Resting Serum Concentration of High-Sensitivity C-Reactive Protein (hs-CRP) in Sportsmen and Untrained Male Adults

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Summary: There is an inverse relationship between regular physical activity and concentration of serum inflammatory markers, with variations in resting CRP in trained and untrained subjects. The effect of acute and prolonged exercises has been studied on inflammatory markers with dearth of information and controversies on the resting serum values of high sensitivity CRP (hs-CRP). Therefore, this study sought to identify and compare variations that occur in serum levels of high sensitivity CRP in groups of sportsmen (6) and physically active untrained subjects. Eighty-one healthy male participants made up of 21 untrained (control), 10 footballers, 10 athletes, 10 karate, 10 volleyballers, 10 basketballers, and 10 baseballers voluntarily participated in the study. Participants rested while in sitting position for about 30 minutes during which blood pressures and heart rates were taken. 5 mls of venous blood was withdrawn from the antecubital vein of the participants (aseptically) between 7:00 and 10:00 am into lithium heparin bottles following an overnight fast. The supernatant was decanted and centrifuged at 3000 rpm, serum was collected and stored at -20°C prior to biochemical assay which was done with the use of enzyme linked immunosorbent assay (ELISA) kits for hs-CRP. Differences in the means within the sporting groups were analysed using one-way ANOVA while the difference between the trained sportsmen and untrained young adults was analysed using the independent T-test. Statistical significance was set at p < 0.05. The Mean ±SEM age, weight, height, and BMI of the participants were 22.0±0.8 years, 64.1±2.2 kg, 1.74±0.3 m, and 20.6±0.2 kg/m² respectively. The resting concentration of hs-CRP (µg/ml) was 1.0±0.2 in the untrained, 2.6±0.7 in footballers, 3.6±2.1 in track athletes, 2.4±0.5 in basketballers, 2.2±0.5 in volleyballers, 2.4±1.3 in baseballers, and 1.7±0.5 in karate respectively. There was no significant difference in the resting hs-CRP amongst the sporting groups, and in the sportsmen and untrained group. Resting serum levels of hs-CRP falls within normal range, and varies insignificantly in groups of sports, and in sportsmen and untrained young male adults. This may suggest that the types of sporting activities or participation in sports has no impact on the resting serum hs-CRP.

Keywords: Resting hs-CRP, Sportsmen, Young male adults

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INTRODUCTION

Physical activity is a routine recommended as a model for studying inflammatory processes and responses (Shek and Shephard, 1998). It has been well established and documented that acute bout of exercise may result in alteration of the circulating levels of a number of pro-inflammatory cells (Semple et al., 2004; Suzuki et al., 1999), and the rise in the inflammatory markers which follows physical activity has been attributed to muscle damage (Semple et al., 2006; Plaisance and Grandjean, 2006). Psychological stress which may be more prevalent in sportsmen has also been shown to initiate an increase in pro-inflammatory cytokines (Maes et al., 1998). Exercised-induced muscle injury is the primary stimulus for IL-6 response (Kasapis and Thompson, 2005) with studies reporting that complex intramuscular signaling stimulates the exercising muscle to release IL-6 which is independent of muscular damage (Pedersen et al., 2001; Febbraio and Pedersen, 2002). IL-6 is central to local and systemic inflammatory processes and its release activates the liver to secrete C-reactive protein (CRP) (Plaisance and Grandjean, 2006). Long term physical activity and good cardio respiratory fitness are inversely associated with CRP levels and this supports the evidence that suggests that regular physical activity has anti-inflammatory properties (Powers et al., 1999). The bulk of exercise training interventions are in accordance with sampling observations that physical activity lowers CRP with an effect that may be as good as or even better than lipid-lowering agents (You et al., 2004). Long term physical training results in a significant reduction in resting serum hs-CRP levels in
C-Reactive protein has been shown to increase dramatically, peaking up to a thousand fold during its acute-phase response usually 24-48 hours following an initial acute inflammatory stimulus such as acute bouts of exercise (Kushner and Rzewnicki, 1994; Pepys, 2003). Recently, there has been more emphasis on ‘high-sensitivity’ or ‘highly-sensitive’ CRP (hs-CRP). High sensitivity C-reactive protein (hs-CRP) is a sensitive marker of low-grade inflammation. It has the assay and analytic characteristics for its clinical uses, with a dose-response relationship to coronary heart disease which is independent of other major predisposing factors. It also adds prognostic information to risk factors of coronary heart disease and other cardiovascular diseases (Pearson et al., 2003; Ridker et al., 2003).

Of recent, there is paucity in research demonstrating possible variations that may occur in the resting serum hs-CRP concentrations between / within sporting groups and untrained group. Therefore, this study sought to investigate and compare variations that may occur in the resting serum hs-CRP concentrations amongst sportsmen from six different sporting activities (Football, Athletics, Basketball, Volleyball, Baseball, and Karate), amongst untrained young male adults, and between sporting and untrained groups. We hypothesized that resting serum hs-CRP would be considerably higher in activities that require all-out sprint performance compared to activities that require more of vigilance and psychomotor performance; and the resting serum hs-CRP concentrations of the trained sportsmen would be higher than that of the untrained young male adults.

MATERIALS AND METHODS

This study was carried out at two centres; Kwara State Stadium, Ilorin, Kwara State, Nigeria and the Department of Physiology Laboratory, College of Health Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria. The ethical approval was given by the ethical review committee of the College of Health Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria. Participants were grouped into seven in which the twenty-one (n=21) untrained (but physically active) male undergraduates from University of Ilorin served as the control group while six other groups were 60 sportsmen from six different sporting activities—football, athletics, volleyball, karate, baseball and basketball. Each group having 10 participants (n=10).

Before the experiment, participants were given information sheets comprising of the details of the study, study design, and all the potential risks associated with the study. The Physical Activity Readiness Questionnaire (PAR-Q) and Health/Lifestyle Questionnaires were also filled by the participants and participants with medical history of respiratory or cardiovascular diseases were excluded from the study.

Materials

Participant’s body weight was measured using a manual weighing scale (Harson Scales Company, USA) with a precision of 0.1kg and the height was measured to the nearest 0.1cm with the use of a heightometer. Blood Pressure was measured with the use of a mercury in-glass sphygmomanometer (Accoson, England) and stethoscope (Littman, USA). Participant’s blood samples were collected using sterile needles and syringes (Menoject, China), latex gloves, wet cotton wool swab with methylated spirit into 5ml lithium heparinized bottles (Micropoint Bioscience Inc, USA) and centrifugation of the samples by a centrifuging machine (Zhangji, China) was done. Supernatant were stored in plain bottles (Silver Health Diagnostics, Nigeria) and kept in a refrigerator (Haier Thermocool, HTF-66H) at -25ºcelsius prior to biochemical analysis. Serum high sensitivity C - reactive protein (hs-CRP) was determined in duplicate using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (Accu-Bind ELISA Microwells, Monobind Inc, USA).

Experimental Protocol

Participants were informed to refrain from strenuous physical activity, smoking and consumption of alcohol and/or caffeine at least 24 hours prior to the venepuncture. On the experimental day, after an overnight fast, between 7:00 am and 10:00 am, anthropometric measurement was taken and participants were told to sit in resting position during which two-reading blood pressure and heart rate was taken with the average recorded. After the 30 minutes of rest period, 5ml of blood sample was collected aseptically from the antecubital vein into lithium heparinized sample bottles. The supernatant was decanted and centrifuged at 3000 rpm, serum was collected and stored at -20ºC prior to biochemical assay which was done with the use of enzyme linked immunosorbent assay (ELISA) kits for hs-CRP. Biochemical analysis was done in the chemical pathology laboratory in the College of Health Sciences, University of Ilorin, Ilorin, Nigeria.
Statistical Analysis
Data are presented as Mean ± SEM. The difference in the mean within the sporting groups was analysed using one-way ANOVA while the difference between the trained sportsmen and untrained young adults was analysed using the independent T-test. Post hoc analysis was performed using the Bonferroni’s test, and values of p < 0.05 are considered to be statistically significant. All analysis was done using SPSS software (version 20; SPSS Inc, Chicago, IL) and Graph Pad Prism software version 5 (Graph Pad software, Inc, San Diego, CA).

RESULTS
Anthropometry and Physiological Variables. Physical and fitness characteristics of the participants were given in Table 1 and 2. Participant’s age, weight, height, and BMI were 22.0±0.8 years, 64.1±2.2 kg, and 1.74±0.3 cm, 20.6±0.2 kg/m² respectively. The ages of volley ball players were significantly higher (p<0.05) than the untrained, football, basketball, and baseball players. The BMI of the participants are within normal range (18.5-24.9 kg/m²) for their ages and sexes. The blood pressures and heart rates of the sportsmen and untrained group. The mean systolic blood pressure, diastolic blood pressure, and heart rate of the sportsmen were insignificantly higher than the untrained group.

Table 1. Selected physiological variables in sportsmen and untrained participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sportsmen (n=60)</th>
<th>Untrained (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>20.1±1.0</td>
<td>21.0±0.3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7±0.2</td>
<td>1.8±0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.1±2.2</td>
<td>64.0±1.1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.6±0.2</td>
<td>20.6±0.3</td>
</tr>
<tr>
<td>Resting SBP (mmHg)</td>
<td>135.7±3.5</td>
<td>124.0±2.9</td>
</tr>
<tr>
<td>Resting DBP (mmHg)</td>
<td>76.2±2.4</td>
<td>72.7±0.2</td>
</tr>
<tr>
<td>Resting Heart Rate (bpm)</td>
<td>83.5±2.5</td>
<td>82.7±3.7</td>
</tr>
</tbody>
</table>

Values expressed as Mean±SEM, SBP: Systolic blood pressure; DBP: Diastolic blood pressure; n: number of participants.

Table 2. Anthropometric data of the participants in the experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yrs)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI(kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Football</td>
<td>19.0±0.6</td>
<td>1.72±0.02</td>
<td>61.7±2.6</td>
<td>20.9±0.6</td>
</tr>
<tr>
<td>Athletics</td>
<td>21.6±1.3</td>
<td>1.74±0.02</td>
<td>66.0±2.2</td>
<td>21.9±0.6</td>
</tr>
<tr>
<td>Basketball</td>
<td>23.1±1.2</td>
<td>1.79±0.03</td>
<td>66.7±3.4</td>
<td>20.6±0.5</td>
</tr>
<tr>
<td>Volleyball</td>
<td>26.3±1.1</td>
<td>1.78±0.01</td>
<td>68.9±1.6</td>
<td>21.7±0.6</td>
</tr>
<tr>
<td>Baseball</td>
<td>19.8±1.8</td>
<td>1.69±0.01</td>
<td>58.4±2.1</td>
<td>20.4±0.4</td>
</tr>
<tr>
<td>Karate</td>
<td>22.8±1.1</td>
<td>1.73±0.02</td>
<td>63.1±2.2</td>
<td>20.9±0.5</td>
</tr>
<tr>
<td>*Untrained</td>
<td>21.0±0.3</td>
<td>1.76±0.01</td>
<td>64.0±1.1</td>
<td>20.6±0.2</td>
</tr>
</tbody>
</table>

Values expressed as Mean±SEM. n: number of participants; * significantly different from Volleyball, p<0.05. BMI: body mass index; a: number of participants in the untrained group is 21, (n=21).

Resting levels of hs-CRP. Figure 1 gives the average resting concentrations of hs-CRP in the seven groups. The average resting hs-CRP of athletes (3.6±2.1 µg/ml) is the highest while that of the untrained group (1.1±0.2 µg/ml) is the lowest. However, there was no significant difference in resting hs-CRP levels in these groups. Figure 2 provides the average resting concentrations of hs-CRP of the sportsmen and untrained group. The increase in hs-CRP observed in the sportsmen (2.46±0.43 µg/ml) was not significantly different from the untrained group (1.06±0.18 µg/ml).

Figure 1: The average resting concentration of hs-CRP in the sporting groups and untrained participants. U - Untrained, F- football A-Athletes, B- basketball, V-volleyball, b- baseball, K- karate.

Figure 2: Mean concentration of hs-CRP of sportsmen and untrained participants.
DISCUSSION

The main objective of this study is to identify, quantify, and compare possible variations that may occur in the resting serum hs-CRP concentrations in different sporting groups, and in sportsmen and untrained (physically active) young men. The result of this study revealed an insignificant variation in resting serum hs-CRP concentrations in the six sporting groups; and in the sportsmen and untrained young male adults.

The normal systemic CRP level is below 5µg/ml, and the average value in sedentary and general population is about 2µg/ml, with no gender, diurnal, or seasonal variations (Pepys, 2003; Gabay and Kushner, 1999). In this study, the average resting hs-CRP levels of all the experimental groups were within normal range with the untrained young adults (1.06±0.18 µg/ml) being insignificantly lower than that of the trained individuals (sportsmen) (2.46±0.43 µg/ml). This differ from study of Hazar (2012) that investigated the resting serum hs-CRP levels of eighteen (18) elite adolescent soccer players (14.3±0.3) and 18 physically active subjects (14.6±0.4). He reported a significantly higher resting serum hs-CRP concentrations in the soccer players compared to the physically active subjects, thus demonstrating that prolonged soccer training increased the resting serum hs-CRP levels. In contrast, this present study agrees with studies of Rawson et al. (2003) and Pitsavos et al. (2003) that demonstrated that physical activity does not influence resting values of CRP. Pitsavos et al. (2003) reported an insignificant difference in the CRP levels of subjects that participated in a leisure-time physical activity compared to the sedentary groups with the CRP concentration of the physically active groups being 33% lower than that of the sedentary groups.

The mechanism by which exercise training reduces inflammation and suppresses level of serum CRP is not well defined. Contracting muscle is the main source of plasma IL-6 (myokine), which is the first cytokine present in response to exercise (Toft et al., 2011), and this muscle derived factor appears to have anti-inflammatory effect. The level of IL-6 released during exercise initiates anti-inflammatory cascade which is proportional to the intensity, duration, and the amount of muscle mass used (Fischer, 2006). IL-6 is central to local and systemic inflammatory processes and its release activates the liver to secrete C-reactive protein (CRP) (Plaisance and Grandjean, 2006). C-reactive protein has been shown to increase dramatically peaking up to a thousand fold during its acute-phase response usually 24-48 hours following an initial acute inflammatory stimulus (Kushner and Rzewnicki, 1994; Pepys, 2003).

Physical activity may cause a reduction in the resting levels of IL-6, Tumour Necrotic Factor (TNF-alpha) and CRP by reducing obesity and leptin as well as increasing adiponectin and insulin sensitivity (Mayer-Davis et al., 1998). However, several factors may also contribute to the exercise-related anti-inflammatory effect, some of which may be mediated by modification of cytokine production from other sites, such as skeletal muscles and mononuclear cells (Smith et al., 1999; Gielen et al, 2003). The results of this study reveal that physically active untrained young adults had lower resting serum hs-CRP values compared to trained sportsmen which agree with the hypothesis made. This implies that participation in long term sport training raise resting serum hs-CRP values (insignificantly) compared to the physical active young adults who do not undergo regular training. The background participation of these untrained individuals in physical activity could be a viable explanation for their resting serum hs-CRP values. The results also indicated that there was no significant difference in the hs-CRP levels within the six sporting activities. However, the karate group had a considerably lower resting hs-CRP level (1.66±0.45 µg/ml) compared to the other sporting groups thus, suggesting that types of sporting activity may influence the resting hs-CRP. The uniqueness of this study is that no previous study examined potential variations in the resting serum hs-CRP in different sporting groups, and between sportsmen and untrained physically active young men. In addition, there was effective screening of cardiovascular diseases with the use of health questionnaires, pre-experiment two-reading blood pressure (with average taken), and heart rates to allow exclusion of patients with cardiovascular diseases which may affect biochemical analysis. Furthermore, blood samples were collected from all the participants at the same time of the day, in the morning (7:00-10:00 am) and after an overnight fast. This study further revealed that while there was a tendency for higher CRP levels in the athletes (3.56±1.06 µg/ml), there was no significant difference in resting hs-CRP in the sporting groups which may be as a result of the low sample size (n=10) in each sporting group. Furthermore, the insignificant difference established in the resting serum of hs-CRP in sportsmen and untrained young men may be justified by the disproportion of the total number of participants in each group who volunteered to participate in this study (n=60; n=21).

Drawbacks were encountered in this study; the sportsmen trained at least 1-2 days before the experimental day, thereby making it difficult to determine whether the levels noted was due to long term training or from the most recent exercise performed the day before; and the untrained group (which are the control) are not entirely sedentary but are physically active young men. Considering these limitations, more studies are essential to fully explore the variations in hs-CRP levels in long term and acute phase response to resistance and endurance exercise. Furthermore, the comparison of hs-CRP levels should include sedentary individuals.
In conclusion, the resting serum concentration of hs-CRP varies insignificantly in groups of sports, and in sportsmen and untrained physically active young men. This may suggest that regular participation in different types of sports and long duration sport training may not raise the resting serum hs-CRP values compared to the physically active young adults who do not undergo regular training.

REFERENCES