2017

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Franzen, Bailey; Greenawalt, Cassandra; and Vlahos, Sloan, "The Effects of Oral Curcumin and Bioperine Supplementation on C-reactive protein In CrossFit Athletes" (2017). Loma Linda University Research Reports. 1.
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The Effects of Oral Curcumin and Bioperine Supplementation on C-reactive protein In CrossFit Athletes

Bailey Franzen, Cassandra Greenawalt & Sloan Vlahos
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PURPOSE. C-reactive protein (CRP) is an acute-phase inflammatory marker which circulates in blood plasma and rises in the presence of systemic inflammation. Strenuous exercise, such as that performed by CrossFit athletes, has been found to acutely raise CRP levels. Curcumin has demonstrated the ability to reduce CRP levels by blunting the release of pro-inflammatory cytokines secondary to the suppression of the NF-κB pathway. Concomitant graduate research administration of bioperine with curcumin improves bioavailability by 2,000%. The aim of this study was to evaluate the effects of oral curcumin and bioperine supplementation on CRP levels in CrossFit athletes.

METHODS. Sixteen CrossFit athletes (Male 56.3%, Female 43.8%) provided pre-intervention CRP samples through a finger stick blood draw. Participants were given a combined curcumin (2g) and bioperine (20μg) supplement to be administered with their first meal of the day following 28 days after initial CRP testing. Participants were instructed to continue their usual exercise, dietary, and lifestyle practices over the duration of the study. On the 28th day of supplementation, participants provided a final CRP blood sample. Pre- and post-intervention CRP samples were evaluated using a paired t-test.

RESULTS. Curcumin and bioperine supplementation resulted in a mean decrease in CRP levels of 0.26 ± 2.27 mg/L; however, this reduction failed to reach statistical significance (p = .65). Overall, five participants (31.3%) showed an increase in CRP at follow-up, five participants (31.3%) showed a decrease in CRP, and six participants (37.5%) showed no observable changes in CRP at follow-up, likely due to CRP levels below the testing sensitivity threshold.

CONCLUSION. The majority of CrossFit athletes evaluated in this study had clinically low or below sensitivity threshold levels of CRP. A lack of measurable data from six participants with CRP levels below testing sensitivity threshold reduced the statistical power of our study. CRP levels in CrossFit athletes should be studied further using larger sample sizes and more sensitive testing devices given the apparent low CRP levels in this population.

Key Words: Curcumin, C-reactive protein, CrossFit, Bioperine
Fifty percent of an individual’s overall health and longevity is associated with health-related behaviors. In addition to a healthy diet and the avoidance of tobacco, physical activity serves as a key practice for maintaining an overall healthy lifestyle. Due to its importance, any hindrance of physical activity may be detrimental to one’s health. One of the most common causes of exercise restriction is exercise-induced inflammation. The inflammation caused by physical activity is most commonly identified as either exercise-induced muscle damage (EIMD) or delayed onset muscle soreness (DOMS). Both EIMD and DOMS can impact subsequent training sessions and activities of daily living, even in regularly active individuals such as CrossFit athletes. With the purpose of helping individuals properly recover from exercise, researchers strive to determine ways in which EIMD and DOMS can be alleviated and prevented.

Several studies have supported a role for nutritional intervention to maintain immune function in the post-exercise period. Turmeric, a bright yellow spice that comes from the root of the *Curcuma longa* plant and that is commonly used in Asian cooking, has been identified as a potential remedy for inflammation caused by exercise. Although turmeric has been used for nearly 4,000 years in southern Asia, its popularity has drastically increased over recent years in the United States. The primary component of turmeric that has been studied is the constituent known as curcumin. Many studies have observed a significant decrease in inflammation in those consuming curcumin versus those receiving a placebo. Though curcumin has shown promise as an anti-inflammatory agent, its bioavailability is poor and requires novel methods of delivery to achieve therapeutic effects. Bioperine, also referred to as piperine, is a major constituent of black pepper and is known to inhibit hepatic and intestinal glucuronidation. Previous studies
found that administering 20 μg of bioperine produced significantly higher serum concentrations, increasing curcumin bioavailability by 2,000% after only 45 minutes. Additionally, it has been shown that concomitant administration of 20 μg of bioperine produced much higher serum concentrations from 15 minutes to 1-hour post administration; the increase in bioavailability was 2,000%. The mechanism for increased bioavailability is that bioperine binds to multiple sights of the CYP3A4 enzyme in cytochrome P450 and intercalates with curcumin to form a hydrogen bonded complex, thereby inhibiting enzymes that cause glucuronosylation of curcumin.

C-reactive protein (CRP) is a protein found in blood plasma; CRP levels rise in response to inflammation. Given the substantial evidence of curcumin’s role in suppressing inflammatory markers, there is valid reason to believe that oral curcumin and bioperine supplementation may aid in the reduction of CRP levels. Thus far, research has primarily focused on curcumin’s impact on circulating cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), in the post-exercise period.

CrossFit athletes regularly partake in varied functional movements performed at relatively high intensity. Such movements reflect, but are not limited to, aspects of gymnastics, weightlifting, running, and rowing. Exercise is an important stressor to the body, leading to an activation of immune responses commonly evoked by other stressors such as trauma and sepsis. This has been verified by elevated levels of serum CRP immediately following high-intensity exercise in healthy adult males. To date, there are no definitive answers as to whether acute exercise related inflammatory markers may have a negative effect over the long term. Some research has demonstrated a reduction in markers, such as CRP, after prolonged exposure to routine exercise, while other studies have shown no such reduction. This variation in results is likely due to concomitant factors such as healthier dietary patterns or weight reduction.
commonly found in conjunction with routine exercise; both factors are known to reduce markers of inflammation. This hypothesis was supported by the Inflammation and Exercise (INFLAME) study, which concluded that exercise without weight loss does not result in the reduction of CRP. CRP is a systemic marker of negative health effects, such as increased risks for various cancers, type two diabetes, and cardiovascular disease. Reducing levels of CRP with inflammatory modulating compounds, like curcumin, may be beneficial over the long term.31-33 Athletes who participate in CrossFit exercise multiple times per week may be in prolonged states of inflammation from frequent and intense physical activity. This graduate student research study aimed to investigate the effects of oral curcumin and bioperine supplementation on CRP levels in CrossFit athletes. To our knowledge, a study involving this intervention amongst CrossFit athletes had not yet been performed. We hypothesized that after four weeks of oral supplementation of 2 grams of curcumin with 20 μg of bioperine, we would observe significantly lowered CRP levels in CrossFit athletes.

Methods

Participants

Twenty healthy, non-pregnant, non-smoking participants were recruited from the CrossFit South Redlands gym in the Inland Empire region of southern California. Upon permission from the CrossFit gym’s owner, researchers recruited in-person by setting up a table in the entryway of the CrossFit gym to inform the athletes about the study. Recruitment took place through brief in-person informative meetings prior to scheduled workout sessions at the CrossFit gym, through word of mouth, and by posting flyers in the gym. Individuals interested in participating were screened for inclusion and exclusion criteria. Participants included in the study were healthy adults between the ages of 18-45 years who had participated in CrossFit exercise at least two
times per week for at least three months prior to the start of the study. Potential participants were excluded for pregnancy, cardiovascular disease, diabetes, arthritis, gingivitis, history of cancer other than small sections of skin, cigarette smoking and/or tobacco use, use of any blood thinning medications, and use of turmeric and/or other herbal supplementation within the last 30 days.

All volunteers were first informed of the study objectives and methods. Upon agreeing to participate in the study, participants signed an informed consent and completed an additional form to provide demographic information. The requested information included height, weight, gender, marital status, education level, and age. All methods and procedures were approved by the Institutional Review Board of Loma Linda University.

Compliance
For each day of the study, participants were asked to record supplement intake on a compliance log. Participants were instructed to take missed pills with the next day’s pills (up to eight at a time, with food) if a day of supplementation was missed. Participants were asked to indicate, “eight pills taken” in the designated notes section on the day the pills were consumed. Participants were asked to record all over-the-counter and prescription medications, as well as the dose of medication, taken during the duration of the study on their supplementation compliance log. Participants were also instructed to record any immunizations received during the study on the compliance log, denoting the type of immunization and the date on which it was received.
Questionnaires

On the first day of testing, participants were asked to complete a demographics form. This form was used to collect information such as name, participant identification, height, weight, contact information, age, gender, highest level of completed education, ethnicity, marital status, sexual activity, and fluid intake. On both testing days, participants were given a Perceived Stress Scale form to indicate their current level of psychogenic stress. They were also asked to complete a Testing Day Questionnaire, in which they were asked to provide information regarding fluid intake, food intake, over-the-counter and prescription medications, sexual activity, and any other information the participants felt may impact their test results.

Supplementation

The supplement used for this study was obtained from Pure Encapsulations, Inc. The dosage instructed for daily consumption was four pills containing a total of 2 grams of curcumin with 20 μg of bioperine and 10 mg of ascorbyl palmitate (a vitamin C ester with antiscorbutic activity). The supplement used for this study contained bioperine to increase the bioavailability of curcumin. The ascorbyl palmitate present in the supplement used for this study acted primarily as a preservative rather than an exogenous antioxidant. Although some research has shown that vitamin C supplementation has beneficial effects on CRP levels, the maximum dosage of vitamin C from the supplement used for this study was only 4-8% of the dose used in studies shown to reduce CRP levels.

High Sensitivity C Reactive Protein (hsCRP) Testing

All hsCRP tests were performed at the designated CrossFit gym by qualified professionals. Participants were informed about the procedure of collecting the blood for measuring hsCRP
levels. Care was taken so that each participant was tested near the same time on each day their hsCRP level was measured. The kit box equilibrated at room temperature for a minimum of 10 minutes to allow material to warm up to room temperature before use.

Assay procedure and Sample Collection (Pre- and Post- Supplementation):

- Diazyme hsCRP Test Kit box was used to analyze capillary blood specimen for hsCRP. The Kit provided a Radio Frequency ID (RFID) card which was inserted into the SMART 700-340 device prior to analysis.

- Prior to acquiring and running participant samples the Research Assistant ran the BioRad developed Diazyme controls.

- One reaction cuvette and one reagent cap from the kit box was used for each participant sample. Each participant had two baseline samples and two post-intervention samples.

- Proper standard precautions were taken to maintain safety and hygiene. Disposable nitrile gloves were worn by the phlebotomist while collecting blood samples.

- The participants were asked to rub their hands together to improve blood circulation. Gentle pressure was applied circumferentially to the mid-finger before the fingerstick to encourage better blood flow. The phlebotomist continued to apply intermittent, gentle, circumferential pressure to the mid-finger immediately after the fingerstick and during the blood acquisition to prevent interstitial fluid concentrated at the tips of the fingers.

- The participant’s non-dominant hand 4th or 5th digit was cleaned with a swab saturated with 70% isopropyl alcohol. The cleaned finger dried completely before the fingerstick was performed.

- An Accu-Check® Safe-T-Pro Plus, sterile, single-use lancet of 1.8mm depth, 23 gauge/0.63 mm width was used. The phlebotomists grasped the lancet between their dominant thumb and forefinger and activated the lancet with their thumb. During the fingerstick the lancet was held perpendicular to the fingertip with firm and even pressure. Immediately after the lancet was activated and discarded in the sharps container, the first drop of blood was cleaned with a sterile cotton swab to minimize collection of interstitial fluid. 20 µL of capillary blood was collected using two EDTA capillaries.

- The participant had a bandage applied to the lanced finger after 40 µL of blood was collected.

- Each 20µl blood sample (40 µl total) was then added into two reaction cuvettes.

- Specimens were labeled with the subject ID number.
- A reagent cap was placed on the top of the reaction cuvettes and snapped into place.

- Specimens were stored at 23°C until analyzed in the LLU Clinical Molecular Psychoneuroimmunology Lab within five hours.

- The participant’s ID number was entered in the Diazyme SMART analyzer.

- The capped reaction cuvettes were inserted into the cuvette holder on the door of the SMART analyzer. Prior to its insertion into the instrument, the cuvette was examined for even blood mixture without bubbles and wiped with lint a free Kimwipe or similar nonabrasive cloth to ensure the cuvette surface was clean.

- The hsCRP assay was performed according to manufacturer guidelines. Specimens were assayed one at a time.

- The results were stored in the device as well as recorded by a researcher in a secure password locked Excel spreadsheet.

**Procedure**

This study was a non-randomized controlled trial. Following completion of the IRB consent form and signed authorization for the use of protected health information, participants were instructed to return the following week for the first day of CRP testing. All recruited participants received a follow-up email and phone call at least 48 hours prior to the first day of testing to remind them of the upcoming study procedures. On the first day of testing, participants met at the CrossFit South Redland’s gym. Upon arrival, participants filled out the designated paperwork (Demographics form, Bill of Rights, Testing Day Questionnaire and Perceived Stress Scale), body weights were recorded, and baseline measurements of CRP were collected by a single fingerstick. Once baseline measurements were taken, participants received verbal instructions, a Compliance Log with written instructions and contact information, and a 28-day supply of supplementation.

Participants were instructed to consume the assigned product daily for four weeks. Instructions provided to the participants indicated that four pills should be taken per day, at about the same time each day, and preferably with the participant’s first meal of the day. In addition,
participants were instructed to maintain their previous dietary and exercise practices during participation.

All participants received an email or text message 48 hours prior to the second day of testing to remind them of the upcoming study procedures. On the final day of supplementation, 16 of the 20 recruited participants returned to the CrossFit gym for the second day of CRP testing. Upon arrival, all participants completed the designated paperwork (Testing Day Questionnaire and Perceived Stress Scale) and submitted their finished Compliance Logs. Additionally, weights were taken on the same scale used for the first day of CRP testing and post-supplementation measurements of CRP were collected by a single fingerstick.

Data Analysis

Collected data was evaluated using SPSS version 24 software using a paired t-test. Frequencies, mean and standard deviation were calculated for the demographic information collected from the participants.

Results

Of 20 participants who were recruited, 16 completed the study in its entirety. Post-supplementation data was not collected for four participants who were lost to follow-up. Analysis was conducted using pre- and post-supplementation data collected for 16 participants (nine males (56.3%) and seven females (43.8%)). Participant demographic data is provided in Table 1.

CrossFit athletes were instructed to have their initial fingerstick blood draw prior to their work out on the first day of data collection. Twelve participants’ pre-supplementation fingerstick blood samples were collected prior to their workout that day. Due to scheduling constraints, four participants were unable to provide fingerstick blood draw samples before working out and
therefore had their baseline CRP data collected after their workout. To control for this variability, on the second day of data collection, the same athletes provided post-supplementation fingerstick blood draw samples after they worked out.

BMI did not differ between the baseline and the follow-up 28 days later (p=.50), during which time the participants were instructed to continue their usual diet and exercise patterns. Similarly, perceived stress did not differ between the baseline and follow-up measures (p=.25).

To assess the effect of curcumin and bioperine supplementation, we examined the inflammatory marker hsCRP using a Point of Care hsCRP assay. As summarized in Table 2, the mean difference in CRP at follow-up decreased by 0.26 +/- 2.27 mg/L; however, this change was not statistically significant (p=.65).

Overall, five participants (31.3%) showed an increase in CRP at follow-up, five participants (31.3%) showed a decrease in CRP, and six participants (37.5%) showed no observable change in CRP at follow-up. A SMART 700-340 point of care device was used to analyze CRP values. The absence of measurable changes for six participants is relative to the decreased sensitivity of the POC assay, where the instrument cannot measure CRP levels <0.50 mg/L. “No change” is used to identify individuals who had CRP levels below the sensitivity threshold for both pre- and post-supplementation measurements. Individual differences between each participant’s baseline and post-supplementation CRP levels are shown in Figure 1.

The change in CRP was tested separately for the pre- and post-exercise measurement groups. Participants in the post-exercise measurement group had a mean CRP difference of +.90 +/- 0.87 mg/L at follow-up. The observed increase was not statistically significant (p=.25). Participants in the pre-exercise measurement group had a mean CRP difference of -0.65 +/- 2.48 mg/L at follow-up. This observed decrease was not statistically significant (p=.25). Moreover,
the variability in CRP difference for the pre-exercise group was largely due to one participant’s CRP that had decreased by 7.97 mg/L at follow-up. A non-parametric test confirmed this participant’s individual measurements did not change the overall results.

**Discussion**

The current study did not reveal a statistically significant difference in CRP level in CrossFit athletes after a 28-day oral curcumin and bioperine supplementation. Our study participants’ low baseline CRP levels support previous research, which suggests that while acute exercise has been shown to transiently increase CRP levels in trained athletes, there is an inverse relationship between elevated CRP and routine strenuous exercise. It is likely that our study sample has acclimated to exercise-induced stress by regularly participating in CrossFit exercise. In turn, half of participants demonstrated no measurable change in CRP from pre- versus post-supplementation. These participants each had baseline and post-supplementation CRP measurements <0.50 mg/L. Scores that were below the POC instrument’s sensitivity threshold of <0.50 mg/L displayed a result of <0.50 mg/L. Therefore, differences in pre- and post-supplementation CRP levels could not be quantified in individual results with lower than the instrument sensitivity results. It is unlikely that CRP levels for these individuals remained unchanged, but with the instrumentation utilized positive and/or negative changes could not be quantified.

One participant’s post-supplementation CRP level was drastically lower than his baseline measurement, which was 941% higher than the mean baseline CRP of other participants. A non-parametric test confirmed that this participant’s individual measurements did not change the overall results. Data collected throughout the study did not provide a clear explanation for his high baseline CRP as an outlier; however, there are several speculations that can be made
regarding explanations for his baseline level. Some previous studies have shown great variance in baseline CRP levels for trained athletes participating in strenuous exercise, ranging from 0.2-13.9 mg/L.36

A primary strength of the current study is its novelty. To our knowledge there are no previous studies evaluating the effects of curcumin and bioperine supplementation on CRP in CrossFit athletes. The study design was strengthened in that it attempted to control for other factors that could influence CRP such as stress, change in body mass index, and pre-existing medical conditions. The supplementation period of 28-days mirrors the average menstrual cycle length for women, controlling for CRP differences associated with menstruation. Additionally, gender was relatively evenly distributed in the study’s sample. Furthermore, pre- and post-supplementation CRP levels were measured within two hours of each other on respective testing days.

The study is limited by the low sensitivity of the point of care device used to analyze hsCRP assays. Of the 16 participants who provided CRP samples, only 10 supplied measurable data, thus limiting the statistical power of our analysis. This acted as a limitation to our already small pilot study sample size. While BMI did not significantly change and general eating pattern information was collected, in-depth food-frequency questionnaires were not conducted. Lastly, information regarding exercise within 24-48 hours of data collection was not assessed. Acquisition of this information would have been beneficial since CRP values may have been impacted by exercise performed 24-48 hours prior to baseline and/or follow-up data collection.

Future research should use laser nephelometry to assess hsCRP levels, which would lower the sensitivity level to as much as 0.04 mg/L. The use of an ultra-sensitive instrument would provide more specific levels and prevent the loss of measurable data. Additionally, future
research should consider using other inflammatory markers such as pre-cursors to CRP. These include IL-6 and TNF-alpha, both of which are the first inflammatory markers produced and released by skeletal muscles during exercise. Prospective research should also investigate the effects of curcumin supplementation on CrossFit athletes with both shorter and longer study durations. Other areas of interest include the effects of curcumin supplementation on athletes versus non-athletes, the effects of varied amounts of curcumin dosages, and the differences between supplementation daily versus supplementation throughout the day.

Conclusion

CrossFit athletes who have been participating in CrossFit activities on a regular basis appear to be more likely to have low CRP levels. In the current study, it is observed that a 28-day supplementation of curcumin and bioperine has reduced overall CRP levels in CrossFit athletes, however, the reduction in CRP was not statistically significant. Previous research supports the use of curcumin as an anti-inflammatory agent; however, its impact on CRP levels in CrossFit athletes warrants additional investigation. We conclude that the effects of curcumin on CRP in CrossFit athletes needs to be explored further with observations using larger sample sizes that are controlled for exercise type, duration, and intensity, in which CRP values are analyzed by an ultra-sensitive testing device.

Acknowledgments

Our research team would like to thank the participants who took part in our study. We would also like to express our appreciation to Loma Linda University and CrossFit South Redlands for the use of their facilities. Lastly, we would like to thank the following individuals for their ongoing assistance, insight, and guidance in the development and successful execution of our
research study: Gurinder Bains, PhD, Lee Berk, DrPH, Kristin Brujhell, B.S., Cory Gheen, M.S.,
RDN, Jeje Noval, PhD, RDN and Grenith Zimmerman, PhD.
1. Sallis R. A Drug Called Exercise: Why You Should Take and Prescribe It. Lecture presented at Loma Linda University; February 16, 2016; Loma Linda, CA.


34. Pure Encapsulation, Inc. 490 Boston Post Rood, sudbary, MA , 01776.

### The effects of oral curcumin and bioperine supplementation on C-reactive protein in CrossFit athletes

Table 1. Baseline Demographic Information for 16 Participants prior to 28-Week Oral Curcumin and Bioperine Supplementation

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<tr>
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Table 2. BMI, Perceived Stress Score, and CRP for 16 Participants pre- and post- 28-Day Oral Curcumin and Bioperine Supplementation

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<tr>
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<th>Post-supplementation</th>
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<td>Mean(SD)</td>
<td>Mean(SD)</td>
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<td>BMI</td>
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<td>0.26(2.27)</td>
<td>.65</td>
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</tbody>
</table>

*Paired t-test

Figure 1. Individual difference in participant baseline CRP and post-supplementation CRP values following 28-day curcumin supplementation.