Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 14/10/2023;
Date Revised: 05/04/2024;
Available Online: 10/07/2024;

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How to Cite:
Attiyah S (2024). Chronic sleep deprivation, noise and high fat diet markedly induce C-reactive protein level in mice model. Adv. Life Sci. 11(3): 629-633.

Keywords:
Sleep deprivation; Noise; High fat diet; Inflammation; CRP

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Chronic sleep deprivation, noise and high fat diet markedly induce C-reactive protein level in mice model

Sama Attiyah

Abstract

Background: C-reactive protein (CRP) is a circulating sign of systemic chronic inflammation that your liver produces if your body is inflamed. Stressful events over a long period of time are linked to inflammatory disorders. Stress pathways are triggered by noise and sleep deprivation, as well as unhealthy habits like fatty meals. Several studies have connected higher CRP levels with plasma levels. The current study aims to explore the potential association's relationship between chronic sleep deprivation, noise, high fat diet, and CRP disorders.

Methods: Three studies were carried out in our study to evaluate C-reactive protein levels in male Swiss albino mice groups after exposure to three different physical stressors for four weeks. The first study mice group were exposed to chronic sleep deprivation; second group was exposed to electrostatic speaker noise stressors; and the third group was fed a high fat diet. Quantitative estimation of C - reactive protein level in plasma using an ELISA kit.

Results: Significant changes in inflammatory CRP levels in the examined plasma were detected in the sleep deprivation group. CRP levels were found to be steadily increasing after two weeks, reaching a significant peak in the fourth week ($p < 0.001$). Moreover, in the fourth week, there were substantial increases in CRP plasma level with independent evidence, in the experimental noise group ($p < 0.001$). As well, the biomarker of inflammation level increased across the dietary high density fat diet, reaching the highest level at the end of the study ($p < 0.05$).

Conclusion: Our findings contribute to the body of evidence indicating a link between external stress and subclinical inflammatory markers. Furthermore, CRP concentrations are triggered by chronic sleep deprivation and noise exposure, and their concentrations rise with prolonged dietary fatty acid consumption.

Introduction

Stress is an adaptive reaction of the human body to numerous stimuli. It is a generic response to any need for change [1]. External stressors are major public health problems that contribute significantly to chronic non-communicable illnesses [1-7]. Noise and sleep deprivation, as well as fat rich meals, induce irritation and begin stress pathways. Stress can be acute or chronic, and numerous studies demonstrate that there are substantial and highly reproducible phenotypic individual differences in response to chronic stress exposure. These investigations have revealed a link between stress and CRP levels [2-4].

Epidemiological research suggests that having a high number of stressful events over a long period of time is linked to poor mental and physical health, as well as death [8]. Sleep loss is a strong stressor that often results in significant rises in irritation with inflammatory responses and a significant raise in CRP level [2]. There is an intimate association between changes in the pro-inflammatory response and sleep deprivation [5]. Furthermore, many studies have revealed that long-term noise exposure is a health risk factor and can contribute significantly to chronic non communicable illnesses as irritation responses [2,5,6]. Noise may raise people's chances of developing cardiovascular diseases as well as persistent upregulation of the inflammatory marker C-reactive protein [9].

Moreover, some prior studies show that the effects of high-fat diet stress on the population are mediated by subclinical inflammation. It reported that inflammatory markers such as CRP rise quickly after consuming an excessive amount of dietary lipids [7,10,11].

CRP is utilized as a sensitive biomarker of inflammation and has been proposed as a valuable tool for monitoring illness development. It has gained significant interest as a diagnostic and prognostic marker in inflammatory disorders; it plays important roles in evaluating the inflammation reactions [12]. Although high plasma CRP levels are not unique to a particular disease process, they are widely employed as a static assessment. CRP levels have been linked to disease progression, severity, and prognosis in a variety of illnesses [13].

Despite the fact that elevated levels of CRP are independently and strongly connected with external stressors, the reason for this relationship is unknown. It is possible that psychological stress produces many alterations in the organism's inflammatory response. [3,14]. Considering the relation between high stress exposure and higher disease risk, it has been linked with stressful life exposures as well as cardiovascular disease [4,6,11].

Our experimental study aims to investigate potential associations' relationships between sleep deprivation, noise, and foods that have lots of fats and elevated CRP candidate biomarkers that may be identified by using mice model.

Methods

Experimental design

56 male, healthy Swiss albino mice (C57BL/6 strain) were between 6 and 8 weeks old and weighed 20 to 30 grams. The animals were sourced from the ECF in Egypt. Mice were thoroughly inspected for microbiological or parasitic infections and kept in plastic cages at 21°C and 60% humidity. Keeping mice in the usual pathogen-free conditions. They were fed a conventional laboratory meal consisting of 24% protein, 4% fat, 4–5% fiber, and water ad-libitum. The animals were fed, housed, and handled in accordance with the Guidelines for the Care and Use of Laboratory Animals.

Three studies were carried out, each in a separate group of mice. Our study duration is four weeks. Briefly, mice were allocated into 3 groups in addition to the control group (n=8), which feeds normally without exposure to any stressors. Group 1: (sleep deprivation experiment) Mice (n=16) received 20 hours of sleep deprivation utilizing a gently revolving wheel (1.0 RPM x 20 hours/day), followed by a 4-hour sleep opportunity in their home cage. The sleep deprivation wheels are plastic rotating wheels with a 9.0-inch diameter. Each one is held together by a sturdy steel plate on one side and a transparent plastic sheet on the other. The wheel cages are intended to facilitate simultaneous spinning of the wheels as well as free access to food and water. The wheel speed was kept constant at 1.0 rotations per minute. Group 2: (noise-exposure experiment) mice (n=16) placed into cages supported by an electrostatic speaker. The white continuous noise was generated using a noise generator supported by a microphone placed at the test mice location for noise output for 12 hours during the experiment daytime. Group 3: (high fat diet experiment) mice (n=16) were continuing to feed with high density (60 kcal% fat diet) for four weeks. More food is usually added as needed. At the end of each week, 4 mice were sacrificed from each group to collect plasma and subjected to CRP evaluation.

CRP level measurement

The blood samples were collected using EDTA tubes at the end of each week from four mice for four weeks from each experimental group, then centrifuged each sample for 15 min at 3000 rpm at 2~8°C within 30 min of collection. Quantitative measurement of C Reactive Protein (CRP) level in plasma was estimated by the

Mouse C Reactive Protein (CRP) ELISA 96-well plate kit (ab222511).

Statistical analysis

Depending on the data distribution, continuous variables are displayed as the median or mean ± SD. The statistical significance of the differences between groups was determined using the Student t-test, with the threshold of significance set at $p < 0.05$.

Results

Fifty-six (56) male, healthy Swiss albino mice allocated into 3 test groups in addition to the control group. Each group has 8 mice. Our study extended for four weeks. At the end of each week, 4 mice were sacrificed from each experimental group to collect plasma using EDTA tubes and subjected to CRP quantitative evaluation using the Mouse C Reactive Protein (CRP) ELISA 96-well plate kit with plate reader.

A significant increase in plasma CRP level was observed in our stressed experimental groups, as shown in Table 1. CRP levels were found to be steadily increasing after two weeks, and gradually elevated in plasma with prolonged time of sleep deprivation in group 1 (sleep deprivation experiment), which is higher than control group mice and most significant after the third and fourth weeks of experimentation procedures (33mg/l ±6, 56mg/l ± 3) ($p < 0.001$) (Fig., 1).

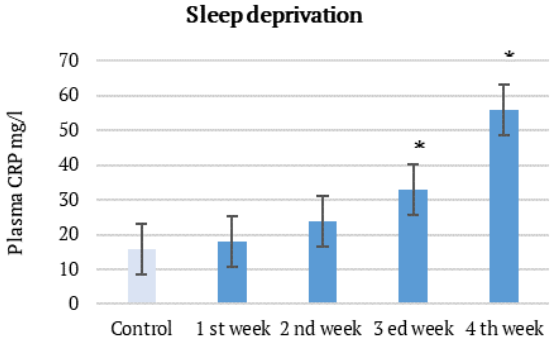

Figure 1: Plasma CRP level in C57BL/6 strain mice under sleep deprivation stress for 4 weeks. The figure shows the pattern of change in CRP over the course of four weeks of the study. CRP level gradually elevated in plasma with prolonged sleep deprivation ($p < 0.001$).

Noise stress becomes more significant as exposure increases throughout the experiment time on the CRP plasma level. The fourth week was the most significant on the noise experiment (71 mg/l ± 4, $p < 0.001$) (Fig., 2). Moreover, the results strongly supported that the plasma CRP levels were exceptionally increased in the noise group by time more than in other groups (groups 1 & 3) from the first week to the end of our experiment. Plasma CRP level was also slightly elevated in (group 3) that fed on a high fat diet for four weeks (60 mg/l ± 6, $p < 0.005$) compared with the control group (50 mg/l ± 6) (Fig.,3).

Duration	Group 1	Group 2	Group 3
1st week	18 ±3	16 ±3	16 ± 2
2nd week	24 ±5	35 ±4	18 ±3
3rd week	33 ±6	44 ±6	20 ±2
4th week	56 ±4	71 ±4	50 ±6

Control versus experimental group; $p < 0.001$

Table 1: Effect of sleep deprivation, noise, and high fat diet on CRP level for four weeks. The table shows the pattern of change in CRP across the study. C-reactive protein changes across the study with increasing exposure time to the stressor (mean ± SD). The most significant group is the noise group (group 2). The protein level increased more than fourfold after four weeks.

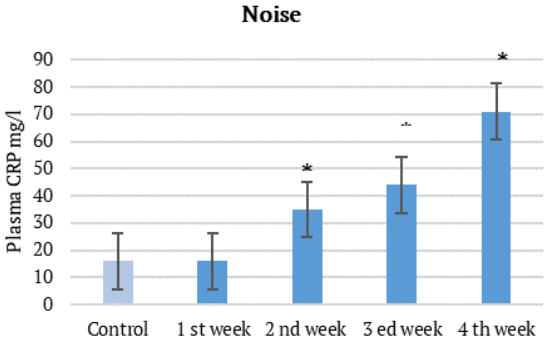

Figure 2: Plasma CRP level in C57BL/6 strain mice under noise stress for 4 weeks. The figure shows CRP concentration changes across the study. CRP showed a significant time effect across the study. CRP was significantly higher in the fourth week ($p < 0.001$).

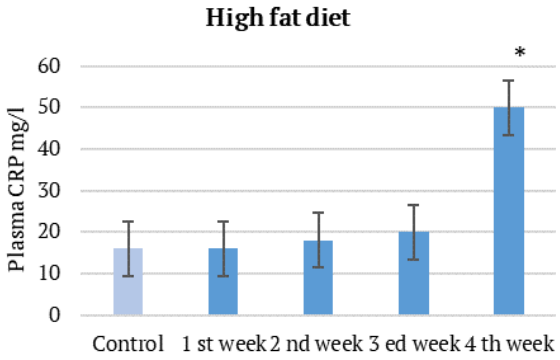

Figure 3: Plasma CRP level in C57BL/6 strain mice with high-fat diet stress for 4 weeks. CRP showed no significant effect in the first three weeks. CRP increased significantly in the fourth week ($p < 0.005$).

Discussion

C-reactive protein (CRP), identified as a protein that precipitates *Streptococcus pneumoniae* C-polysaccharide, has long been recognized as a sensitive indicator of inflammation [12]. CRP has several pro- and anti-inflammatory actions. Although not unique to a particular disease process, CRP is routinely employed as a static assessment, and CRP levels have been linked to a variety of diseases [13].

Despite substantial studies, the effects of sleep deprivation on C-reactive protein (CRP), are

inconclusive, whereas other studies found no difference in CRP levels [14]. Furthermore, studies found decreases in CRP levels [15], whereas others found increases in CRP levels [16, 17] which is consistent with our findings. The relation between CRP level and noise is extensively documented in different studies. C-reactive protein was significantly reduced in the noise exposed rats' experiment [18], while its concentrations have been reported to rise after prolonged noise stress exposure [19]. Our study linked elevated CRP levels to noise stress.

There is an association between fatty acid consumption and CRP inflammatory indicators. Glial cells are activated by a high-fat diet, causing them to generate and release cytokines and C-reactive protein as inflammatory mediators [20]. Our study aims to assess if dietary fatty acid consumption corresponds with serum high-sensitivity C-reactive protein levels. Consistent with our findings, epidemiological studies linked chronic stress resulting from a high fat diet with inflammatory markers [21, 22]. Murakami et al., [23] found no significant link between a high fat diet and elevated CRP. Whereas other study found no significant link between a high fat diet and a high CRP level [24]. Therefore, a high intake of meals reached with fat could increase inflammation, as reflected in CRP level.

Our studies directly implicate the stress induced increase the level of CRP. Its level increased in mice exposed to stressful events over a long period of time, stressors of sleep deprivation, noise, as well as high fat diet intake, in accordance with previous epidemiological research. Collectively, these findings suggest that alterations in CRP explain the increased inflammation risk linked to our experimental designed stressors.

In conclusion, our findings support the concept that some anxiety and stress-related disorders are associated with elevated levels of inflammatory markers like CRP. The relationship between CRP and sleep deprivation and noise, as well as high fat diet intake, was more likely to show elevated plasma CRP levels. More research is needed to determine the potential causative and mechanistic ties involved in the triggering of CRP with stressors, as well as to correlate external stresses with other subclinical inflammatory indicators.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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