CHANGED C-REACTIVE PROTEIN AND SERUM AMYLOID-A LEVELS IN BLOOD SERUM, ADIPOSE TISSUE AND LIVER OF OBESITY INDUCED RATS

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ABSTRACT

C-reactive protein (CRP) and serum amyloid A (SAA) are acute-phase proteins. These proteins are synthesized predominantly by liver during the acute-phase of inflammation. Recent studies have demonstrated that CRP and SAA are synthesized and secreted by adipose tissue as well, and increase in the mass of adipose tissue during obesity leads to elevated CRP and SAA levels in blood serum. A model of animal obesity was created using high-fat diet. Sixty, eight-weeks-old male Wistar rats were randomly divided into two groups – 1) 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 CRP and SAA concentrations were quantified by ELISA method. Higher blood CRP and SAA concentration and lower liver CRP and SAA levels were found for the group of obese animals compared to the control group. The concentration of SAA in adipose tissue was significantly higher in obese compared with the lean animals. No significant difference was found for the levels of CRP in adipose tissues obtained from the two experimental groups. No correlations exist between CRP and SAA levels in blood circulation, liver and adipose tissue. The changes in the
concentration of CRP and SAA in blood serum, adipose tissue and liver, in experimental obesity might be an initial step in the development of low-grade, chronic inflammation.

KEYWORDS: Obesity, low-grade inflammation, C-reactive protein, serum amyloid A, Wistar rats.

INTRODUCTION

Obesity has become epidemic in many countries worldwide as a consequence of sustained overnutrition and changed lifestyle. The prevalence rates are continuing to increase, most rapidly in developing countries, and affect all age groups. Obesity predisposes individuals to an increased risk of developing several diseases, including atherosclerosis, diabetes, non-alcoholic fatty liver disease and some types of cancers.[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 initial 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] Baseline levels of CRP are being used by some investigators as a predictor of inflammation leading to atherosclerosis vascular disease.[9]

Similarly to CRP, the serum amyloid A (SAA) is an acute-phase protein with a high degree of homology in mammals. It is a plasma protein whose concentration increases by as much as 1 000-fold or more, during the acute phase response following bacterial infection, tissue damages, and inflammation.[10, 11] Acute-phase serum amyloid A (A-SAA) is secreted by hepatocytes in response to injury and is regulated by proinflammatory cytokines (IL-6, TNF-α). The expression level of A-SAA is often used as a potential biomarker to monitor the health status of animals exhibiting an acute or chronic inflammation.[11] Recent studies have
demonstrated that adipocytes are also capable of producing SAA. Comparison between the levels of gene expression of A-SAA in human tissues has shown that adipose tissue (AT) is a major site of A-SAA production in obesity.[12-14] The production of A-SAA by enlarged adipose tissue might contribute to the growing pool of circulating SAA in obesity.[15]

Chronic, low-grade inflammation is different from acute inflammation.[17] It has been hypothesized that the chronic inflammation could be detected by minor increase in CRP levels.[5] In the context of obesity related low-grade, chronic inflammation, the level of circulating SAA remains slightly high, about 6-fold higher in obese compared with individuals with normal weight.[17, 15]

The aim of the present study was to compare the CRP and SAA concentrations in the white adipose tissue (WAT), liver and blood serum in experimentally induced obesity in rats. Commonly used diets inducing obesity are rich in fat and carbohydrates, either from fructose or sucrose, and closely mimic the human diet. Diet-induced obese rodents, including Wistar rats, can be considered a valid animal model that reflects the effects observed in humans.[18]  

MATERIALS AND METHODS
This study has an approval for the usage of laboratory animals in experiments from Bulgarian Agency for Food Safety (BAFS resolution №21/19.03.2012), and it is in accordance with the ethical standards of the Medical University of Plovdiv (resolution of the University Ethic Committee №P4004/28.06.2013).

Sixty, eight-weeks-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 cm², temperature: 22 ±2°C, 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 treated with overdose of the anesthetic ketamine (87.5 mg/kg)/xylazine (12.5mg/kg). After cervical dislocation, blood serum, adipose tissue and liver specimens were collected and immediately frozen at -18°C.

On the day of analysis, the tissues excised from experimental animals were brought to room temperature. The samples, prepared for the quantification of SAA, were homogenized by
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mechanical homogenizer in PBS+Triton 100x, pH-7.4. The samples prepared for measurements of CRP, were homogenized by mechanical homogenizer in 0.05M Tris buffer+Tween 20, pH-7.4. All solid particles were removed from both reagents by centrifugation for 10 minutes at 10 000 rpm. 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 rpm and water phases containing purified protein solutions were collected.

The levels of SAA in blood serum, white adipose tissue (WAT) and liver specimens were quantified by ELISA method using commercially available kits from MyBioSource (Rat Serum amyloid A (SAA) ELISA kit – MyBioSource Inc., San Diego, CA, USA). 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). Both processes were maintained according to manufacturer’s recommendations. The quantitative analyses were performed by an ELISA microplate reader (HumanReader).

The results for serum levels of SAA were presented as ng/ml. CRP levels are presented as μg/ml. The total protein concentration in WAT and liver homogenates was quantified by colorimetric Lowry method,[19] and the results were expressed as ng SAA or ng CRP per mg Protein.

All assays were carried out in duplicates. The comparison between both groups of animals was made by independent-samples t-test for parametric data (body weight), and by Mann-Whitney test for non-parametric data (SAA and CRP concentrations). The results obtained are presented as mean ±SD for parametric and as median, 95%CI for non-parametric data. Spearmen’s rank correlation coefficient was used for the determination of CRP and SAA correlations in all samples measured. Differences with p<0.05 were considered as statistically significant.

RESULTS

All animals were randomly divided in two groups, each consists of thirty animals. The initial body weight of the experimental animals was 167 ±11 g; and fourteen weeks later body weights were 270.4 ±11 and 317 ±16 g for the control and obese group, respectively.
(p<0.0001). No data for diseases among the animals has been reported by our veterinary surgeon during the experiment duration.

Figure 1. Statistical evaluation of the differences in CRP levels in: A. blood samples, B. liver, and C. white adipose tissues obtained from the control group (left bars) and the obese group (right bars).
Figure 2. Statistical evaluation of the differences in SAA levels in: a) blood samples, b) liver, and c) white adipose tissues obtained from the control group (left bars) and the obese group (right bars).
Serum CRP concentration was significantly elevated in the group with obesity, 963.41 μg/ml, 95% CI 898.35-1063.88, compared to the control group – 649.34 μg/ml, 95% CI 558.02-688.45 (p<0.0001). The liver CRP concentration of the obese animals, was lower 3.21 ng/mg, 95% CI 3.09-4.16 than the concentration in control animals 5.16 ng/mg, 95% CI 3.26-6.71 (p=0.048). The concentration of CRP in WAT doesn’t show any significant deference among the obese 504.45, 95% CI 395.33-637.05, and the control animals 430.12 ng/mg, 95% CI 294.72-601.03 (p=0.841). All comparisons are illustrated in Figure 1.

The concentrations of SAA, detected in blood serum, were elevated in the group with obesity – 10.37 ng/ml, 95% CI 9.97-11.02, compared with the control group – 6.58 ng/ml, 95% CI 5.64-7.23 (p<0.0001). The changes of SAA concentrations in liver homogenates were similar to those found for CRP, that is shown as decreased SAA levels in obese – 12.17 ng/mg, 95% CI 11.85-13.69 – compared with the control animals – 16.05 ng/mg, 95% CI 13.01-21.21 (p=0.005). SAA levels were significantly higher (p=0.001) in WAT obtained from obese animals (1049.45 ng/mg, 95% CI 1041.49-1387.17) compared to the adipose tissue samples from the control group (529.91 ng/mg, 95% CI 339.52-770.16). All statistical comparisons are presented in Figure 2.

We were unable to find any correlations between the changed levels of CRP and SAA in blood serum, WAT and liver. All correlations are presented in Table 1.

Table 1. Correlations between CRP and SAA in blood serum, liver and adipose tissue homogenates (Spearnen rank correlation).

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<thead>
<tr>
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<th>Blood serum</th>
<th>Liver homogenates</th>
<th>Adipose tissue homogenates</th>
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<tr>
<td>Controls, n=30</td>
<td>p=0.180</td>
<td>p=0.760</td>
<td>p=0.397</td>
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<tr>
<td>Experiment, n=30</td>
<td>p=0.331</td>
<td>p=0.842</td>
<td>p=0.995</td>
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DISCUSSION

Obesity is a chronic, multifactorial, and complex disease resulting from a long-term positive energy balance, in which both genetic and environmental factors are involved.[20, 21] It was recently suggested that some forms of obesity are associated with chronic low-grade inflammation[22] which was shown by this study as well. The mean serum CRP level in obese animals was three-fold higher than the reference values for healthy rats provided by the manufacturer of the assay and approximately two-fold higher than the levels measured for our control animals. This confirms that in obesity-induced, low-grade inflammation, the
levels of serum CRP are slightly elevated and both, WAT and liver contribute to the elevated serum levels.\cite{5,8}

The concentration of SAA in blood serum in control group was lower compared with the group consisted of obese animals. In our experimental group we observed slightly elevated levels, less than two-fold. Our model of obesity induced rats was unable to confirm the slightly elevated, approximately six-fold, SAA levels in chronic, low grade inflammation, reported for obese human patients.\cite{15,17}

Liver is the main source of CRP\cite{5} and SAA\cite{11} in blood circulation. We found a decreased level of liver CRP and SAA in obese compared with lean animals. This observation may reflect the rate of secretion of CRP and SAA from the liver of obese animals and worth further studies.

An increase in adipose tissue mass characterizes overweight and obesity where both adipocytes and infiltrated macrophages synthesize and secrete proinflammatory cytokines and adipokynes.\cite{23} CRP levels in the white adipose tissue were higher than in the liver, but there was not any significant difference between the CRP concentration in obese and lean animals. Most likely, the increased serum CRP levels depend on the increasing of only adipose tissue mass and decrease with its reduction.\cite{24,25} We detected that the concentration of SAA in adipose tissue is higher than the concentration in liver. Moreover, there is statistically significant difference between SAA levels measured in adipose tissue samples, collected from experimental and control group of animals. This difference shows that the adipose tissue synthesizes and stores SAA, acting as an active endocrine organ.\cite{26}

**CONCLUSION**

Obesity, as a state of low-grade inflammation changes the concentrations of the proinflammatory CRP and SAA molecules in blood serum. But these changes are insufficient and not enough evident markers to state that healthy, obesity induced animals develop systemic, chronic inflammation.

Both, the liver and the white adipose tissue are active participants in synthesis and secretion of CRP and SAA. The absence of correlations between CRP and SAA levels shows that these two molecules are independent inflammatory markers. Additional investigations are
needed for more accurate outlining the origin and the sources of the inflammatory process in obesity.

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CONFLICT OF INTEREST
The authors do not have any conflict of interest related to this research.

REFERENCES


