# Poor Dietary Habits Increase Proinflammatory Factors in College Students

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## Introduction

Obesity levels are on the rise for Americans, reaching up to 41.9% of the population as of 2021. Obesity has many long-term adverse health consequences, and one of the mechanisms by which obesity disrupts health and brain function is through the release of proinflammatory cytokines contributing to chronic low-grade inflammation. However, there is some evidence that poor diet, which can lead to obesity, can increase inflammation even in the absence of weight gain. This suggests that diet is a critical risk factor for future health outcomes.
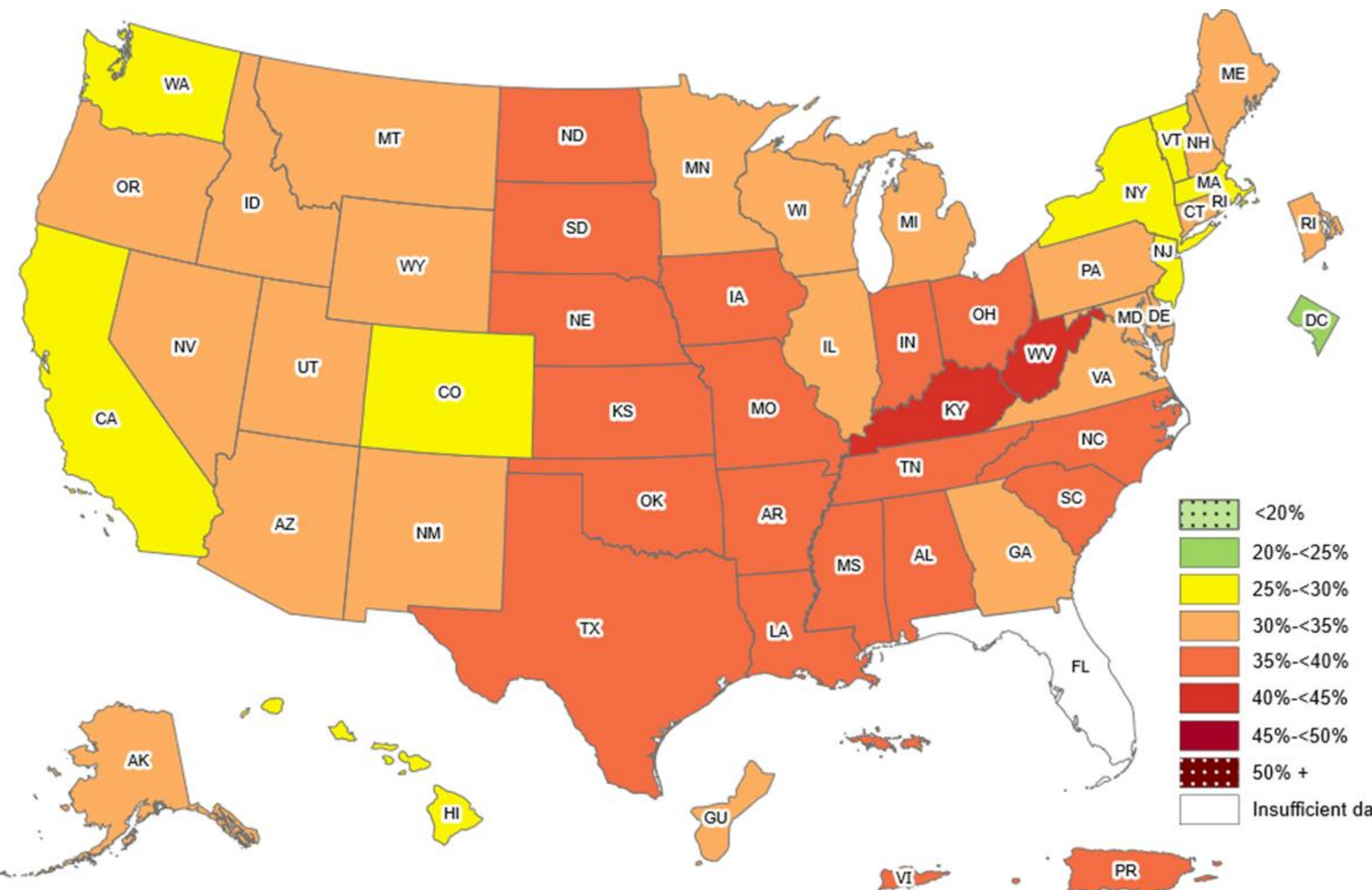


Figure 1. 2021 Obesity Prevalence Map According the Centers for Disease Control Behavioral Risk Factor Surveillance System. Obesity is measured as a body mass index (BMI) at or greater than 30. While increases in obesity have increased across all age groups, long-term health outcomes can be particularly detrimental for rising obesity trends in children and young adults due to ongoing brain development.

College-students are an adolescent aged group particularly susceptible to poor diet and nutrition, and as the brain is still developing during this time, dietary choices can have long-term consequences on the brain and body. To investigate whether poor dietary choices were related to changes in proinflammatory markers, we measured interleukin (IL)-6, a cytokine prevalent in areas of inflammation, and C-reactive protein (CRP), which is produced by the liver to indicate high inflammation in the body in college students at the University of North Carolina. These two cytokines, when present in high amounts, are involved in low-grade inflammation of the brain.
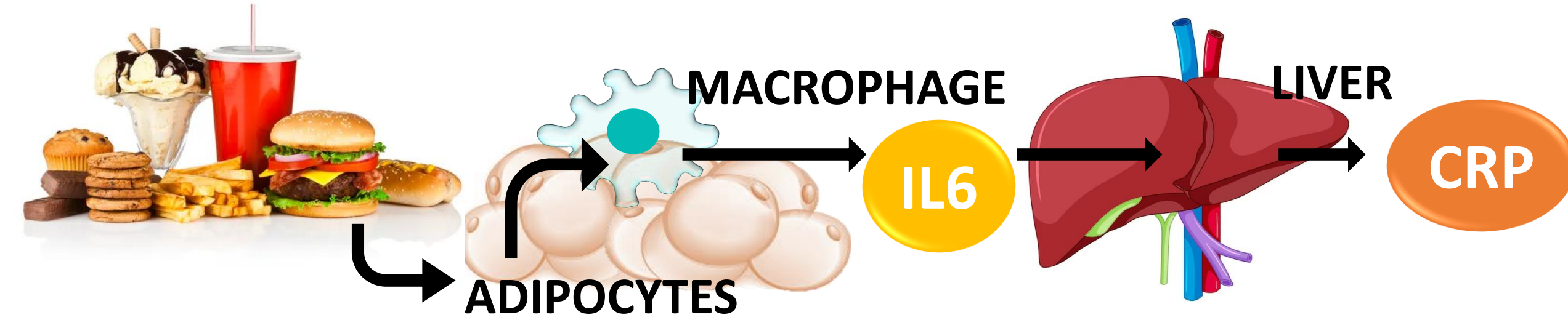


Figure 2. Poor diet leads to low grade proinflammatory markers in college aged students. We hypothesized that individuals with poor nutrition and high fat intake would have heightened levels of IL-6 and CRP, which could lead to negative long-term effects on health and cognition..

## Methods

Participants: Participants were voluntarily recruited from the University of North Carolina at Chapel Hill NSCI 439 Neuroimmunology course in Fall 2022 as part of the CURE curricula.

| Race | Sample Size |
| --- | --- |
| White | n= 16 |
| Black or African American | n= 3 |
| American Indian or Alaska Native | n= 0 |
| Asian | n= 12 |
| Native Hawaiian or Pacific Islander | n= 0 |
| Other | n= 5 |

| Sex | Sample Size |
| --- | --- |
| Male | n = 12 |
| Female | n = 24 |

Questionnaire: Each participant responded to a series of surveys examining the nutritional quality of their diets and fat intake with questions adapted from the Nutrition Questionnaire by the Weight Loss Center at University of Florida Health and the Individual Dietary Assessment by the Food Nutrition Information Center at the US Department of Agriculture. Results to the surveys on nutrition and fat intake were quantified based on each question's relative estimated health impact. Participants were divided into healthy and unhealthy groups based on pre-set cutoffs for each data set. For nutrition data, a score greater than 10 and, for fat intake, a score greater than 30 was classified as unhealthy.

IL-6 and CRP analysis: Blood was individually collected on index cards and stored for analysis of IL6 and CRP using ELISAs catalog number SPCKB-PS-000328 (IL6) and catalog number SPCKB-PS-000200 which were each analyzed using the Ella platform. Samples for IL6 were diluted 1:1 in a 100 µL solution of wash buffer and assay. Samples for CRP were diluted 1:20 in a 100 µL solution with sample and diluent. For each sample, 50ul of solution was plated into the ELISA wells.

Statistical analysis: A t-test was conducted between healthy and unhealthy groups for amounts of plasma CRP and IL6 for both nutritional quality and fat intake. Pending statistically significant results, follow up correlational analyses were run.

## Results

### Overall Nutritional Scores

Here the overall nutrition score represents the score on the nutritional quality survey, with a score greater than 10 being deemed "unhealthy".
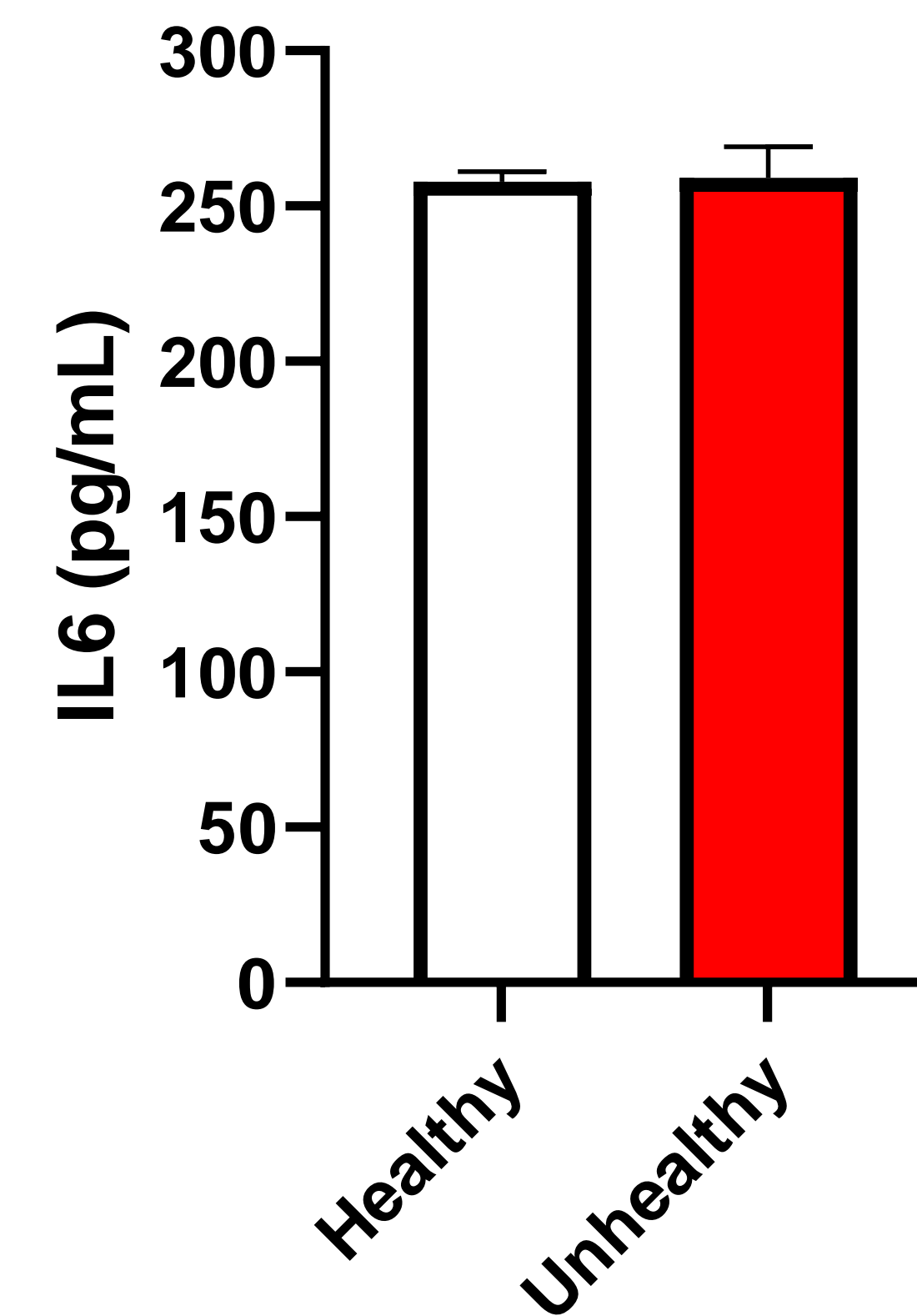






$r = 0.41$
$p = 0.01$
$y = 9833.2x - 8833.6$

Figure 3. Overall nutritional diet does not impact IL6 values (pg/mL) in college students. Nutritional diet, whether categorized as healthy or unhealthy, did not impact plasma IL6 in this cohort, $t(34)= -0.127$, $p= .899$. These results suggest that a modestly unhealthy diet in college is insufficient to influence plasma IL6. Results are expressed as mean ± SEM. *: $p < 0.05$
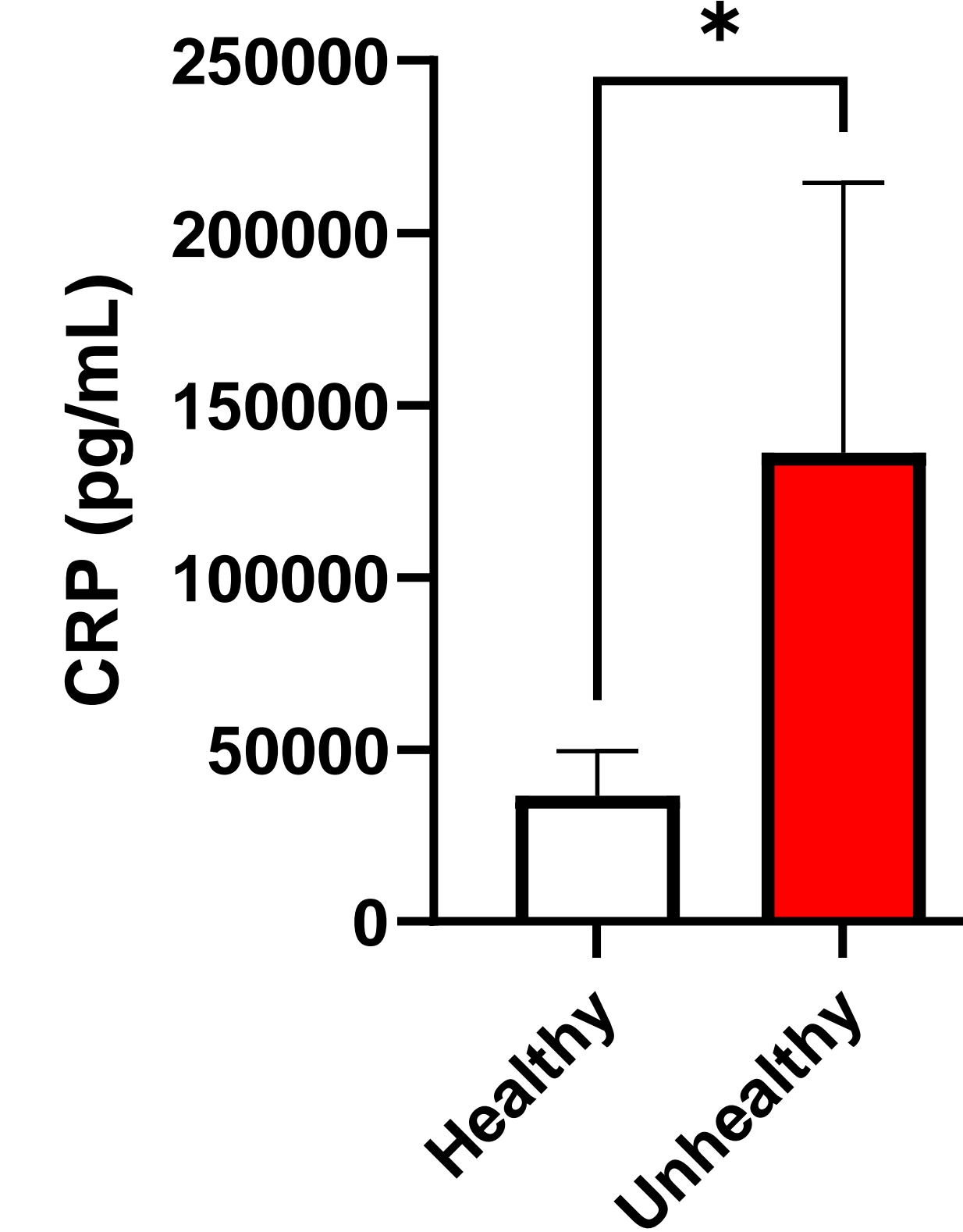
Figure 4. Unhealthy nutritional diet increases CRP values (pg/mL) in college students. College students with a generally unhealthy nutritional diet exhibited significantly higher CRP levels than corresponding classmates with healthier diets, $t(29)= -2.212$, $p= 0.035$ Results are expressed as mean ± SEM. *: $p < 0.05$
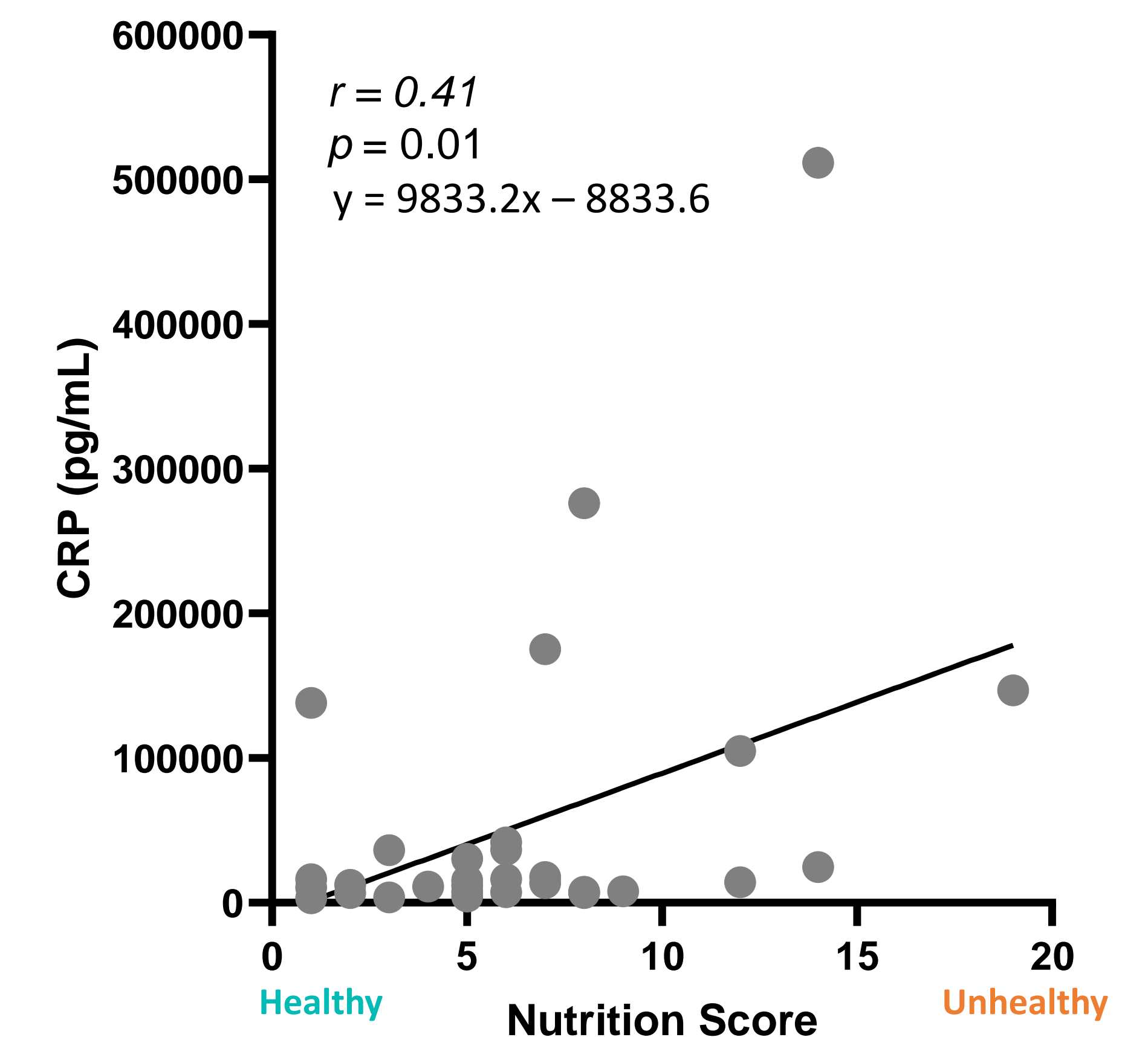
Figure 5. CRP increases in correlation with increasingly poor overall nutritional scores. Relationship between overall nutritional intake and plasma CRP (pg/mL) modeled through correlational analysis. CRP was found to have a significant positive correlation with increasing poor nutrition scores, $r(29)= 0.41$, $p= 0.011$. These data suggest that increasingly poor nutritional choices results in a linear increase in circulating levels of the acute phase protein CRP.
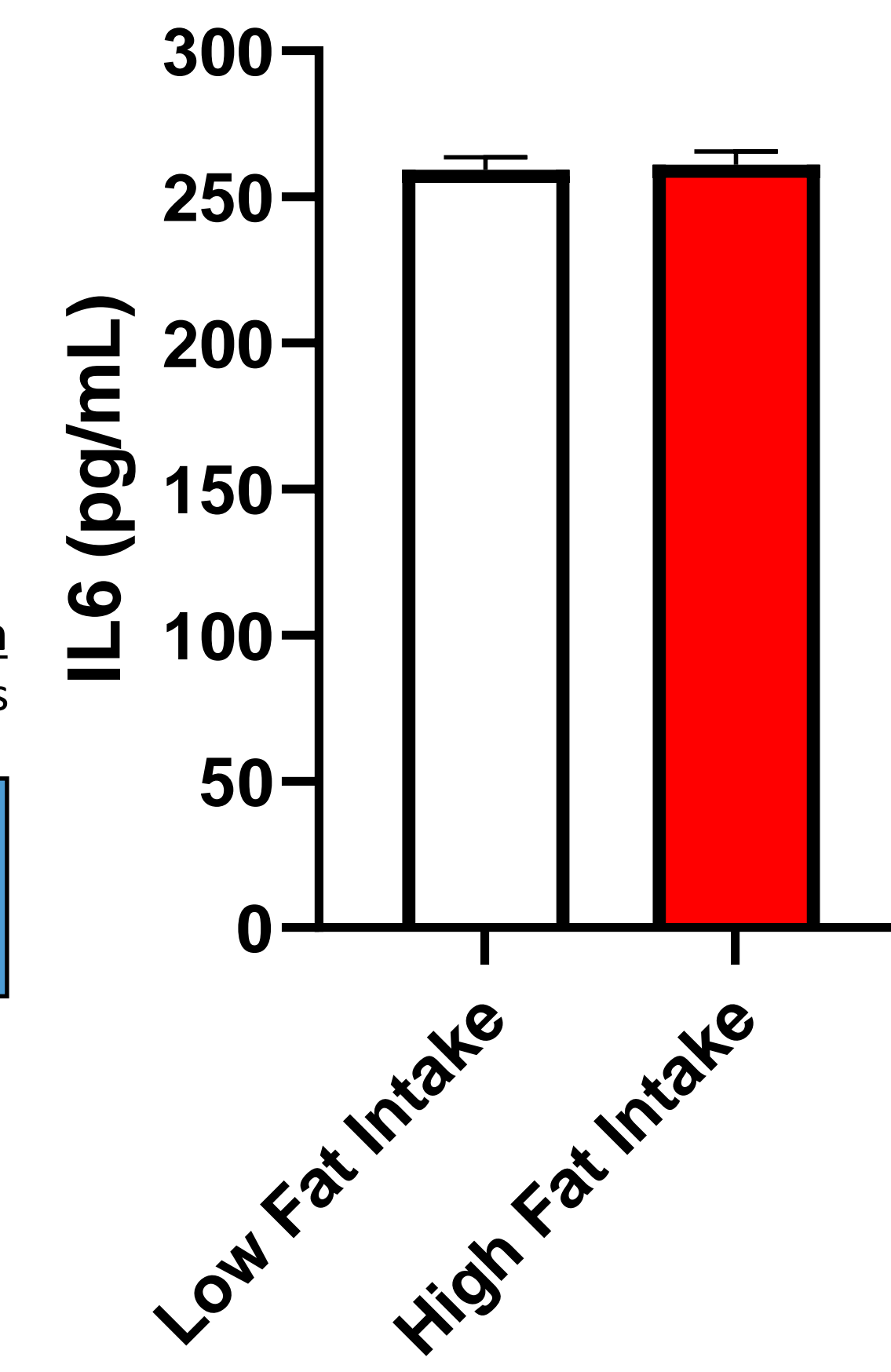
### Fat Intake





Figure 7. High fat intake did not significantly impact plasma IL6 levels, $t(34)= -0.617$, $p= 0.541$. Results are expressed as mean ± SEM. *: $p < 0.05$
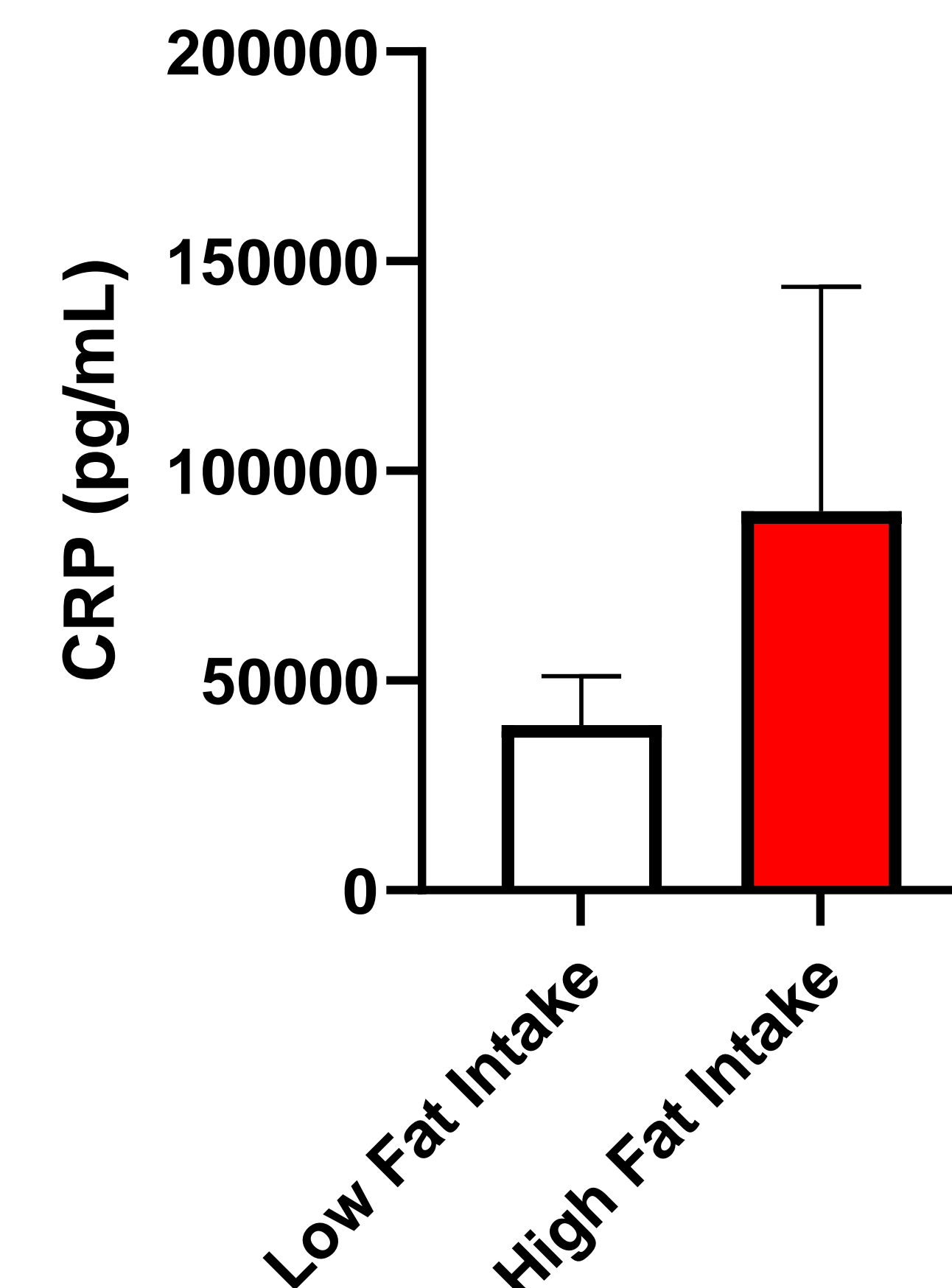
Figure 8. High fat intake did not significantly impact plasma CRP levels, $t(29)= -1.273$, $p= 0.213$. Results are expressed as mean ± SEM. *: $p < 0.05$

### Sex Differences



A.
$r = 0.49$
$p = 0.01$
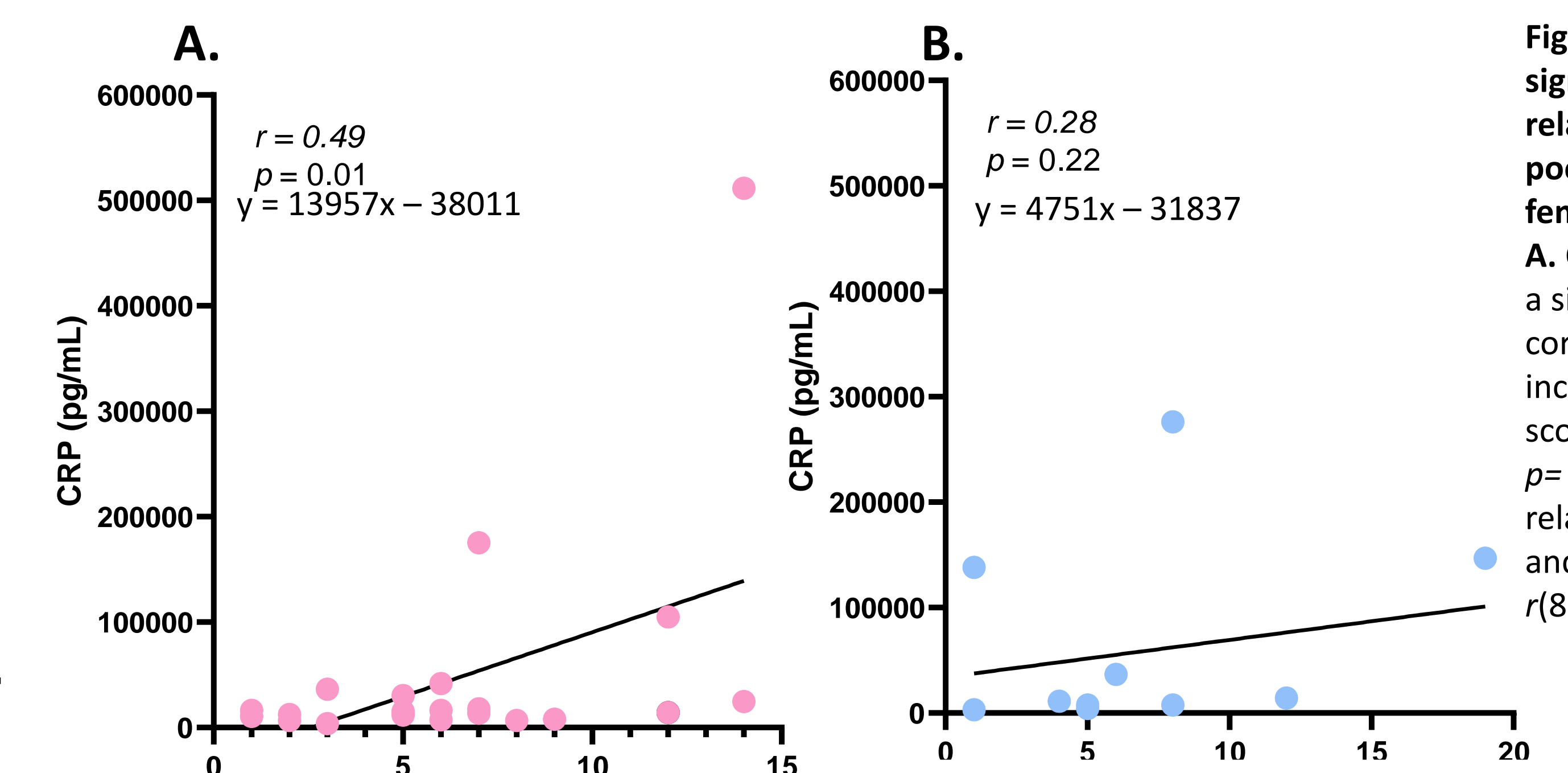$y = 13957x - 38011$

B.
$r = 0.28$
$p = 0.22$
$y = 4751x - 31837$

Figure 6. CRP significantly increased in relation to increasingly poor dietary choices in females but not males. A. CRP was found to have a significant positive correlation with increasing poor nutrition scores with a $r(19)= 0.49$, $p= 0.012$ B. There was no relationship between CRP and overall diet in males, $r(8)= 0.28$, $p= 0.220$.

## Conclusions

- Basal IL6 is not significantly impacted by overall nutritional intake or overall fat content in diet in college students.
- Circulating levels of the acute-phase protein CRP are significantly elevated in college students with poor dietary habits. This especially applies to females, as they seem to be disproportionately affected by the correlation between poor nutrition and greater levels of inflammation relative to males.

## Limitations and Future Directions

- This was a limited sample size of college-educated individuals. Expanding the sample of participants to include students from other universities to further generalize the results. In addition, including non-college educated individuals would further expand this study's value as data indicate that education level is inversely associated with poor dietary choices.
- Distinctions between healthy and unhealthy were determined based on mean data values to ensure relatively equal n-sizes across groups. However, this division was somewhat arbitrary and does not reflect the full distribution of dietary habits.
- No questions assessed dietary intake during childhood, which could influence dietary intake in college and resulting inflammatory factors.