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CONCENTRATION OF C-REACTIVE PROTEIN IN WHITE ADIPOSE TISSUE, LIVER AND BLOOD SERUM OF MALE WISTAR RATS

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ABSTRACT

PURPOSE: The aim of the present study is to make a quantitative analyze of C-reactive protein (CRP) concentration in the white adipose tissue, liver and blood serum in experimentally induced obesity in rats.

METHODS: A model of animal obesity was created using high-fat diet. Forty, eight-weeks-old male Wistar rats were randomly divided into two groups – 1) normal, control group fed with standard rodent food and 2) experimental group fed with a high-fat diet. They were subjected on these regiments for fourteen weeks. Blood serum, liver and adipose tissue specimens were obtained from each animal at the end of the experimental period. The concentration of CRP in all samples was quantified by ELISA method.

RESULTS: Serum CRP concentration was elevated in the group with obesity, med.947.51 (95% CI 868.09 – 1049.93) μg/ml compared with the control group med.649.34 (95% 558.02 – 688.45) μg/ml, $p<0.0001$. The liver CRP concentration in obese animals was lower than the concentration in control animals, $p=0.048$. The concentration of CRP in adipose tissue doesn't show any significant deference among the obese and control animals, $p=0.826$.

CONCLUSION: The liver is an active participant in the development of low-grade, systemic inflammation in obese male rats, rather than adipose tissue.

Key words: animal model of obesity, low-grade inflammation, C-reactive protein, liver, adipose tissue

INTRODUCTION

Obesity has become epidemic in many countries worldwide as a consequence of sustained overnutrition.The prevalence rates are continuing to increase, most rapidly in developing countries, and affect all age groups. (1). Obesity is characterized by an increase in adipose tissue mass that may function as an active endocrine organ synthesizing acute-phase proteins (2). These molecules are blood proteins that can be used to assess the innate immune system's response to infection, inflammation or trauma (3). Their concentration could be increased (positive acute-phase proteins) or decreased (negative acute-phase proteins) (4).

C-reactive protein (CRP) is an acute-phase plasma protein, which is phylogenetically highly conserved across different species and participates in the systemic response to inflammation. After an acute inflammatory stimulus its concentration may increase rapidly and markedly as much as 1 000-fold (5). CRP is synthesized and released primarily by hepatocytes, although reported data suggest that local CRP synthesis and secretion may occur at other sites, such as macrophages (6), smooth muscle cells (7) and adipocytes (8).

Clinically, CRP has been used to detect acute infections. It has also been used to evaluate the

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inflammatory response in chronic diseases such as rheumatoid arthritis. Baseline levels of CRP are being used by some investigators as a predictor of inflammation leading to atherosclerosis vascular disease (9). Many epidemiological studies have shown associations between slightly increased CRP levels and increased risk for cardiovascular disease, diabetes mellitus, and multiple components of the metabolic syndrome, including obesity, insulin resistance, dyslipidemia, and increased blood pressure (10, 5).

Chronic, low-grade inflammation is different from acute inflammation (11). It has been hypothesized that the chronic inflammation could be detected by minor increase in CRP levels (5). Several metabolically active tissues, including liver, white adipose tissue (WAT), muscles, and more recently gut and its intestinal flora, participate as sources and sites of inflammation (12).

The aim of the present study was to make a quantitative analyze of C-reactive protein (CRP) concentration in the white adipose tissue (WAT), liver and blood serum in experimentally induced obesity in rats.

MATERIALS AND METHODS

This study has an approval for the usage of laboratory animals in experiments from Bulgarian Agency for Food Safety and it is in accordance with the ethical standards of the Medical University of Plovdiv.

Forty, eight-week-old male Wistar rats (weight 152 – 186 g) were obtained from University vivarium. They were randomly divided into two groups – 1) normal, control group, fed with standard rodent food and 2) experimental group, fed with a high-fat diet (D12451 – Research Diets, Inc.). The animals of the both control and experimental groups were orally fed and had free access to water. All of the animals were maintained under standard housing conditions – living space: 350 cm^2, temperature: 22 ±2ºC, humidity: 55 ±10% and 12/12h light/dark cycles. They were subjected on these regiments for fourteen weeks. At the end of the fourteenth week they were fasted overnight, afterwards were anaesthesized and decapitated. Blood serum, adipose tissue and liver specimens were collected and immediately frozen at -18ºC.

Tissues excised from experimental animals were brought to room temperature. They were homogenized by mechanical homogenizer in Tris buffer + Tween20, pH-7.4. All solid particles were removed by centrifugation for 10 minutes at 10 000 g. Dichloromethane (0.4 ml) was added to 1 ml of supernatant to eliminate all lipid contaminations. The process was followed by second centrifugation for 10 minutes at 10 000 g and water phases containing purified protein solutions were collected.

CRP levels in blood serum, WAT and liver specimens were quantified by ELISA method using commercially available kits from BioVendor (Rat hsCRP ELISA kit – BioVendor, Laboratorni Medicina, a.s.). The whole process was maintained according to manufacturer's recommendations. CRP analyzes were performed by an ELISA microplate reader (HumanReader).

The total protein concentration in WAT and liver homogenates was quantified by Lowry method (13), and the results were expressed as ng CRP per mg Protein.

All assays were performed in duplicates. The comparison between both groups of animals was performed by independent-samples t-test for parametric data (body weight), and by Mann-Whitney test for non-parametric data (CRP concentration). The results are presented as mean ±SD for parametric and as median, 95%CI for non-parametric data.

RESULTS

Compared with the starting body weight of 167 ±11g, body weights were 270.4 ±11 and 317 ±16 g in control and experimentally induced obesity groups, $p<0.0001$. Serum CRP concentration was elevated in the group with obesity 947.51, 95% CI 868.09 – 1049.93 μg/ml compared with the control group 649.34, 95% CI 558.02 – 688.45 μg/ml, $p<0.0001$. The liver CRP concentration in obese animals was lower 3.210, 95% CI 3.093 – 4.159 than the concentration in control animals 5.162, 95% CI 3.256 – 6.713 ngCRP/mgProtein, $p=0.048$. The concentration of CRP in WAT doesn't show any significant deference between the obese 0.532, 95% CI 0.353 – 0.712 and control animals 0.336, 95% CI -0.586 – 2.391 ngCRP/μgProtein, $p=0.826$. There is statistical difference between CRP

concentration in liver and adipose tissue in both control (p=0.001) and experimental (p<0.0001) groups.

DISCUSSION

The rat model is preferred to document the pro-inflammatory effects of CRP (14). In rat models, high CRP level stimulates pro-inflammatory, pro-oxidant and pro-coagulant states via increased macrophage activity (15). Obesity-induced inflammation is a result of overnutrition and pathways that drive abnormal metabolic homeostasis. Previous studies have shown that the obesity-induced, low-grade inflammation is characterized with much lower levels of circulating cytokines compared with acute inflammation (16).

Firstly, we found that feeding rats with high-fat diet resulted in an increase of body weight (17). Further, we quantified CRP levels in blood serum, white adipose tissue and liver. In rats, basal level of CRP in blood serum is 300 – 500 μg/ml (18). In our control group the level is insignificantly higher than normal – 649.34 μg/ml, but in our experiment group we observed an increasing approximately two-fold than normal **(Figure 1)**. This confirms that in obesity-induced, low-grade inflammation, the levels of serum CRP are slightly elevated.

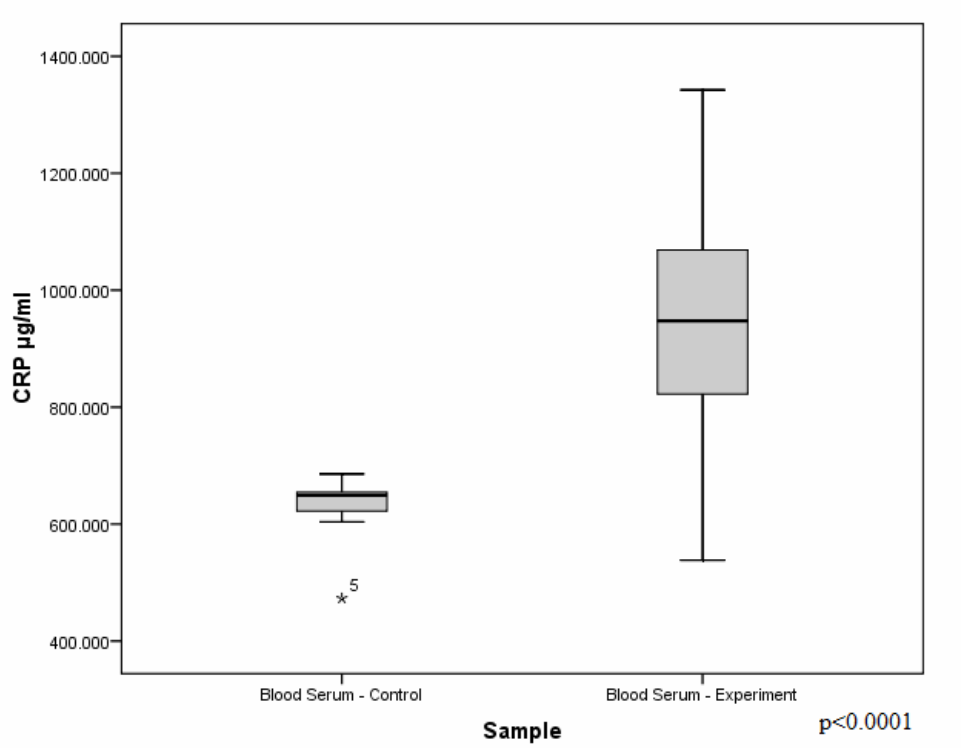

Figure 1. Concentration of CRP in blood serum of control and experimental groups

Overweight and obesity are characterized with an increase in adipose tissue mass, where both adipocytes and infiltrated macrophages synthesize and secrete proinflammatory cytokines and adipokynes (19). CRP levels in the white adipose tissue were higher than in the liver, but there was not any significant difference among the CRP concentration in obese and lean animals **(Figure 2).**

Most likely, the increased serum CRP levels don't depend on the changed metabolism, but on the increasing of only adipose tissue mass and decrease with its decreasing (20, 21).

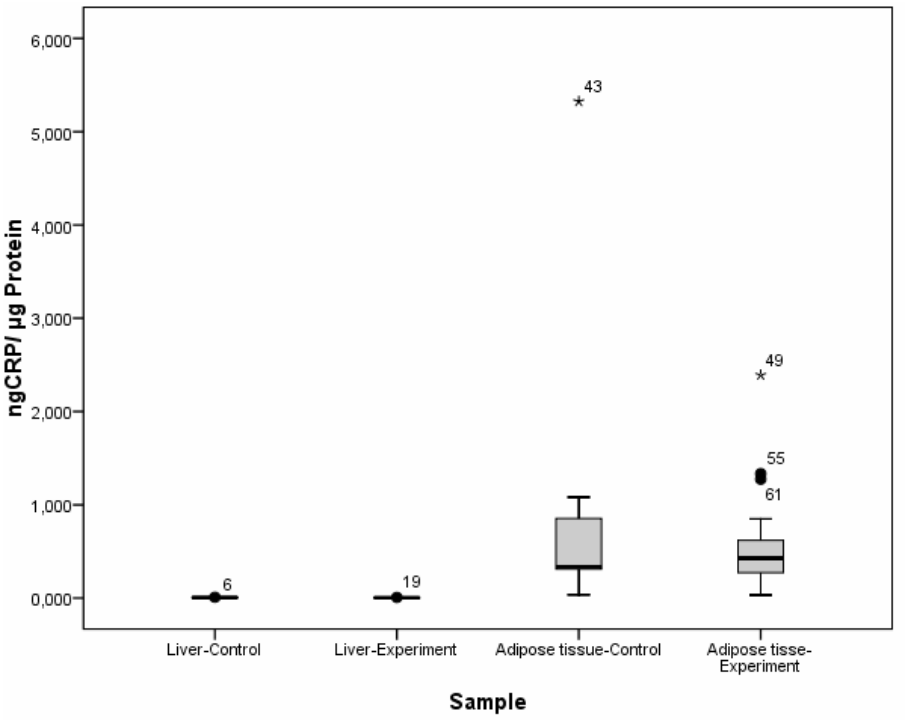

Figure 2. CRP concentration in liver and WAT in control and experimental animals

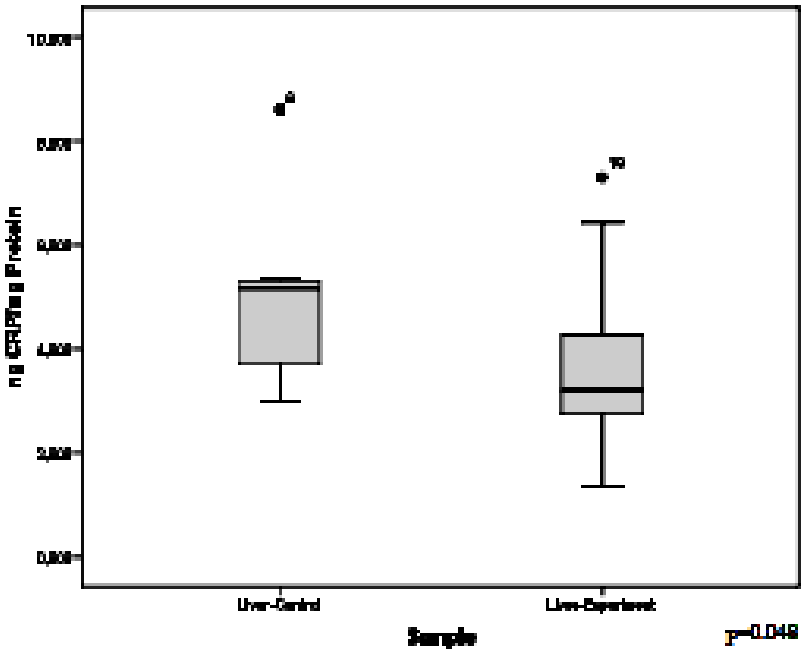

Figure 3. CRP concentration in liver of control and experimental groups

Liver is the main source of CRP in plasma (5). We found a decreased level of liver CRP in obese compared with lean animals **(Figure 3)**. This observation may reflect the rate of secretion of CRP from the liver of obese animals and worth further studies.

CONCLUSION

Obesity, as low-grade, systemic inflammation is characterized with slightly increased CRP levels in blood. The liver is an active participant in the development of such type of inflammation, rather than adipose tissue. Additional

investigations are needed for more accurate outlining the origin and the sources of the inflammatory process in obesity.

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