to determine the strength of the association between CRF levels with circulating levels of plasma high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α); (2) to determine the strength of the association between adiposity levels and circulating levels of plasma hs-CRP, IL-6, and TNF- α ; and (3) to test the moderating effect of adiposity on the strength of the association between CRF levels and circulating levels of plasma hs-CRP, IL-6, and TNF- α .

Significance: Atherosclerotic cardiovascular disease is a significant public health concern. Low grade chronic vascular inflammation plays a key role in atherosclerosis. CRF has shown health benefits by reducing circulating levels of inflammatory markers. However, it is uncertain whether the beneficial effect of CRF derives from the CRF itself or from inadequate adjustment for adiposity. Moreover, the evidence examining adiposity as a modifier of this association in young adults is lacking.

Methods: A cross-sectional analytic design will be conducted to achieve the three specific aims. Participants will be recruited through flyers and emails. Inclusion criteria include young adults aged 20-34 years old, healthy (without diagnosis of disease), being capable of completing the CRF testing, and not using any hormonal therapy. After screening for eligibility, 88 participants will be enrolled and they will be scheduled for a data collection session. Data will be collected on each participant through CRF testing, anthropometric measurement and blood sampling of inflammatory biomarkers in one

session at the University of Texas Health Science Center at Houston School of Nursing. CRF will be estimate by a submaximal treadmill test and adiposity will be assessed by body mass index and waist circumference. Inflammatory markers will be measured by enzyme-linked immunosorbent assay (ELISA). Separate analyses will be conducted with each of the inflammatory markers (hs-CRP, IL-6, and TNF- α) as the dependent variable in multiple linear regression for aims one and two. Multiple linear regression models, with interaction terms (CRF $\times$ BMI) (CRF $\times$ waist circumference) will be addressed to assess aim three.

Nursing Implications: This information provides more accurate evaluation and interpretation of inflammatory biomarkers in clinical practice as related to CRF and adiposity. Additionally, these finding will be useful in understanding the physiological mechanisms of CRF, adiposity, and inflammatory biomarkers, which contributes to decreased atherosclerosis risk. Lastly, these findings will be useful in in planning preventive strategies to reduce low-grade chronic inflammation in obese as well as healthy weight young adults, which in turn delay the progression of atherosclerosis and reduce the risk of CVD events.

Specific Aims

Chronic, low-grade vascular inflammation plays a key role in the initiation and progression of atherosclerosis (Packard & Libby, 2008). C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) have been documented as the major inflammatory markers associated with atherosclerotic progression and events (Packard & Libby, 2008). It has demonstrated that atherosclerotic lesions begin early in life albeit clinical manifestations are not observed until symptoms occur in later life (Loria et al., 2007).

Cardiorespiratory fitness (CRF) has shown health benefits in reducing circulating levels of inflammatory markers, but it is still uncertain whether there is a definitive link between CRF and inflammatory biomarkers in healthy young adults (Hamer, 2007). There is also evidence that adiposity might be a factor attenuating the link of CRF with low grade inflammation (Hamer, 2007). However, no previous studies have examined the interplay of CRF and adiposity on inflammatory biomarkers in healthy young adults. In this proposed study, the principal investigator (PI) will examine the associations of CRF and adiposity with inflammatory biomarkers and the interaction of CRF and adiposity on inflammatory biomarkers in healthy young adults aged 20 to 34 years. Adiposity is defined as body mass index (BMI) and waist circumference in this study. The PI will pursue the main purpose of this study through three specific aims as follows:

Aim1. To determine the strength of the association between CRF levels with circulating levels of plasma hs-CRP, IL-6, and TNF- α . Hypothesis: Higher levels of CRF will be related to lower levels of circulating plasma hs-CRP, IL-6, and TNF- α .

Aim2. To determine the strength of the association between adiposity levels and circulating levels of plasma hs-CRP, IL-6, and TNF- α . Hypothesis: *2a.* Higher levels of BMI will be related to higher levels of circulating plasma hs-CRP, IL-6, and TNF- α . *2b.* Higher levels of waist circumference will be related to higher levels of circulating plasma hs-CRP, IL-6, and TNF- α .

Aim3. To test the moderating effect of adiposity on the strength of the association between CRF levels and circulating levels of plasma hs-CRP, IL-6, and TNF- α . Hypothesis: *3a.* Individuals with higher levels of BMI and higher levels of CRF will have lower levels of circulating plasma hs-CRP, IL-6, and TNF- α compared with individuals with higher levels of BMI and lower levels of CRF. *3b.*

Individuals with higher waist circumference and higher levels of CRF will have lower levels of circulating plasma hs-CRP, IL-6, and TNF- α compared with individuals with higher waist circumference and lower levels of CRF.

For public health strategies and improvements of cardiovascular health, the proposed work is innovative in that it will test the central hypothesis that young adults with higher levels of CRF will have lower levels of inflammatory markers than those with lower levels of CRF, even among individuals who have greater adiposity as measured by BMI and waist circumference. Additionally, understanding the interplay of CRF, adiposity and inflammatory markers is of great importance because it will contribute to more accurate evaluation and interpretation of inflammatory markers in clinical practice. Finally, these findings will be useful in planning interventions to reduce low-grade chronic inflammation in obese as well as healthy weight young adults, which in turn may delay the progression of atherosclerosis and reduce the risk of CVD.

Research Strategy

Significance

Atherosclerotic cardiovascular disease (CVD) is a significant public health burden in the United States (Go et al., 2013). Atherosclerosis, a systemic disease process in which amassment of plaque builds up in the arterial wall, is the major underlying cause of the clinical CVD and CVD events (Go et al., 2013; Libby et al, 2009). It has been demonstrated that low-grade systemic vascular inflammation plays a pivotal role in the initiation and progression of atherosclerosis (Libby et al., 2009) by impairing endothelial function (Hansson, 2005; Ferri et al., 2007). Among the many inflammatory biomarkers, hs-CRP, IL-6, and TNF- α are well-recognized markers of inflammation in relation to atherogenesis (Calabro, Golia, & Yeh, 2009; Haddy et al., 2003). Hs-CRP, a primitive acute phase inflammatory protein, is secreted by the liver in reaction to IL-6 and has been demonstrated to be represented in atherosclerotic plaque (Clearfield, 2005; Wilson, Ryan, & Boyle, 2006). The “high sensitivity (hs)” term means “the measurement of CRP in serum or plasma sample using immunoassay methods with sufficient sensitivity” in asymptomatic individuals (Bajpai, Goyal, & Sperling, 2010, p.191). IL-6 is a pro-inflammatory cytokine and produced by fibroblasts, endothelial cells, monocytes, and adipose tissue (Schuett, Luchtefeld, Grothusen, Grote, & Schieffer, 2009). IL-6 plays an important role in controlling hepatic production of inflammatory proteins, including hs-CRP and fibrinogen (Antuna-Puente, Fève, Fellahi, & Bastard, 2008). TNF- α , a pro-inflammatory cytokine, is secreted by macrophages, lymphocyte, and adipose tissue (Petersen & Pedersen, 2005). TNF- α activates the production of IL-6 in adipose tissue and blood mononuclear cells (Petersen & Pedersen, 2005).

Although clinical symptoms of atherosclerotic CVD, such as chest pain or discomfort, shortness of breath or fatigue, numbness, and blurred vision are not commonly detected until adulthood it is a progressive atherosclerotic process that occurs early in life and has clinical indications, including acute coronary syndrome and acute stroke usually later in life (Loria et al., 2007; Frank et al., 2010). Hence, young adults could be a main target for preventive endeavors, such as individual's health behaviors because these efforts might minimize or delay atherosclerotic process, which could eventually decrease the incidence of clinical CVD throughout middle and older adulthood (Loria et al., 2007).

CRF refers to the health-related component of physical fitness (Caspersen, Powell, & Christenson, 1985). CRF defined as “the ability to perform large muscle, dynamic, moderate-to-high intensity exercise for prolonged periods of time” (American College of Sports Medicine [ACSM], 2010b, p.71). CRF is “an individual's capacity of the cardiovascular, respiratory, and muscle systems to supply oxygen during sustained physical activity” (ACSM, 2010a, p.3). It has reported that higher levels of CRF are closely related to higher levels of habitual physical activity (Blair et al., 1995). The “gold standard” or criterion measure of CRF is maximal oxygen uptake ($\text{VO}_{2\text{max}}$) (ACSM, 2010). The $\text{VO}_{2\text{max}}$ reflects the capacity of the heart, lungs, and blood to deliver oxygen to the working muscles during dynamic exercise involving large muscle mass (Heyward, 2010). The $\text{VO}_{2\text{max}}$ commonly estimates either a maximal or submaximal exercise test. CRF in this study will be estimated by using a submaximal treadmill walking protocol because maximal exercise testing is not always feasible or practical in the health and research settings (Heyward, 2010). Additionally, maximal exercise testing with analysis

of expired gases requires expensive laboratory equipment, considerable amount time, trained personnel and possible physician oversight. Further, there is considerable participant burden, associated safety considerations, and obtaining a valid estimate requires participant motivation (Heyward, 2010). The selected submaximal treadmill walking test protocol will utilize individual's heart rate (HR) response to submaximal treadmill work rates to estimate VO_{2max} (Ebbeling et al., 1991). It has reported that CRF is inversely associated with CRP in young adults (Kuo, Yen, Chen, Yu, & Bean, 2007; Williams et al., 2005). However, it is remain uncertain whether an inverse association between CRF and inflammatory biomarkers is due to the health benefits of CRF or results from changes in adiposity (Harmer, 2007).

Adiposity is defined as “the quality or state of being fat” (*Merriam-Webster Dictionary*). The adiposity is a synonym for obesity or fatness (*Merriam-Webster Dictionary*). Adiposity has been assessed by anthropometric measures in public health and clinical practices. Adiposity in this study will be assessed by BMI and waist circumference (WC). BMI (kg/m^2) has been recommended for the identification of measuring body fat (WHO, 2000) and the most widely accepted measures of overall adiposity (Stevens, McClain, & Truesdale, 2008); however, BMI does not differentiate fat mass from bone and muscle mass and does not provide any information on the distribution of body fat, particularly central body fat (Stevens, McClain, & Truesdale, 2008). Thus, WC will be assessed for measuring central adiposity (Stevens, McClain, & Truesdale, 2008). A growing body of evidence has supported that there is positive association of adiposity with inflammatory biomarkers (Park, Park, & Yu, 2005; Wang, Reed, Goli, & Goswami, 2011). However, there is not enough evidence to identify the

strength and patterns of association of anthropometric adiposity measures with CRP, IL-6 and TNF- α .

Although there is evidence that CRF is inversely associated with inflammatory biomarkers (Hamer, 2007), it is still under debate whether the beneficial effect of CRF derives from the CRF itself or from inadequate adjustment for adiposity (Hamer, 2007), which adiposity also may influence in inflammatory processes (Rocha & Libby, 2009). More importantly, only two studies have examined the association of CRF and CRP in young adults over the past decade (Kuo et al., 2007; Williams, Milne, Hancox, & Poulton, 2005). One study measured CRP using a low sensitivity CRP assay, which may underestimate the strength of the association between CRF and inflammation (Williams et al., 2005). The other study was population-based study, which included study participants with CVD risk factors, such as hypertension, diabetes and current smokers or participants with anti-inflammatory or CVD medications (Kuo et al., 2007), thus, the findings cannot be generalized to healthy young adults. Neither study examined adiposity as moderator of the association between inflammation and CRF, and what extent that adiposity influences the association between CRF and inflammation. Furthermore, these two previous studies only focused on CRP as a biomarker of inflammation. It is essential to investigate other inflammatory markers, such as IL-6 and TNF- α in order to better understand inflammatory processes in relation to adiposity and CRF. Therefore, this proposed study is significant as it will provide data on the role of adiposity as a moderator of the strength of the association between CRF and three inflammatory biomarkers. This will fill an important gap in knowledge. Figure1 shows a schematic diagram of the conceptual framework and specific aims of this proposed study.

The proposed study is significant as it will

- (1) Provide a basis for more accurate interpretation and evaluation of inflammatory biomarkers in clinical settings as related to CRF and adiposity assessment.
- (2) Contribute a better understanding inflammatory process in relation to CRF and adiposity in a young adult population.
- (3) Provide knowledge for the design of preventive strategies to reduce low grade chronic inflammation in obese as well as healthy weight young adults, which inturn may delay the progression of atherosclerosis risk and reduce the risk of CVD.

Figure 1. Conceptual framework for cardiorespiratory fitness, adiposity and inflammatory biomarkers in young adults

Innovation

The proposed study is innovative in that it will

- (1) No previous studies have examined the interplay of CRF and adiposity on inflammatory makers in young adults.
- (2) Be one of the few studies that assessed CRF in young adults without known diagnosis of disease using a single-stage submaximal treadmill walking test in community setting.
- (3) Provide CRF levels, adiposity levels and plasma inflammatory biomarkers levels not only hs-CRP but it also IL-6 and TNF- α in young adults. To our knowledge, this is the first study to include IL-6 and TNF- α in relation to CRF in healthy young adults. There is limited data on these variables in young adults. This information will expand scientific knowledge on the inflammatory mechanisms related to CRF and adiposity, which may contribute to atherosclerosis risk in this population.

Approach

Preliminary study.

Effects of exercise intervention on inflammatory markers in healthy young and middle aged adults: A systematic review. The PI conducted a systematic review of effects of exercise interventions on inflammatory markers in healthy young and middle aged adults (Park &Meininger). The aim of this systematic review was to assess the effects of exercise interventions on circulating levels of inflammatory markers CRP, IL-6, and TNF- α in healthy young and middle aged adults. Method of this study was that Medline and PubMed databases were searched for studies published between 1966 and

July 2011 using keywords related to “exercise”, “C-reactive protein”, “interleukin-6”, and “tumor necrosis factor-alpha”. Inclusion criteria were (a) randomized controlled trials (RCTs), (b) exercise interventions with a minimum duration of 4 weeks, and (c) participants’ ages 18 to 65 years old. Main findings were as follows: Of 1,490 identified publications, 93 were retrieved for detailed evaluation and 8 met inclusion criteria. Of 6 RCTs on CRP, 1 study reported decreased CRP ($P<0.05$) in aerobic exercise. Of 3 RCTs on IL-6, 1 study showed decreased IL-6 ($P\leq 0.02$) in aerobic exercise. Of 4 RCTs on TNF- α , 1 study reported elevated TNF- α ($P<0.05$) in aerobic exercise among females, and 1 study demonstrated decreased TNF- α ($P<0.01$) in high-intensity aerobic exercise. The evidence supporting the effects of exercise interventions on CRP, IL-6 and TNF- α is sparse and inconsistent, and degree of adiposity was not considered. Further research is needed on the potential role of body fat as it relates to the effects of exercise and CR fitness on inflammatory biomarkers.

Anthropometric adiposity measures and inflammatory biomarkers in adolescents and young adults: A systematic review. The purpose of the systematic review was to determine the associations between anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF%) and inflammatory biomarkers (CRP, IL-6, and TNF- α) in healthy adolescents and young adults without a known diagnosis of diseases. Method of this study was that MEDLINE (from 1946 to June 08, 2012), CINAHL (from 1995 to June 08, 2012), and SCOPUS (from 1960 to June 08, 2012) using keywords related to anthropometric adiposity measures; “body mass index,” “waist circumference,” and inflammatory biomarkers; “C-reactive protein”, “interleukin-6”, and “tumor necrosis factor-alpha”. Inclusion criteria were (1) adolescents and young adults aged 13 to 40

years; (2) investigated the association between at least one anthropometric adiposity measures and CRP, IL-6, and/or TNF- α ; (3) anthropometric adiposity measured by BMI, WC, WHR, WHtR, and/or body percentage assessing a skinfold thickness; (4) CRP, IL-6, and TNF- α levels measured by blood; (5) if cross-sectional data from the same study cohort was published in more than one studies, then only results from the report that most directly evaluated the association of anthropometric adiposity and inflammation was included; (6) all types of research designs; (7) reported in an original study; (8) no country restriction; and (9) published study in English language. Exclusion criteria were: (1) adolescent and young adults with diagnosed diseases and infectious diseases; (2) pregnant women; (3) reported adiposity measured only by DEXA or MRI; (4) reported a review or an editorial article; and (5) reported in an abstract. Main findings were as follows: Of 3,714 identified publications, 166 full text studies were retrieved for detailed evaluation and 27 met inclusion criteria. The main findings of the systematic review showed that there was significant and positive association of anthropometric adiposity measured by both total adiposity and central adiposity (BMI, WC, WHtR, WHR, and BF%) with CRP in adolescents and young adults in 23 studies (96%) ($p < 0.05$) and there was no significant association in adolescents in one study (4%). The findings of the review in the associations between anthropometric adiposity measures (BMI, WC, WHR, and BF%) with IL-6 and TNF- α in adolescents and young adults remained inconsistent. Further research is needed to focus on the interplay of diet, physical activity, or weight loss with CRP, IL-6 and TNF- α for developing appropriate and effective lifestyle modification in reducing circulating levels of CRP, IL-6 and TNF- α and in preventing further development of atherosclerotic CVD and acute CVD events in later life.

Methods

Research design. The proposed cross-sectional analytic study is designed to achieve three specific aims. After screening for eligibility, health young adults 20-34 years old will be enrolled. Data will be collected on each participant through CRF testing, anthropometric measurement and blood sampling of inflammatory biomarkers in one session at the University of Texas Health Science Center at Houston (UTHSC-H) School of Nursing (SON).

Participants and setting. The target population for this study is healthy young adults aged 20 to 34 years residing in Houston, a large metropolitan city and with an ethnically diverse population in southeast Texas. Adults can be divided into young adulthood (the 20s and 30s), middle adulthood (the 40s and 50s), and later adulthood (age 60 or 65 and up) based on developmental periods in the human lifespan (Craig & Dunn, 2010). The focus of this study is on young adults aged 20 to 34 without a known diagnosis of diseases because women's menopause occurs after age of 40 to 45 and perimenopause typically begins several years before menopause. A sample of the population will be accessed using non-probability quota sampling approach in the area of UTHSC-H.

Sample size. Sample size was estimated based on a multiple linear regression model, regression coefficient (R^2) with interactions using nQuery Advisor version 7.0 (nQuery, Saugus, MA). The estimated sample size was 88 subjects to detect a medium effect size ($0.059 \leq R^2 < 0.138$) according to Jacob Cohen (1998), a power of 80%, and an alpha level of 0.050 for a two-tailed test. With a sample size of 88, the interaction of adiposity and CRF would be stated by at $p < 0.05$ if the change in R^2 with the addition of

the interaction term were as lower at 0.069. To address possible attrition up to 20%, 110 participants will be recruited. Since there were no previous studies to estimate for the interaction term, the PI chose the medium (R^2) of 0.069 for interactions for a multiple linear regression model in this study.

Inclusion and exclusion criteria. Inclusion criteria are (1) young adults age 20-34 years; (2) healthy (defined as “not currently having acute or chronic diseases” as specified in exclusion criteria); (3) capable of completing the CRF test and there is no minimal or maximal value for $VO_{2\text{ max}}$ as long as participants are physically able to do CRF testing safely (ACSM, 2010a; ACSM, 2010b); and (4) able to speak and read English. Exclusion criteria are as follows in both males and females: (1) currently non-smoker or smoking cessation greater than 1 year because smoking status may increase hs-CRP, IL-6 and possibly certain cytokine receptors antagonists (O’Connor et al., 2009); (2) alcohol dependence or regular consumption of more than 7 servings per week because alcohol intake alters inflammatory biomarkers (O’Connor et al., 2009); (3) acute infections, including a current or recent influenza illness, recent flu shot recipients, acute respiratory infection within 2 weeks; (4) dental infection and problem within 2 weeks; (5) CVD, such as hypertension, heperlipidemia and diabetes mellitus type I or II; (6) rheumatoid arthritis and immune disorders because of the possible alteration of inflammatory markers; (7) history of inflammatory bowel disease, such as Crohn’s disease and ulcerative colitis; (8) recent surgery within 1 month; (9) currently taking medications, such as hormone replacement therapy, antihypertensive medications, allergy shots or systemic corticosteroids, aspirin, statin, and selective serotonin uptake inhibitors (SSRIs), and anti-inflammatory medications, all of which may impact levels of

circulatory inflammatory biomarkers (O'Connor et al., 2009); (10) history of orthopedic injury in the past and limitations due to the musculoskeletal demands of CRF test (11) other medical conditions and medications that would increase inflammatory markers and would prohibit CRF test; and (12) the maximum hs-CRP cut off point will be set at 10.0 mg/L which may have potentially reflected inflammation due to sepsis and an acute infection or other inflammatory conditions (Pearson et al., 2003). For females, inclusion criteria are as follows: (1) self-reported menstrual cycle length of 24-35 days during the 2 months before entering the study; and (2) self-reported without a menstrual period at the time of data collection because menses may impact increased levels of CRP (Gaskins et al., 2012). Exclusion criteria are as follows: (1) natural or surgical menopausal status; (2) use of hormone replacement therapy (HRT); (3) not currently or recently (past 6 months) pregnant status; (4) not currently or recently (past 2 months) lactating females because breast feeding may influence CRP (Williams, Williams & Poulton, 2006); (5) history of gynecologic problems, such as fibroids, endometriosis, or polycystic ovary syndrome because these gynecologic problems have been reported to increase CRP (Escobar-Morreale, Luque-Ramírez, & González, 2011); (6) hormonal contraception use within the past three months, such as hormone patch and vaginal ring methods; and (7) IUDs with hormones and Depo-Provera within the past 12 months, which of all may increase inflammatory biomarkers (Gaskins et al., 2011; O'Connor et al., 2009). Those taking oral contraceptives will not be excluded from the study, but data on this variable will be collected so that it can be included as a covariate in the analysis.

Setting for data collection. The data will be collected by the PI at the Center for Nursing Research, and a biological sciences laboratory in the UTHSC-H SON. Data

collection will be performed in a CRF study room in the UTHSC-H SON. The room will be maintained between 68 and 76 degrees Fahrenheit, with a humidity level between 20 to 60 percent using a room temperature thermometer (Q-Track IAQ monitor, TSI Incorporated, Shoreview, MN) throughout the data collection period based on the Occupational Safety and Health Administration recommendations (2003).

Recruitment of subject and data collection procedure. Participants will be recruited by posting fliers in the UTHSC-H and surrounding communities of UTHSC-H as well as by sending e-mails to students and employees of the UTHSC-H. The recruitment will begin from the end of November 2012 through December 2013. Time frame of project activity is provided (Appendix F). A flyer and email will describe the need for healthy young adult volunteers, age 20-34 years, of all fitness and weight levels. The flyers will include a very brief description of the study, and will provide the PI's phone number and e-mail address for further information (Appendix A). If participants are interested in the study, they will contact the PI. The PI will provide detailed information on the study, including the purpose of the study, study procedures, data collection plan, and potential risks and benefits. During a telephone interview, the PI will screen participant's eligibility. When participants meet the inclusion criteria and they show an interest in participating in the study, they will be scheduled to attend a data collection session in the UTHSC-H SON. For preparation of data collection, participants will be instructed (1) not to eat after 8 pm the night or 12 hours before the testing; (2) not to do strenuous exercise, consume alcohol, or use anti-inflammatory drugs 24 hours prior to blood sampling; (3) to arrive at the UTHSC-H SON bioscience laboratory between 8am and 10 am; (4) to wear comfortable exercise-type clothing; (5) to avoid tobacco and

caffeine 3 hours prior to test; (6) to consume plenty of fluids; and (7) to obtain an adequate amount of sleep the night before the test (ACSM a, 2010; ACSM b, 2010). Because inflammatory markers are elevated during menstruation, women participants will be scheduled for a day when they are not menstruating (Gaskins et al., 2011). Before collecting the data, informed consent will be obtained by the PI. The PI will thoroughly explain and will clarify any information on this study. After obtaining informed consent, each participant will complete an interview guided pre-assessment health questionnaire (ACSM a, 2010). The questionnaire was designed to identify whether the participants are safe to test for CRF (ACSM a, 2010). If the participant will not be safe to perform CRF testing, he or she will be excluded such as high blood pressure, short of breath, chest pain, heart palpitation, heart murmur, fainting, back pain and intermittent leg pain. Once the participant is eligible to perform CRF testing, the eligible participant's blood test will be collected between 8am and 10am. After collecting blood samples, anthropometric characteristics (height, weight and waist circumference) will be measured. A light snack and water will be provided. After a brief resting period, the PI will conduct CRF testing. The CRF test will take 20-30 minutes of walking on the treadmill including warm-up and cool down stages. Participants who complete all procedures will be compensated with a \$10 gift card. All procedures will be conducted by the PI. The results of CRF levels, body anthropometric measurements and levels of inflammatory markers will be released for participants who show an interest in knowing their results. Procedures for obtaining informed consent and all protocols will be approved by the Committee for the Protection of Human Subjects at the UTHSC-H.

Instruments.

Demographic characteristics. The PI will complete pre-assessment questionnaire regarding demographic and health-related information, lifestyle, and activity levels from participant's self-report. The pre-assessment questionnaire was developed by ACSM (2010a). The demographic information includes information on age, gender, ethnicity, marital status, and education. Age, gender, ethnicity and education will be described participants' characteristics and be used as covariates in the multiple regression analysis.

Assessment of CRF. CRF will be assessed by a single-stage submaximal treadmill walking test (SSTWT). The SSTWT was developed by Ebbeling et al. (1991) for estimating VO_2max of low-risk, healthy adults 20 to 50 years. The SSTWT was validated by correlating the indirectly estimated VO_2max based on individual's walking speed, heart rate (HR), age and sex and the directly measured respiratory gas exchange (VO_2max) in the cross-validation group ($N = 22$) (Ebbeling et al., 1991). A correlation (r) of .96 was reported, with multiple correlation (R^2) of .86 ($\text{SEE} = 4.85 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) (Ebbeling et al., 1991).

Single-stage submaximal treadmill walking test protocol. The SSTWT consisted of a 4-minute warm-up stage, 4-minute workload stage, and a 2- 5minute cooldown stage. The treadmill will be calibrated before CRF test to ensure the accuracy of the test. Prior to beginning the SSTWT, participants will be familiarized with the treadmill (Precor 956i, Precor, Inc., Woodinville, WA), including a visual demonstration in order to maximize participant safety.

In the first warm-up stage, participants will walk on the treadmill for a 4-minute at 0% incline and a walking speed (2.0 to 4.5 mph) that brings the HR to between 50%

and 70% beats per minute (bpm) age-predicted maximal HR by the PI. In workload stage, the treadmill incline will increase to 5% at the same speed for a 4-to 5-minute. Following a workload stage, the participants will complete a cooldown stage at a slow walk and 0% incline for a 2-5minute. Resting BP and HR using the oscillometric technique (HEM-907SL, Omron Healthcare, Inc., Bannockburn, IL) will be assessed prior to a warm-up stage, twice and after a cool-down stage one time. If the participant's resting blood pressure $\geq 130/90$ mmHg or resting HR ≥ 100 per minute, the participant will not be allowed to have a SSTWT. Polar HR (Vintage NV model, Polar CIC, Inc., Port Washington, NY) will be recorded during the SSTWT protocol and the Borg Ratings of Perceived Exertion scale (RPE) will be documented at the end of the warm-up stage and the workload stage. The Borg Scale takes into account a participant's fitness level. It matches how hard he or she feels he or she is working with numbers from 6 (very, very light) to 20 (very, very hard) (Borg, 1970). Steady-state HR defines as a HR within ± 5 bpm during the last 2minute of the workload stage.

The main outcome of the CRF test will be estimated maximal oxygen uptake (VO_2max). The SSTWT prediction equation will be used to estimate VO_2max based on age, sex, walking speed (mph), and steady-state HR.

Estimated VO_2 max ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) = $15.1 + 21.8$ (speed in mph) - 0.327 (steady state HR in bpm) - 0.263 (speed x age in years) + 0.00504 (SS HR in bpm age in years) + 5.98 (sex; female = 0, male = 1)

Assessment of adiposity. Adiposity will be assessed using BMI and waist circumference. BMI will be calculated by dividing the participant's weight in kilograms by the height in meters squared (kg/m^2). Height will be measured without shoes to the

nearest 0.1 cm using a wall-mounted stadiometer (Accustat, Genentech Inc.). Weight will be measured to the nearest 1/4th lb with a calibrated balance beam scale. Height and weight will be measured with minimal clothing and without shoes and hats (NHANES, 2010). Waist circumference (WC) is a measure of the degree of central of body fat. WC will be measured to the nearest 0.1 cm using a flexible, nonstretchable tape. The tape measure will be placed just above the uppermost lateral border of the right ilium crest and will be extended around the waist in a horizontal plane (NHANES, 2010).

Measurement of hs-CRP, IL-6, and TNF- α . Prior to blood sample collection, the PI will ascertain that the participant is comfortable with instructions and procedures for collecting blood sample. A 10 ml fasting blood will be collected from an antecubital vein into two vacutainers containing ethylene diamine tetra acetic acid (EDTA) vacutainers by the PI. After collecting two EDTA vacutainers, the blood samples will be transported to the Bioscience Laboratory in the UTHSC-H SON on dry ice pellets with ice designed for frozen temperature to maintain that temperature. The blood samples will be immediately centrifuged at 1,630 relative centrifugal force (rcf) at -4°C for 30 minutes (Centrifuge5810R, Eppendorf AG Hamburg, Germany). After centrifugation, plasma samples will be divided into eight aliquots. Each cryo-tube will be labeled in numerical order of the participant study identification, date, year, and stored at -80°C for up to a maximum of one year.

Plasma hs-CRP will be determined by a sandwich ELISA (Alpco C-reactive protein (hs-CRP), EIA, Salem, N.H., USA). Plasma hs-CRP will present in milligram per liter (mg/L). Intra- and inter-assay coefficients of variation are less than 2%. Plasma IL-6 concentration will be measured using the BIOSOURCE Enzyme Amplified Sensitivity

Immunoassay (EASIA) kit (BioSource, Nivelles, Belgium). Plasma TNF- α will be quantified using a solid phase sandwich ELISA (ALPCO Diagnostics). Plasma IL-6 and TNF- α will be represent in pictogram per milliliter (pg/ml). Plates of biomarkers will be read on a microplate reader using absorbance (Bioteck instruments Inc., Winooski, VT). WorkOutTM software (version 2.5, Perkin Elmer Life and Analytical Sciences) will be used for analyzing assay results. Intra- and inter-assay coefficients of variation will be below 10%. All inflammatory biomarkers will be assayed at the Bioscience Laboratory at the UTHSC-H SON for this study.

Data Analysis

Statistical analyses will be performed by using statistical software SPSS version 22.0 (SPSS Inc., Chicago, IL). Descriptive statistics will be used to summarize characteristics of study participants, including mean, standard deviation, frequency and percentage. For continuous variables, histograms, box plots, and normality probability plot will be used to identify normality of distribution, outliers, and missing data.

Appropriate transformations will be performed where necessary. All analyses will be two-tailed and P-value < 0.05 will be considered statistically significant.

Separate analysis will be conducted with each of the inflammatory markers (hs-CRP, IL-6, and TNF- α) as the dependent variable in multiple linear regression analysis for aim 1: the strength of the association between CRF and inflammatory biomarkers, and the aim 2: the strength of the association between adiposity and inflammatory biomarkers. Analyses for aim 1 and 2 will be adjusted for possible confounders including age, sex, ethnicity and education.

Aim1. To determine the strength of the association between CRF levels with circulating levels of plasma hs-CRP, IL-6, and TNF- α . Hypothesis: Higher levels of CRF will be related to lower levels of circulating plasma hs-CRP, IL-6, and TNF- α .

Aim2. To determine the strength of the association between adiposity levels and circulating levels of plasma hs-CRP, IL-6, and TNF- α . Hypothesis: 2a. Higher levels of BMI will be related to higher levels of circulating plasma hs-CRP, IL-6, and TNF- α . 2b. Higher levels of waist circumference will be related to higher levels of circulating plasma hs-CRP, IL-6, and TNF- α .

Multiple linear regression models, with interaction terms (CRF $\times$ BMI) (CRF $\times$ waist circumference) will be examined to assess aim 3: the moderating effect of adiposity (moderator) on the strength of the association of CRF (independent variable) with inflammatory biomarkers (dependent variable). Analyses for aim 3 will be adjusted for possible confounders including age, gender, ethnicity and education.

Aim3. To test the moderating effect of adiposity on the strength of the association between CRF levels and circulating levels of plasma hs-CRP, IL-6, and TNF- α . Hypothesis: 3a. Individuals with higher levels of BMI and higher levels of CRF will have lower levels of circulating plasma hs-CRP, IL-6, and TNF- α compared with individuals with higher levels of BMI and lower levels of CR fitness. 3b. Individuals with higher waist circumference and higher levels of CRF will have lower levels of circulating plasma hs-CRP, IL-6, and TNF- α compared with individuals with higher waist circumference and lower levels of CRF.

Limitations

Firstly, a weakness of this study is the difficulty of establishing causal relationships from data collected in a cross-sectional time frame. Secondly, a non-probability sampling method in a university setting will limit generalization of the findings of this study. In spite of these limitations, the findings will provide important knowledge about inflammatory markers in young adults and will be used for designing longitudinal studies and interventions to improve cardiovascular health in this target population.

Facilities/Resources/Consultants

Center for Nursing Research for CR fitness and bioscience laboratory for handling and analyzing inflammatory biomarkers at the UTHSC-H SON are available for the conduct of this project. Additionally, statisticians to assist with data management and analysis are available. Lastly, fitness experts at UTHSC-H Recreation Center and School of Public Health are available for consultation.

Human Subjects Concerns

Protection of human subjects. Protection of human subjects' rights will be assured with obtaining appropriate approval from the University of Texas Health Science Center at Houston Committee for Protection of Human Subjects (UTHSC-H CPS). The PI will be trained on Collaborative Institutional Training Initiative (CITI) and iRIS. The PI will thoroughly explain the purpose and overall procedure of the study, and potential benefits and risks of participating in the study prior to obtaining informed consent. Participants will be informed that if they are unwilling to participate in this study, they can withdraw at any time. Participants will be allowed and encouraged to ask any

questions regarding the study before completing the informed consent process with a signature. No data will be collected before informed consent. Information regarding protection of privacy and confidentiality, and how the data will be handled with confidentiality will be provided to the participants.

Potential benefits and risk of the proposed study to the subjects. Participants may benefit from learning about physical inactivity, body fatness and weight as related to risk for cardiovascular disease. Participants will be informed of potential discomforts and risks as related to CR fitness and blood sampling. With regards to blood test, participants may feel some pain and have a bruise at the needle site. There might be a risk for developing an infection at the needle site but this is rare. In relation to CR fitness, participants may feel some discomforts during and after fitness test. These include dizziness and fainting but these effects are rare. The trained professional is available to assist with unusual situations that may arise. If participants are interested in their results, they will be received a copy of their results.

Data and safety monitoring plan. The PI will be responsible for monitoring and managing data safety and analysis procedures. Each participant will be given a study identification number. Forms and documents will be kept separate from the data file and kept in a locked cabinet. Data files and blood samples will be coded by the study identification numbers. Confidentiality of the data will be assured through coding systems without specific participant's identifiers. Protected data will be entered into a double password protected computer database with restricted access to the system.

Inclusion of minorities, women, and children. The target population of this proposed study is healthy young adults aged 20-34 years. This study population will

include diverse ethnic groups, such as African American, Asian, and Hispanic. This study will exclude children, adolescents, elderly, and pregnant women.

References

- Adiposity. (n.d.). In *Merriam-Webster's online dictionary* (11th ed.). Retrieved from <http://www.merriam-webster.com/medical/adiposity>
- American College of Sports Medicine (2010a). *ACSM's health-related physical fitness assessment manual*. 3rd ed. Philadelphia, PA: Lippincott, Williams & Wilkins.
- American College of Sports Medicine (2010b). *Guidelines for exercise testing and prescription*. 8th ed. Philadelphia, PA: Lippincott, Williams & Wilkins.
- Antuna-Puente, B., Feve, B., Fellahi, S., & Bastard, J. P. (2008). Adipokines: The missing link between insulin resistance and obesity. *Diabetes & Metabolism*, 34(1), 2-11. doi:10.1016/j.diabet.2007.09.004
- Bajpai, A., Goyal, A., & Sperling, L. (2010). Should we measure C-reactive protein on earth or just on JUPITER? *Clinical Cardiology*, 33(4), 190-198. doi: 10.1002/clc.20681
- Blair, S. N., Kohl, H. W., 3rd, Barlow, C. E., Paffenbarger, R. S., Jr, Gibbons, L. W., & Macera, C. A. (1995). Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *JAMA : The Journal of the American Medical Association*, 273(14), 1093-1098.
- Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitation Medicine*, 2(2), 92-98.
- Calabro, P., Golia, E., & Yeh, E. T. (2009). CRP and the risk of atherosclerotic events. *Seminars in Immunopathology*, 31(1), 79-94. doi: 10.1007/s00281-009-0149-4

- Caspersen, C. J., Powell, K. E., & Christenson, G. (1985). Physical activity, exercise and physical fitness: Definitions and distinctions for health-related research. *Public Health Reports*, 100(2), 126-131.
- Clearfield, M. B. (2005). C-reactive protein: A new risk assessment tool for cardiovascular disease. *The Journal of the American Osteopathic Association*, 105(9), 409-416. Retrieved from <http://www.jaoa.org/content/105/9/409.full.pdf+html>
- Cohen, J. (1998). Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Erlbaum.
- Craig, G. J. ,& Dunn, W. L. (2010). *Understanding human development*. 2nd ed. Upper Saddle River, NJ: Pearson Education, Inc
- CRP ELISA Kit for the in vitro determination of c-reactive protein in serum, plasma, stool and urine. (2012). ALPCO Diagnostic.
- Ebbeling, C. B., Ward, A., Puleo, E. M., Widrick, J., & Rippe, J. M. (1991). Development of a single-stage submaximal treadmill walking test. *Medicine and Science in Sports and Exercise*, 23(8), 966-973.
- Escobar-Morreale, H. F., Luque-Ramírez, M., & González, F. (2011). Circulating inflammatory markers in polycystic ovary syndrome: A systematic review and metaanalysis. *Fertility and Sterility*, 95(3),1048-1058.e2. doi: 10.1016/j.fertnstert.2010.11.036
- Ferri, C., Croce, G., Cofini, V., De Berardinis, G., Grassi, D., Casale, R., . . . Desideri, G. (2007). C-reactive protein: Interaction with the vascular endothelium and possible

role in human atherosclerosis. *Current Pharmaceutical Design*, 13(16), 1631-1645.

Franks, P. W., Hanson, R. L., Knowler, W. C., Sievers, M. L., Bennett, P. H., & Looker, H. C. (2010). Childhood obesity, other cardiovascular risk factors, and premature death. *The New England Journal of Medicine*, 362(6), 485-493.
doi:10.1056/NEJMoa0904130

Gaskins, A. J., Wilchesky, M., Mumford, S. L., Whitcomb, B. W., Browne, R. W., Wactawski-Wende, J., . . . Schisterman, E. F. (2012). Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: The BioCycle study. *American Journal of Epidemiology*, 175(5), 423- 431.

Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., . . . American Heart Association Statistics Committee and Stroke Statistics Subcommittee. (2013). Heart disease and stroke statistics--2013 update: A report from the american heart association. *Circulation*, 127(1), e6-e245. doi: 10.1161/CIR.0b013e31828124ad; 10.1161/CIR.0b013e31828124ad

Haddy, N., Sass, C., Drosch, S., Zaiou, M., Siest, G., Ponthieux, A., . . . Visvikis, S. (2003). IL-6, TNF- α and atherosclerosis risk indicators in a healthy family population: The STANISLAS cohort. *Atherosclerosis*, 170(2), 277-283. doi: 10.1016/S0021-9150(03)00287-9

Hamer, M. (2007). The relative influences of fitness and fatness on inflammatory factors. *Preventive Medicine*, 44(1), 3-11. Retrieved from <http://dx.doi.org.10.1016/j.ypmed.2006.09.005>

Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *The New England Journal of Medicine*, 352(16), 1685-1695. doi: 10.1056/NEJMra043430

Heyward, H. V. (2010). *Advanced fitness assessment and exercise prescription*. 6th ed. Champaign, IL; Human Kinetics.

IL-6 EIA for the quantitative determination of IL-6 in serum, plasma, buffered solution or cell culture medium.(2008). ALPCO Diagnostic.

Isasi, C. R., Deckelbaum, R. J., Tracy, R. P., Starc, T. J., Berglund, L., & Shea, S. (2003). Physical fitness and C-reactive protein level in children and young adults: The Columbia university BioMarkers study. *Pediatrics*, 111(2), 332-338.

Kuo, H. K., Yen, C. J., Chen, J. H., Yu, Y. H., & Bean, J. F. (2007). Association of cardiorespiratory fitness and levels of C-reactive protein: Data from the National Health and Nutrition Examination Survey 1999-2002. *International Journal of Cardiology*, 114(1), 28-33. doi:10.1016/j.ijcard.2005.11.110

Libby, P., Ridker, P. M., & Hansson, G. K. (2009). Inflammation in atherosclerosis: From pathophysiology to practice. *Journal of the American College of Cardiology*, 54(23), 2129-2138. doi:10.1016/j.jacc.2009.09.009

Loria, C. M., Liu, K., Lewis, C. E., Hulley, S. B., Sidney, S., Schreiner, P. J., . . . Detrano, R. (2007). Early adult risk factor levels and subsequent coronary artery calcification: The CARDIA study. *Journal of the American College of Cardiology*, 49(20), 2013-2020. doi:10.1016/j.jacc.2007.03.009

National Health and Nutrition Examination Survey (NHANES) (2009). Anthropometric procedures manual. Center for Disease Prevention. Retrieved from

www.cdc.gov/nchs/data/nhanes/nhanes_03_04/BM.pdf

nQuery Advisor (2011). nQuery advisor sample size software. Saugus, MA: Statistical Solution.

O'Connor, M. F., Bower, J. E., Cho, H. J., Creswell, J. D., Dimitrov, S., Hamby, M.

E., . . . Irwin, M. R. (2009). To assess, to control, to exclude: Effects of biobehavioral factors on circulating inflammatory markers. *Brain, Behavior, &*

Immunity, 23(7), 887-897. Retrieved from

<http://dx.doi.org/10.1016/j.bbi.2009.04.005>

Occupational Safety and Health Administration (2003). OSHA Policy on Indoor Air Quality: Office Temperature/Humidity and Environmental Tobacco Smoke.

Retrieved from

http://www.osha.gov/pls/oshaweb/owadisp.show_document?ptable=INTERPRETATIONS&p_id=24602

Packard, R. R., & Libby, P. (2008). Inflammation in atherosclerosis: From vascular

biology to biomarker discovery and risk prediction. *Clinical Chemistry*, 54(1), 24-38. doi:10.1373/clinchem.2007.097360

Park, E. & Meininger, J. C. Effects of exercise interventions on inflammatory markers in young and middle aged adults: A systematic review (In preparation for submission).

- Park, H. S., Park, J. Y., & Yu, R. (2005). Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. *Diabetes Research and Clinical Practice*, 69(1), 29-35. doi:10.1016/j.diabres.2004.11.007
- Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O., 3rd, Criqui, M., . . . American Heart Association. (2003). 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*, 107(3), 499-511.
- Petersen, A. M., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, 98(4), 1154-1162. doi:10.1152/jappphysiol.00164.2004
- Rocha, V. Z., & Libby, P. (2009). Obesity, inflammation, and atherosclerosis. *Nature Reviews.Cardiology*, 6(6), 399-409. doi:10.1038/nrcardio.2009.55
- Schuett, H., Luchtefeld, M., Grothusen, C., Grote, K., & Schieffer, B. (2009). How much is too much? interleukin-6 and its signalling in atherosclerosis. *Thrombosis and Haemostasis*, 102(2), 215-222. doi:10.1160/TH09-05-0297
- SPSS. (2011). Statistical package for the social sciences. Chicago, IL:IBM.
- Stevens, J., McClain, J. E., & Truesdale, K. P. (2008). Selection of measures in epidemiologic studies of the consequences of obesity. *International Journal of Obesity (2005)*, 32 Suppl 3, S60-6. doi:10.1038/ijo.2008.88
- TNF- α ELISA for the quantitative determination of TNF- α in serum, plasma, buffered solution or cell culture medium. (2011). ALPCO Diagnostic.

- Wang, S., Reed, D. B., Goli, S., & Goswami, D. (2011). Blood leptin and C-reactive protein provide more sensitive assessment than blood lipids and other inflammatory biomarkers in overweight university students. *Nutrition Research*, 31(8), 586-593. doi:10.1016/j.nutres.2011.07.006
- WHO (2000). Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization Technical Report Series, 894, i-xii, 1-253.
- Williams, M. J., Milne, B. J., Hancox, R. J., & Poulton, R. (2005). C-reactive protein and cardiorespiratory fitness in young adults. *European Journal of Cardiovascular Prevention and Rehabilitation: Official Journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology*, 12(3), 216-220.
- Williams, M. J. A., Williams, S. M., & Poulton, R. (2006). Breast feeding is related to C reactive protein concentration in adult women. *Journal of Epidemiology and Community Health*, 60(2), 146-148, doi: 10.1136/jech.2005.039222
- Wilson, A. M., Ryan, M. C., & Boyle, A. J. (2006). The novel role of C-reactive protein in cardiovascular disease: Risk marker or pathogen. *International Journal of Cardiology*, 106(3), 291-297. doi: 10.1016/j.ijcard.2005.01.068

Anthropometric Adiposity Measures and Inflammatory Biomarkers in

Adolescents and Young Adults: A Systematic Review

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Abstract

Background

Overweight and obesity are major risk factors for cardiovascular disease (CVD) morbidity and mortality. Low- grade chronic inflammation may be a mediating factor in the association of overweight and obesity with development of atherosclerosis and higher risk for CVD and CVD events. C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α) are well-recognized markers of inflammatory response to atherosclerosis.

Purpose

The purpose of the systematic review was to determine the associations between anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF%) and inflammatory biomarkers (CRP, IL-6, and TNF- α) in adolescents and young adults.

Methods

The electronic databases Ovid MEDLINE, CINAHL, and SCOPUS were searched for studies published between 1946 and July 2013. Inclusion criteria were (a) adolescents and young adults aged 13 to 40 years without a known diagnosis of diseases (b) examined the association between at least one anthropometric adiposity measures and CRP, IL-6, and /or TNF- α , and (c) published study in English language.

Results

Of 4,876 identified publications, 176 were retrieved for detailed evaluation and 28 met inclusion criteria. There is a significant and positive association between anthropometric adiposity measured by total and central adiposity (BMI, WC, WHtR, WHR, and BF%) and CRP in adolescents and young adults in 22 out of 23 studies (96%). Findings of

anthropometric adiposity measures (BMI, WC, WHR, and BF%) and IL-6 and TNF- α in adolescents and young adults remain inconclusive. No single anthropometric adiposity measures in adolescents and young adults would appear to be superior in relation to CRP, IL-6 and TNF- α .

Conclusions

The evidence supports that anthropometric adiposity measures are positively associated with CRP in adolescents and young adults. However, anthropometric adiposity measures and IL-6 and TNF- α in adolescents and young adults is inconclusive. Additional studies are needed to identify the strength and patterns of association of anthropometric adiposity measures with CRP, IL-6 and TNF- α . Further studies are needed to identify sex and ethnicity differences in the association of anthropometric adiposity measures and inflammatory biomarkers.

Keywords: body mass index; waist circumference; waist to hip ratio; waist to height ratio; body fat percentage; c-reactive protein; interleukin-6; tumor necrosis factor- α ; adolescents; young adults

Anthropometric Adiposity Measures and Inflammatory Biomarkers in Adolescents and Young Adults: A Systematic Review

Atherosclerosis is a systemic disease process in which accumulation of plaque builds up in the arterial wall and a main pathophysiological determinant of clinical cardiovascular events, morbidity and mortality (Go et al., 2013). It has been shown that chronic, low-grade inflammation plays an important role in the initiation and progression of atherosclerosis by impairing endothelial function (Libby, Ridker, & Hansson, 2009; Widlansky, Gokce, Keaney, & Vita, 2003). The endothelial dysfunction promotes development, progression, and clinical expression of atherosclerosis, such as an acute atherosclerotic event (Bucdkley, Rongwei, Freeman, Rogers, & Helfand, 2009; Packard & Libby, 2008; Wildansky et al., 2003). C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) are well-recognized biomarkers of inflammation in relation to atherogenesis (Libby et al., 2009). CRP, a primitive acute phase inflammatory protein, plays an essential role in innate immune response and inflammatory processes (Wilson, Ryan, & Boyle, 2006). CRP is primarily produced in the liver and is synthesized by hepatocytes in response to inflammatory cytokines, mainly IL-6 (Wilson et al., 2006). IL-6 is a pro-inflammatory cytokine that play a major role in the acute phase response in relation to infection and is produced by various cells, such as fibroblasts, endothelial cells, monocytes, and adipocytes (Schuett, Luchtefeld, Grothusen, Grote, & Schieffer, 2009). The important role of IL-6 is controlling hepatic production of inflammatory proteins, including CRP (Schuett et al., 2009). TNF- α is a pro-inflammatory cytokine that is secreted by various cells, such as macrophages, lymphocyte, and adipocytes (Petersen & Pedersen, 2005).

The prevalence of overweight and obesity is a significant public health problem worldwide, and it has grown substantially over the past few decades (Lobstein, Baur, & Uauy, 2004; World Health Organization [WHO], 2012). It is estimated that worldwide obesity has more than doubled since 1980 (WHO, 2012). Overweight and obesity are considered major risk factors for cardiovascular disease (CVD) mortality and morbidity (Roger et al., 2012; WHO, 2012). The underlying mechanisms for the relationship of overweight and obesity with CVD have not been fully elucidated. A growing body of evidence indicates that chronic inflammation may be a mediating factor in the association of obesity with development of atherosclerosis (Mathieu, Lemieux & Deprès, 2010; Rocha & Libby, 2009; Wang & Nakayama, 2010). More specifically, excess adipose tissue induces pro-inflammatory cytokines, including IL-6 and TNF- α (Calabrò et al., 2009) which precipitate the release of CRP from the liver (Wang & Nakayama, 2010; Yudkin, Stehouwer, Emeis & Coppack, 1999). In this way, obesity itself promotes inflammatory process which induces endothelial dysfunction and plaque formation, thus contributing atherosclerosis and higher risk for future CVD and CVD events (McGill, McMahan, Herderick et al., 2002; Meyers & Gokce, 2007; Ikeoka, Mader, & Pieber, 2010).

Albeit clinical symptoms related to atherosclerosis, such as chest pain or discomfort, shortness of breath or fatigue, numbness, and blurred vision commonly are not detected in adolescents and young adults, atherosclerotic changes in the vessel wall already begin early in life, and these changes progress and predispose to the development of atherosclerotic CVD and acute CVD events in middle-aged and older adult populations (Järvisalo et al., 2002). In addition, the growing overweight and obesity in adolescents

and young adults accelerate atherosclerotic inflammatory processes (McGill, McMahan, Herderick et al., 2002; Rocha & Libby, 2009). Therefore, knowledge of inflammatory biomarkers in relation to adiposity in adolescents and young adults may lead to new avenues to deter atherosclerotic processes that increase the risk of atherosclerotic CVD and acute CVD events in middle and older adults.

Although dual-energy x-ray absorptiometry (DEXA), computed tomography (CT), magnetic resonance imaging (MRI), and underwater weighing are more precise and reliable measures of adiposity, these require greater cost and are not feasible in routine public health and clinical practices (Stevens et al., 2008). For these reasons, adiposity has been assessed by anthropometric measures (Body mass index [BMI], body fat percentage [BF%], waist circumference [WC], waist to hip ratio [WHR], and waist to height ratio [WHtR]) in public health and clinical practices. BMI is the most widely used anthropometric measure and is calculated as body weight in kilograms divided by height in meters squared (kg/m^2) (Cornier et al., 2011). BF%, which can be estimated from measurement of skinfold thickness or bioelectrical impedance, is also used for assessing fat mass, or adiposity, as a proportion of total body mass (Stevens, McClain, & Truesdale, 2008). BMI and BF% are measures of total adiposity whereas WC, WHR, and WHtR are measures of central adiposity (Cornier et al., 2011). WC has been measured in different ways. The National Institutes of Health National Heart, Lung, and Blood Institute (HNHLB) (2000), currently recommends using an inelastic tape in a horizontal plane at the end of normal expiration in United States for the measurement of WC. The WHO (2011) recommends the use of the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. WHR is the circumference of the waist divided

by the circumference of the hip and has been used for assessing body fat distribution (Cornier et al., 2011). Hip circumference is measured at the level of the widest portion of the buttocks (Cornier et al., 2011; WHO, 2011). WHtR is the circumference of the waist divided by the height (Cornier et al., 2011).

To date, there is only one systematic review and meta-analysis addressing the obesity and CRP in various populations (Choi, Joseph, & Pilote, 2012); however, this review only focused on the CRP as a biomarker of inflammation. It is important to evaluate other inflammatory markers, such as IL-6 and TNF- α in order to better understand inflammatory processes in relation to anthropometric total and central adiposity measures. Therefore, the purpose of the systematic review was to determine the associations between anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF%) and inflammatory biomarkers (CRP, IL-6, and TNF- α) in adolescents and young adults without a known diagnosis of diseases. The goal of this systematic is to provide better understanding of the evidence linking anthropometric adiposity measures with inflammatory processes in young individuals free of co-morbidities. This review may provide an insight for early intervention strategies, such as a lifestyle modification and weight loss for improving long-term outcomes of reducing inflammation as related to adiposity. Additionally, this systemic review identifies gaps in current knowledge and provides future directions for research and practice.

Methods

Literature Search

A systematic literature search was conducted to identify potentially relevant studies examining the association between anthropometric adiposity measures and

circulating levels of CRP, IL-6, and TNF- α in adolescents and young adults. The electronic databases Ovid MEDLINE (from 1946 to July 03, 2013), CINAHL (from 1995 to July 05, 2013), and SCOPUS (from 1960 to July 05, 2013) were searched. Reference lists of articles that met the inclusion criteria were searched manually for any additional studies. Search terms included Medical Subject Headings (MeSH) and keywords related to anthropometric adiposity measures (“body mass index,” “waist circumference,” “abdominal obesity,” “abdominal adiposity,” “abdominal diameter,” “waist to hip ratio,” “waist to height ratio,” “skinfold thickness,” “body fat percentage,” “body fat distribution,” “body composition”); inflammatory markers (“inflammation,” “C-reactive protein”, “interleukin-6”, “tumor necrosis factor-alpha”); and “adolescent,” “teen,” “university student,” “college student,” and “young adult.” Each category of variables was combined with each population using the “OR” Boolean operator. The variables and population were then combined using the “AND” Boolean operator. Literature searches were limited to the English language and human subjects. The process of the search strategy is shown in Figure 1.

Inclusion and Exclusion Criteria

Inclusion criteria were: (1) adolescents and young adults aged 13 to 40 years; (2) investigated the association between at least one anthropometric adiposity measures and CRP, IL-6, and/or TNF- α ; (3) anthropometric adiposity measured by BMI, WC, WHR, WHtR, and/or body fat percentage assessing a skinfold thickness or bioelectrical impedance ; (4) CRP, IL-6, and TNF- α levels measured from blood samples; (5) all types of research designs; (6) an original study; (7) no country restriction; and (8) published study in English language. Exclusion criteria were: (1) adolescent and young adults with

diagnosed diseases and infectious diseases; (2) pregnant women; (3) adiposity measured only by DEXA or MRI; (4) a review or an editorial article; and (5) an abstract.

Data Extraction and Synthesis

This review extracted year of publication, country, participants, setting, design, study period, anthropometric measures, measures of CRP, IL-6 and TNF- α , results, analysis and significance of the association, and adjustment of covariates (Table 1 and Table 2). Mean, range, and proportion of age, sex, ethnicity, BMI, and sample size were reported. The statistical analyses and significance of associations or correlations between anthropometric adiposity measures and CRP, IL-6 and TNF- α for each study were presented as odds ratios, correlation coefficients, and/or regression coefficients with *p*-value and 95% confidence interval if original studies were reported in this way. If the results of the studies were not reported in this way, the study findings were summarized for this review. Due to variation in growth rates, hormonal changes and pubertal stage of adolescents and young adults, the two groups were considered separately in this review.

Study Quality Assessment

Study method and quality were assessed using an evaluation checklist, which was developed by the first author based on the guidelines for assessing observational studies (Pocock et al., 2004; von Elm et al., 2007; Zaccai, 2004). This evaluation checklist included 11 questions to evaluate the main issues and potential biases related to design, sampling, setting, measurements, analyses, and peer-review of each study (Table 3 and Table 4). Each item was scored as “Yes=1,” “No=0,” and “Not reported=0”, allowing the range of the score to be 0 to 11.

Results

Study Selection

As can be seen in Figure 2, a total of 4,876 studies were found from the Ovid MEDLINE, CINAHL, SCOPUS databases, and manual search of references. Of these, 264 studies were identified by title and abstract. After the removal of duplicates, 176 relevant studies remained for further detailed evaluation of the full text. Of the 176 studies, 139 studies were excluded: 61 were not relevant to the research questions; 42 studies were excluded for not meeting the criteria of age 13-40 years; 27 did not report associations or correlations between anthropometric adiposity measures and inflammatory markers; in four studies adiposity was measured by only DEXA or MRI; and in two studies participants had diagnosed diseases. In addition, two studies were excluded since dependent variables were not related to inflammatory biomarkers (CRP, IL-6, and TNF- α). Three cross-sectional studies were excluded due to same data from the same study cohort were collected. Four studies were a review or editorial and two studies reported an abstract only. Lastly, one systematic review and meta-analysis was excluded (Choi, Joseph & Pilote, 2013). Thus, 28 studies were included in this systematic review. The specific search strategies are illustrated in Figure 2.

Study Characteristics

Of 28 studies, six were conducted in United States, 17 were conducted in Australia, Egypt, Germany, Spain, Brazil, Italy, United Kingdom, Columbia, Finland, and New Zealand, and five studies were conducted in Korea, China, Philippines and India. Age of participants ranged from 13 to 39 years. The studies enrolled a total of 15,862 participants; 4,859 adolescents and 11,003 young adults. Sample size ranged from

30 to 3,289 per study. Of the 28 studies, five studies consisted of males only (Jung et al., 2008; Vikram et al., 2006; Bo, Raspo, Morra, Isaia et al., 2004; Bo, Raspo, Morra, Cassader et al., 2004; Perez et al., 2003), four studies consisted of females only (El-Wakkad et al., 2013; Serrano et al., 2010; Brydon et al., 2008; Morrison et al., 2011), and 19 studies consisted of both males and females. Of the 28 studies, nine studies reported participant's ethnicity (Huang et al., 2011; Jung et al., 2008; Khan et al., 2010; Moon et al., 2004; Petty et al., 2010; Brydon et al., 2008; Mills et al., 2008; Morrison et al., 2011; Wang, Christoffel et al., 2011).

For study design, only one out of 28 studies had a prospective design (Khan et al., 2010), whereas the other remaining 27 studies used cross sectional designs. Although the data of the two studies were from the same study cohort (McDade et al., 2009; McDade et al., 2011), this review included these two studies because the outcomes of the inflammatory biomarkers are different; One study focused on CRP only (McDade et al., 2009) and the other study examined IL-6 only (McDade et al., 2011).

Of the 28 studies, nine studies analyzed data combining both males and females (Herder et al., 2007; Khan et al., 2010; Martinez-Gomez et al., 2010; Moon et al., 2004; Hermsdorff et al., 2011; McDade et al., 2009; McDade et al., 2011; Mills et al., 2008; Orri et al., 2010). Eight studies conducted a separate analysis for males and females (Denney-Wilson et al., 2008; Huang et al., 2011; Petty et al., 2010; Wang et al., 2011; Wärnber et al., 2006; Nazmi et al., 2008; Raitakari et al., 2005; Williams et al., 2002). Two studies reported data for a combined analysis for males and females as well a separate analysis for males and females (Hermsdorff et al., 2012; Wang, Reed et al., 2011). In addition, none of the studies reflected ethnic influences or ethnic differences in

the association between anthropometric adiposity and inflammatory biomarkers in this review. Two studies reported ethnicity, but they did not determine the association between anthropometric adiposity and inflammatory biomarkers (Khan et al., 2010; McDade et al., 2011). The association between adiposity and inflammatory biomarkers was analyzed using odds ratio, Pearson coefficient, Spearman coefficient, or regression coefficient. The detailed characteristics of each study were summarized in Table 1 and Table 2.

Anthropometric Adiposity Measures

Anthropometric adiposity was assessed by BMI, WC, WHR, WHtR, and BF. The BF (mm) or (%) was examined by sum of skinfold thickness using a skinfold caliper or by bioelectrical impedance. Of the total 28 studies, 24 studies included two or more anthropometric measures; two studies used BMI only (Khan et al., 2010; McDade et al., 2011); one study used WC only (Jung et al., 2008); and one study used BF% only (Hermsdorff et al., 2012). Twenty six studies considered adiposity as a continuous variable and two studies analyzed adiposity as a categorical variable (Denney-Wilson et al., 2008; Nazmi et al., 2008). Of these two studies, one study categorized BMI into non-obese, overweight and obesity based on the International Obesity Task Force and the United Kingdom Child Growth Foundation Charts (Denney-Wilson et al., 2008) and the other study categorized BMI into WHO recommendation: $< 18.5\text{kg/m}^2$ (underweight), $18.5\text{-}24.9\text{ kg/m}^2$ (normal), $25.0\text{-}29.9\text{ kg/m}^2$ (overweight), and 30kg/m^2 (obese) (Nazmi et al., 2008) (Table 1 and Table 2).

Measurement of CRP, IL-6 and TNF- α

CRP was measured using diverse immunoassay tests. Five studies used enzyme-linked immunosorbent assay (ELIZA), 5 studies used immune-turbidimetry, 2 studies used high-sensitivity immunoassay, 2 studies used automated DPC immulite chemiluminescent immunoassay, and other studies used ultrasensitive assay, high sensitive-monoclonal antibody assay, latex enhanced immune-turbidimetry, sandwich immunoassay, high sensitivity-Denka-Seiken assay, high sensitivity-latex enhanced immune-nephelometric assay and Cardio high sensitivity-CRP assay. For IL-6, 6 studies used ELIZA, and other studies used high sensitivity-human cytokine, and high sensitivity-multiplex immunoassay. For TNF- α , 4 studies used ELIZA. Of the 28 studies, 21 studies have an overnight fast.

Study Quality Assessment

None of the studies met all of the evaluation quality criteria. Of the 28 studies, eight studies met high quality (10 out of 11); eight studies met nine out of 11; six studies met eight out of 11; five studies met seven out 11; and one studies met low quality (six out of 11). Of the 28 studies, 25 studies reported study designs either in method section, discussion or limitation section. Of the 28 studies, 10 studies did not specify inclusion and exclusion criteria, and only one study reported a sample size estimate (Nazmi et al., 2008). Of the 28 studies, six studies (Jung et al., 2009; Bo et al., 2004; Mills et al., 2008; Morrison et al., 2011; Orri et al., 2010; Raitakari et al., 2005) did not report how anthropometric adiposity was measured in detail and three studies (Hermsdorff et al., 2012; Hermsdorff et al., 2011; McDade et al., 2009) provided protocols and guidelines on the measurements of anthropometric adiposity in their reference section (Table 3, Table

4). Of the 28 studies, 12 adjusted for covariates in data analysis. Covariates included age, gender, ethnicity, lipids, family income, physical activity, pubertal status, and homeostasis model assessment of insulin resistance (HOMA-IR) in adolescent studies, whereas, age, gender, smoking status, physical activity, skin color, family income, years of education were included in young adult studies.

Associations of Anthropometric Adiposity Measures with Inflammation in Adolescents

Thirteen studies identified associations between anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF) and inflammatory biomarkers (CRP, IL-6, and TNF- α) in adolescents. The descriptive values on anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF) and inflammatory biomarkers (CRP, IL-6, and TNF- α) were reported in Table 1.

Anthropometric adiposity and CRP. Of these 13 studies, 10 studies examined the association of anthropometric adiposity measures and CRP levels (Denney-Wilson et al., 2008; Huang et al., 2011; Jung et al., 2009; Khan, Rieder, Cohen, Coupey, & Wildman, 2010; Martinez-Gomez et al., 2010; Petty et al., 2010; Serrano et al., 2010; Vikram et al., 2006; Wang, Christoffel et al., 2011; Wärnberg et al., 2006). Of the 10 studies, nine studies found significantly positive associations between one or more anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF) and CRP (Denney-Wilson et al., 2008; Huang et al., 2011; Jung et al., 2009; Khan, Rieder, Cohen, Coupey, & Wildman, 2010; Martinez-Gomez et al., 2010; Petty et al., 2010; Vikram et al., 2006; Wang, Christoffel et al., 2011; Wärnberg et al., 2006). However, one study did not find any significant association of BMI and WC with CRP (Petty et al., 2010). Of the

nine studies, five studies included males and females separately (Denney-Wilson et al., 2008; Huang et al., 2011; Petty et al., 2010; Wang, Christoffel et al., 2011; Wärnberg et al., 2006). Of the five studies, four studies revealed that there were significantly positive associations between two and more anthropometric adiposity measures (BMI, WC, WHtR, and BF) and CRP in both males and females (Denney-Wilson et al., 2008; Huang et al., 2011; Wang, Christoffel et al., 2011; Wärnberg et al., 2006). However, one study revealed that there were significantly positive associations of BMI and WC with CRP only for females but not males (Petty et al., 2010).

Anthropometric adiposity and IL-6. Of these 13 studies, three studies determined associations of anthropometric adiposity measures (BMI, WC, and BF) and IL-6 levels (Herder et al., 2007; Martinez-Gomez et al., 2010; Petty et al., 2010). Only one study revealed that there were significantly positive associations between anthropometric adiposity measures (BMI and WC) and IL-6 levels (Herder et al., 2007). In contrast, the remaining two studies revealed that no significant association between anthropometric adiposity measures and IL-6 (Martinez-Gomez et al., 2010; Petty et al., 2010). Of the three studies, only one study included a separate analysis for males and females (Petty et al., 2010). This study found that there were no significant associations of BMI and WC with IL-6 in males or females (Petty et al., 2010).

Anthropometric adiposity and TNF- α . Of these 13 studies, three examined associations of anthropometric adiposity measures (BMI, WC, WHR, and BF) and TNF- α . Two studies found that there were significant positive associations of BMI, WC, and WHR with TNF- α (El-Wakkad et al., 2013; Moon et al., 2004). However, one study did

not find significant associations of BMI and WC with TNF- α (Herder et al., 2007). None of these studies included sex differences.

Association of Anthropometric Adiposity Measures and Inflammation in Young Adults

Fifteen studies found associations between anthropometric measures (BMI, WC, WHR, WHtR, and BF) and inflammatory biomarkers (CRP, IL-6, and TNF- α) in young adults. The descriptive values on anthropometric adiposity measures (BMI, WC, WHR, WHtR, and BF) and inflammatory biomarkers (CRP, IL-6, and TNF- α) were reported in Table 2.

Anthropometric adiposity and CRP. Of the 15 studies, 13 studies determined the association of anthropometric adiposity measures and CRP (Bo, Raspo, Morra, Cassader, et al., 2004; Bo, Raspo, Morra, Isaia, et al., 2004; Hermsdorff et al., 2012; Hermsdorff, Zulet, Puchau, & Martinez, 2011; McDade et al., 2009; Mills et al., 2008; Morrison et al., 2011; Nazmi et al., 2008; Orri, Carter, & Howington, 2010; Perez et al., 2003; Raitakari et al., 2005; Wang, Reed, Goli, & Goswami, 2011; Williams et al., 2002). All of the 13 studies found that there were significant positive associations between one or more anthropometric adiposity measures (BMI, WC, WHR, and BF) and CRP. Of the 13 studies, five studies included a sex differences (Hermsdorff et al., 2012; Nazmi et al., 2008; Raitakari et al., 2005; Wang, Reed et al., 2011; Williams et al., 2002). Of the five studies, four found that there were significantly positive associations between two or more anthropometric adiposity measures (BMI, WC, WHR, and BF) and CRP in both males and females (Hermsdorff et al., 2012; Nazmi et al., 2008; Raitakari et al., 2005; Williams et al., 2002). However, remaining one study showed that there were significant

positive associations of BMI and BF% with CRP in females but not males (Wang, Reed et al., 2011).

Anthropometric adiposity and IL-6. Of these 15 studies, five studies focused on associations between anthropometric adiposity measures (BMI, WC, WHR, and BF%) and IL-6. Of the five studies, four reported a significant positive associations between one or more anthropometric adiposity measures (BMI, WHR, and BF%) and IL-6 (Brydon et al., 2008; Hermsdoff et al., 2011; McDade et al., 2011; Mills et al., 2008). However, one study did not find significant associations of BMI and BF% with IL-6 (Wang, Reed et al., 2011). Of the five studies, sex differences were examined only in one study, in which no significant associations of BMI and BF% with IL-6 in either males or females were found (Wang, Reed et al., 2011).

Anthropometric adiposity and TNF- α . Of these 15 studies, only one study examined associations between anthropometric adiposity measures (BMI and BF%) and TNF- α (Wang, Reed et al., 2011). The study did not find significant associations of BMI and BF% with TNF- α (Wang, Reed et al., 2011). In sex differences, the study did not find significant associations of BMI and BF% with TNF- α in either males or females (Wang, Reed et al., 2011).

Discussion

Synthesis of Main Findings and Knowledge

The major finding of this systematic review is that there is a significant and positive association between anthropometric adiposity measured by total and central adiposity (BMI, WC, WHtR, WHR, and BF%) and CRP in adolescents and young adults in 22 out of 23 studies (96%). These positive associations are consistent with the findings

of a previous review on the positive association between abdominal adiposity and CRP in adults (Brooks et al., 2010) and a previous systematic review and meta-analysis on obesity and CRP in various populations (Choi, Joseph, & Pilote, 2012). In addition, elevated circulating levels of CRP were found in obese participants when compared with non-obese counterparts, which supported evidence for an association of obesity and CRP (Denney-Wilson et al., 200; Nazmi et al., 2008) ($p < 0.05$). The finding of a significant association between obesity and CRP is in agreement with previous results showing that weight loss was associated with a decline in circulating levels of CRP in obese individuals aged 20 to 46 years (Esposito et al., 2003) and systematic review (Selvin, Paynter, & Erlinger, 2007) which provide additional supportive evidence for a link of obesity and CRP. Only one study did not indicate any significant associations between the anthropometric measures (BMI, WC, WHR, and BF%) and CRP in female adolescents in Brazil (Serrano et al., 2010). This study did not report detailed information about the measurement of CRP assay and coefficients of variation (CV), and did not discuss any limitations regarding the findings.

The present systematic review examined additional inflammatory biomarkers, including IL-6 and TNF- α and anthropometric adiposity. The findings of the review in the associations between anthropometric adiposity measures (BMI, WC, WHR, and BF%) with IL-6 from eight studies and TNF- α from four studies in adolescents and young adults have shown inconsistent results. Of the eight studies of IL-6, five studies revealed significant positive associations ($p < 0.05$) in adolescents (Herder et al., 2007) and young adults (Brydon et al., 2008; Hermsdoff et al., 2011; McDade et al., 2011; Mills et al., 2008), whereas three studies showed no significant associations in adolescents

(Martinez-Gomez et al., 2010; Petty et al., 2010) and young adults (Wang, Reed et al., 2011). Of the four studies in TNF- α , two studies revealed significantly positive associations ($p < 0.05$) in adolescents (El-Wakkad et al., 2013; Moon et al., 2004) and two studies found no significant associations in adolescents and young adults (Herder et al., 2007; Wang et al., 2011). One of the possible reasons for the discrepancy may be due to the different sampling strategies. For instance, some studies consisted primarily of non-obese participants (Herder et al., 2007; Martinez-Gomez et al., 2010; Petty et al., 2010; Wang, Reed et al., 2011), whereas other studies had a preponderance of obese participants (El-Wakkad et al., 2013; Moon et al., 2004). The latter might be better able to identify an obesity-induced inflammatory association than the former. Another possible reason for the discrepant findings may be attributable to low blood concentrations of IL-6 and TNF- α if some studies have participants who are mostly from a non-obese population. Of studies showing the positive association of anthropometric measures with CRP, IL-6 and TNF- α in adolescents and young adults, there was no single anthropometric adiposity measure that suggested a stronger correlation than others with CRP, IL-6 and TNF- α in adolescents and young adults.

Sex

Sex has been reported to influence the association between anthropometric adiposity measures and inflammatory biomarkers. For sex differences, only two studies reported there were significant positive associations of anthropometric adiposity measures (BMI, WC, and BF%) with CRP in female but not males (Petty et al., 2010; Wang et al., 2011). The findings are congruent with previous systematic review and meta-analysis showing that r for BMI and $\ln(\text{CRP})$ was greater in women than men by

0.24 (CI, 0.09-0.37) in adults (Choi, Joseph, & Pilote, 2012). In a previous population-based study of middle-aged men and women, adiposity was strongly associated with higher CRP levels in women as compared to men (Thorand et al., 2006). It could be explained in several ways. First of all, women have more total adiposity and subcutaneous fat than men (Lohman, 1981). In addition, women have higher levels of CRP as compared to men (Khera et al., 2005). Moreover, women who take oral contraceptives have higher levels of CRP compared with those taking a placebo (Eilertsen et al., 2005). Furthermore, women during menses have higher levels of CRP (median, 0.74mg/L), than CRP during the follicular phase of the menstrual cycle and CRP during ovulation is lowest (Gaskins et al., 2011). In these cases, study designs could control use of oral contraceptives as a covariate. Also, data on CRP could be collected at times other than the menstrual phase. Thus, the associations between anthropometric adiposity measures and inflammatory biomarkers might be modified by sex. However, further studies are needed to identify sex differences while controlling use of oral contraceptives and menses. None of studies determined sex differences in anthropometric adiposity measures and IL-6 and TNF- α .

Ethnicity

For ethnicity differences, none of the studies reflected ethnic and race differences in associations between anthropometric adiposity measures and inflammatory markers in this review. Only two studies out of 28 studies reported ethnicity, but these studies did not determine differences by ethnicity (Khan et al., 2010; Mills et al., 2008). Previous systematic review and meta-analysis found that the association (r) between obesity (BMI) and ln (CRP) was greater in North Americans/Europeans than Asians by 0.15 (CI, 0-

0.28), which indicated that ethnicity may be a potential moderator between anthropometric adiposity measures and inflammatory markers. Therefore, information on ethnicity should be obtained and tested as a moderating variable in the analysis of future studies.

Study Methodological Quality

The majority of studies used a cross-sectional design, from which causality between adiposity and inflammation could not be determined. Additionally, this cross-sectional design does not allow a prospective evaluation of these associations. Ten studies of a total of 28 studies did not address specific inclusion and exclusion criteria of participants. Of the 10 studies, seven studies were conducted in nationally representative samples and the participants were relatively healthy adolescents and young adults (Denney-Wilson et al., 2008; Huang et al., 2011; Martinex-Gomez et al., 2010; McDade et al., 2009; Morrison et al., 2011; Nazmi et al., 2008; Wang et al., 2011); however, studies without considering inclusion and exclusion criteria had reduced internal validity. Interestingly, most studies did not report any power calculation to justify their sample size, although most of these studies included a large sample size and were population-based studies. Studies conducted with a small sample size without achieving sufficient power also may reduce generalizability and external validity.

In addition ten of these 28 studies did not report CV of the assay, so the degree of measurement error is unknown. Moreover, one study addressed that CRP was measured by non-high sensitive assay, which potentially could lead to underestimation of the association of anthropometric adiposity and CRP (Williams, et al., 2002). More sensitive biomarkers should be required to accurately detect circulating levels of the inflammation

especially in healthy populations in order to avoid reducing internal validity.

Furthermore, the majority of studies assessed a single CRP, IL-6 and TNF- α measurement, which may not accurately reflect long term-low grade inflammatory status. Sixteen of the 28 studies did not report potential confounders, such as age, sex, ethnicity, acute smoking/current smokers, and alcohol dependence for their statistical analyses (O'Connor et al., 2009). This lack of identification and control of potential confounding variables also reduced internal validity.

Gaps and Future Directions

In order to further advance knowledge in the area of anthropometric adiposity measures and inflammation in adolescents and young adults, gaps are identified and recommendations are proposed based on this review. First of all, there is not enough evidence to arrive at the conclusion of anthropometric adiposity measures with IL-6 and TNF- α . More evidence is required for anthropometric adiposity measures with IL-6 and TNF- α , particularly using continuous variables for more accurate estimation of the strength of associations. Also, stratified sampling or quota sampling strategies regarding non-obese, overweight, and obese participants should be considered for better detection of associations between adiposity and inflammatory processes. Secondly, of the 28 studies, ten studies included a sex breakdown and a statistical analysis was performed for males and females separately. Of the 28 studies, four studies did not control sex either separately or as a covariate (Moon et al., 2004; McDade et al., 2009; Mills et al., 2008; Orri et al., 2010), and none of the studies determined ethnicity differences or influences. Future studies should routinely obtain and assess sex and ethnicity because of the potential impact of sex and ethnicity in the association between adiposity and

inflammation (Choi et al., 2012; O'Connor et al., 2009). Thirdly, other important variables, such as a smoking status, alcohol dependence, acute exercise, and selective serotonin reuptake inhibitor (SSRI) use, which increase in inflammatory markers and may contribute to the heterogeneous results, should be assessed, controlled and possibly excluded (O'Connor et al., 2009), and explain how and why they chose covariates for adjustment or exclusion criteria in order to rule out their effects on the association between anthropometric adiposity measures and inflammation. In addition, two studies suggested that diet and physical activity/fitness play an important role in reducing inflammatory markers (Petty et al., 2010; Vikram et al., 2006), thus future studies need to focus on the interplay of diet and physical activity/fitness, and how those variables influence the association between anthropometric adiposity measures and inflammation. Fourthly, most studies did not provide the justification of their sample size. Future studies should consider sufficient power calculation to justify their enough sample size or a larger sample of both genders in order to confirm these findings. Fifthly, since there is a limitation of cross-sectional design, the significant association between anthropometric adiposity measures and inflammation need to be evaluated in prospective studies in adolescents and young adults. Sixthly, most studies pointed out the use of a single measurement of inflammatory biomarkers, which may reduce a precision to reflect a long-term inflammatory status. Future studies should consider repeated measurements in order to ascertain a more stable estimate of inflammatory status. Lastly, in order to understand underlying inflammatory mechanisms in relation to anthropometric adiposity, other inflammatory markers, such as IL-1 β , should be investigated.

Review Limitations

This systematic review has some limitations. First of all, since this review was limited by studies published in English language, a reporting bias may exist. Some of the included studies did not consider their inclusion and exclusion criteria, which may reduce internal validity. In addition, this review only focused on indirect measures of excess adiposity; BMI, WC, WHR, WHtR, and BF%, which has less accuracy than direct measures, such as DEXA, CT, or MRI. Furthermore, heterogeneity of study populations and diverse statistical analyses for associations limited the consolidation for synthesis of main findings. The evaluation checklist for study quality assessment was developed based on the guidelines for assessing observational studies (Atlantis & Baker, 2008; Pocock et al., 2004; von Elm et al., 2007; Zaccai, 2003). The quality score did not establish cutoff score to determine strong, moderate, or weak of included studies. Finally, the populations of this review only focused on adolescents and young adults therefore the findings could be different from middle and older adulthood due to physical and biological changes. In spite of these limitations, findings of this review have significant implications for assessing inflammatory biomarkers in relation to adiposity in public health and clinical practices. More importantly, although associations between obesity and CRP has been explored in a systematic review and meta-analysis in various populations (Choi et al., 2012), our review included empirical literature investigating the associations of anthropometric approach assessing adiposity measures (BMI, WC, WHR, WHtR, and BF%) and inflammatory biomarkers, including CRP IL-6 and TNF- α in adolescent and young adult populations.

Conclusions

This systematic review overall identified that there are consistently positive associations of anthropometric adiposity measures (BMI, WC, WHR, and BF%) and CRP in adolescents and young adults without a known diagnosis of diseases. Findings of anthropometric adiposity measures (BMI, WC, WHR, and BF%) and IL-6 and TNF- α in adolescents and young adults remain inconclusive. No single anthropometric adiposity measures in adolescents and young adults would appear to be superior in relation to CRP, IL-6 and TNF- α . Further studies are needed to identify the strength and patterns of association of anthropometric adiposity measures with CRP, IL-6 and TNF- α . Moreover, further studies are needed to identify ethnicity differences in the association of anthropometric adiposity measures and inflammatory biomarkers. Lastly, research is needed to focus on the interplay of diet, physical activity/fitness, or weight loss with CRP, IL-6 and TNF- α for developing appropriate and effective lifestyle modification in reducing circulating levels of CRP, IL-6 and TNF- α and in preventing further development of atherosclerotic CVD and acute CVD events in later life.

References

- Adiposity. (n.d.). In *Merriam-Webster's online dictionary* (11th ed.). Retrieved from <http://www.merriam-webster.com/medical/adiposity>
- American College of Sports Medicine (2010). *Guidelines for exercise testing and prescription* (8th ed.). Philadelphia, PA: Lippincott, Williams & Wilkins.
- Bajpai, A., Goyal, A., & Sperling, L. (2010). Should we measure C-reactive protein on earth or just on JUPITER? *Clinical Cardiology*, 33(4), 190-198.
doi:10.1002/clc.20681
- Bo, M., Raspo, S., Morra, F., Cassader, M., Isaia, G., & Poli, L. (2004). Body fat is the main predictor of fibrinogen levels in healthy non-obese men. *Metabolism: Clinical and Experimental*, 53(8), 984-988. doi: 10.1016/j.metabol.2003.12.009
- Bo, M., Raspo, S., Morra, F., Isaia, G., Cassader, M., Fabris, F., & Poli, L. (2004). Body fat and C-reactive protein levels in healthy non-obese men. *Nutrition, Metabolism and Cardiovascular Diseases*, 14(2), 66-72. doi:10.1016/S0939-4753(04)80012-7
- Brooks, G. C., Blaha, M. J., & Blumenthal, R. S. (2010). Relation of C-reactive protein to abdominal adiposity. *The American Journal of Cardiology*, 106(1), 56-61.
doi:10.1016/j.amjcard.2010.02.017
- Brydon, L., Wright, C. E., O'Donnell, K., Zachary, I., Wardle, J., & Steptoe, A. (2008). Stress- induced cytokine responses and central adiposity in young women. *International Journal of Obesity*, 32(3), 443-450. doi:10.1038/sj.ijo.0803767
- Buckley, D. I., Fu, R., Freeman, M., Rogers, K., & Helfand, M. (2009). C-reactive protein as a risk factor for coronary heart disease: A systematic review and meta-

- analyses for the U.S. preventive services task force. *Annals of Internal Medicine*, 151(7), 483-495.
- Calabro, P., Golia, E., Maddaloni, V., Malvezzi, M., Casillo, B., Marotta, C., . . . Golino, P. (2009). Adipose tissue-mediated inflammation: The missing link between obesity and cardiovascular disease? *Internal and Emergency Medicine*, 4(1), 25-34. doi: 10.1007/s11739-008-0207-2
- Choi, J., Joseph, L., & Pilote, L. (2013). Obesity and C-reactive protein in various populations: A systematic review and meta-analysis. *Obesity Reviews*, 14(3), 232-244. doi:10.1111/obr.12003
- Cornier, M. -, Després, J. -, Davis, N., Grossniklaus, D. A., Klein, S., Lamarche, B., . . . Poirier, P. (2011). Assessing adiposity: A scientific statement from the American Heart Association. *Circulation*, 124(18), 1996-2019. doi:10.1161/CIR.0b013e318233bc6a
- de Koning, L., Merchant, A. T., Pogue, J., & Anand, S. S. (2007). Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: Meta-regression analysis of prospective studies. *European Heart Journal*, 28(7), 850-856. doi:10.1093/eurheartj/ehm026
- Denney-Wilson, E., Hardy, L. L., Dobbins, T., Okely, A. D., & Baur, L. A. (2008). Body mass index, waist circumference, and chronic disease risk factors in Australian adolescents. *Archives of Pediatrics and Adolescent Medicine*, 162(6), 566-573. doi:10.1001/archpedi.162.6.566
- Eilertsen, A. L., Hoibraaten, E., Os, I., Andersen, T. O., Sandvik, L., & Sandset, P. M. (2005). The effects of oral and transdermal hormone replacement therapy on C-

- reactive protein levels and other inflammatory markers in women with high risk of thrombosis. *Maturitas*, 52(2), 111-118. doi: 10.1016/j.maturitas.2005.01.004
- El-Wakkad, A., Hassan, N. E. M., Sibaii, H., & El-Zayat, S. R. (2013). Proinflammatory, anti-inflammatory cytokines and adiponkines in students with central obesity. *Cytokine*, 61(2), 682-687.
- Esposito, K., Pontillo, A., Di Palo, C., Giugliano, G., Masella, M., Marfella, R., & Giugliano, D. (2003). Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: A randomized trial. *JAMA : The Journal of the American Medical Association*, 289(14), 1799-1804. doi: 10.1001/jama.289.14.1799
- Gaskins, A. J., Wilchesky, M., Mumford, S. L., Whitcomb, B. W., Browne, R. W., Wactawski-Wende, J., . . . Schisterman, E. F. (2012). Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: The BioCycle study. *American Journal of Epidemiology*, 175(5), 423-431. doi: 10.1093/aje/kwr343
- Gelber, R. P., Gaziano, J. M., Orav, E. J., Manson, J. E., Buring, J. E., & Kurth, T. (2008). Measures of obesity and cardiovascular risk among men and women. *Journal of the American College of Cardiology*, 52(8), 605-615. doi:10.1016/j.jacc.2008.03.066
- Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., . . . American Heart Association Statistics Committee and Stroke Statistics Subcommittee. (2013). Heart disease and stroke statistics--2013 update: A report from the american heart association. *Circulation*, 127(1), e6-e245. doi: 10.1161/CIR.0b013e31828124ad; 10.1161/CIR.0b013e31828124ad

Herder, C., Schneitler, S., Rathmann, W., Haastert, B., Schneitler, H., Winkler, H., . . .

Martin, S. (2007). Low-grade inflammation, obesity, and insulin resistance in adolescents. *Journal of Clinical Endocrinology and Metabolism*, 92(12), 4569-4574.

Hernsdorff, H. H., Volp, A. C., Puchau, B., Barbosa, K. B., Zulet, M. A., Bressan, J., &

Martinez, J. A. (2012). Contribution of gender and body fat distribution to inflammatory marker concentrations in apparently healthy young adults. *Inflammation Research*, 61(5), 427-435.

Hernsdorff, H. H. M., Zulet, M. Á., Puchau, B., & Martínez, J. A. (2011). Central adiposity rather than total adiposity measurements are specifically involved in the inflammatory status from healthy young adults. *Inflammation*, 34(3), 161-170. doi: 10.1007/s10753-010-9219-y

Huang, R., De Klerk, N., Mori, T. A., Newnham, J. P., Stanley, F. J., Landau, L. I., . . .

Beilin, L. J. (2011). Differential relationships between anthropometry measures and cardiovascular risk factors in boys and girls. *International Journal of Pediatric Obesity*, 6(2-2), e271-e282. doi: 10.3109/17477166.2010.512388

Ikeoka, D., Mader, J. K., & Pieber, T. R. (2010). Adipose tissue, inflammation and cardiovascular disease. *Revista Da Associacao Medica Brasileira (1992)*, 56(1), 116-121.

Järvisalo, M. J., Harmoinen, A., Hakanen, M., Paakkunainen, U., Viikari, J., Hartiala, J., .

Raitakari, O. T. (2002). Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 22(8), 1323-1328. doi: 10.1161/01.ATV.0000024222.06463.21

Jung, C., Fischer, N., Fritzenwanger, M., Pernow, J., Brehm, B. R., & Figulla, H. R.

(2009). Association of waist circumference, traditional cardiovascular risk factors, and stromal-derived factor-1 in adolescents. *Pediatric Diabetes*, 10(5), 329-335.

doi: 10.1111/j.1399-5448.2008.00486.x

Khan, U. I., Rieder, J., Cohen, H. W., Coupey, S. M., & Wildman, R. P. (2010). Effect of

modest changes in BMI on cardiovascular disease risk markers in severely obese, minority adolescents. *Obesity Research and Clinical Practice*, 4(3), e231-e237:

<http://dx.doi.org.10.1016/j.orcp.2010.03.001>

Khera, A., McGuire, D. K., Murphy, S. A., Stanek, H. G., Das, S. R., Vongpatanasin, W.,

... de Lemos, J. A. (2005). Race and gender differences in C-reactive protein levels. *Journal of the American College of Cardiology*, 46(3), 464-469. doi:

10.1016/j.jacc.2005.04.051

Lear, S. A., James, P. T., Ko, G. T., & Kumanyika, S. (2010). Appropriateness of waist

circumference and waist-to-hip ratio cutoffs for different ethnic groups. *European Journal of Clinical Nutrition*, 64(1), 42-61. doi: 10.1038/ejcn.2009.70

Libby, P., Ridker, P. M., & Hansson, G. K. (2009). Inflammation in atherosclerosis:

From pathophysiology to practice. *Journal of the American College of*

Cardiology, 54(23), 2129-2138. doi:10.1016/j.jacc.2009.09.009

Lobstein, T., Baur, L., Uauy, R. for the IASO International Obesity TaskForce. (2004).

Obesity in children and young people: A crisis in public health. *Obesity Reviews:*

An Official Journal of the International Association for the Study of Obesity, 5

Suppl 1, 4-104. doi: 10.1111/j.1467-789X.2004.00133.x

- Lohman, T. G. (1981). Skinfolts and body density and their relation to body fatness: A review. *Human Biology*, 53(2), 181-225.
- Mathieu, P., Lemieux, I., & Després, J. -. (2010). Obesity, inflammation, and cardiovascular risk. *Clinical Pharmacology and Therapeutics*, 87(4), 407-416. doi: 10.1038/clpt.2009.311
- Martinez-Gomez, D., Eisenmann, J. C., Wärnberg, J., Gomez-Martinez, S., Veses, A., Veiga, O. L., & Marcos, A. (2010). Associations of physical activity, cardiorespiratory fitness and fatness with low-grade inflammation in adolescents: The AFINOS study. *International Journal of Obesity*, 34(10), 1501-1507. doi:10.1038/ijo.2010.114
- McGill, H. C., Jr, McMahan, C. A., Herderick, E. E., Zieske, A. W., Malcom, G. T., Tracy, R. E., . . . Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. (2002). Obesity accelerates the progression of coronary atherosclerosis in young men. *Circulation*, 105(23), 2712-2718.
- McDade, T. W., Rutherford, J. N., Adair, L., & Kuzawa, C. (2009). Population differences in associations between C-reactive protein concentration and adiposity: Comparison of young adults in the Philippines and the United States. *American Journal of Clinical Nutrition*, 89(4), 1237-1245. doi: 10.3945/ajcn.2008.27080
- McDade, T. W., Tallman, P. S., Adair, L. S., Borja, J., & Kuzawa, C. W. (2011). Comparative insights into the regulation of inflammation: Levels and predictors of interleukin 6 and interleukin 10 in young adults in the Philippines. *American Journal of Physical Anthropology*, 146(3), 373-384. doi: 10.1002/ajpa.21586

- Meyers, M. R., & Gokce, N. (2007). Endothelial dysfunction in obesity: Etiological role in atherosclerosis. *Current Opinion in Endocrinology, Diabetes, and Obesity*, 14(5), 365-369. doi: 10.1097/MED.0b013e3282be90a8
- Mills, P. J., Shapiro, D., Goldstein, I. B., Ottaviani, C., Pung, M. A., Khandrika, S., . . . Rutledge, T. R. (2008). Metabolic predictors of inflammation, adhesion, and coagulability in healthy younger-aged adults. *Obesity*, 16(12), 2702-2706. doi: 10.1038/oby.2008.420
- Moon, Y. S., Kim, D. H., & Song, D. K. (2004). Serum tumor necrosis factor-alpha levels and components of the metabolic syndrome in obese adolescents. *Metabolism: Clinical & Experimental*, 53(7), 863-867. doi: 10.1016/j.metabol.2004.02.007
- Morrison, J. A., Glueck, C. J., Daniels, S. R., Wang, P., Stroop, D. M., & Horn, P. S. (2011). High-sensitivity C reactive protein: Associations with cardiovascular risk factors and tracking in female adolescents and young adults. *Isrn Pediatrics Print*, 2011, 707206. doi:10.5402/2011/707206
- National Institutes of Health National Heart, Lung, and Blood Institute (2000). *The practical guide, Identification, evaluation, and treatment of overweight and obesity in adults*. Retrieved from www.nhlbi.nih.gov/guidelines/obesity/prctgd_c.pdf
- Nazmi, A., Oliveira, I. O., & Victora, C. G. (2008). Correlates of C-reactive protein levels in young adults: A population-based cohort study of 3827 subjects in Brazil. *Brazilian Journal of Medical and Biological Research*, 41(5), 357-367.

Retrieved from http://www.scielo.br/scielo.php?script=sci_pdf&pid=S0100-879X2008000500003&lng=en&nrm=iso&tlng=en

- O'Connor, M. F., Bower, J. E., Cho, H. J., Creswell, J. D., Dimitrov, S., Hamby, M. E., . . . Irwin, M. R. (2009). To assess, to control, to exclude: Effects of biobehavioral factors on circulating inflammatory markers. *Brain, Behavior, & Immunity*, 23(7), 887-897: <http://dx.doi.org/10.1016/j.bbi.2009.04.005>
- Orri, J. C., Carter, S. R., & Howington, E. B. (2010). Gender comparison of C-reactive protein and cardiovascular disease risk in college students and intercollegiate athletes. *Journal of Sports Medicine and Physical Fitness*, 50(1), 72-78.
- Packard, R. R., & Libby, P. (2008). Inflammation in atherosclerosis: From vascular biology to biomarker discovery and risk prediction. *Clinical Chemistry*, 54(1), 24-38. doi: 10.1373/clinchem.2007.097360
- Perez, M., Casas, J. P., Cubillos-Garzón, L. A., Serrano, N. C., Silva, F., Morillo, C. A., & López-Jaramillo, P. (2003). Using waist circumference as a screening tool to identify Colombian subjects at cardiovascular risk. *European Journal of Cardiovascular Prevention and Rehabilitation*, 10(5), 328-335. doi:10.1097/01.hjr.0000095050.46631.6f
- Petersen, A. M., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, 98(4), 1154-1162. doi:10.1152/jappphysiol.00164.2004
- Petty, K. H., Li, K., Dong, Y., Fortenberry, J., Stallmann-Jorgensen, I., Guo, D., & Zhu, H. (2010). Sex dimorphisms in inflammatory markers and adiposity in African-

American youth. *International Journal of Pediatric Obesity*, 5(4), 327-333.

doi: 10.3109/17477160903497019

Pocock, S. J., Collier, T. J., Dandreo, K. J., de Stavola, B. L., Goldman, M. B., Kalish, L.

A., . . . McCormack, V. A. (2004). Issues in the reporting of epidemiological studies: A survey of recent practice. *BMJ (Clinical Research Ed.)*, 329(7471), 883. doi:10.1136/bmj.38250.571088.55

Raitakari, M., Mansikkaniemi, K., Marniemi, J., Viikari, J., & Raitakari, O. T. (2005).

Distribution and determinants of serum high-sensitive C-reactive protein in a population of young adults. the cardiovascular risk in young Finns study. *Journal of Internal Medicine*, 258(5), 428-434. doi: 10.1111/j.1365-2796.2005.01563.x

Rocha, V. Z., & Libby, P. (2009). Obesity, inflammation, and atherosclerosis. *Nature Reviews. Cardiology*, 6(6), 399-409. doi:10.1038/nrcardio.2009.55

Schuett, H., Luchtefeld, M., Grothusen, C., Grote, K., & Schieffer, B. (2009). How much is too much? interleukin-6 and its signaling in atherosclerosis. *Thrombosis and Haemostasis*, 102(2), 215-222. doi:10.1160/TH09-05-0297

Selvin, E., Paynter, N. P., & Erlinger, T. P. (2007). The effect of weight loss on C-reactive protein: A systematic review. *Archives of Internal Medicine*, 167(1), 31-39. doi: 10.1001/archinte.167.1.31

Serrano, H. M. S., Carvalho, G. Q., Pereira, P. F., Do Carmo Gouveia Peluzio, M., Do Carmo Castro Franceschini, S., & Priore, S. E. (2010). Body composition, biochemical and clinical changes of adolescents with excessive adiposity. [Composição corpórea, alterações bioquímicas e clínicas de adolescentes com

excesso de adiposidade] *Arquivos Brasileiros De Cardiologia*, 95(4), 464-472:

<http://dx.doi.org/10.1590/S0066-782X2010005000109>

Stevens, J., McClain, J. E., & Truesdale, K. P. (2008). Selection of measures in epidemiologic studies of the consequences of obesity. *International Journal of Obesity* (2005), 32 Suppl 3, S60-6. doi:10.1038/ijo.2008.88

Thorand, B., Baumert, J., Doring, A., Herder, C., Kolb, H., Rathmann, W., . . . KORA Group. (2006). Sex differences in the relation of body composition to markers of inflammation. *Atherosclerosis*, 184(1), 216-224.

doi:10.1016/j.atherosclerosis.2005.04.011

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., GÅtzsche, P. C., & Vandembroucke, J. P. (2007). The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies*. *Bulletin of the World Health Organization*, 85(11), 867-872. doi:10.2471/BLT.07.045120

Vikram, N. K., Misra, A., Pandey, R. M., Dwivedi, M., Luthra, K., Dhingra, V., & Talwar, K. K. (2006). Association between subclinical inflammation & fasting insulin in urban young adult north Indian males. *Indian Journal of Medical Research*, 124(DEC.), 677-682.

Wang, G., Christoffel, K. K., Brickman, W. J., Hong, X., Arguelles, L., Zhang, S., . . .

Wang, X. (2011). C-reactive protein in adolescent twins: Patterns and relationship to adiposity. *Journal of Clinical Endocrinology and Metabolism*, 96(10), 3226-3233. doi:10.1210/jc.2011-0590

- Wang, S., Reed, D. B., Goli, S., & Goswami, D. (2011). Blood leptin and C-reactive protein provide more sensitive assessment than blood lipids and other inflammatory biomarkers in overweight university students. *Nutrition Research*, 31(8), 586-593. doi: 10.1016/j.nutres.2011.07.006
- Wang, Z., & Nakayama, T. (2010). Inflammation, a link between obesity and cardiovascular disease. *Mediators of Inflammation*, 2010, 535918. doi:10.1155/2010/535918
- Wärnberg, J., Nova, E., Moreno, L. A., Romeo, J., Mesana, M. I., Ruiz, J. R., . . . Blay, M. G. (2006). Inflammatory proteins are related to total and abdominal adiposity in a healthy adolescent population: The AVENA study. *American Journal of Clinical Nutrition*, 84(3), 505-512.
- WHO (2000). Obesity: Preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization Technical Report Series, 894, i-xii, 1-253.
- WHO (1995). Physical status: The use and interpretation of anthropometry report of a WHO expert committee. World Health Organization Technical Report Series, 854, 1-452.
- Widlansky, M. E., Gokce, N., Keaney, J. F., Jr, & Vita, J. A. (2003). The clinical implications of endothelial dysfunction. *Journal of the American College of Cardiology*, 42(7), 1149-1160. doi: 10.1016/S0735-1097(03)00994-X
- Williams, M. J., Poulton, R., & Williams, S. (2002). Relationship of serum ferritin with cardiovascular risk factors and inflammation in young men and women. *Atherosclerosis*, 165(1), 179-184. doi: 10.1016/S0021-9150(02)00233-2

- Wilson, A. M., Ryan, M. C., & Boyle, A. J. (2006). The novel role of C-reactive protein in cardiovascular disease: Risk marker or pathogen. *International Journal of Cardiology*, 106(3), 291-297. doi: 10.1016/j.ijcard.2005.01.068
- World Health Organization (2012). *Obesity and overweight*. Retrieved from <http://www.who.int/mediacentre/factsheets/fs311/en/>
- World Health Organization (2011). *Waist circumference and waist- hip ratio. Report of a WHO Expert Consultation, Geneva, 8-11 December 2008* Retrieved from http://www.who.int/nutrition/publications/obesity/WHO_report_waistcircumference_and_waisthip_ratio/en/
- Yudkin, J. S., Stehouwer, C. D., Emeis, J. J., & Coppel, S. W. (1999). C-reactive protein in healthy subjects: Associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? *Arteriosclerosis, Thrombosis, and Vascular Biology*, 19(4), 972-978. doi: 10.1161/01.ATV.19.4.972
- Zaccai, J. (2004). How to assess epidemiological studies. *Postgraduate Medical Journal*, 80(941), 140-147. doi:10.1136/pgmj.2003.012633

Ovid Medline search

(1946 to July 03 2013)

1. exp Inflammation/
2. exp cytokines/ or exp interleukin-6/ or exp tumor necrosis factor-alpha/
3. exp C-Reactive Protein/
4. (crp or c-reactive or interleukin 6 or il-6 or tnf-alpha or tnf).ti,ab.
5. 1 or 2 or 3 or 4
6. exp adolescent/ or exp young adult/
7. exp young adult/
8. (aged adj2 ("24" or "25" or "26" or "27" or "28" or "29" or "30" or "31" or "32" or "33" or "34" or "35" or "36" or "37" or "38" or "39" or "40")).ab.
9. (youth or adolescen* or teen* or young adult* or university student*).ti,ab.
10. 6 or 7 or 8 or 9
11. body mass index/ or (body mass or bmi).ti,ab.
12. skinfold thickness/ or skinfold*.ti,ab.
13. body fat distribution/ or adiposity/
14. body fat percentage.ti,ab. or body composition/
15. (anthropometric adiposity or anthropometric measure*).ti,ab.
16. waist to height ratio/ or waist to height ratio.ti,ab.
17. waist circumference/ or abdominal obesity/ or abdominal adiposity.ti,ab. or abdominal diameter.ti,ab.
18. waist to hip ratio/ or waist to hip ratio.ti,ab.
19. 5 and 10

20. 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18

21. 19 and 20

22. limit 21 to English language

2,325 articles

CINAHL search

(1995 to July 05, 2013)

S1. ((MH "Inflammation") OR (MH "Cytokines") OR (MH "Tumor Necrosis Factor")
OR (MH "C-Reactive Protein")) OR TI (crp or c-reactive or interleukin 6 or tnf or il-6)

OR AB (crp or c-reactive or interleukin 6 or tnf or il-6)

S2. ((MH "Adolescence") OR (MH "Young Adult")) OR TI (youth or adolescen* or
teen* or young adult*) OR AB (youth or adolescen* or teen* or young adult*)

S3. S1 and S2

S4. ((MH "Waist-Hip Ratio") OR (MH "Waist Circumference")) OR TI (adominal
adiposity or abdominal diameter) OR AB (adominal adiposity or abdominal diameter)

S5. (MH "body mass index") OR TI (body mass or bmi) OR AB (body mass or bmi)

S6. (MH "Skinfold Thickness") OR TI skinfold* OR AB skinfold*

S7. (MH "Adipose Tissue Distribution") OR (MH "Adipose Tissue")

S8. TI "waist to height ratio" OR AB "waist to height ratio"

S9. (MH "Body Composition+") OR TI body fat percentage OR AB body fat percentage

S10. s4 or s5 or s6 or s7 or s8 or s9

S11.s3 and s10

441 articles

SCOPUS search

(1960 to July 05, 2013)

TITLE-ABS-KEY("inflammation" OR "c-reactive protein" OR "CRP" OR "interleukin-6" OR "IL-6" OR "tumor necrosis factor-alpha" OR "TNF-alpha") AND TITLE-ABS-KEY("body mass index" OR " BMI" OR "skinfold thickness" OR "body fat distribution" OR "adiposity" OR "body fat percentage" OR "waist circumference" OR "waist to hip ratio" OR "waist to height ratio" OR "abdominal obesity" OR "abdominal adiposity") AND TITLE-ABS-KEY("adolescent*" OR "university student*" OR "college student*" OR "young adult*") AND (LIMIT-TO(LANGUAGE, "English"))

2,108 articles

Figure 1. Database search strategy

Figure 2. Flowchart of study selection for systematic review

Table 1

Studies reporting the association of anthropometric adiposity and inflammatory biomarkers in adolescents

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Denney-Wilson et al, 2008, Australia	To determine the association between measures of adiposity (BMI, WC) and risk factors for heart disease, type2 diabetes, fatty liver disease, and the clustering of risk factors in Australian adolescents.	N = 496 (M/F =290/206) Age 15.4 ± 0.4y	Secondary schools in Sydney	Cross-sectional Feb 2004-May 2004 Substudy of the New South Wales Schools Physical Activity and Nutrition Survey (SPANS 2004)	BMI (kg/m ²) non-overweight vs. overweight (≥ 25) vs. obese (≥ 30) (International Obesity Task Force Definition cut points) WC (cm) non-overweight vs. overweight (91 st percentile) vs. obese (95 th percentile) (UK Child Growth Foundation Charts 1997 cut points) : measured at the narrowest point between	Hs-CRP (mg/L) Plasma Ultrasensitive assay Intra-assay CV = Not reported Inter-assay CV = 7.4% Cut-off level = 3.0 mg/L > 3.0 (high) ≤ 3.0 (low or normal) Overnight fast	Hs-CRP was significantly associated with overweight and obese (defined by BMI and WC) in both adolescent's boys and girls ($p < 0.001$).	Logistic regression OR (95% CI) BMI (M) Non-overweight 1 (ref) (N = 210) Overweight (N = 61) 4.7 (1.2-18.5) Obese (N = 19) 16.6 (3.5-79.2) WC (M) Non-overweight 1 (ref) (N = 232) Overweight (N = 29) 3.0 (0.6-15.6) Obese (N = 29) 12.7 (5.0-31.9) BMI (F) Non-overweight 1 (ref) (N = 166) Overweight (N = 31) 6.8 (2.4-19.0) Obese (N = 9) 6.8 (1.7-27.3) WC (F) Non-overweight 1 (ref) (N = 169) Overweight (N = 20) 3.2 (1.1-9.4)	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
					the lower costal border and the iliac crest			Obese (N = 17) 5.3 (0.8-12.1)	
El-Wakkad, et al., 2013, Egypt	To investigate the effect of central obesity in obese adolescent girls on the promotion of the secretion of pro-inflammatory (TNF-α, IL-1β, leptin) and anti-inflammatory adiponectin (IL-4, IL-5) adiponkines in adolescent Egyptian girls.	N = 86 (F) Age 13-18y BMI > 95 percentile Group I (N = 43) (WHR < 0.80) WHR: mean ± SE 0.74±0.00045 TNF-α: mean ± SE 2.24±0.59 pg/ml Group II (N = 43) (WHR ≥ 0.80) WHR: mean ± SE 0.89±0.017 TNF-α: mean ± SE 30.4±1.735 pg/ml	Students from four local public schools Cairo in Egypt	Cross-sectional (Study design: not reported) Study period: not reported	WHR: calculated by dividing WC (cm) by hip circumference (cm). WC (cm): measured the smaller circumference between the iliac crest and first rib. Hip (cm): measured at the most prominent area of the buttocks at the level of the symphysis pubis. Abdominal obesity: WHR ≥ 0.80	TNF-α (pg/mL) Serum ELISA CV= Not reported Sensitivity = 2.3 pg/mL Fasting not reported	There was a significant positive correlation between WHR and TNF-α of group II.	Pearson correlation TNF-α WHR (≥ 0.80): $r = 0.559, p < 0.001$ in group II	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Herder et al, 2007, Germany	To characterize the pattern of subclinical immune activation that is associated with indices of obesity and insulin resistance in youth and analyze whether this association is explained by obesity as assessed by BMI and WC in adolescents.	N = 519 (M/F = 293/226) Age 15.5 ± 0.8y BMI (M) 22.4 ± 4.3 WC (M) 78.2 ± 11.8cm IL-6 (M): median (IQR) 0.9 (0.6; 1.5) pg/mL TNF-α (M): median (IQR): 1.8 (1.5; 2.2) pg/mL BMI (F) 23.3 ± 4.9 WC (F) 76.8 ± 10.7cm IL-6 (M): median (25 th ; 75 th percentiles) 0.9 (0.6; 1.6) pg/mL TNF-α (M) 1.7 (1.4; 2.2) pg/mL	Students from selected secondary schools for a medical check-up by Public Health Office in Düsseldorf	Cross-sectional In 2005	BMI (kg/m ²) WC (cm): measured at a level midway between the lower rib margin and iliac crest to the nearest 0.5cm.	IL-6 (pg/mL) Serum ELISA Intra-assay CV = 5.2% Inter-assay CV = 15.5% Sensitivity = 0.08pg/ml TNF-α (pg/mL) Serum ELISA Intra-assay CV = 6.7% Inter-assay CV = 8.8% Sensitivity = 0.25pg/ml Overnight fast	IL-6 was significantly associated with both higher BMI and higher WC ($p \leq 0.001$). TNF-α was not significantly associated with both BMI and WC ($p = \text{NS}$).	Linear regression β : regression coefficients IL-6 (ln-transformed) BMI: $\beta = 0.033$ ($p < 0.001$) WC: $\beta = 0.011$ ($p = 0.001$) TNF-α (ln-transformed) BMI: $\beta = -0.003$ ($p = 0.395$; NS) WC: $\beta = -0.003$ ($p = 0.114$; NS)	Age, sex, lipids

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Huang et al, 2011, Australia	To determine whether individual CV risk factors have a different pattern of relationships to used anthropometric measures.	N = 1,149 (M/F = 604/545) Age 14y Caucasian BMI (M) 21.0 ± 4.1 WC (M) 76.3 ± 11.2cm WHtR (M) 0.46 ± 0.07 CRP (M) 2.2 ± 0.6 mmol/L BMI (F) 21.8 ± 4.3 WC (F) 75.1 ± 10.4cm WHtR (F) 0.46 ± 0.06 CRP (F) 2.4 ± 0.6 mmol/L	The West Australian pregnancy cohort (Raine) study follow-up their children at age 14	Cross-sectional Study period: not reported	BMI (kg/m ²) WC (cm): measured at umbilicus level WHtR	CRP (mmol/L) Serum Hs- monoclonal antibody assay Intra-assay CV = Not reported Inter-assay CV = 2.1-2.6% Overnight fast	CRP had equally and most highly correlated with WC, WHtR and BMI in adolescents. No single anthropometric measures best predicted CRP.	Stepwise linear regression B: regression coefficient indicates the amount of change in units of the outcome variable (CRP) that is associated with a single unit change in WC, WHtR, and BMI. CRP (log) BMI (M) B= 0.11 (<i>p</i> < 0.001) R ² = 0.34 B= 0.12 (<i>p</i> < 0.001) R ² = 0.35 B= 0.12 (<i>p</i> < 0.001) R ² = not reported	Unadjusted Adjusted for Age, family income, physical activity Additional adjusted for pubertal staging

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location	Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
							WHtR (M) B= 5.55($p = 0.003$) R ² = not reported B= 4.84 ($p = 0.02$) R ² = not reported B= 5.00 ($p = 0.02$) R ² = not reported WC (F) B = 3.23 ($p = 0.002$) R ² = 0.17 B= 3.10 ($p = 0.003$) R ² = 0.18	Unadjusted Adjusted for Age, family income, physical activity Additional adjusted for pubertal staging Unadjusted Adjusted for Age, family income, physical activity

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
								B = 3.05 ($p = 0.01$) R ² = NA	Additional adjusted for pubertal staging
Jung et al, 2008, Germany	To investigate whether WC, BMI standard deviation score predict classical and new markers of CV risk and diabetes in male Caucasian adolescents.	N = 79 (M) Age 13-17y Caucasian, BMI (Median (IQR)) 22.8 (20.5-30.5) WC (Median (IQR)) 81 (73-91) cm 48% (n = 38) had a WC ≥ 90 th Percentile Hs-CRP (Median (IQR)) 0.60 (0.10-1.60) mg/L	Volunteers from Schools of the region of Jena, Germany	Cross-sectional (Design: not reported) Study period: not reported	WC (cm): Not reported	Hs-CRP (mg/L) Serum ELISA CV=Not reported Overnight fast	WC predicts hs-CRP ($p < 0.001$).	Linear regression HsCRP WC: R ² = 0.214 ($p < 0.001$)	No
Khan et al, 2010, USA	To examine the association of a five percent change in BMI over a six-month time period on levels of traditional and newer CVD risk markers in a clinical population of severely obese,	N = 83 (baseline) (M/F=33/50) Age 15.1 ± 2.0y AA = 47 Hispanic = 36 Severely obese (BMI > 95 th percentile for age and sex) adolescents BMI : 42.3 ± 7.8 CRP (median (IQR))	A consecutive sampling from Children's hospital weight loss clinic at initial visit, and six-month At 6 month follow up	A six-month longitudinal study	BMI (kg/m ²): At baseline	CRP (mg/dL): At baseline Measurement site = Not reported Latex enhanced immune-turbidometry Intra-assay CV < 5% Inter-assay CV = Not reported Overnight fast	BMI directly correlated with CRP ($p \leq 0.001$) at baseline. BMI remained significantly associated with CRP after adjusting for age, sex, and ethnicity	Bivariate correlation (at baseline) BMI: Spearman's rho = 0.50 ($p < 0.001$) Multivariate regression (at baseline) BMI: $\beta = 0.04$ 95%CI: 0.02-0.04 ($p < 0.001$)	Adjusted for age, sex, ethnicity

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
	AA and Hispanic adolescents.	0.5 (0.1, 1.1) mg/dL	N = 50		BMI (kg/m ²): 6 month follow up	CRP (mg/dL): 6 month follow up	(<i>p</i> = 0.001) at baseline. At 6 month follow up, comparison of changes in CRP level with change in BMI, there was a significant (<i>p</i> = 0.05 for trend) between the three groups of BMI change.	At 6 month follow up CRP (mg/dL) changes: median (IQR) ≥ 5% BMI increase 0.1 (0, 0.5) (N = 8) BMI within 5% 0 (-0.2, 0.2) (N = 32) ≥ 5% BMI decrease 0 (-0.4, 0) (N = 10) (<i>p</i> = 0.05: for trend)	Not reported
Martinez-Gomez, et al, 2010, Spain	To examine the independent associations of objective measured physical activity, cardiorespiratory fitness, and fatness with low-grade inflammatory markers in adolescents.	N = 192 (M/F = 98/94) Age 13-17y BMI (M+F) 21.7 ± 3.5 WC (M+F) 73.4 ± 9.4 cm BF (M+F) 33.5 ± 12.1 mm CRP (M+F) 0.7 ± 1.3 mg/dL IL-6 (M+F) 16.6 ± 28.6 pg/mL	A sub-sample from the AFINOS surveillance study: volunteer adolescents in the region of Madrid	Cross-sectional November 2007-February 2008	BMI (kg/m ²) WC (cm): Not reported BF (mm) measured by sum of six SFs (triceps, biceps, subscapular, suprailiac, thigh, calf) using skinfold caliper	CRP (mg/L) Serum Immuno-turbidimetry CV= Not reported IL-6 (pg/mL) Serum Hs-Human Cytokine MILLIPLEX MAP kit CV= Not reported Overnight fast	BMI, WC and BF were significantly associated with CRP (<i>p</i> < 0.001). BMI, WC, and BF were not significant association with IL-6.	Partial correlations CRP (log) BMI: <i>r</i> = 0.250 (<i>p</i> < 0.001) WC: <i>r</i> = 0.301 (<i>p</i> < 0.001) BF: <i>r</i> = 0.236 (<i>p</i> < 0.001) IL-6 (square-root-transformed) BMI: <i>r</i> = -0.014 (<i>p</i> = NS) WC: <i>r</i> = 0.031 (<i>p</i> = NS) BF: <i>r</i> = 0.050 (<i>p</i> = NS)	Adjusted for age, sex, and pubertal status

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
								Multiple regression CRP (log) BF: $\beta = 0.241$ ($p = 0.002$) $R^2 = 0.040$ IL-6 (square-root-transformed) BF: $\beta = -0.016$ ($p = 0.842$) $R^2 = 0.005$	Adjusted for age, sex, pubertal status, and HOMA-IR
Moon et al, 2004, South Korea	To evaluate the relationship between serum TNF-α, its receptors, and metabolic syndrome components and anthropometric indices in obese and non-obese Asian adolescents.	N = 71 (M/F = 26/45) Age 15-16y Asian Non-obese (BMI < 23): N = 32 (M/F = 14/25) BMI 20.6 ± 0.86 WC 74.83 ± 3.96 cm Obese (BMI > 25): N = 39 (M/F = 12/20) BMI 29.44 ± 2.92 WC 93.83 ± 5.55 cm using Asia- Pacific criteria of obesity TNF-α was not reported	Sampling/setting/location were not reported	Cross-sectional Study period: Not reported	BMI (kg/m ²) WC (cm): measured midway between the lowest rib margin and the iliac crest at the end of gentle expiration	TNF-α (pg/mL) Serum ELISA Intra-assay CV < 7% Inter-assay CV < 9% Sensitivity = 3pg/ml Overnight fast	The serum TNF-α concentration was positively correlated with both the BMI ($p < 0.005$) and WC ($p < 0.001$).	Linear correlation BMI: $r = 0.346$ ($p < 0.005$) WC: $r = 0.525$ ($p < 0.001$)	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Petty et al, 2010, USA	To examine the sex-specific association of adiposity variables with five inflammation markers: IL-6, leptin, CRP, adiponectin, and MCP1 in AA adolescents.	N = 166 (M/F = 78/88) Age 14-19y AA BMI (M) 24.35 ± 4.7 WC (M) 80.32 ± 11.43 cm CRP (M) 1.01 ± 1.8 ug/mL IL-6 (M) 1.29 ± 1.3 pg/mL BMI (F) 26.16 ± 6.6 WC (F) 78.46 ± 11.34 cm CRP (F) 1.27 ± 1.8 ug/mL IL-6 (F) 1.80 ± 1.7 pg/mL	AA volunteers in local public high schools in the Augusta Richmond Country	Cross-sectional June 2006-July 2008	BMI (kg/m ²) WC (cm): measured with thin clothing on, at the midpoint between the lowest rib and the iliac crest.	Hs-CRP (ug/mL) Plasma ELISA Inter-assay CV < 7.0% Intra-assay CV < 8.3% IL-6 (pg/mL) Plasma ELISA Intra-assay CV < 7.8% Inter-assay CV < 9.6% Non-fasting	In males, CRP and IL-6 were not significantly associated with BMI and WC. In females, CRP was significantly associated with BMI ($p < 0.01$) and WC ($p < 0.001$), whereas IL-6 was not significantly associated with BMI and WC.	Partial correlations CRP (M) (log) BMI: $r = 0.179$ ($p = NS$) WC: $r = 0.173$ ($p = NS$) IL-6 (M) (log) BMI: $r = 0.130$ ($p = NS$) WC: $r = 0.093$ ($p = NS$) CRP (F) (log) BMI: $r = 0.442$ ($p < 0.01$) WC: $r = 0.447$ ($p < 0.001$) IL-6 (F) (log) BMI: $r = 0.164$ ($p = NS$) WC: $r = 0.155$ ($p = NS$)	Adjusted for age
Serrano et al, 2010, Brazil	To evaluate body composition, anthropometric changes, biochemical and clinical characteristics of female	N = 113 (F) Age 14-18y BMI 22.90 ± 4.1 WC 71.01 ± 7.78 cm WHR 0.80 ± 0.04 BF 28.72 ± 5.14% CRP 3.572 ± 3.732 mg/dL	Public schools in Viçosa, Minas Gerais	Cross-sectional Study period: Not reported	BMI (kg/m ²) WC (cm): measured at the minimum circumference between the iliac crest and the rib cage WHR(cm/cm)	CRP (mg/dL) Site, assay, and CV= Not reported Overnight fast	CRP was not correlated with BMI, WC, WHR, and BF (%).	Pearson correlation CRP (positive values) BMI: $r = -0.056$ ($p = 0.823$) WC: $r = -0.05$ ($p = 0.845$) WHR: $r = -0.144$ ($p = 0.569$) BF%: $r = 0.112$	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
	adolescents.				BF%: measured by bioelectrical impedance Hip (cm): measured at the maximum protuberance of the buttocks			($p = 0.659$)	
Vikram et al, 2006, India	To examine the association of serum hs-CRP with fasting insulin and insulin resistance in urban adolescent and young adult males in north India.	N = 324 (M) Age 14-25y BMI 20.1 ± 3.5 WC 70.7 ± 7.8 cm WHR 0.82 ± 0.04 CRP (Geometric mean (95%CI)) 0.63 (0.56-0.71) $\mu\text{g/mL}$	Participants selected randomly from schools and colleges in southwest New Delhi, multistage cluster sampling	Cross-sectional In 2001-2003	BMI (kg/m^2) WC (cm): measured midway between the iliac crest and the lower-most margin of the ribs WHR BF%: measured by bioelectrical impedance Hip (cm): measured at the maximum circumference of the buttocks, subject standing with feet placed together	Hs-CRP ($\mu\text{g/mL}$) Serum ELISA Intra-assay CV = 1.7% Inter-assay CV = 3.3% Overnight fast	Hs-CRP levels correlated significantly with BMI, WC, and WHR ($p < 0.05$).	Pearson correlation Hs-CRP (log) BMI: $r = 0.14$ ($p < 0.01$) WC: $r = 0.17$ ($P < 0.01$) WHR: $r = 0.16$ ($p < 0.01$) BF%: $r = 0.07$ ($p = \text{NS}$)	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF- α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Wang et al, 2011, China	To examine the impact of pubertal development, gender, and adiposity distribution on CRP levels in adolescent twins.	N = 1,180 (M/F = 632/548) Age 13-21y Asian BMI (M) 18.7 ± 2.3 WC (M) 68.4 ± 6.1 cm CRP (M) (Median (IQR)) 0.85 (0.39-1.80) mg/L BMI (F) 19.8 ± 2.4 WC (F) 66.1 ± 6.3 cm CRP (M) (Median (IQR)) 0.75 (0.34-1.72) mg/L	Large community-based Chinese twin cohort in Anging, China	Cross-sectional In 2005-2006	BMI (kg/m ²) WC (cm): measured at the level of the umbilicus to the nearest millimeter	CRP (mg/L) Plasma Sandwich Immunoassay Intra-assay CV < 5.1% Inter-assay = Not reported Fasting: Not reported	BMI and WC were linearly associated with log (CRP) levels both males and females ($p < 0.05$).	Linear regression β : coefficient CRP (log) BMI (M): $\beta = 0.23$ ($p < 0.0001$) Partial $r^2 = 0.0516$ WC (M): $\beta = 0.22$ ($p < 0.0001$) Partial $r^2 = 0.0366$ BMI (F): $\beta = 0.31$ ($p < 0.0001$) Partial $r^2 = 0.0665$ WC (F): $\beta = 0.31$ ($p < 0.0001$) Partial $r^2 = 0.0788$	Adjusted for active/passive smoking, Tanner stage, and age

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Wärnber et al, 2006, Spain	To establish the possible relations of serum inflammatory proteins with body fat estimates and body fat distribution in an apparently healthy Spanish adolescent population.	N = 472 (M/F = 248/224) Age 13-18.5y BMI (M) < 25 BMI 20.13 ± 2.06 WC 70.60 ± 5.16 cm WHR 0.81 ± 0.04 BF 57.88 ± 18.74mm BMI (M) ≥ 25 BMI 26.50 ± 2.99 WC 86.99 ± 8.34 cm WHR 0.85 ± 0.05 BF 118.97 ± 36.43mm BMI (F) < 25 BMI 20.42 ± 1.75 WC 68.90 ± 5.61 cm WHR 0.75 ± 0.05 BF 88.47 ± 20.96mm BMI (F) ≥ 25 BMI 26.95 ± 3.67 WC 81.27 ± 8.79 cm WHR 0.79 ± 0.07 BF 141.94 ± 33.57mm	Multiple-step random sampling and then by random selection of classrooms from public and private schools in each city, and then stratified by age and sex: the multicenter AVENA study	Cross-sectional In 2000-2002	BMI (kg/m ²) WC (cm): Measured midway between the lowest rib margin and the iliac crest after gentle expiration WHR BF (mm) :6SF (triceps, biceps, subscapular, suprailiac, thigh and calf) Hip (cm): measured at the point yielding the maximum circumference over the buttocks	Hs-CRP (mg/L) Serum Immuno-turbidimetry Intra-assay CV = Not reported Inter-assay CV < 2% Overnight fast	Hs-CRP was significantly correlated with BMI, WC, and BF (sum of 6 SF Thicknesses) ($p < 0.05$).	Partial correlation ln CRP (M) BMI: $r = 0.268$ ($p = 0.01$) WC: $r = 0.226$ ($p = 0.01$) WHR: $r = 0.107$ ($p = NS$) BF: $r = 0.280$ ($p = 0.01$) ln CRP (F) BMI: $r = 0.265$ ($p = 0.01$) WC: $r = 0.220$ ($p = 0.01$) WHR: $r = 0.004$ ($p = NS$) BF: $r = 0.274$ ($p = 0.01$)	Adjusted for age and Tanner stage

Abbreviations: N, number; M, male; F, female; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; WHtR, waist to height ratio; BF, body fat; CRP, c-reactive protein; hs, high sensitivity; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; y, year; CV = coefficient of variation; OR, Odds Ratio; CI, confidence interval; NA, not applicable; NS, not significance; CV, cardiovascular; CVD, cardiovascular disease; HOMA-IR, homeostasis model assessment of insulin resistance; SF, skinfold; ref, reference; AA, African American; log, log transformed (logarithmically transformed); IQR, interquartile range

Table 2

Studies reporting the association of anthropometric adiposity and inflammatory biomarkers in young adults

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity/obesity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Bo, Raspo, Morra, Isaia et al, 2004, Italy	To investigate the relationships between body fat, CRP levels and metabolic variables in healthy, non-obese sons of patients affected by MS.	N = 85 (M) Age 31.5 ± 7.5y BMI 23.8 ± 3.5 WC 86.8 ± 9.9 cm WHR 0.9 ± 0.1 CRP (Median (IQR)) 0.65 (0.25-1.02) mg/L	Healthy, non-obese without overt MS from among the sons of MS patient at the Lipid Clinic of the Department of Geriatrics of a University teaching hospital in Turin	Cross-sectional Study period: not reported	BMI (kg/m ²) WC (cm): measured midway between the lower margin of the last rib and the iliac crest WHR Hip (cm): measured around the pelvis at the point of maximum buttock protrusion	CRP (mg/L) Plasma HS-immunoassay with a monoclonal antibody coated with polystyrene particles CV= Not reported Overnight fast	CRP levels were significantly associated with the measures of obesity (BMI, WC and WHR) ($p < 0.001$) in healthy, non-obese sons of MS patients.	Univariate linear regression CRP (log) BMI: $r = 0.525$ ($p < 0.0001$) WC: $r = 0.617$ ($p < 0.0001$) WHR: $r = 0.486$ ($p < 0.0001$)	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Bo, Raspo, Morra, Cassader et al, 2004, Italy	To evaluate whether body fat is an independent predictor of hs-CRP levels in healthy, young adult non-obese subjects.	N = 87 (M) Age 30 ± 3.5y Healthy, non-obese young adults BMI 24.1 ± 3.5 WC 86.8 ± 9.9 cm WHR 0.9 ± 0.1 CRP (Median (IQR)) 0.65 (0.25-1.02)mg/dL	Volunteers of young adults men of the hospital staff at the Lipid Clinic of the Department of Geriatrics of a University teaching hospital in Turin.	Cross-sectional Study period: Not reported	BMI (kg/m ²) WC (cm): Not reported WHR and hip: Not reported	Hs-CRP (mg/L) Plasma HS-immunoassay with a monoclonal antibody coated with polystyrene particles CV= Not reported Overnight fasting	Hs-CRP level was strongly associated with BMI, WC and WHR ($p < 0.001$).	Univariate linear regression CRP (log) BMI: $\beta = 0.147$ ($p < 0.001$) WC: $\beta = 0.061$ ($p < 0.001$) WHR: $\beta = 7.454$ ($p < 0.001$)	No
Brydon et al., 2008, UK	To investigate the relationship between measures of adiposity and cytokine, CV and neuroendocrine response to stress in healthy young women.	N = 67 (F) Age 21.3y (18-25y) White: 66.7%, Non-white: 33.3% BMI 23.2 ± 3.1 WC 70.3 ± 7.9 cm BF 25.7 ± 5.4% IL-6 0.71 ± 0.4 pg/mL	Volunteers of healthy females in the University College London	Cross-sectional Study period: Not reported	BMI (kg/m ²) WC (cm): measured midway between the lowest rib and iliac crest BF%: measured by bioelectrical impedance and % fat was calculated as fat wt divided by total body wt (fat + lean) at baseline	IL-6 (pg/mL): At baseline Plasma ELISA Intra-assay CV = 5.3% Inter-assay CV = 9.2% Non-fasting	IL-6 was significantly related to BF%, and BMI at baseline.	Partial correlation (at baseline) BMI: $r = 0.31$ ($p < 0.05$) WC: $r = 0.17$ ($p = \text{NS}$) BF%: $r = 0.28$ ($p < 0.05$)	Adjusted for age, ethnicity, and smoking status

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Hermesdorff et al., 2012, Brazil and Spain	To assess the potential contribution of gender, body fat distribution, and their interactions to some inflammatory marker concentrations (CRP, complement factor 3 (C3), and ceruloplasmin (Cp) in young adults.	N = 317 (M/F = 122/195) Age: 22±3y (18-35y) BMI (M+F) 22.1 ± 2.8 BF (M+F) 26.1 ± 6.4% CRP (M+F) (Median (IQR)) 0.83 (0.47-1.58) mg/dL	Volunteers from the Federal University of Viçosa, Brazil (M/F = 67/92) and the University of Navarra, Spain (M/F = 55/103)	Cross-sectional Study period: Not reported clearly	BF%: estimated by the equations using four skinfold thickness (biceps, triceps, subscapular, and suprailiac)	CRP (mg/L) Plasma ELISA Intra-assay CV < 10% Inter-assay CV < 10% Overnight fast	BF significantly presented R ² for CRP (11%) in both sex. BF significantly presented CRP in men (13%) and women (7%).	Multiple regression analyses CRP (log) (M+F) BF: R ² = 0.11 (p < 0.001) B-coefficient (95% CI) = 0.023 (0.016; 0.030) (p < 0.001) CRP (log) (M) BF: R ² = 0.13 (p < 0.001) B-coefficient (95% CI) = 0.035 (0.019; 0.051) (p < 0.001) CRP (log) (F) BF: R ² = 0.07 (p < 0.001) B-coefficient (95% CI) = 0.032 (0.014; 0.042) (p < 0.001)	Multiple linear regression models were performed for sex separately.
Hermesdorff et al., 2011, Spain	To assess the potential association of some selected plasma inflammatory marker concentrations with adiposity (total and central) and	N = 154 (M/F = 53/101) Age 21± 3y BMI (M+F) 22.1 ± 2.7 WHR (M+F) 0.74 ± 0.06 BF (M+F) 20.0 ± 6.5% CRP (M+F)	Volunteers from the University of Navarra, Spain	Cross-sectional Study period: Not reported clearly	WC (cm): measured midway between the lowest rib and the iliac crest Hip (cm): Measured at the maximal hip circumference, without gluteal	CRP (mg/L) Plasma ELISA Intra-assay CV < 10% Inter-assay CV < 10% IL-6 (pg/mL) Plasma	Those subjects with higher WC and WHR (central adiposity) Showed higher CRP and IL-6 (p < 0.05). Only IL-6 concentration	Multiple linear regression for trend (r was not reported p for trend (WC, WHR, and BF% were categorized into tertiles to assess trends.), from linear regression model adjusted for covariates) CRP WC (p = 0.015)	Adjusted for age, sex, smoking, and physical activity.

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
	metabolic features in apparently healthy young adults.	(Median (IQR)) 0.8(0.6-1.4) mg/l IL-6 (M+F) (Median (IQR)) 1.1(0.8-1.5) pg/l			contraction. WHR BF%: measured by a bioelectric impedance and sum of four SF thickness: biceps, triceps, subscapular, and suprailiac)	ELISA Intra-assay CV < 10% Inter-assay CV < 10% Overnight fast	was positively associated with total BF% ($p < 0.05$). Central adiposity-related indicators (WC/WHR) correlated better than those assessing total adiposity with plasma proinflammatory markers.	WHR ($p = 0.020$) BF% ($p = 0.053$) IL-6 WC ($p = 0.001$) WHR ($p = 0.002$) BF% ($p = 0.001$)	
McDade et al, 2009, Philippines	To investigate the predictors of CRP in young adults living in the Philippines.	N = 1,648 (M/F = 916/732) Age 20.9 ± 0.3 y BMI (M+F) 20.7 ± 3.2 WC (M+F) 70.3 ± 7.8 cm 3SF (M+F) 48.5 ± 22.3 mm CRP (M+F) (Median (IQR)) $0.2 (0.1, 0.9)$ mg/L	The Cebu Longitudinal Health and Nutrition Survey follow up Cebu, metropolitan area	Cross-sectional In 2005	BMI (kg/m ²) WC (cm): Not reported 3SF thickness: triceps, subscapular, and suprailiac	CRP (mg/L) Plasma Hs-immuno-turbidimetric method Intra-assay CV = Not reported Inter-assay CV < 7.6% Overnight fast Cut-off level = 3.0 mg/L > 3.0 (high) ≤ 3.0 (low or normal) >10 mg/L: excluded due to	In female, BMI and WC were significant predictors of elevated CRP (p : not reported) although WC was a strong predictor when all measures were considered simultaneously. A 1cm increase in WC was associated with a 6.0% increase in the likelihood of CRP > 3mg/L	A series of maximum likelihood logistic regression : Detailed data were not reported	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
						infectious diseases	(OR: 1.060, $p < 0.001$). In males, SF thickness was significant in predicting elevated CRP. A 1mm increase in the sum of 3SF measures was associated with a 1.1% increase in the likelihood of elevated CRP (OR: 1.011, $p = 0.09$).		
McDade et al, 2011, Philippines	To report concentrations of IL-6 and to evaluate adiposity, infectious, and socioeconomic predictors of individual variation in IL-6 in young adults.	N = 1,569 (M/F = 866/703) Age 20.9 ± 0.3y (20-22y) BMI (M+F) 20.7 ± 3.2 IL-6 (M+F) (Median (IQR)) 1.00 (0.28, 3.21) pg/mL	The Cebu Longitudinal Health and Nutrition Survey follow up Cebu, metropolitan area	Cross-sectional In 2005	BMI (kg/m ²)	IL-6 (pg/mL) Plasma Hs-multiplex Immunoassay Intra-assay CV =13.3% Inter-assay CV for low (14.7%) and high (12.4%) Overnight fast	BMI was positively associated with IL-6 concentration ($p < 0.05$).	Tobit regression model IL-6 (log) BMI: B = 0.039 (SE = 0.016, $p < 0.05$)	Adjusted for sex

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Mills et al, 2008, USA	To determine the unique contributions of metabolic factors as predictors of CRP and IL-6 in healthy younger-aged adults.	N = 175 (M/F = 83/92) AA = 28 Asian = 56 White = 68 Hispanic = 23 Age 30.4 ± 4.8y (22-39y) BMI(M+F) 24.4 ± 3.35 WHR (M+F) 0.823 ± 0.07 CRP (M+F) 0.855 ± 1.24 mg/L IL-6 (M+F) 0.864±0.75 pg/mL	Consecutive sample, all volunteers were employed in full-time day shifts at the University of California, Los Angeles or the surrounding Los Angeles area	Cross-sectional (Design: not reported)	BMI (kg/m ²) WHR and hip Not reported	CRP (mg/L) Plasma Hs Denka-Seiken assay Intra-and inter-assay CV < 10% Sensitivity < 0.05 mg/L IL-6 (pg/mL) Plasma ELISA Intra-and inter-assay CV < 10% Sensitivity < 0.72 pg/ml Overnight fast	CRP was positively associated with BMI and WHR ($p < 0.05$). IL-6 was positively associated with WHR ($p < 0.01$).	Univariate correlation CRP BMI: $r = 0.340$ ($p < 0.01$) WHR: $r = 0.141$ ($P < 0.05$) IL-6 BMI: $r = 0.070$ ($p = \text{NS}$) WHR: $r = 0.306$ ($p < 0.01$)	No
Morrison et al, 2011, USA	To assess hs-CRP tracking in black and white females from mid-adolescence to young adulthood and to identify factors associated with hs-CRP.	N = 392 (F) Age 26y Caucasian and AA BMI, WC, and hs-CRP were not reported	The National Growth and Health Study (NGHS), a prospectively followed up	Cross-sectional	BMI (kg/m ²) WC (cm): Not reported	Hs-CRP (mg/L) Serum N hs latex enhanced immune-nephelometric assay CV = Not Reported Overnight fast	Hs-CRP was Significantly correlated with BMI and WC ($p < 0.001$).	Bivariate correlation CRP (log) BMI: $r = 0.56$ ($p < 0.001$) WC: $r = 0.55$ ($p < 0.001$)	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Nazmi et al, 2008, Brazil	To identify factors associated with CRP levels in a young adult Brazilian population.	N = 3,289 (M/F = 1,919/1,370) Age 22.8y (21.9-23.7y) BMI and hs-CRP were not reported	The 1982 Pelotas Birth Cohort study in the city of Pelotas, prospectively followed up	Cross-sectional In 2004-2005	BMI (kg/m ²) WC (cm): measured at the narrowest girth of the trunk or halfway between the costal margin and iliac crest	Hs-CRP (mg/L) Plasma automated DPC (Siemens) Immulite Chemiluminescent immunoassay Intra-assay CV = 10% Inter-assay CV = 7% Sensitivity < 0.1mg/L Non-fasting	Hs-CRP was significantly associated with BMI and WC in men and women ($p < 0.001$).	Crude analyses (p -values for trend by linear regression) Hs-CRP (log) BMI (M): β (95%CI) <18.5: $\beta = 0.57$ (0.43-0.75) 18.5-24.9: 1 (ref) 25.0-29.9: $\beta = 1.54$ (1.34-1.76) ≥ 30.0 : $\beta = 3.03$ (2.44-3.76) ($p < 0.001$) WC (M): 1 (ref) ≥ 94 : $\beta = 2.33$ (1.94-2.82) ($p < 0.001$) BMI (F): β (95%CI) <18.5: $\beta = 0.73$ (0.56-0.96) 18.5-24.9: 1 (ref) 25.0-29.9: $\beta = 1.68$ (1.37-2.05) ≥ 30.0 : $\beta = 3.34$ (2.56-4.34) ($p < 0.001$) WC (F): 1 (ref) ≥ 80 : $\beta = 2.29$ (1.93-2.74) ($p < 0.001$)	Adjusted for skin color, age, family income, and years of education

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Orri et al, 2010, USA	To determine the relationship between CAD risk factors and CRP among apparently healthy college-aged men and women.	N = 30 (M/F = 16/14) Age 22.1 ± 3.1y BMI (M) 26.5 ± 4.4 WHR (M) 0.83 ± 0.05 BF (M) 14.5 ± 6.5% CRP (M) 0.55 ± 0.51 mg/L BMI (F) 24.2 ± 1.5 WHR (F) 0.73 ± 0.03 BF (F) 24.3 ± 4.5% CRP (F) 0.77 ± 1.16 mg/L	Volunteers of college men and women (4M and 8F intercollegiate athletes)	Cross sectional Over 2week period	BMI (kg/m ²) WHR and hip: Not reported in detail BF%: measured by sum of SFs	CRP (mg/L) Plasma Cardio hs-CRP assay CV= Not reported Sensitivity = 0.2 mg/L Overnight fast	CRP was positively correlated with BMI ($r = 0.47, p < 0.05$) and BF% ($r = 0.58, p < 0.05$).	Pearson correlation CRP (log) BMI: $r = 0.47$ ($p < 0.05$) WHR: $r = 0.26$ ($p = \text{NS}$) BF%: $r = 0.58$ ($p < 0.05$)	No
Perez et al, 2003, Colombia	To determine and evaluate the level of WC and CRP levels in a Colombian population.	N = 145 (M) Age 28.9y (17-38y) BMI 24.08 ± 3.97 WC 83.32 ± 10.11 cm WHR 0.86 ± 0.06 CRP (N=145) was not reported.	Young healthy males recruited from different levels of tertiles of WC the health service of the	Cross sectional Study period: Not reported	BMI (kg/m ²) WC (cm): measured midway between the iliac crest and the lower portion of the rib cage WHR and hip: Not reported	CRP (mg/L) Plasma Chemiluminescent enzyme-labeled immunometric assay CV = Not Reported Overnight fast	CRP was significantly correlated with BMI, WC and WHR ($p < 0.00001$).	Pearson correlation BMI: $r = 0.08$ ($p < 0.00001$) WC: $r = 0.11$ ($p < 0.00001$) WHR: $r = 0.11$ ($p < 0.00001$)	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
			Colombian army						
Raitakari et al, 2005, Finland	To investigate the distribution of CRP and the population determinants of CRP levels in young adults.	<p>N = 2,120 (M/F = 975/1,046) Age 31.7 ± 5.1y (24-39y)</p> <p>BMI (M) 25.7 ± 4.1 WC (M) 89.6 ± 10.7 cm WHR (M) 0.90 ± 0.06 CRP (M) 1.43 ± 3.26 mg/L</p> <p>BMI (F) 24.4 ± 4.7 WC (F) 78.8 ± 11.4 cm WHR (F) 0.79 ± 0.06 CRP (F) 2.01 ± 3.90 mg/L</p>	The Cardiovascular Risk in Young Finn Study, five centre follow up	Cross-sectional In 2001	<p>BMI (kg/m²) WC (cm): Not reported WHR and hip: Not reported</p>	<p>CRP (mg/L) Serum Hs turbidimetric immunoassay Intra- assay CV = Not reported Inter-assay CV = 3.33% Overnight fast</p>	CRP was significantly correlated with BMI, WC, and WHR in men and women ($p < 0.0001$).	<p>Linear regression β: regression coefficients CRP (log) BMI (M): $\beta = 0.0505$ ($p < 0.0001$) WC (M): $\beta = 0.0020$ ($p < 0.0001$) WHR (M): $\beta = 2.8674$ ($p < 0.0001$)</p> <p>BMI (F): $\beta = 0.0498$ ($p < 0.0001$) WC (F): $\beta = 0.0019$ ($p < 0.0001$) WHR (F): $\beta = 2.0162$ ($p < 0.0001$)</p>	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Wang et al, 2011, USA	To assess BMI and BF% as measures of overweight and obesity and to determine their relationship to inflammatory markers in university students.	N = 110 (M: 28.2%, F: 71.80%) Age 21.3y (18-28y) BMI (M+F) 24.13 ± 4.29 BF (M+F) 25.23 ± 8.64 CRP (M+F) 2.34 ± 2.44 µg/mL IL-6 (M+F) 0.97 ± 1.56 pg/mL TNF-α (M+F) 1.02 ± 0.84 pg/mL	Volunteers from the university students enrolled at Texas Tech University, Lubbock, Texas	Cross-sectional In 2009-2010	BMI (kg/m ²) BF%: measured by bioelectric impedance	CRP (µg/mL) Plasma ELISA CV = Not reported IL-6 (pg/ml) Plasma ELISA CV = Not reported TNF-α (pg/ml) Plasma ELISA CV = Not reported Overnight fast	CRP was significantly correlated with BMI ($p < 0.01$) and BF% ($p < 0.001$) in university students. M vs. F CRP was not significantly correlated with BMI and BF% in male, whereas CRP was significantly correlated with BMI ($p < 0.01$) and BF% ($p < 0.001$) in females. IL-6 and TNF-α were not significant correlated with BMI and BF% in university students as well as both genders.	Spearman correlation (r_s) CRP BMI M+F: $r_s = 0.288$ ($p < 0.01$) M: $r_s = 0.206$ ($p = NS$) F: $r_s = 0.422$ ($p < 0.001$) BF% M+F: $r_s = 0.393$ ($p < 0.001$) M: $r_s = 0.112$ ($p = NS$) F: $r_s = 0.362$ ($p < 0.01$) IL-6 BMI M+F: $r_s = 0.090$ ($p = NS$) M: $r_s = 0.242$ ($p = NS$) F: $r_s = 0.108$ ($p = NS$) BF% M+F: $r_s = 0.097$	No

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location	Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF-α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
							<p>($p = \text{NS}$) M: $r_s = 0.015$ ($p = \text{NS}$) F: $r_s = 0.054$ ($p = \text{NS}$)</p> <p>TNF-α (log) BMI M+F: $r_s = 0.072$ ($p = \text{NS}$) M: $r_s = -0.056$ ($p = \text{NS}$) F: $r_s = 0.140$ ($p = \text{NS}$)</p> <p>BF% M+F: $r_s = 0.105$ ($p = \text{NS}$) M: $r_s = -0.035$ ($p = \text{NS}$) F: $r_s = 0.135$ ($p = \text{NS}$)</p>	

Author, year, country	Purpose	Participants (N/sex/age/adiposity/inflammatory markers: mean±SD), setting/location		Design, study period	Anthropometric adiposity measures (BMI, WC, WHR, WHtR, BF%)	Inflammatory markers (CRP, IL-6, TNF- α) measurement site, assay, CV	Results	Analysis and significance	Adjustments for covariates
Williams et al, 2002, New Zealand	To evaluate the relationship between ferritin, metabolic cardiovascular risk factors, CRP, and anthropometric measurements in young men and women.	N = 815 (M/F = 436/379) Age 26y BMI (M) 24.8 ± 4.7 WC (M) 84.2 ± 8.3 cm CRP (M) 2.4 ± 4.4 mg/L BMI (F) 25.2 ± 3.9 WC (F) 75.4 ± 9.3 cm CRP (F) 5.3 ± 6.8 mg/L	The Dunedin Multidisciplinary Health and Development Study, a longitudinal follow up study, Dunedin, New Zealand	Cross-sectional At age 26y	BMI (kg/m ²) WC (cm): Measured the half way between the coastal border and the iliac crest	CRP (mg/L) Serum Immune-turbidimetric assay using non-hs assay Intra-assay CV = Not reported Inter-assay CV = 5.6-12.9% Non-fasting	CRP was significantly correlated with BMI and WC in young men and women ($p < 0.05$).	Univariate correlation BMI (M): $r = 0.11$ ($p < 0.05$) WC (M): $r = 0.10$ ($p < 0.05$) BMI (F): $r = 0.29$ ($p \leq 0.0001$) WC (F): $r = 0.27$ ($p \leq 0.0001$)	No

Abbreviations: N, number; M, male; F, female; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; WHtR, waist to height ratio; BF, body fat; CRP, c-reactive protein; hs, high sensitivity; IL-6, interleukin 6; TNF- α , tumor necrosis factor alpha; log, log transformed (logarithmically transformed); ELISA, enzyme-linked immunosorbent assay; RIA, radioimmunoassay; y, year; CV = coefficient of variation; OR, Odds Ratio; CI, confidence interval; NA, not applicable; NS, not significance; CV, cardiovascular; CVD, cardiovascular disease; HOMA-IR, homeostasis model assessment of insulin resistance; SF, skinfold; wt, weight; min, minimum; max, maximum; MS, metabolic syndrome; UK, United Kingdom; ref, reference

Table 3

Quality assessment of included studies in Adolescents

Study, year, journal	Design:	Sample			Setting:	Measurements		Analyses			Peer review journal:	Quality Indicators met
	Were study design clearly described?	Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?	Was setting clearly described?	Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?	Was this publication peer-reviewed?	
Denney-Wilson et al, 2008, <i>Archives of Pediatrics and Adolescent Medicine</i>	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	8/11
El-Wakkad et al., <i>Cytokine</i>	Not reported	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	8/11
Herder et al, 2007, <i>Journal of Clinical Endocrinology and Metabolism</i>	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/11

Study, year, journal	Design:	Sample			Setting:	Measurements		Analyses			Peer review journal:	Quality Indicators met
	Were study design clearly described?	Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?	Was setting clearly described?	Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?	Was this publication peer-reviewed?	
Huang et al, 2011, <i>International Journal of Pediatric Obesity</i>	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/11
Jung et al, 2009, <i>Pediatric Diabetes</i>	Not reported	Yes	Yes	No	Yes	Not reported	No	Yes	Yes	No	Yes	6/11
Khan et al, 2010, <i>Obesity Research and Clinical Practice</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/11
Martinex-Gomez et al, 2010, <i>International Journal of Obesity</i>	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/11

Study, year, journal	Design:	Sample			Setting:	Measurements		Analyses			Peer review journal:	Quality Indicators met
	Were study design clearly described?	Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?	Was setting clearly described?	Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?	Was this publication peer-reviewed?	
Moon et al, 2004, <i>Metabolism: Clinical & Experimental</i>	Yes	Yes	Not reported	No	Not reported	Yes	Yes	Yes	Yes	No	Yes	7/11
Petty et al, 2010, <i>International Journal of Pediatric Obesity</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/11
Serrano et al, 2010, <i>Arquivos Brasileiros De Cardiologia</i>	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	7/11
Vikram et al, 2006, <i>Indian Journal of Medical Research</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	9/11

Study, year, journal	Design: Were study design clearly described?	Sample			Setting: Was setting clearly described?	Measurements		Analyses			Peer review journal: Was this publication peer-reviewed?	Quality Indicators met
		Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?		Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?		
Wang, Christoffel et al, 2011, <i>Journal of Clinical Endocrinology and Metabolism</i>	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9/11
Wärnberg et al, 2006, <i>American Journal of Clinical Nutrition</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/11

Table 4

Quality assessment of included studies in Young Adults

Study, year, journal	Design:	Sample			Setting:	Measurements		Analyses			Peer review journal:	Quality Indicators met
	Were study design clearly described?	Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?	Was setting clearly described?	Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?	Was this publication peer-reviewed?	
Bo et al., 2004, <i>Metabolism: Clinical and Experimental</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	9/11
Bo et al., 2004, <i>Nutrition, Metabolism and Cardiovascular Diseases</i>	Yes	Yes	Yes	No	Yes	Not reported	Yes	Yes	Yes	No	Yes	8/11
Brydon et al., 2008, <i>International Journal of Obesity</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/11

Study, year, journal	Design:	Sample			Setting:	Measurements		Analyses			Peer review journal:	Quality Indicators met
	Were study design clearly described?	Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?	Was setting clearly described?	Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?	Was this publication peer-reviewed?	
Hermesdorff et al., 2012, <i>Inflammation Research</i>	Yes	Yes	Yes	No	Yes	Reported in a previous reference	Yes	Yes	Yes	Yes	Yes	10/11
Hermesdorff et al., 2011, <i>Inflammation</i>	Yes	Yes	Yes	No	Yes	Reported in a previous references	Yes	Yes	Yes	Yes	Yes	10/11
McDade et al., 2009, <i>American Journal of Clinical Nutrition</i>	Yes	No	Yes	No	Yes	Reported in a previous references	Yes	Yes	Yes	No	Yes	8/11
McDade et al., 2011, <i>American Journal of Physical Anthropology</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/11

Study, year, journal	Design: Were study design clearly described?	Sample			Setting: Was setting clearly described?	Measurements		Analyses			Peer review journal: Was this publication peer-reviewed?	Quality Indicators met
		Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?		Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?		
Mills et al., 2008, <i>Obesity</i>	Not reported	Yes	Yes	No	Yes	Not reported	Yes	Yes	Yes	No	Yes	7/11
Morrison et al., 2011, <i>ISRN Pediatrics</i>	Yes	No	Yes	No	Yes	Not reported	Yes	Yes	Yes	No	Yes	7/11
Nazmi et al., 2008, <i>Brazilian Journal of Medical and Biological Research</i>	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10/11
Orri et al., 2010, <i>Journal of Sports Medicine and Physical Fitness</i>	Yes	No	Yes	No	Yes	Not reported	Yes	Yes	Yes	No	Yes	7/11

Study, year, journal	Design: Were study design clearly described?	Sample			Setting: Was setting clearly described?	Measurements		Analyses			Peer review journal: Was this publication peer-reviewed?	Quality Indicators met
		Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?		Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?		
Perez et al., 2003, <i>European Journal of Cardiovascular Prevention and Rehabilitation</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	9/11
Raitakari et al., 2005, <i>Journal of Internal Medicine</i>	Yes	Yes	Yes	No	Yes	Not reported	Yes	Yes	Yes	No	Yes	8/11
Wang, Reed et al., 2011, <i>Nutrition Research</i>	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	9/11

Study, year, journal	Design: Were study design clearly described?	Sample			Setting: Was setting clearly described?	Measurements		Analyses			Peer review journal: Was this publication peer-reviewed?	Quality Indicators met
		Were eligibility criteria specified?	Were any sources and methods of selection of participants clearly described?	Was sample size clearly justified and determined?		Were anthropometric adiposity variables reliably measured and described?	Were the inflammatory markers measured using valid instruments?	Were the statistical methods to infer the independent (exposure) variable and dependent (outcome) variables associated/correlated/covary?	Were the statistical methods of data analysis appropriate for the levels of measurement of the variable?	Was the adequate adjustment for confounding in the analyses?		
Williams et al., 2002, <i>Atherosclerosis</i>	Yes	Yes	Yes	No	Yes	Yes	No (measuring non-high sensitivity CRP assay)	Yes	Yes	No	Yes	8/11

Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in

Young Adults

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Abstract

Background

Low grade systemic inflammation plays a key role in atherosclerosis, and C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α), markers of systemic inflammation, are associated with cardiovascular events and adiposity. Cardiorespiratory fitness has shown health benefits by reducing circulating levels of inflammatory markers. However, it remains uncertain whether the negative association between cardiorespiratory fitness and inflammatory biomarkers is due to cardiorespiratory fitness itself or results from lower levels of adiposity. Moreover, the evidence examining the interaction between cardiorespiratory fitness and adiposity in inflammation in young adults is lacking.

Purpose

The aims of this study were to (1) determine the strength of the associations of cardiorespiratory fitness and adiposity (body mass index [BMI], waist circumference [WC]) with circulating levels of plasma hs-CRP, IL-6, and TNF- α ; and (2) test the moderating effect of adiposity on the strength of the association between cardiorespiratory fitness and circulating levels of plasma hs-CRP, IL-6, and TNF- α .

Methods

A cross-sectional study was conducted with 88 young adults aged 20-34 years without diagnosed diseases. A submaximal treadmill walking test was used to assess cardiorespiratory fitness. BMI and WC were measured to assess adiposity. The hs-CRP, IL-6 and TNF- α were assayed and were $\log_{10}$ -transformed. For aim one, a separate multiple regression analysis was conducted with each of hs-CRP, IL-6, and TNF- α as

dependent variables and adjusted for confounders. Analysis of covariance (ANCOVA) was used and adjusted for confounders for aim two. Confounding variables tested were sex, ethnicity, oral contraceptive use, and education level.

Results

Aim1: Cardiorespiratory fitness was not significantly associated with $\log_{10}$ hs-CRP after adjustment for BMI or WC and confounders. Cardiorespiratory fitness was not significantly associated with $\log_{10}$ IL-6 after adjustment for BMI and confounders.

However, cardiorespiratory fitness was significantly and negatively associated with $\log_{10}$ IL-6 after adjustment for WC and confounders (Model adjusted $R^2 = .273$, $p < .0001$; $\beta = -.341$, $t = -1.995$, $p = .049$). Aim 2: Cardiorespiratory fitness $\times$ BMI or WC interaction was not significantly associated with $\log_{10}$ hs-CRP after adjustment for confounders.

Similarly, cardiorespiratory fitness $\times$ BMI interaction was not significantly associated with $\log_{10}$ IL-6 after adjustment for confounders. However, cardiorespiratory fitness $\times$ WC interaction was significantly associated with $\log_{10}$ IL-6 after adjustment for confounders (Model adjusted $R^2 = .258$, $p < .0001$; partial $\eta^2 = .056$, $F = 4.730$, $p = .033$). There were no associations of cardiorespiratory fitness, adiposity, and $\log_{10}$ TNF- α .

Conclusions

In young adults, higher cardiorespiratory fitness is significantly associated with lower levels of IL-6, particularly in young adults with central adiposity. Further studies are warranted to determine if experimentally induced increases in cardiorespiratory fitness reduce inflammatory markers in young adults. Longitudinal studies are needed to understand the underlying inflammatory mechanisms related to interaction between

cardiorespiratory fitness and adiposity, and its influence on cardiovascular disease risk in young adults.

Keywords: cardiorespiratory fitness; body mass index; waist circumference; c-reactive protein; interleukin-6; tumor necrosis factor- α ; young adults

Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Young Adults

Atherosclerotic cardiovascular disease (CVD) is a significant public health burden in the United States (Go et al., 2013). Atherosclerosis, a systemic disease process in which amassment of plaque builds up in the arterial wall, is the major underlying cause of the clinical CVD and CVD events (Go et al., 2013; Libby et al, 2009). It has been demonstrated that low-grade systemic inflammation plays a pivotal role in the initiation and progression of atherosclerosis (Libby et al., 2009) by impairing endothelial function (Ferri et al., 2007). Among the many inflammatory biomarkers, high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumor necrosis factor –alpha (TNF- α) are well-recognized markers of inflammation in relation to atherogenesis (Calabro, Golia, & Yeh, 2009; Haddy et al., 2003).

C-reactive protein (CRP) is a major acute phase inflammatory protein, which is mainly produced by the liver in response to numerous inflammatory cytokines, particularly IL-6 (Bajpai, Goyal, & Sperling, 2010). The “high sensitivity (hs)-CRP” term is defined as “the measurement of CRP in serum or plasma sample using immunoassay methods with sufficient sensitivity to quantify CRP throughout its normal range” in asymptomatic individuals (Bajpai, Goyal, & Sperling, 2010, p.191). Hs-CRP detects the same CRP molecule as CRP test, but it detects substantially lower levels of inflammation (Bajpai, Goyal, & Sperling, 2010). Numerous prospective studies have indicated that plasma level of hs-CRP is a strong independent predictor for future cardiovascular events in individual with no prior history of CVD (Buckley, Fu, Freeman, Rogers, & Helfand, 2009; Ridker, 2001; Ridker, 2003).

IL-6 is a pro-inflammatory cytokine and produced by fibroblasts, endothelial cells, monocytes, and adipose tissue (Schuett, Luchtefeld, Grothusen, Grote, & Schieffer, 2009). IL-6 plays an important role in controlling hepatic production of inflammatory proteins, including hs-CRP and fibrinogen (Antuna-Puente, Fève, Fellahi, & Bastard, 2008). A previous prospective study has reported that elevated levels of IL-6 are associated with increased risk of future myocardial infarction in 14,916 apparently healthy men (Ridker, Rifai, Stampfer, & Hennekens, 2000). TNF- α , a pro-inflammatory cytokine, is secreted by macrophages, lymphocytes, and adipose tissue (Petersen & Pedersen, 2005). TNF- α activates the production of IL-6 in adipose tissue and blood mononuclear cells (Petersen & Pedersen, 2005). It has been reported that TNF- α is associated with degree of early atherosclerosis and correlates with metabolic and cellular perturbations in atherogenesis (Skoog et al., 2002).

Although clinical symptoms of atherosclerotic CVD, such as chest pain or discomfort, shortness of breath or fatigue, numbness, and blurred vision are not commonly detected until adulthood it is a progressive atherosclerotic process that occurs early in life and has clinical indications for acute coronary syndrome and acute stroke usually later in life (Franks et al., 2010; Loria et al., 2007). Hence, young adults could be an optimal target for preventive endeavors to minimize or delay atherosclerotic process, which could eventually decrease the incidence of clinical CVD throughout middle and older adulthood (Loria et al., 2007).

This study is focused on cardiorespiratory fitness and adiposity of young adults in relation to hs-CRP, IL-6 and TNF- α . Cardiorespiratory fitness refers to “the ability of the circulatory and respiratory systems to supply oxygen and nutrients during sustained

physical activity” (Caspersen, Powell, & Christenson, 1985). Cardiorespiratory fitness is related to “the ability to perform large muscle dynamic, moderate-to-high intensity exercise for prolonged periods of time” (American College of Sports Medicine [ACSM], p.71). It has been reported that higher levels of cardiorespiratory fitness are closely related to higher levels of habitual physical activity (Blair et al., 1995). Maximal oxygen uptake (VO_{2max}) is the gold standard or criterion measure of cardiorespiratory fitness (ACSM, 2010) and is commonly assessed with either a maximal or submaximal exercise test, using a treadmill or cycle ergometer (ACSM, 2010). Cardiorespiratory fitness in this present study was estimated using a submaximal treadmill walking protocol, which utilizes individual’s heart rate (HR) response to submaximal treadmill work rates to estimate VO_{2max} (Ebbeling et al., 1991). Although direct measurement of cardiorespiratory fitness using a maximal exercise test (with or without collection of expired gases) is more precise than a submaximal exercise test, the maximal exercise test is not always feasible or practical in health and research settings due to the need for expensive laboratory equipment, trained personnel, possible physician oversight, participant burden, and associated safety considerations (Heyward, 2010).

Cardiorespiratory fitness has shown health benefits in lowering circulating levels of inflammatory biomarkers in various populations (Church, Barlow, Earnest, Kampert, Priest, & Blair, 2002; Kullo, Khaleghi, & Hensrud, 2007, Kuo, Yen, Chen, Yu, & Bean, 2007; Isasi, Deckelbaum, Tracy, Starc, Berglund, & Shea, 2003; Williams et al., 2005). However, it remains uncertain whether the negative association between cardiorespiratory fitness and inflammatory biomarkers is due to the health benefits of

cardiorespiratory fitness itself or results from its association with lower levels of adiposity (Harmer, 2007).

Adiposity is defined as “the quality or state of being fat” (*Merriam-Webster Dictionary*), and a synonym for obesity or fatness (*Merriam-Webster Dictionary*). Adiposity has been assessed by anthropometric measures in public health and clinical practice. Adiposity in this study was assessed by BMI and waist circumference (WC). BMI (kg/m^2) has been recommended for the identification of measuring total body fat and the most widely accepted measures of overall adiposity (Stevens, McClain, & Truesdale, 2008); however, BMI does not differentiate fat mass from bone and muscle mass and does not provide any information on the distribution of body fat, particularly central body fat (Stevens, McClain, & Truesdale, 2008). Thus, this study included WC for assessing central adiposity (Stevens, McClain, & Truesdale, 2008). Adiposity has been positively associated with CRP in various populations (Brook, Blaha, & Blumenthal, 2010; Choi, Joseph, & Pilote, 2013).

Only two studies have reported the association of cardiorespiratory fitness and inflammation measured by CRP in young adults over the past decade (Kuo et al., 2007; Williams, Milne, Hancox, & Poulton, 2006). Both studies reported that cardiorespiratory fitness was negatively associated with CRP in young adults independent of BMI, age, race, and cardiovascular risk factors, such as blood pressure, smoking, etc. In the study by Williams et al., investigators measured CRP using a low sensitivity CRP assay, which may underestimate the strength of the association between cardiorespiratory fitness and CRP (Williams et al., 2005). In the population-based study by Kuo et al., they included study participants with CVD risk factors, such as hypertension, diabetes and current

smokers or participants with anti-inflammatory or CVD medications (Kuo et al., 2007), which may influence levels of inflammatory biomarkers. Neither study examined adiposity as moderator of the association between inflammation and cardiorespiratory fitness, nor the extent to which adiposity influences the association between cardiorespiratory fitness and inflammation. In addition, neither study assessed central adiposity in detail. Furthermore, these two previous studies focused only on CRP as a biomarker of inflammation. It is essential to investigate other inflammatory markers, such as IL-6 and TNF- α in order to better understand inflammatory processes in relation to adiposity and cardiorespiratory fitness.

This study aimed to (1) determine the strength of the association of cardiorespiratory fitness and adiposity (BMI and WC) with circulating levels of plasma hs-CRP, IL-6, and TNF- α ; and (2) test the moderating effect of adiposity (BMI and WC) on the strength of the association between cardiorespiratory fitness levels and circulating levels of plasma hs-CRP, IL-6, and TNF- α in young adults aged 20 to 34 years. This study may fill an important gap in the literature by providing data on the role of adiposity as a moderator of the strength of the association between cardiorespiratory fitness and three inflammatory biomarkers.

Methods

Design

This present study utilized a cross-sectional analytic design. Data were collected on each young adult through cardiorespiratory fitness testing, anthropometric measurement and blood sampling of inflammatory biomarkers in one session, lasting

approximately one and half hours, at the University of Texas Health Science Center at Houston (UTHSC-H) School of Nursing (SON).

Participant, sampling plan and recruitment

The target population for this study was young adults aged 20 to 34 years residing in Houston, a large metropolitan city with an ethnically diverse population in southeast Texas. A sample of the population was accessed using a non-probability quota sampling approach in order to include approximately equal numbers of males and females of normal weight ($BMI < 25 \text{ kg/m}^2$), and overweight or obese ($BMI \geq 25 \text{ kg/m}^2$). The estimated sample size was 88 subjects to detect a medium effect size ($0.059 \leq R^2 < 0.138$) according to Cohen (1998), a power of 80%, and an alpha level of .05 for a two-tailed test using nQuery Advisor version 7.0 (nQuery, Saugus, MA).

The inclusion criteria were: (1) young adults aged 20-34 years; (2) no diagnosed diseases; (3) physically capacity for completing the cardiorespiratory fitness testing safely (ACSM, 2010); and (4) ability to speak and read English. Exclusion criteria were: (1) current smoker; (2) alcohol dependence or regular consumption of more than 7 servings per week; (3) acute infections, including a current or recent influenza illness, flu shot recipients within last 4weeks, acute respiratory infection within last 3weeks; (4) dental infection and problems; (5) cardiometabolic diseases, such as hypertension, hyperlipidemia and diabetes mellitus type I or II; (6) rheumatoid arthritis or immune disorders; (7) history of inflammatory bowel disease, such as Crohn's disease and ulcerative colitis; (8) a surgery within the past one month; (9) currently taking medications, such as use of hormone replacement therapy, antihypertensive medications, allergy shots or systemic corticosteroids, aspirin, statin, and selective serotonin uptake

inhibitors (SSRIs), and anti-inflammatory medications; (10) history of orthopedic injury in the past and limitations due to the musculoskeletal demands of cardiorespiratory test ; and (11) other medical conditions and medications that would increase inflammatory markers or would prohibit cardiorespiratory fitness testing (O'Connor et al., 2009). For females, additional inclusion criteria were: (1) self-reported regular menstrual cycle length of 24-35 days; and (2) self-report of no menstruation at the time of data collection because menses may affect the levels of hs-CRP (Gaskins et al., 2012). Additional exclusion criteria were: (1) natural or surgical menopause; (2) current or recent pregnancy (past 6 months); (3) currently or recently (past 2 months) lactating females (Williams, Williams & Poulton, 2006); (4) history of gynecologic problems, such as fibroids, endometriosis, or polycystic ovary syndrome (Escobar-Morreale, Luque-Ramírez, & González, 2011); (5) use of contraceptive hormone patch or vaginal ring methods of contraception currently or within the past three months; and (6) intrauterine device (IUD) with hormones or Depo-Provera currently or within the past 12 months (Gaskins et al., 2011; O'Connor et al., 2009). This study included females who currently or in the past have taken oral contraceptives, and included as a covariate in the analysis. Participants were recruited by posting fliers in the UTHSC-H, surrounding communities of the UTHSC-H, and multiple campuses in Houston. The recruitment began at the end of March 2013 and continued through December 2013. Individuals interested in the study contacted the principal investigator (PI) by phone or e-mail and were screened by interview. Participants that met the selection criteria were invited to a data collection session.

Procedures

The data were collected by the PI at the Center for Nursing Research in the UTHSC-H, school of nursing (SON). The fitness room was maintained between 68 and 76 degrees Fahrenheit, with a humidity level between 20 to 60 percent using a room temperature thermometer (Q-Track IAQ monitor, TSI Incorporated, Shoreview, MN) throughout the data collection period (ACSM, 2010).

To prepare for data collection, participants were instructed (1) to fast from eating food for 12-hours prior to test but were allowed to drink water only; (2) not to engage in strenuous exercise, consume alcohol, or use anti-inflammatory drugs 24 hours prior to blood sampling; (3) to arrive at the UTHSC-H SON bioscience laboratory between 8am and 10 am; (4) to wear comfortable exercise-type clothing; (5) to avoid caffeine 12- hours prior to test; (6) to consume plenty of fluids; and (7) to obtain an adequate amount of sleep the night before the test (ACSM, 2010). The informed consent was obtained from each participant. All procedures were administered by the PI. Participants received \$10.00 and a free sandwich for completion of data collection protocol. All study procedures were approved by the Committee for the Protection of Human Subjects at the UTHSC-H.

Instruments and Data Collection

Assessment of cardiorespiratory fitness. Cardiorespiratory fitness was assessed by a single-stage submaximal treadmill walking test (SSTWT). The SSTWT was developed by Ebbeling et al. (1991) for estimating VO_2max of low-risk, healthy adults 20 to 50 years old. The SSTWT was validated by correlating the indirectly estimated VO_2max based on individual's walking speed, heart rate (HR), age and sex and the

directly measured respiratory gas exchange ($\text{VO}_{2\text{max}}$) in the cross-validation group ($n = 22$) (Ebbeling et al., 1991). A correlation (r) of .96 was reported, with multiple correlation (R^2) of .86 ($\text{SEE} = 4.85 \text{ ml/kg}\cdot\text{min}$) (Ebbeling et al., 1991).

Single-stage submaximal treadmill walking test protocol. The SSTWT consisted of a 4-minute warm-up stage, 4-minute workload stage, and a 2- 5minute cool-down stage. The treadmill was calibrated before cardiorespiratory fitness test to ensure the accuracy of the test. Prior to beginning the SSTWT, participants were familiarized with the treadmill (Precor 956i, Precor, Inc., Woodinville, WA), including a visual demonstration in order to maximize participant safety. In the first warm-up stage, participants walked on the treadmill for 4-minutes at 0% incline and a walking speed (2.0 to 4.5 mph) that brings the HR to between 50% and 70% beats per minute (bpm) age-predicted maximal HR by the PI. In workload stage, the treadmill incline was increased to 5% at the same speed for a 4-to 5-minute period. Following a workload stage, the participants completed a cool-down stage at a slower walking pace and 0% incline for a 2-5minute period. Resting BP and HR using the oscillometric technique (HEM-907SL, Omron Healthcare, Inc., Bannockburn, IL) was assessed prior to a warm-up stage, twice and after a cool-down stage one time. If the participant's resting blood pressure was $\geq 130/90 \text{ mmHg}$ or resting HR $\geq 100 \text{ bpm}$, the participant was not allowed to continue with the SSTWT protocol. Polar HR (Vintage NV model, Polar CIC, Inc., Port Washington, NY) was recorded during the SSTWT protocol and the Borg Ratings of Perceived Exertion scale (RPE) was documented at the end of the warm-up stage and the workload stage. RPE provides a subjective indication of how hard the participant feels he or she is working with numbers, rating from 6 (very, very light) to 20 (very, very hard)

(Borg, 1970). Steady-state HR is defined as a HR within 5 bpm during the last 2 minute of the workload stage. The main outcome of the cardiorespiratory test estimated maximal oxygen uptake (VO_2max). The SSTWT prediction equation was used to estimate VO_2max based on age, sex, walking speed (mph), and steady-state HR.

$$\begin{aligned} \text{Estimated } \text{VO}_2 \text{ max (ml/kg}\cdot\text{min)} = & 15.1 + 21.8 (\text{speed in mph}) - 0.327 (\text{steady} \\ & \text{state HR in bpm}) - 0.263 (\text{speed} \times \text{age in years}) + 0.00504 (\text{SS HR in bpm age in} \\ & \text{years}) + 5.98 (\text{sex; female} = 0, \text{male} = 1) \end{aligned}$$

Assessment of adiposity. Adiposity was assessed using BMI and WC. BMI was calculated by dividing the participant's weight in kilograms by the height in meters squared (kg/m^2). Height was measured without shoes to the nearest 0.1 cm using a wall-mounted stadiometer (Accustat, Genentech Inc.). Weight was measured to the nearest $1/4^{\text{th}}$ lb with a calibrated balance beam scale. Height and weight were measured with minimal clothing and without shoes and hats (National Health and Nutrition Examination Survey [NHANES], 2010). WC was a measure of the degree of central of body fat. WC was measured to the nearest 0.1 cm using a flexible, nonstretchable tape. The tape measure was placed just above the uppermost lateral border of the right ilium crest and was extended around the waist in a horizontal plane (NHANES, 2010). Height, weight, and waist circumference were repeated, and the mean of two measures was calculated.

Measurement of hs-CRP, IL-6, and TNF- α . Blood collection was taken after a 12-hour overnight fast. A 10 ml blood sample was collected from an antecubital vein into two vacutainers containing ethylene diamine tetra acetic acid (EDTA) by the PI. The blood samples were immediately centrifuged at 1,630 relative centrifugal force (rcf) at -4°C for 30 minutes (Centrifuge5810R, Eppendorf AG Hamburg, Germany). After

centrifugation, plasma samples were divided into eight aliquots and stored at -80°C until batch assayed. Plasma hs-CRP was determined by the CRP Ultra Wide Range Reagent kit (Sekisui Diagnostics, Inc., PE, Canada), a latex-enhanced turbidimetric in vitro immunoassay (AU480 automated platform, Beckman Coulter, Marietta, GA) at the Atherosclerosis Clinical Research Laboratory at the Baylor College Medicine. The plasma hs-CRP samples exceeding the upper limit of linearity were diluted and repeated. The new values were multiplied by the dilution factor to generate a final reportable value. The detectable levels of hs-CRP ranged from 0.05 to 160 mg/L. Intra-assay coefficient of variations (CV) for hs-CRP was < 3.3%. Plasma IL-6 and TNF- α were determined using Quantikine high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (R&D System, MN, USA) at the Bioscience Laboratory in the UTHSC-H SON. The detectable levels of IL-6 ranged from 0.156 to 10 pg/mL, and the detectable levels of TNF- α ranged from 0.5 to 32 pg/mL. IL-6 and TNF- α were run on the WorkOut™ software (version 2.5, Perkin Elmer Life and Analytical Sciences). The intra-assay CV and inter-assay CV for IL-6 were 9.4% and 2.3%, and inter-assay CV for TNF- α were 11.0% and 1.0% respectively.

Age, sex, ethnicity, education, oral contraceptive use were considered as possible confounders related to inflammation based on the previous studies (Cauci et al., 2008; Dreon, Slavin, & Phinney, 2003; Friedman & Herd, 2010; Ferrucci et al., 2005; Kelley-Hedgpeeth et al., 2008; O'Connor et al., 2009; Paalani, Lee, Haddad, & Tonstad, 2011).

Statistical Analysis

Statistical analyses were performed by using statistical software SPSS version 22.0 (SPSS Inc., Chicago, IL). Descriptive data were presented as mean $\pm$ standard

deviation (SD), frequency and percentage. Data were checked for normality of distribution before analyses and were transformed as necessary. The distributions of hs-CRP, IL-6, and TNF- α were positively skewed and were $\log_{10}$ -transformed. All statistical tests were two-tailed and p -value $< .05$ was considered statistically significant. Multiple regression analysis was used to examine aim 1: the strength of the association of cardiorespiratory fitness and adiposity with inflammatory biomarkers. For aim 2, analysis of covariance (ANCOVA) was used to examine the association of cardiorespiratory fitness (continuous variable) $\times$ adiposity (categorical variables) interaction term with inflammatory biomarkers (continuous variable) as the outcome variable. Categories for adiposity were as follows; BMI was divided into $< 25 \text{ kg/m}^2$ (normal weight) and $\text{BMI} \geq 25 \text{ kg/m}^2$ (overweight or obese), and WC was divided at the 50th percentile (median value = 86.4 cm); $\text{WC} < 86.4 \text{ cm}$ and $\text{WC} \geq 86.4 \text{ cm}$. Separate analyses were conducted with each of the inflammatory markers (hs-CRP, IL-6, and TNF- α) as the dependent variable. Analyses for aim 1 and 2 were adjusted for possible confounders including age, sex, or oral contraceptives (females only), ethnicity and education. The dichotomous variables of sex and use of oral contraceptives were considered as an interaction term, which resulted in three categories (males, females without oral contraceptives, and female with oral contraceptives). Variables that were statistically significantly related to the dependent variable ($p < .05$) were included as confounding variables.

Results

Descriptive Characteristics of Participants

One hundred and thirty young adults were assessed for eligibility; 88 participants completed the study as shown in Figure 1. The descriptive characteristics of the

participants are presented in Table 1. All participants ($n = 88$) had complete data for cardiorespiratory fitness, BMI, WC, hs-CRP, and TNF- α , and 87 participants had IL-6 data. The mean of age of participants was 25.97 ± 3.84 (SD) years (range, 20-34 years), and 48.9% was males. The anthropometric and health characteristics of the sample are presented for the sample and for males and females separately in Table 2. Males had higher mean levels of cardiorespiratory fitness, and WC than females whereas female had higher mean levels of hs-CRP than males. Males and females had similar mean levels in BMI, IL-6, and TNF- α .

Associations of Cardiorespiratory Fitness and Adiposity (BMI and WC) with $\text{Log}_{10}\text{hs-CRP}$ and $\text{Log}_{10}\text{IL-6}$

Multiple linear regression analysis was used with $\text{log}_{10}\text{hs-CRP}$ as the outcome variable and cardiorespiratory fitness as predictor variables in Table 3. In multiple linear regression analysis, age, ethnicity, and education were not significant confounding variables related to $\text{log}_{10}\text{hs-CRP}$ ($p < .20$). This study identified sex and oral contraceptives as important confounders related to $\text{log}_{10}\text{hs-CRP}$ ($p < .20$).

Cardiorespiratory fitness was significantly and negatively associated with $\text{log}_{10}\text{hs-CRP}$ after adjustment for sex $\times$ oral contraceptive interaction term (Model1: adjusted $R^2 = .300$, $F(2, 84) = 13.430$, $p < .0001$; β -coefficient = $-.639$, $t = -4.920$, $p < .0001$).

However, after BMI was added to this model, cardiorespiratory fitness was not significantly associated with $\text{log}_{10}\text{hs-CRP}$ (Model 2: adjusted $R^2 = .429$, $F(4, 83) = 17.324$, $p < .0001$; β -coefficient = $-.178$, $t = -1.137$, $p = .259$). Likewise, after WC was added as a predictor, cardiorespiratory fitness was not significantly associated with

$\log_{10}$ hs-CRP either (Model 3: adjusted $R^2 = .418$, $F(4, 83) = 16.641$, $p < .0001$; β -coefficient = $-.202$, $t = -1.291$, $p = .200$).

In terms of $\log_{10}$ IL-6, multiple linear regression analysis was used with $\log_{10}$ IL-6 as the outcome variable and cardiorespiratory fitness as predictor variable in Table 4. In multiple linear regression analysis, age and ethnicity were not significant confounding variables related to $\log_{10}$ IL-6 ($p < .20$). Therefore, this study identified sex and education as confounding variables in the statistical analysis of $\log_{10}$ IL-6 ($p < .20$).

Cardiorespiratory fitness was significantly and negatively associated with $\log_{10}$ IL-6 after adjustment for sex and education (Model 1: adjusted $R^2 = .216$, $F(3, 82) = 6.921$, $p < .0001$; β -coefficient = $-.638$, $t = -4.673$, $p < .0001$). When BMI was added to this model, cardiorespiratory fitness was not significantly associated with $\log_{10}$ IL-6 (Model 2: adjusted $R^2 = .283$, $F(5, 81) = 7.801$, $p < .0001$; β -coefficient = $-.316$, $t = -1.854$, $p = .067$). However, cardiorespiratory fitness was significantly and negatively associated with $\log_{10}$ IL-6 after WC was added to this model (Model 3: adjusted $R^2 = .273$, $F(5, 81) = 7.459$, $p < .0001$; β -coefficient = $-.341$, $t = -1.995$, $p = .049$).

Interaction between Cardiorespiratory Fitness and Adiposity on $\log_{10}$ hs-CRP

The results of the analysis of covariance (ANCOVA) to examine the association of cardiorespiratory fitness (continuous variable) $\times$ BMI (categorical variables) interaction term with $\log_{10}$ hs-CRP (continuous variable) as the outcome variable were presented in Table 5. There was a statistically significant cardiorespiratory fitness $\times$ BMI interaction term for $\log_{10}$ hs-CRP (Model 1: adjusted $R^2 = .229$, $F(3, 84) = 9.607$, $p < .0001$; interaction term, partial $\eta^2 = .051$, $F(1, 84) = 4.553$, $p = .036$). The interaction effect is illustrated in Figure 2. For example, young adults with $BMI \geq 25 \text{ kg/m}^2$ and a

high cardiorespiratory fitness level ($55\text{mL/kg}\cdot\text{min}$) had a lower level of hs-CRP (0.60 mg/L) compared with the hs-CRP level (2.39 mg/L) of young adults with $\text{BMI} \geq 25\text{ kg/m}^2$ and a low cardiorespiratory fitness level ($35\text{mL/kg}\cdot\text{min}$). This suggests that higher levels of cardiorespiratory fitness are associated with lower levels of hs-CRP in young adults with BMI in the overweight/ obese range. In contrast, among those with $\text{BMI} < 25\text{ kg/m}^2$, the levels of hs-CRP did not vary by the level of cardiorespiratory fitness. For instance, as illustrated in Figure 2 young adults with $\text{BMI} < 25\text{ kg/m}^2$ and a high cardiorespiratory fitness level ($55\text{mL/kg}\cdot\text{min}$) had a hs-CRP (0.45mg/L), which is very similar to those with $\text{BMI} < 25\text{ kg/m}^2$ and a low cardiorespiratory fitness level ($35\text{mL/kg}\cdot\text{min}$) hs-CRP (0.49mg/L). This suggests that higher levels of cardiorespiratory fitness are associated with lower levels of hs-CRP in young adults in the presence of high $\text{BMI} \geq 25\text{ kg/m}^2$ group. However, the cardiorespiratory fitness $\times$ BMI interaction term was not statistically significant on hs-CRP after adjustment for sex $\times$ oral contraceptive interaction ($p = .127$) in model 2, Table 5. This means that the sex $\times$ oral contraceptive interaction has an important confounding effect in relation to cardiorespiratory fitness, BMI and hs-CRP.

Table 6 shows the analysis of covariance (ANCOVA) to examine the association of cardiorespiratory fitness (continuous variable) $\times$ WC (categorical variables) interaction term with $\log_{10}\text{hs-CRP}$ (continuous variable) as an outcome variable. There was not a statistically significant cardiorespiratory fitness $\times$ WC interaction term on $\log_{10}\text{hs-CRP}$ (Model 1: adjusted $R^2 = .314$, $F(3, 84) = 14.283$, $p < .0001$; interaction term partial $\eta^2 = .012$, $F(1, 84) = 1.059$, $p = .306$ in Table 6). Similarly, there was not a statistically

significant cardiorespiratory fitness $\times$ WC interaction term on $\log_{10}$ hs-CRP after adjustment for sex $\times$ oral contraceptive interaction in model 2, Table 6.

Interaction between Cardiorespiratory Fitness and Adiposity (BMI and WC) on $\log_{10}$ IL-6

The results of the analysis of covariance (ANCOVA) to examine the association of cardiorespiratory fitness (continuous variable) $\times$ BMI (categorical variables) interaction term with $\log_{10}$ IL-6 (continuous variable) as an outcome variable were shown in Table 7. Model 1 shows that there was not a statistically significant cardiorespiratory fitness $\times$ BMI interaction term on $\log_{10}$ IL-6 (Model1: adjusted $R^2 = .190$, $F(3, 83) = 7.705$, $p < .0001$; interaction term partial $\eta^2 = .033$, $F(1, 83) = 2.791$, $p = 0.099$). Similarly, the cardiorespiratory fitness $\times$ BMI interaction term after adjustment for sex and education was not statistically significant on $\log_{10}$ IL-6 (Model 2: adjusted $R^2 = .240$, $F(6, 80) = 5.528$, $p < .0001$; interaction term partial $\eta^2 = .022$, $F(1, 80) = 1.820$, $p = .181$).

Table 8 shows the analysis of covariance (ANCOVA) to examine the association of cardiorespiratory fitness (continuous variable) $\times$ WC (categorical variables) interaction term with $\log_{10}$ IL-6 (continuous variable) as the outcome variable. There was a statistically significant cardiorespiratory fitness $\times$ WC interaction term on $\log_{10}$ IL-6 (Model 1: adjusted $R^2 = .228$, $F(3, 83) = 9.456$, $p < .0001$; interaction term partial $\eta^2 = .080$, $F(1, 83) = 7.254$, $p = .009$). The interaction effect is illustrated in Figure 3. For instance, young adults with $WC \geq 86.4$ cm and a high cardiorespiratory fitness ($55\text{mL/kg}\cdot\text{min}$) had lower levels of IL-6 (0.83 pg/ml) compared with the IL-6 level (1.83 pg/ml) of young adults with $WC < 86.4$ cm and a low cardiorespiratory fitness

(35mL/kg·min). In contrast, young adults with WC < 86.4cm and a high cardiorespiratory fitness (55mL/kg·min) had a 0.84 IL-6 level, which was similar to the level of IL-6 (0.88 pg/ml) of young adults WC < 86.4 cm and a high cardiorespiratory fitness (35mL/kg·min). This significant interaction between cardiorespiratory fitness and WC on log₁₀IL-6 was sustained after adjustment for sex and education (Model 2: adjusted R² = .258, F (6, 80) = 5.991, *p* < .0001; interaction term partial eta² = .056, F (1, 80) = 4.730, *p* = .033). Thus, the association between cardiorespiratory fitness and log₁₀IL-6 depends on which levels of WC are being considered.

TNF-α

There were no associations of cardiorespiratory fitness and adiposity (BMI and WC) with log₁₀ TNF-α in young adults.

Discussion

The finding of the present study was that cardiorespiratory fitness was not significantly associated with hs-CRP after adjustment for BMI (WC) and sex × oral contraceptive interaction. Cardiorespiratory fitness was not significantly associated with IL-6 after adjustment for BMI, sex and education. However, higher levels of cardiorespiratory fitness were significantly and negatively associated with lower IL-6 levels after adjustment for WC, sex and education in young adults aged 20 to 34 years. For aim two, neither BMI nor WC significantly modified the strength of the associations of cardiorespiratory fitness with hs-CRP levels after adjustment for sex × oral contraceptives interaction. However, WC significantly modified the strength of the association of cardiorespiratory fitness with IL-6 after adjustment for sex and education. Specifically, young adults with WC ≥ 86.4cm (median split) and high levels of

cardiorespiratory fitness had significantly lower levels of IL-6 compared with young adults with WC ≥ 86.4 cm with lower levels of cardiorespiratory fitness. This provides evidence that cardiorespiratory fitness may act as an anti-inflammatory role in lowering levels of circulating IL-6 particularly in young adults with central adiposity.

In relation to the first aim, the findings of the associations of cardiorespiratory fitness and adiposity (BMI and WC) with hs-CRP of this present study were inconsistent with previous two studies (Kuo et al., 2007; Williams et al., 2005). Williams and colleagues reported that cardiorespiratory fitness levels are negatively associated with CRP levels after adjustment for BMI, systolic blood pressure, smoking, and combined oral contraceptive use in 26-year-old young adults ($p \leq 0.01$). This inconsistent result could be explained by differences between the two samples in mean BMI. The means of BMI in the previous study were male (25.2kg/m^2) and females (24.8kg/m^2), whereas the means of BMI in the present study were males ($26.06 \pm 5.03\text{kg/m}^2$) and females ($26.23 \pm 6.81\text{kg.m}^2$). It has been reported that cardiorespiratory fitness is negatively associated with fatness (Martinez-Gomez et al., 2010). Moreover, higher levels of BMI are associated with high levels of hs-CRP (Choi, Joseph, & Pilote, 2013). The other previous study by Kuo and colleagues also reported that a significantly negative association between cardiorespiratory fitness and CRP after adjustment for age, race, BMI category, cardiovascular risk factors and anti-inflammatory medication or CVD medications in 1438 adults aged 20-49 from the NHANES 1999-2002. This inconsistent result could be explained by statistically different adjustment for BMI variable. The previous study adjusted for BMI as categorical variables whereas this present study adjusted BMI as a continuous variable. This statistically different adjustment for adiposity (BMI or WC)

could explain the different results. The findings of present study, however, were consistent with previous study by Martinez-Gomez et al. (2010), which showed that cardiorespiratory fitness was negatively associated with CRP, but this association did not remain statistically significant after adjustment for body fat in 192 adolescents aged 13-17 years (Martinez-Gomez et al., 2010). Previous review by Hamer (2007) has also reported that high levels of fitness are associated with lower levels of inflammation, although the associations were mixed after adjusting for measures of fatness.

In terms of IL-6, no previous studies examined the association of cardiorespiratory fitness and adiposity (BMI and WC) with IL-6 in young adults aged 20-34 years. To our best knowledge, the present study is the first study to examine the association of levels of cardiorespiratory fitness and adiposity (BMI and WC) with IL-6 levels particularly in young adult aged 20-34 years. The present study found that higher levels of cardiorespiratory fitness are significantly associated with lower levels of IL-6 in young adults when controlling for WC, sex and education.

In relation to the second aim, this present study is the first to examine the interactions of cardiorespiratory fitness and adiposity (BMI and WC) in relation to hs-CRP and IL-6 levels in young adults. For circulating hs-CRP concentration, this present study did not find significant interactions between cardiorespiratory fitness and either BMI or WC levels with hs-CRP levels after adjustment for sex $\times$ oral contraceptive interaction in young adults. This finding may suggest that both cardiorespiratory fitness and adiposity (BMI and WC) could be independently related to hs-CRP. In a previous literature review including diverse populations by Hamer (2007) reported that both fitness and fatness were associated with inflammatory factors although the relative

contributions of both may be dependent on sex, age, and disease status. Thus, both maintaining high levels of cardiorespiratory fitness and low levels of BMI and WC could be important factors for maintaining low hs-CRP levels in young adults.

For circulating IL-6 concentration, however, cardiorespiratory fitness $\times$ WC interaction was significantly associated with IL-6 after adjustment for sex and education in young adults. This means that young adults with central adiposity may have lower levels of IL-6 if they have higher levels of cardiorespiratory fitness, indicating that higher levels of cardiorespiratory fitness may exert an anti-inflammatory effect and thereby protect against low-grade inflammation (Wilund, 2007). The mechanisms for the potential anti-inflammatory roles of fitness are not completely understood. The possible mechanisms have suggested that regular physical exercise could improve endothelium-dependent vasodilation by enhancing blood flow and shear stress, resulting in increased the bioavailability of nitric oxide production (Di Francescomarino, Sciartilli, Di Valerio, Di Baldassarre, & Gallina, 2009). In contrast, this present study did not find a significant interaction between cardiorespiratory fitness and BMI on IL-6 after adjustment for sex and education. That is, both cardiorespiratory fitness and BMI could be independently related to IL-6 after adjustment for sex and education. No previous findings can be compared with findings of the present study in interaction between cardiorespiratory fitness and adiposity (measured by BMI and WC) on IL-6 in young adults.

Another important finding of this present study was that there was a significant interaction between cardiorespiratory fitness and WC with regards to IL-6, but there was no significant interaction between cardiorespiratory fitness and WC with regards to hs-CRP. This difference may be explicated by differences in hs-CRP and IL-6 secretion. IL-

6 is produced in adipose tissue, and induces hepatic production of CRP (Shoelson, Herrero, & Naaz, 2007). CRP is an acute-phase reactant synthesized mainly in the liver and is regulated by IL-6 levels (Clearfield, 2005). At rest, approximately 30% of circulating IL-6 is released from adipose tissue (Mohamed-All et al., 1997), and visceral adipose tissue secretes 2-3times more IL-6 than subcutaneous adipose tissue (Fried, Bunkin, & Greenberg, 1998). This present study found that higher levels of cardiorespiratory fitness were significantly related to lower levels of IL-6 after adjustment for WC, sex and education. This study also found that higher levels of cardiorespiratory fitness were significantly associated with lower levels of IL-6, particularly young adults with WC $\geq$ 86.4cm. Taken together, IL-6 levels influenced by visceral adipose tissue and higher levels of cardiorespiratory fitness are significantly related to reducing IL-6 levels in young adults with WC $\geq$ 86.4 cm.

This present study found that use of oral contraceptive was a significant confounding variable in relation to hs-CRP. Previous studies also have reported that hs-CRP levels are significantly higher among oral contraceptive user versus non-users (Cauci et al., 2008; Dreon et al., 2003). The underlying mechanisms by which oral contraceptives induces higher levels of hs-CRP have not been fully clarified (Dreon et al., 2003). Previous investigations have suggested that exogenous estrogenic hormones may have directly modulate hepatic synthesis and therefore may affect pro-inflammatory pathways (van Rooijen, Hansson, Frostegard, Silveira, Hamsten, & Bremme, 2006), but further investigations are required to clarify the pathways.

In relation to TNF- α , this present study did not find any associations of cardiorespiratory fitness and BMI (WC) with TNF- α for aim 1 and aim 2. This result is

possibly due to low concentrations of TNF- α in young adults. In previous studies of the association between adiposity and TNF- α , Wang and colleagues (2011) reported that TNF- α was not significantly related to BMI and percent body fat in university students, and this present study did not find any associations. In contrast, Moon et al and colleagues (2004) reported that TNF- α was significantly and positively associated with both BMI ($r = .346$; $p < .005$) and WC ($r = .525$; $p < .001$) in obese adolescents using a cross-sectional study.

There are several limitations in this present study. Firstly, it is the inability to establish causal relationships from data collected cross-sectional time frame. Secondly, a non-probability quota sampling method in university and community settings limited generalization of the findings of this study to young adults. Thirdly, indirect estimate of VO_{2max} through HR response to a submaximal single stage treadmill test estimated VO_{2max} instead of direct measurement of VO_{2max} through open circuit spirometry during maximal treadmill test (ACSM, 2010). Lastly, this present study found that 6 participants who had hs-CRP levels from 10.4 to 21.11mg/L, and these participants were not excluded from the data analysis. Albeit the American Heart Association (AHA)/Centers for Disease Control and Prevention (CDC) recommends that an hs-CRP level of $>10\text{mg/L}$ should be repeated in 2 weeks due to the possible presence of an acute infection (Pearson et al., 2003), the 6 participants of this present study were unlikely to have acute infectious conditions. This present study very carefully assessed an acute infection or any trauma through inclusion and exclusion criteria and before blood testing. In addition, the 6 participants were all females that were overweight or obese (BMI from 28 to 44.1 kg/m^2). Except for one participant, they all had taken oral contraceptive medications.

This suggests that more extreme levels of adiposity may be directly associated with acute chronic inflammation, and oral contraceptives also may contribute to higher levels of hs-CRP. However, further study is needed to clarify this association. Moreover, exclusion of the 6 participants with an hs-CRP >10mg/L did not significantly change the findings (aim1 and 2) although the data were not shown in this paper.

The main strengths of this present study includes careful selection of participants using rigorous inclusion and exclusion criteria, and control of important confounders in relation to each biomarker. In addition, this present study extends previous studies by including inflammatory biomarkers in addition to CRP, such as IL-6 and TNF- α in young adults. Furthermore, central adiposity assessed by waist circumference regarding this association is included, which has not been examined in young adults previously.

Prospective studies are needed to ascertain the influences of cardiorespiratory fitness and adiposity on inflammatory biomarkers during young adulthood. Exercise intervention studies for improving cardiorespiratory fitness and weight reduction are also warranted to reduce hs-CRP and IL-6 and to improve cardiovascular health in young adults. Moreover, research is needed to investigate the associations of cardiorespiratory fitness and adiposity with TNF- α in overweight and obese young adults.

In conclusion, cardiorespiratory fitness is significantly and negatively associated with IL-6 in young adults aged 20-34 years after adjustment for WC, sex, and education. Moreover, there is a significant interaction between cardiorespiratory fitness and WC in predicting IL-6 levels after adjustment for sex and education in young adults aged 20-34 years old. Therefore, cardiorespiratory fitness may decrease risk of atherosclerosis, CVD events, and CVD by reducing inflammatory biomarkers. Further studies are warranted to

determine if experimentally induced increases in cardiorespiratory fitness reduce inflammatory markers in young adults. Longitudinal studies are needed to understand the inflammatory mechanisms related to cardiorespiratory fitness and adiposity, and its influence on cardiovascular disease risk in young adults.

References

- American College of Sports Medicine (2010). *Guidelines for exercise testing and prescription*. 8th ed. Philadelphia, PA: Lippincott, Williams & Wilkins.
- Antuna-Puente, B., Feve, B., Fellahi, S., & Bastard, J. P. (2008). Adipokines: The missing link between insulin resistance and obesity. *Diabetes & Metabolism*, 34(1), 2-11. doi:10.1016/j.diabet.2007.09.004
- Bajpai, A., Goyal, A., & Sperling, L. (2010). Should we measure C-reactive protein on earth or just on JUPITER? *Clinical Cardiology*, 33(4), 190-198. doi: 10.1002/clc.20681
- Blair, S. N., Kohl, H. W., 3rd, Barlow, C. E., Paffenbarger, R. S., Jr, Gibbons, L. W., & Macera, C. A. (1995). Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *JAMA : The Journal of the American Medical Association*, 273(14), 1093-1098.
- Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitation Medicine*, 2(2), 92-98.
- Brooks, G. C., Blaha, M. J., & Blumenthal, R. S. (2010). Relation of C-reactive protein to abdominal adiposity. *The American Journal of Cardiology*, 106(1), 56-61. doi:10.1016/j.amjcard.2010.02.017
- Buckley, D. I., Fu, R., Freeman, M., Rogers, K., & Helfand, M. (2009). C-reactive protein as a risk factor for coronary heart disease: A systematic review and meta-analyses for the U.S. preventive services task force. *Annals of Internal Medicine*, 151(7), 483-495. doi:151/7/483 [pii]

Calabro, P., Golia, E., & Yeh, E. T. (2009). CRP and the risk of atherosclerotic events.

Seminars in Immunopathology, 31(1), 79-94. doi: 10.1007/s00281-009-0149-4

Caspersen, C. J., Powell, K. E., & Christenson, G. (1985). Physical activity, exercise and

physical fitness: Definitions and distinctions for health-related research. *Public*

Health Reports, 100(2), 126-131.

Cauci, S., Di Santolo, M., Culhane, J. F., Stel, G., Gonano, F., & Guaschino, S. (2008).

Effects of third-generation oral contraceptives on high-sensitivity C-reactive protein and homocysteine in young women. *Obstetrics and Gynecology*, 111(4),

857-864. doi:10.1097/AOG.0b013e31816a2476 [doi]

Choi, J., Joseph, L., & Pilote, L. (2013). Obesity and C-reactive protein in various

populations: A systematic review and meta-analysis. *Obesity Reviews*, 14(3), 232-

244. doi:10.1111/obr.12003

Church, T. S., Barlow, C. E., Earnest, C. P., Kampert, J. B., Priest, E. L., & Blair, S. N.

(2002). Associations between cardiorespiratory fitness and C-reactive protein in

men. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 22(11), 1869-1876.

Clearfield, M. B. (2005). C-reactive protein: A new risk assessment tool for

cardiovascular disease. *The Journal of the American Osteopathic Association*,

105(9), 409-416.

Cohen, J. (1998). Statistical power analysis for the behavioral sciences (2nd ed.). Hills

dale, NJ: Erlbaum.

Di Francescomarino, S., Sciartilli, A., Di Valerio, V., Di Baldassarre, A., & Gallina, S.

(2009). The effect of physical exercise on endothelial function. *Sports Medicine*

(Auckland, N.Z.), 39(10), 797-812. doi:10.2165/11317750-000000000-00000

[doi]

Dreon, D. M., Slavin, J. L., & Phinney, S. D. (2003). Oral contraceptive use and increased plasma concentration of C-reactive protein. *Life Sciences*, 73(10), 1245-1252. doi:S0024320503004259 [pii]

Ebbeling, C. B., Ward, A., Puleo, E. M., Widrick, J., & Rippe, J. M. (1991). Development of a single-stage submaximal treadmill walking test. *Medicine and Science in Sports and Exercise*, 23(8), 966-973.

Escobar-Morreale, H. F., Luque-Ramírez, M., & González, F. (2011). Circulating inflammatory markers in polycystic ovary syndrome: A systematic review and meta-analysis. *Fertility and Sterility*, 95(3), 1048-1058.e2. doi:10.1016/j.fertnstert.2010.11.036

Ferri, C., Croce, G., Cofini, V., De Berardinis, G., Grassi, D., Casale, R., . . . Desideri, G. (2007). C-reactive protein: Interaction with the vascular endothelium and possible role in human atherosclerosis. *Current Pharmaceutical Design*, 13(16), 1631-1645.

Ferrucci, L., Corsi, A., Lauretani, F., Bandinelli, S., Bartali, B., Taub, D. D., . . . Longo, D. L. (2005). The origins of age-related proinflammatory state. *Blood*, 105(6), 2294-2299. doi:10.1182/blood-2004-07-2599

Franks, P. W., Hanson, R. L., Knowler, W. C., Sievers, M. L., Bennett, P. H., & Looker, H. C. (2010). Childhood obesity, other cardiovascular risk factors, and premature death. *The New England Journal of Medicine*, 362(6), 485-493. doi:10.1056/NEJMoa0904130

- Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoid. *The Journal of Clinical Endocrinology and Metabolism*, 83(3), 847-850. doi:10.1210/jcem.83.3.4660 [doi]
- Friedman, E. M., & Herd, P. (2010). Income, education, and inflammation: Differential associations in a national probability sample (the MIDUS study). *Psychosomatic Medicine*, 72(3), 290-300. doi:10.1097/PSY.0b013e3181cfe4c2; 10.1097/PSY.0b013e3181cfe4c2
- Gaskins, A. J., Wilchesky, M., Mumford, S. L., Whitcomb, B. W., Browne, R. W., Wactawski-Wende, J., . . . Schisterman, E. F. (2012). Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: The BioCycle study. *American Journal of Epidemiology*, 175(5), 423-431.
- Go, A. S., Mozaffarian, D., Roger, V. L., Benjamin, E. J., Berry, J. D., Borden, W. B., . . . American Heart Association Statistics Committee and Stroke Statistics Subcommittee. (2013). Heart disease and stroke statistics--2013 update: A report from the American Heart Association. *Circulation*, 127(1), e6-e245. doi: 10.1161/CIR.0b013e31828124ad; 10.1161/CIR.0b013e31828124ad
- Haddy, N., Sass, C., Droesch, S., Zaiou, M., Siest, G., Ponthieux, A., . . . Visvikis, S. (2003). IL-6, TNF- α and atherosclerosis risk indicators in a healthy family population: The STANISLAS cohort. *Atherosclerosis*, 170(2), 277-283. doi: 10.1016/S0021-9150(03)00287-9
- Hamer, M. (2007). The relative influences of fitness and fatness on inflammatory factors. *Preventive Medicine*, 44(1), 3-11.

Heyward, H. V. (2010). *Advanced fitness assessment and exercise prescription*. 6th ed.

Champaign, IL; Human Kinetics.

Isasi, C. R., Deckelbaum, R. J., Tracy, R. P., Starc, T. J., Berglund, L., & Shea, S. (2003).

Physical fitness and C-reactive protein level in children and young adults: The Columbia university BioMarkers study. *Pediatrics*, 111(2), 332-338.

Kelley-Hedgpeth, A., Lloyd-Jones, D. M., Colvin, A., Matthews, K. A., Johnston, J.,

Sowers, M. R., . . . Chae, C. U. (2008). Ethnic differences in C-reactive protein concentrations. *Clinical Chemistry*, 54(6), 1027-1037.

Kullo, I. J., Khaleghi, M., & Hensrud, D. D. (2007). Markers of inflammation are

inversely associated with VO₂ max in asymptomatic men. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, 102(4), 1374-1379. doi:01028.2006 [pii]

Kuo, H. K., Yen, C. J., Chen, J. H., Yu, Y. H., & Bean, J. F. (2007). Association of

cardiorespiratory fitness and levels of C-reactive protein: Data from the National Health and Nutrition Examination Survey 1999-2002. *International Journal of Cardiology*, 114(1), 28-33. doi:10.1016/j.ijcard.2005.11.110

Libby, P., Ridker, P. M., & Hansson, G. K. (2009). Inflammation in atherosclerosis:

From pathophysiology to practice. *Journal of the American College of Cardiology*, 54(23), 2129-2138. doi:10.1016/j.jacc.2009.09.009

Loria, C. M., Liu, K., Lewis, C. E., Hulley, S. B., Sidney, S., Schreiner, P. J., . . .

Detrano, R. (2007). Early adult risk factor levels and subsequent coronary artery calcification: The CARDIA study. *Journal of the American College of Cardiology*, 49(20), 2013-2020. doi:10.1016/j.jacc.2007.03.009

- Martinez-Gomez, D., Eisenmann, J. C., Wärnberg, J., Gomez-Martinez, S., Veses, A., Veiga, O. L., & Marcos, A. (2010). Associations of physical activity, cardiorespiratory fitness and fatness with low-grade inflammation in adolescents: The AFINOS study. *International Journal of Obesity*, 34(10), 1501-1507.
- Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., . . . Coppel, S. W. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo. *The Journal of Clinical Endocrinology and Metabolism*, 82(12), 4196-4200. doi:10.1210/jcem.82.12.4450 [doi]
- Moon, Y. S., Kim, D. H., & Song, D. K. (2004). Serum tumor necrosis factor- α levels and components of the metabolic syndrome in obese adolescents. *Metabolism: Clinical & Experimental*, 53(7), 863-867. doi: 10.1016/j.metabol.2004.02.007
- National Health and Nutrition Examination Survey (NHANES) (2009). Anthropometric procedures manual. Center for Disease Prevention. Retrieved from www.cdc.gov/nchs/data/nhanes/nhanes_03_04/BM.pdf
- O'Connor, M. F., Bower, J. E., Cho, H. J., Creswell, J. D., Dimitrov, S., Hamby, M. E., . . . Irwin, M. R. (2009). To assess, to control, to exclude: Effects of biobehavioral factors on circulating inflammatory markers. *Brain, Behavior, & Immunity*, 23(7), 887-897. Retrieved from <http://dx.doi.org/10.1016/j.bbi.2009.04.005>
- Paalani, M., Lee, J. W., Haddad, E., & Tonstad, S. (2011). Determinants of inflammatory markers in a bi-ethnic population. *Ethnicity & Disease*, 21(2), 142-149.

- Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O., 3rd, Criqui, M., . . . American Heart Association. (2003). 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*, 107(3), 499-511.
- Petersen, A. M., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, 98(4), 1154-1162.
doi:10.1152/jappphysiol.00164.2004
- Ridker, P. M. (2003). Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*, 107(3), 363-369.
- Ridker, P. M. (2001). High-sensitivity C-reactive protein: Potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation*, 103(13), 1813-1818.
- Ridker, P. M., Rifai, N., Stampfer, M. J., & Hennekens, C. H. (2000). Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*, 101(15), 1767-1772
- Schuett, H., Luchtefeld, M., Grothusen, C., Grote, K., & Schieffer, B. (2009). How much is too much? interleukin-6 and its signalling in atherosclerosis. *Thrombosis and Haemostasis*, 102(2), 215-222. doi:10.1160/TH09-05-0297
- Shoelson, S. E., Herrero, L., & Naaz, A. (2007). Obesity, inflammation, and insulin resistance. *Gastroenterology*, 132(6), 2169-2180.
doi:http://dx.doi.org.ww5.sph.uth.tmc.edu:2048/10.1053/j.gastro.2007.03.059

Skoog, T., Dichtl, W., Boquist, S., Skoglund-Andersson, C., Karpe, F., Tang, R., . . .

Hamsten, A. (2002). Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *European Heart Journal*, 23(5), 376-383. doi:10.1053/euhj.2001.2805 [doi]

Stevens, J., McClain, J. E., & Truesdale, K. P. (2008). Selection of measures in epidemiologic studies of the consequences of obesity. *International Journal of Obesity* (2005), 32 Suppl 3, S60-6. doi:10.1038/ijo.2008.88

van Rooijen, M., Hansson, L. O., Frostegard, J., Silveira, A., Hamsten, A., & Bremme, K. (2006). Treatment with combined oral contraceptives induces a rise in serum C-reactive protein in the absence of a general inflammatory response. *Journal of Thrombosis and Haemostasis : JTH*, 4(1), 77-82. doi:JTH1690 [pii]

Wang, S., Reed, D. B., Goli, S., & Goswami, D. (2011). Blood leptin and C-reactive protein provide more sensitive assessment than blood lipids and other inflammatory biomarkers in overweight university students. *Nutrition Research*, 31(8), 586-593. doi: 10.1016/j.nutres.2011.07.006

Williams, M. J., Milne, B. J., Hancox, R. J., & Poulton, R. (2005). C-reactive protein and cardiorespiratory fitness in young adults. *European Journal of Cardiovascular Prevention and Rehabilitation: Official Journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology*, 12(3), 216-220.

Williams, M. J. A., Williams, S. M., & Poulton, R. (2006). Breast feeding is related to C reactive protein concentration in adult women. *Journal of Epidemiology and Community Health*, 60(2), 146-148, doi: 10.1136/jech.2005.039222

Wilund, K. (2007). Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? *Clinical Science*; 112(11-12): 543-555.

Figure 1. Young adult recruitment flow diagram

Table 1

Participant characteristics

	All (N = 88)	
	n	% or Mean $\pm$ SD
Age (years)	88	25.97 $\pm$ 3.84
Sex		
Male	43	48.9% 26.30 $\pm$ 3.90
Female	45	51.1% 25.66 $\pm$ 3.80
Ethnicity		
Caucasian	24	27.3%
African American	18	20.5%
Hispanic	19	21.6%
Asian	20	22.7%
Mixed Race	7	8.0%
Marital status		
Married or living with partner	18	20.5%
Single	70	79.5%
Occupation		
Employee	16	18.2%
Doctoral student	9	10.1%
Master student	28	31.8%
College student	33	37.5%
Other	2	2.3%
Education		
Graduate degree	17	19.3%
Undergraduate degree	49	55.7%
High school degree	22	25.0%
Oral contraceptives	45	
Yes	12	26.6%
No	33	73.4%

Table 2

Descriptive statistics for anthropometric and inflammatory biomarkers in young adult aged 20-34 years (N = 88)

	All (N = 88)		Males (n = 43)		Females (n = 45)	
	Mean $\pm$ SD (Range)	n (%)	Mean $\pm$ SD (Range)	n (%)	Mean $\pm$ SD (Range)	n (%)
Height (cm)	169.93 $\pm$ 9.42		176.22 $\pm$ 7.49		163.93 $\pm$ 6.83	
Weight (kg)	75.66 $\pm$ 18.76		81.00 $\pm$ 17.52		70.55 $\pm$ 18.68	
BMI (kg/m ²)	26.15 $\pm$ 5.97		26.06 $\pm$ 5.03		26.23 $\pm$ 6.81	
Normal weight < 25	21.90 $\pm$ 1.74	44 (50)	22.42 $\pm$ 1.51	21 (48.8)	21.43 $\pm$ 1.83	23 (51.1)
Overweight/Obese $\geq$ 25	30.39 $\pm$ 5.69	44 (50)	29.53 $\pm$ 4.76	22 (51.2)	31.25 $\pm$ 6.50	22 (48.9)
WC (cm)	90.06 $\pm$ 14.30		91.03 $\pm$ 12.96		89.12 $\pm$ 15.57	
<86.4	80.01 $\pm$ 4.78	44 (50)	81.93 $\pm$ 4.33	18 (41.9)	78.67 $\pm$ 4.69	26 (57.8)
$\geq$ 86.4	100.11 $\pm$ 13.59	44 (50)	97.59 $\pm$ 13.18	25 (58.1)	103.42 $\pm$ 13.74	19 (42.2)
SBP (mm Hg)	114.31 $\pm$ 9.18		119.59 $\pm$ 5.89		109.26 $\pm$ 8.95	
DBP (mm Hg)	73.23 $\pm$ 8.66		74.97 $\pm$ 7.92		71.57 $\pm$ 9.08	
HR (per min)	68.72 $\pm$ 11.00		67.14 $\pm$ 9.73		70.22 $\pm$ 12.00	
CRF	45.72 $\pm$ 9.03	88	52.27 $\pm$ 5.06	43	39.47 $\pm$ 7.41	45
(VO ₂ max(mL/kg·min))	(15.42-59.84)		(38.42-59.84)		(15.42-56.53)	
hs-CRP (mg/L)	2.16 $\pm$ 3.81	88	1.35 $\pm$ 1.98	43	2.69 $\pm$ 4.87	45
	(0.067-21.11)		(0.067-9.41)		(0.085-21.11)	
IL-6 (pg/ml)	1.26 $\pm$ 1.17	87	1.07 $\pm$ 0.68	43	1.45 $\pm$ 1.48	44
	(0.33-8.69)		(0.40-3.70)		(0.33-8.69)	
TNF- α (pg/ml)	1.09 $\pm$ 1.19	88	1.16 $\pm$ 0.99	43	1.03 $\pm$ 1.36	45

(0.42-9.72)

(0.48-5.71)

(0.42-9.72)

Abbreviations: CRF, cardiorespiratory fitness; BMI, body mass index; WC, waist circumference; CRP, c-reactive protein; IL-6, interleukin-6; TNF- α , tumor necrosis factor-alpha; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

Note: WC was divided into two categories based on the 50th percentile (median value = 86.4cm).

Table 3

Multiple linear regression of cardiorespiratory fitness and adiposity with log₁₀hs-CRP (N = 88)

Predictor variables	Outcomes								
					Log ₁₀ hs-CRP (mg/L)				
	Models				Coefficients				
	<i>R</i> ²	adj <i>R</i> ²	F	<i>p</i>	<i>B</i>	<i>SE B</i>	β	<i>t</i>	<i>p</i>
Model 1	.324	.300	13.430	.000					
Intercept					2.028	.457		4.435	.000
CRF (mL/kg·min)					-.042	.009	-.639	-4.920	.000
Sex × OC									.000
Male (ref)									
Female (non-OC)					-.577	.166	-.468	-3.475	.001
Female (OC)					.194	.186	.111	1.040	.301
Model 2	.455	.429	17.324	.000					
Intercept					-.838	.763		-1.098	.275
CRF (mL/kg·min)					-.012	.010	-.178	-1.137	.259
BMI (kg/m ²)					.049	.011	.484	4.464	.000
Sex × OC									.001
Male (ref)									
Female (non-OC)					-.144	.178	-.117	-.806	.423
Female (OC)					.443	.177	.255	2.498	.014
Model 3	.445	.418	16.641	.000					
Intercept					-1.253	.877		-1.429	.157
CRF (mL/kg ⁻¹ ·min ⁻¹)					-.013	.010	-.202	-1.291	.200
WC (cm)					.019	.005	.462	4.252	.000
Sex × OC									.001
Male (ref)									
Female (non-OC)					-.125	.185	-.102	-.679	.499
Female (OC)					.484	.183	.279	2.647	.010

Abbreviations: CRF, cardiorespiratory fitness; BMI, body mass index; WC, waist circumference; hs-CRP, high sensitive C-reactive protein; adj, adjusted; B, unstandardized coefficients; SE, standard error; β , standardized coefficients; OC, oral contraceptives; ref, reference.

Model 1: Multiple linear regression predicting CRF from log₁₀hs-CRP after adjustment for sex × oral contraceptive interaction term

Model 2: Multiple linear regression predicting CRF from log₁₀hs-CRP after adjustment for BMI and sex × oral contraceptive interaction term

Model 3: Multiple linear regression predicting CRF from log₁₀hs-CRP after adjustment for WC and sex × oral contraceptive interaction term

Note: hs-CRP was transformed into log₁₀hs-CRP (mg/L). CRF, BMI, and WC, continuous variables

Table 4

Multiple linear regression of cardiorespiratory fitness and adiposity with log₁₀IL-6 (N = 87)

Predictor variables	Outcomes								
					Log ₁₀ IL-6 (pg/ml)				
	Models				Coefficients				
	<i>R</i> ²	adj <i>R</i> ²	F	<i>p</i>	<i>B</i>	<i>SE B</i>	β	<i>t</i>	<i>p</i>
Model 1	.252	.216	6.921	.000					
Intercept					.933	.185		5.037	.000
CRF (mL/kg·min)					-.020	.004	-.638	-4.673	.000
Sex									
Male (ref)									
Female					-.196	.077	-.346	-2.548	.013
Education									.081
Graduate degree (ref)									
Undergraduate degree					-.162	.071	-.285	-2.275	.026
High school degree					-.113	.082	-.175	-1.386	.170
Model 2	.325	.283	7.801	.000					
Intercept					.137	.405		.337	.737
CRF (mL/kg·min)					-.010	.005	-.316	-1.854	.067
BMI (kg/m ²)					.017	.006	.361	2.952	.004
Sex									
Male (ref)									
Female					-.069	.085	-.122	-.810	.421
Education									.160
Graduate degree (ref)									
Undergraduate degree					-.129	.069	-.226	-1.868	.065
High school degree					-.123	.078	-.189	-1.571	.120
Model 3	.315	.273	7.459	.000					
Intercept					.022	.465		.047	.963
CRF (mL/kg·min)					-.011	.005	-.341	-1.995	.049
WC (cm)					.007	.002	.334	2.727	.008
Sex									
Male (ref)									
Female					-.063	.088	-.112	-.715	.477
Education									.160
Graduate degree (ref)									
Undergraduate degree					-.131	.069	-.231	-1.889	.062
High school degree					-.120	.079	-.184	-1.517	.133

Abbreviations: CRF, cardiorespiratory fitness; BMI, body mass index; WC, waist circumference; IL-6, interleukin-6; adj, adjusted; B, unstandardized coefficients; SE, standard error; β , standardized coefficients; ref, reference.

Model 1: Multiple linear regression predicting CRF from $\log_{10}$ IL-6 after adjustment for sex and education

Model 2: Multiple linear regression predicting CRF from $\log_{10}$ IL-6 after adjustment for BMI, sex and education

Model 3: Multiple linear regression predicting CRF from $\log_{10}$ IL-6 after adjustment for WC, sex and education

Note: IL-6 (pg/mL) was transformed into $\log_{10}$ IL-6 (pg/mL); CRF, BMI, and WC, continuous variables

Table 5

Analysis of covariance for log₁₀hs-CRP as an interaction of CRF and BMI (N = 88)

Predictor variables	<i>R</i> ²	adj <i>R</i> ²	<i>p</i>	<i>B</i>	<i>SE</i> <i>B</i>	df	<i>F</i>	<i>p</i>	Partial <i>eta</i> ²
Model 1	.255	.229	.000			3	9.607	.000	.255
Intercept				-.235	.506	1	3.630	.060	.041
CRF(mL/kg·min)				-.002	.010	1	5.802	.018	.065
BMI(<25kg/m ²) (ref)									
BMI(≥25kg/m ²)				1.664	.626	1	7.061	.009	.078
CRF(mL/kg·min)×BMI(≥25kg/m ²)				-.028	.013	1	4.553	.036	.051
Model 2	.385	.348	.000			5	10.280	.000	.385
Intercept				.753	.666	1	9.257	.003	.101
CRF(mL/kg·min)				-.020	.013	1	9.802	.002	.107
BMI(<25kg/m ²) (ref)									
BMI(≥25kg/m ²)				1.172	.594	1	3.894	.052	.045
CRF(mL/kg·min)×BMI(≥25kg/m ²)				-.019	.012	1	2.379	.127	.028
Sex × OC						2	8.662	.000	.174
Male (ref)									
Female (non-OC)				-.400	.172				
Female (OC)				.293	.184				

Abbreviations: CRF, cardiorespiratory fitness; BMI, body mass index; hs-CRP, high sensitive C-reactive protein; oral contraceptives, OC; adj, adjusted; B, unstandardized coefficients; SE, standard error; OC, oral contraceptive; ref, reference.

Model1: ANCOVA testing an interaction of CRF and BMI with log₁₀hs-CRP without adjustment for sex × OC

Model 2: ANCOVA testing an interaction of CRF and BMI with log₁₀hs-CRP after adjustment for sex × OC.

Note: hs-CRP was transformed into log₁₀hs-CRP (mg/L); CRF, continuous variable; BMI was divided into two categories (normal weight <25; overweight and obese ≥ 25).

	Low Fitness (35 mL/kg·min)	High Fitness (55 mL/kg·min)
BMI ≥ 25	Log ₁₀ hs-CRP = 0.379 Hs-CRP = 2.39 mg/L	Log ₁₀ hs-CRP = -0.221 Hs-CRP = 0.60 mg/L
BMI < 25	Log ₁₀ hs-CRP = -0.305 Hs-CRP = 0.49 mg/L	Log ₁₀ hs-CRP = -0.345 Hs-CRP = 0.45 mg/L

Figure 2. Model1: ANCOVA testing an interaction of CRF and BMI with log₁₀hs-CRP without adjustment for confounders

Table 6

Analysis of covariance for log₁₀hs-CRP as an interaction of CRF and WC (N = 88)

Predictor variables	<i>R</i> ²	adj <i>R</i> ²	<i>p</i>	<i>B</i>	<i>SE B</i>	df	<i>F</i>	<i>p</i>	Partial eta ²
Model 1	.338	.314	.000			3	14.283	.000	.338
Intercept				.153	.440	1	6.212	.015	.069
CRF(mL/kg·min)				-.012	.009	1	8.745	.004	.094
WC (<86.4cm) (ref)									
WC (≥86.4cm)				1.109	.568	1	3.821	.054	.044
CRF(mL/kg·min)×WC(≥86.4cm)				-.012	.012	1	1.059	.306	.012
Model 2	.446	.412	.000			5	13.207	.000	.446
Intercept				.753	.631	1	7.490	.008	.084
CRF(mL/kg·min)				-.023	.012	1	7.456	.008	.083
WC (<86.4cm) (ref)									
WC (≥86.4cm)				.626	.552	1	1.287	.260	.015
CRF(mL/kg·min)×WC(≥86.4cm)				-.003	.012	1	.079	.779	.001
Sex × OC						2	8.015	.001	.164
Male (ref)									
Female (non-OC)				-.244	.173				
Female (OC)				.406	.178				

Abbreviations: CRF, cardiorespiratory fitness; WC, waist circumference; hs-CRP, high sensitive C-reactive protein; adj, adjusted; B, unstandardized coefficients; SE, standard error; OC, oral contraceptive; ref, reference.

Model1: ANCOVA testing an interaction of CRF and WC with log₁₀hs-CRP without adjustment for sex × OC

Model 2: ANCOVA testing an interaction of CRF and WC with log₁₀hs-CRP after adjustment for sex × OC.

Note: hs-CRP (mg/L) was transformed into log₁₀hs-CRP (mg/L); CRF, continuous variable; WC was divided into two categories based on the 50th percentile (median value = 86.4 cm).

Table 7

Analysis of covariance for log₁₀ IL-6 as an interaction of CRF and BMI (N = 87)

Predictor variables	<i>R</i> ²	adj <i>R</i> ²	<i>p</i>	<i>B</i>	<i>SE B</i>	df	<i>F</i>	<i>p</i>	Partial eta ²
Model 1	.218	.190	.000			3	7.705	.000	.218
Intercept				.080	.250	1	6.533	.012	.073
CRF(mL/kg·min)				-.004	.005	1	7.569	.007	.084
BMI(<25kg/m ²) (ref)									
BMI(≥25kg/m ²)				.626	.307	1	4.154	.045	.048
CRF(mL/kg·min)×BMI(≥25kg/m ²)				-.011	.007	1	2.791	.099	.033
Model 2	.293	.240	.000			6	5.528	.000	.293
Intercept				.637	.338	1	10.644	.002	.117
CRF(mL/kg·min)				-.011	.006	1	10.731	.002	.118
BMI(<25kg/m ²) (ref)									
BMI(≥25kg/m ²)				.501	.305	1	2.698	.104	.033
CRF(mL/kg·min)×BMI(≥25kg/m ²)				-.009	.006	1	1.820	.181	.022
Sex									
Male				.143	.080	1	3.195	.078	.038
Female (ref)									
Education							2.696	.074	.063
Graduate degree (ref)									
Undergraduate degree				-.163	.081	1			
High school degree				-.127	.070	1			

Abbreviations: CRF, cardiorespiratory fitness; BMI, body mass index; IL-6, interleukin-6; adj, adjusted; B, unstandardized coefficients; SE, standard error; ref, reference.

Model1: ANCOVA testing an interaction of CRF and BMI with log₁₀IL-6 without adjustment for sex and education

Model 2: ANCOVA testing an interaction of CRF and BMI with log₁₀IL-6 after adjustment for sex and education.

Note: IL-6 (pg/mL) was transformed into log₁₀IL-6 (pg/mL); CRF, continuous variable; BMI was divided into two categories (normal weight <25kg/m²; overweight and obese ≥ 25kg/m²).

Table 8

Analysis of covariance for log₁₀ IL-6 as an interaction of CRF and WC (N = 87)

Predictor variables	<i>R</i> ²	adj <i>R</i> ²	<i>p</i>	<i>B</i>	<i>SE B</i>	df	<i>F</i>	<i>p</i>	Partial eta ²
Model 1	.255	.228	.000			3	9.456	.000	.255
Intercept				-.017	.224	1	8.596	.004	.094
CRF(mL/kg·min)				-.001	.005	1	9.823	.002	.106
WC (<86.4cm) (ref)									
WC (≥86.4cm)				.876	.287	1	9.306	.003	.101
CRF(mL/kg·min)×WC(≥86.4cm)				-.016	.006	1	7.254	.009	.080
Model 2	.310	.258	.000			6	5.991	.000	.310
Intercept				.511	.332	1	10.763	.002	.119
CRF(mL/kg·min)				-.009	.006	1	10.918	.001	.120
WC (<86.4cm) (ref)									
WC (≥86.4cm)				.705	.292	1	5.818	.018	.068
CRF(mL/kg·min)×WC(≥86.4cm)				-.013	.006	1	4.730	.033	.056
Sex									
Male				.128	.083	1	2.386	.126	.029
Female (ref)									
Education							2.039	.137	.048
Graduate degree (ref)									
Undergraduate degree				-.141	.070	1			
High school degree				-.116	.080	1			

Abbreviations: CRF, cardiorespiratory fitness; WC, waist circumference; IL-6, interleukin-6; adj, adjusted; B, unstandardized coefficients; SE, standard error; ref, reference.

Model1: ANCOVA testing an interaction of CRF and WC with log₁₀IL-6 without adjustment for sex and education

Model 2: ANCOVA testing an interaction of CRF and WC with log₁₀IL-6 after adjustment for sex and education.

Note: IL-6 (pg/mL) was transformed into log₁₀IL-6 (pg/mL); CRF, continuous variable; WC was divided into two categories based on the 50th percentile (median value = 86.4cm).

	Low Fitness (35 mL/kg·min)	High Fitness (55 mL/kg·min)
WC ≥ 86.4 cm	log ₁₀ IL-6 = 0.264 IL-6 = 1.83 pg/ml	log ₁₀ IL-6 = -0.076 IL-6 = 0.83 pg/ml
WC < 86.4 cm	log ₁₀ IL-6 = -0.052 IL-6 = 0.88 pg/ml	log ₁₀ IL-6 = -0.072 IL-6 = 0.84 pg/ml

Figure 3. Model1: ANCOVA testing an interaction of cardiorespiratory fitness and WC with log₁₀IL-6

Appendix A

Study Flyers

ATTENTION!!

Individuals between the **ages of 20 and 34** are needed to participate in a new research study!!

We are seeking individuals **without** heart disease, respiratory conditions and chronic disease for a study of young adults.

During one visit to the research center participants in this study will:

- Treadmill walk test to monitor heart rate
- Have a blood test
- Answer some basic questions about yourself.
- Be compensated for your participation in this study.

If you are interested or have further questions please call
716-909-4121 or email: **Eunduck.Park@uth.tmc.edu**
 Eunduck Park, MSN, ANP, RN
 University of Texas Health Science Center, School of Nursing

Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu
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IRB NUMBER: HSC-SN-12-0323
 IRB APPROVAL DATE: 3/7/2013

ATTENTION!!

Individuals between the **ages of 20 and 34** are needed to participate in a new research study!!

We are seeking young males and females who are in the **green color (Body Mass Index (BMI) > 25)** without smoking, heart disease, respiratory conditions and other chronic diseases.

During one visit to the research center, participants in this study will:

- Have treadmill walk test.
- Have a blood test.
- Answer some basic questions about yourself.
- Be compensated for your participation.

If you are interested or have further questions please call email:

Eunduck.Park@uth.tmc.edu or call **716-909-4121**

Eunduck Park, MSN, RN, ANP, The University of Texas School of Nursing

Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adult Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu	Young Adults Study 716-909-4121 Eunduck.Park@uth.tmc.edu
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IRB NUMBER: HSC-SN-12-0323
IRB APPROVAL DATE: 10/03/2013

Appendix B
Eligibility Criteria Check List

Participants' Initial Eligibility Checklist

Identification

1. Interviewers name	2. Date (mm/dd/yy)
3. Participant Initials: <u> </u> <u> </u> First Last	

Inclusion Criteria		
(A check "No" in any of following questions (Q4-Q8) indicates that the potential participant is not eligible for the study)		
<i>If the participant "Don't know", check "I" on NO.</i>		
	Yes	No
4. Young adults aged 20-34		
5. Is capable of cardiorespiratory fitness test (Treadmill walk test)?		
6. Is able to speak, read, comprehend and respond in English?		
<i>For Women only</i>		
7. Menstrual cycle length of 24-35 days during the last 2 months		
8. Are you having your period now? (If a participant is on a period now, she will be scheduled a day when she is not on her menstruating period).		

Exclusion Criteria (A check "yes" in any of flowing questions (Q9-Q27) indicates that the potential participant is not eligible for the study) <i>If the participant "Don't know", check "I" on NO.</i>		
	Yes	No
9. Acute smoking, current smokers or smoking cessation less than 1 year		
10. Alcohol dependence or regular alcohol consumption > 7 servings per week		
11. Acute infection, a current or recent influenza illness or a recent flu shot recipient, acute respiratory infection within 2weeks		
12. Dental infection or problem within 2 weeks		
13. Chronic diseases: cardiovascular diseases: hypertension, lipidemia, and type 2 diabetes		
14. Immune disorders: rheumatoid arthritis		
15. History of osteoporotic injury and limitations due to the musculoskeletal demands of cardiorespiratory fitness test, or other bone diseases		
16. History of osteoporosis		
17. Recent surgery within 1 month		
18. Currently taking medications: hormone replacement therapy, anti-hypertensive medications, anti-platelet or coagulation medications, allergy shots or systemic corticosteroids, or selective serotonin uptake inhibitors		
19. Anti-inflammatory medications: statin, aspirin or had taken these medications in the past 48 hours		
20. Other medical conditions or medications that would prohibit participation in exercise and blood sampling (If yes, please list below)		

<i>For women only,</i>	Yes	No
21. Menopausal status		
22. Use of hormone replace therapy		
23. Is currently pregnant		
24. Lactating female		
25. History of gynecologic problems, such as endometriosis or polycystic ovary syndrome		
26. Hormonal contraception use except oral contraception: patch. depo-provera		
27. Intra Uterine Devices with hormones and Depo-Provera within past 12 months		

Participant's Eligibility: Yes _____ No _____

Appendix C
Informed Consent

THE UNIVERSITY OF TEXAS HEALTH SCIENCE CENTER - HOUSTON**Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Healthy Young Adults****HSC-SN-12-0323****INFORMED CONSENT TO JOIN A RESEARCH STUDY****INVITATION TO TAKE PART**

You are invited to take part in a research project called, "Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Healthy Young Adults," conducted by Eunduck Park, MSN, ANP, RN of the University of Texas Health Science Center. For this research project, she will be called the Principal Investigator or PI.

You have been invited to join this research study because healthy young adult volunteers are needed to conduct this research project. Your decision to take part is voluntary and you may refuse to take part, or choose to stop from taking part, at any time. A decision not to take part or to stop being a part of the research project will not change the services available to you from the medical community. You may refuse to answer any questions asked or written on any forms.

This research project has been reviewed by the Committee for the Protection of Human Subjects (CPHS) of the University of Texas Health Science Center at Houston as HSC-SN-12-0323.

DESCRIPTION OF RESEARCH:

PURPOSE: The purpose of this research study is to look at firstly, how your physical fitness (based on a treadmill walk test) is associated with inflammatory markers in your body. Secondly, how body weight is associated with inflammatory markers in your body. Lastly, how your fitness and weight are associated with inflammatory markers in your body.

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IRB APPROVAL DATE: 10/31/2012

Scientists have debated whether fitness is more associated with inflammatory markers or if body weight is more associated with inflammatory markers in your body. High levels of inflammation have been shown to be related to heart disease. The findings of this study may help to improve health care and to design programs to maintain and improve the health of young adults. However, the tests performed in this study are not diagnostic and will not predict if you will get heart disease.

This is a local study that will enroll a total of 88 subjects in Houston.

PROCEDURE:

If you agree and are able to take part in this study, you will undergo the following procedures after signing this informed consent:

- You will be asked to fill out a pre-assessment health questionnaire to identify if it is safe for you to take the fitness test. If it is not safe to test your fitness, you will be excluded from this study. The demographic questionnaire includes age, gender, ethnicity, marital status, and education. You will also be interviewed about your health conditions.
- If you are eligible to participate in this study, you will be scheduled for blood testing, body measurements, such as height and weight, and physical fitness test. This test will be performed one day at the UTHSC-H, School of Nursing and taken about 3 hours.
- For your scheduled test day, you are asked not to eat after 8 pm the night before the test. Additionally, you are asked not to do strenuous exercise or physical activity, drink alcohol, or use anti-inflammatory drugs 24 hours prior to the blood draw. You are asked to arrive at the laboratory at the School of Nursing between 8am and 10am for blood test. We will draw 10 ml (about 2 teaspoons) of blood for inflammation testing. For the fitness test, you are asked to wear comfortable exercise-type clothing, and avoid tobacco and caffeine 3 hours prior to test. You are also asked to have an adequate amount sleep the night before the test.
- After blood test, study professional staff will measure your height, weight and waist circumference. These measures are to assess your body size. You will be asked to remove any outer clothing, such as a sweater, as well as hat and shoes.
- After these measurements, you will be given a light free lunch with juice or water.
- After having a light lunch and brief resting period, the investigator will test your fitness. This test assesses how your body handles physical activity. This test will take 20-30 minutes of walking on the treadmill with warm-up and cool down stages. You will be asked to wear a heart rate monitor around your chest. Study staff will guide you during the test.

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Appendix D

Pre-Assessment Health Questionnaire

Pre-assessment Health Questionnaire

Pre-assessment Health Questionnaire consists of two sections: section 1. demographic information and section 2. pre-assessment questionnaires. Section 1 demographic information includes information on age, gender, ethnicity, marital status, and education. Section 2 pre-assessment health questionnaire will be used to determine the individual's risk related to cardiorespiratory fitness participation. The questionnaire is designed to identify whether the participants are appropriate and safe to test for cardiorespiratory fitness.

The objectives of the pre-assessment health questionnaire are:

- To identify those with a medical contraindication.
- To identify those with other health risk/medical concerns that may influence the decision about performing a cardiorespiratory fitness test (sub-maximal treadmill test).

Pre-Health Assessment Questionnaire

Interviewer's name:

Study unit identifier:

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Date of Interview: / /
 (Month) (Date) (Year)

Question #	Item
Section 1: Demographic Information (Q1-Q6)	
1	<p>Respondent's gender:</p> <p>_____ MALE [1] _____ FEMALE [2]</p>
2	<p>"How old did you turn on your last birthday?"</p> <p> _____ _____ years old</p>

3	<p>“What is your ethnicity?”</p> <p>_____ Caucasian [01]</p> <p>_____ African American [02]</p> <p>_____ Hispanic [03]</p> <p>_____ Native American [04]</p> <p>_____ Asian [05]</p> <p>_____ Pacific Islander [06]</p> <p>_____ Mixed Race [07]</p> <p>_____ DON'T KNOW [97]</p>
4	<p>“What is your martial status?”</p> <p>_____ Married or living with partner [01]</p> <p>_____ Single [02]</p> <p>_____ Divorced or Widowed [03]</p> <p>_____ Refused [97]</p>
5	<p>“What is your education level?”</p> <p>_____ Post doctoral →Skip question #6 [01]</p> <p>_____ PhD →Continue with question #6 [02]</p> <p>_____ Master's degree →Continue with question #6 [03]</p> <p>_____ Bachelor's degree→ Continue with question #6 [04]</p> <p>_____ High school's degree →Skip question #6 [05]</p> <p>_____ Middle school's degree →Skip question #6 [06]</p> <p>_____ Don't know →Skip question #6 [07]</p>
6	<p>“If you are student, what year are you in?”</p>

Section 2: Pre-Assessment Questionnaires

Reference: American College of Sports Medicine (b). ACSM's health-related physical fitness assessment manual. 3rd ed. Philadelphia (PA): Lippincott, Williams & Wilkins: 2010 (p.20-21)

Please provide responses (YES or NO) to the following concerning family history, your own history, and any symptoms you have had:

FAMILY HISTORY			PERSONAL HISTORY			SYMPTOMS		
Have any immediate family members had a:			Have you ever had:			Have you ever had:		
	YES	NO		YES	NO		YES	NO
heart attack	☐	☐	High blood pressure	☐	☐	Chest pain	☐	☐
heart surgery	☐	☐	High cholesterol	☐	☐	Shortness of breath	☐	☐
coronary stent	☐	☐	Diabetes	☐	☐	Heart palpitations	☐	☐
cardiac catheterization	☐	☐	Any heart problems	☐	☐	Skipped heartbeats	☐	☐
congenital heart defect	☐	☐	Disease of arteries	☐	☐	Heart murmur	☐	☐
stroke	☐	☐	Thyroid disease	☐	☐	Intermittent leg pain	☐	☐
Other chronic disease: _____			Lung disease	☐	☐	Dizziness or fainting	☐	☐
_____			Asthma	☐	☐	Fatigue – usual activities	☐	☐
_____			Cancer	☐	☐	Snoring	☐	☐
_____			Kidney disease	☐	☐	Back pain	☐	☐
_____			Hepatitis	☐	☐	Orthopedic problems	☐	☐
_____			Other: _____			Other: _____		

STAFF COMMENTS: _____

Have you ever had your cholesterol measured? Yes ☐ No ☐ If yes, value _____ Where: _____

Are you taking any prescription (include birth control pills) or nonprescription medications? Yes ☐ No ☐

For each of your current medications, provide the following information:

MEDICATION	Dosage—times/day	Time taken	Years on medication	Reason for taking
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

HOSPITALIZATIONS: Please list recent hospitalizations (Women: do not list normal pregnancies)

Year	Location	Reason

Any other medical problems/concerns not already identified? Yes ☐ No ☐ If so, please list: _____

LIFESTYLE HABITS

Do you ever have an uncomfortable shortness of breath during exercise or when doing activities?

Yes ☐ No ☐

Do you ever have chest discomfort during exercise? Yes ☐ No ☐

If so, does it go away with rest? Yes ☐ No ☐

Do you currently smoke? Yes ☐ No ☐ If so, what? Cigarettes ☐ Cigars ☐ Pipe ☐

How long have you smoked? _____ years

How much per day: < 1/2 pack ☐ 1/2 to 1 pack ☐ 1 to 1 1/2 packs ☐ 1 1/2 to 2 packs ☐ >2 packs ☐

Have you ever quit smoking? Yes ☐ No ☐ When? _____

How many years and how much did you smoke? _____

Do you drink any alcoholic beverages? Yes ☐ No ☐ If yes, how much in 1 week? (indicate below)

Beer _____ (cans) Wine _____ (glasses) Hard liquor _____ (drinks)

Do you drink any caffeinated beverages? Yes ☐ No ☐ If yes, how much in 1 week? (indicate below)

Coffee _____ (cups) Tea _____ (glasses) Soft drinks _____ (cans)

Are you currently following a weight reduction diet plan? Yes ☐ No ☐

If so, how long have you been dieting? _____ months

Is the plan prescribed by your doctor? Yes ☐ No ☐

Have you used weight reduction diets in the past? Yes ☐ No ☐ If yes, how often and what type? _____

ACTIVITY LEVEL EVALUATION

What is your occupational activity level? Sedentary ☐ Light ☐ Moderate ☐ Heavy ☐

Do you currently engage in vigorous physical activity on a regular basis? Yes ☐ No ☐

If so, what type(s)? _____ How many days per week? _____

How much time per day? <15 min ☐ 15-30 min ☐ 31-60 min ☐ >60 min ☐

How long have you engaged in this type of activity? <3 months ☐ 3-12 months ☐ >1 year ☐

Do you engage in any recreational or leisure-time physical activities on a regular basis? Yes ☐ No ☐

If so, what activities? _____

On average: How often? _____ times/week; for how long? _____ time/session

How long have you engaged in this type of activity? <3 months ☐ 3-12 months ☐ >1 year ☐

Appendix E

Flow Chart for Sampling and Data Collection

Appendix F

Time Frame for Project 2012-2013

Appendix G
Notification Approval

Committee for the Protection of Human Subjects

6419 Farnam Street, Suite 1100
Houston, Texas 77030

NOTICE OF APPROVAL TO IMPLEMENT REQUESTED CHANGES

May 28, 2013

HSC-SN-12-0323 - Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Healthy Young Adults
PI: Eun Duck Park

Reference Number: 096182

PROVISIONS: Unless otherwise noted, this approval relates to the research to be conducted under the above referenced title and/or to any associated materials considered at this meeting, e.g. study documents, informed consent, etc.

APPROVED: By Expedited Review and Approval

CHANGE APPROVED: Posting CPHS approved flyers at Harris County Community College and University of Houston

REVIEW DATE: May 27, 2013

APPROVAL DATE: May 28, 2013

CHAIRPERSON: John C. Ribble, MD

Upon receipt of this letter, and subject to any provisions noted above, you may now implement the changes approved.

CHANGES: The principal investigator (PI) must receive approval from the CPHS before initiating any changes, including those required by the sponsor, which would affect human subjects, e.g. changes in methods or procedures, numbers or kinds of human subjects, or revisions to the informed consent document or procedures. The addition of co-investigators must also receive approval from the CPHS. **ALL PROTOCOL REVISIONS MUST BE SUBMITTED TO THE SPONSOR OF THE RESEARCH.**

INFORMED CONSENT: Informed consent must be obtained by the PI or designee(s), using the format and procedures approved by the CPHS. The PI is responsible to instruct the designee in the methods approved by the CPHS for the consent process. The individual obtaining informed consent must also sign the consent document. **Please note that if revisions to the informed consent form were made and approved, then old blank copies of the ICF MUST be destroyed. Only copies of the appropriately dated, stamped approved informed consent form can be used when obtaining consent.**

UNANTICIPATED RISK OR HARM, OR ADVERSE DRUG REACTIONS: The PI will immediately inform the CPHS of any unanticipated problems involving risks to subjects or others, of any serious harm to subjects, and of any adverse drug reactions.

RECORDS: The PI will maintain adequate records, including signed consent documents if required, in a manner that ensures subject confidentiality.

Committee for the Protection of Human Subjects

6419 Fannin Street, Suite 1103
Houston, Texas 77030

NOTICE OF APPROVAL TO IMPLEMENT REQUESTED CHANGES

October 31, 2012

HSC-SN-12-0323 - Association of Cardiorespiratory Fitness and Adiposity with
Inflammatory Biomarkers in Healthy Young Adults
PI: Dr. Eunduck Park

Reference Number: 088871

PROVISIONS: Unless otherwise noted, this approval relates to the research to be conducted under the above referenced title and/or to any associated materials considered at this meeting, e.g. study documents, informed consent, etc.

APPROVED: By Expedited Review and Approval

CHANGE APPROVED: Revised Protocol Version 1.4. (dated 9/29/2012)
And Consent Document Version 1.1 (dated 10/31/2012)

REVIEW DATE: October 30, 2012

APPROVAL DATE: October 31, 2012

CHAIRPERSON: Richard Kirkeeide, PhD

Upon receipt of this letter, and subject to any provisions noted above, you may now implement the changes approved.

CHANGES: The principal investigator (PI) must receive approval from the CPHS before initiating any changes, including those required by the sponsor, which would affect human subjects, e.g. changes in methods or procedures, numbers or kinds of human subjects, or revisions to the informed consent document or procedures. The addition of co-investigators must also receive approval from the CPHS. ALL PROTOCOL REVISIONS MUST BE SUBMITTED TO THE SPONSOR OF THE RESEARCH.

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RECORDS: The PI will maintain adequate records, including signed consent documents if required, in a manner that ensures subject confidentiality.

Eunduck Park, MSN, RN

EDUCATION

The University of Texas Health Science Center at Houston (UTHSC-H)	2010-2014	PhD	Nursing
The State University of New York at Buffalo	2008-2010	MS	Nursing Adult Nurse Practitioner
The State University of New York at Buffalo	2005-2007	BS	Nursing
Suwon Women's College Suwon, South Korea	1993-1996	AD	Nursing

LICENSURE & CERTIFICATION

State	Active or Inactive
Nurse Practitioner in Adult Health New York State	Inactive May 2010- May 2013
Registered Nurse Texas State	Active 2010-Present
Registered Nurse New York State	Active 2003-Present
Registered Nurse Republic of Korea	Active 1996-Present
Basic Life Support for Health Care Provider, 2008-Present	
Collaborative IRB Training Initiative (CITI), 2009-Present	
HIPPA Certificate, 2009	
Teacher's Certificate, School Nurse in Republic of Korea, 1996-Present	

PROFESSIONAL EXPERIENCE

Institution Dates	Position Title	Inclusive
ST. Mary's Hospital CATHOLIC UNIVERSITY Seoul, South Korea Medical Unit	Registered Nurse (Full time)	May 1996-July 2001
Buffalo General Hospital Orthopedic Unit Buffalo, New York	Registered Nurse (Full time)	August 2007-July 2008
The University of Texas Health Science Center at Houston School of Nursing	Teaching Assistant	May 2013-August 2014

HONORS AND AWARDS

Award	Award Organization	Date
National Nursing Honor Society	Sigma Theta Tau International, Gamma Kappa Chapter The State of New York at Buffalo School of Nursing	March 2007
	Zeta Pi Chapter The UTHSC-H School of Nursing	March 2011
PARTNERS Scholarship Full tuition support for Three years of the PhD program	The UTHSC-H School of Nursing	August 2010-May 2013
James T. and Nancy B. Willerson Endowment Scholarship	The UTHSC-H School of Nursing	August 2013
Sigma Theta Tau International Zeta Pi Chapter, PhD Excellent Award	The UTHSC-H School of Nursing	May 2014

RESEARCH GRANTS

Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Young Adults (Dissertation), Principal Investigator

Sigma Theta Tau International, Zeta Pi Chapter, \$2,000, May 2012

Center for Nursing Research (Treadmill Equipment), October 2012
The UTHSC-H, School of Nursing

Global Korean Nursing Foundation (GKNF)-US, \$2,000, August 2013

Dr. Meininger's Professorship Research Support (Assay kits), December 2013
The UTHSC-H, School of Nursing

PUBLICATIONS AND PAPERS

Park, E., Meininger, J. C., Kang, D., & Gabriel, K. P. Association of cardiorespiratory fitness and adiposity with inflammatory biomarkers in young adults. In preparation for submission.

Park, E., Meininger, J. C., Baun, M., & Kang, D. Anthropometric adiposity measures and inflammatory biomarkers in adolescents and young adults: A systematic review. In preparation for submission.

Park, E. & Meininger, J. C. Effects of exercise interventions on inflammatory markers in healthy adults: A systematic review. In preparation for submission.

Master Thesis: The prevalence of cardiovascular risk factor in type 2 diabetes in an inner-city primary care practice, The State University of New York at Buffalo.

PRESENTATIONS

Park, E., Meininger, J. C., Kang, D., & Gabriel, K. P. (August 2013). Association of cardiorespiratory fitness and adiposity with inflammatory biomarkers in young adults. Presented at the Sigma Zeta Pi conference, The UTHSC-H, School of Nursing (podium presentation).

Park, E. & Meininger, J. C. (April 2012). Effects of exercise interventions on inflammatory markers in healthy adults: A systematic review. Presented at the Nursing Research Day, The UTHSC-H, School of Nursing/Sigma Zeta Pi (poster presenter).

Tukov, M. S., **Park, E.**, & Rejman, K. P. (April 2010). The prevalence of cardiovascular risk factors in type 2 diabetes in an inner-city primary care practice. Presented at the Sigma Xi Research Day, The State University of New York at Buffalo (poster presenter).

PROFESIONAL MEMBERSHIPS

Sigma Theta Tau International Society of Nursing, Zeta Pi Chapter, 2011-Present
 Sigma Theta Tau International Society of Nursing, Gamma Kappa Chapter, 2007-2011
 American College Nurse Practitioner (ACNP), 2010-Present
 Southern Nursing Research Society (SNRS), 2012-Present
 Council for the Advancement of Nursing Science (CANS), 2012-Present
 Preventive Cardiovascular Nursing Association (PCNA), 2011-Present
 American Heart Association (AHA), 2012-Present
 American College of Sports Medicine (ACSM), 2014-Present
 Obesity Society, 2014-Present

COMMUNITY SERVICE

Volunteer, ST. JOSEPH'S OFFICE, May 1996 – July 2001, Seoul, South Korea
 -Nonprofit organization
 -Provided comprehensive health care and support services to homeless
 -Presented nursing care and emotional support for those living on the margins of society or are forsaken by society as a registered nurse volunteer

RESEARCH EXPERIENCE AND TRAINING RELEVANT PROJECTS

Ph.D. Dissertation, “Association of Cardiorespiratory Fitness and Adiposity with Inflammatory Biomarkers in Young Adults”
 -Trained for **Biological Laboratory Practicum for Biobehavioral Research**, the UTHSC-H, School of Nursing, Biological Laboratory, May 2012-Present
 -Trained for **Sub-Maximal Cardiorespiratory Fitness Testing**, the UTHSC-H, Health Recreation Center, Houston, Texas, January 2012-Present

Master Thesis, “The Prevalence of Cardiovascular Risk Factor in Type 2 Diabetes in an Inner-City Primary Care Practice”
 -***Data Collector***, JUDGE METTINA COMMUNITY HEALTH CENTER, Buffalo, New York, May 2009-December 2009
 -Collected data using chart reviews on patients with Type 2 diabetes
 -Data coding and entering using SPSS soft program

Nursing Research Day: Moderator in Community Section
 The UTHSC-H, School of Nursing/ Sigma Zeta Pi

April 2013

Cardiometabolic Journal Club

January 2011-June 2013

Chair: Dr. Meininger, Co-chair: Dr. Gallagher

The UTHSC-H, School of Nursing

TEACHING EXPERIENCE*Teaching Assistant*, Department of Acute and Continuing Care, The UTHSC-H, School of Nursing, May 2013-August 2014**Management Patients in High Acuity Settings**

May 2013-August 2014

Adult Health Care I

May 2013

*Teaching Activities***Curriculum Planning and Development**

Fall 2013

-Presented and demonstrated "Providing Tracheostomy Care" to peers and faculty