C-Reactive Protein in the Initial Phase of Postprandial Lipemia in Subjects with Central Obesity

Djeyne Silveira Wagmacker¹,², Jefferson Petto¹, Fabiano Leichsenring Silva¹, Alan Carlos Nery dos Santos¹, Ana Marice Teixeira Ladeia¹

¹Escola Bahiana de Medicina e Saúde Pública - Salvador, BA - Brazil
²Faculdade Adventista da Bahia - Cachoeira, BA - Brazil

Abstract

**Background:** Studies indicate that during postprandial lipemia (PPL), free radical formation occurs, stimulating the endothelium to secrete cytokines that mediate inflammatory responses. Excess adipose tissue, especially in the abdominal region, is positively correlated with C-reactive protein (CRP) values. However, little is known about CRP variations during the initial phase of PPL, especially among obese individuals.

**Objective:** To determine if there are variations in CRP plasma concentrations among individuals with central obesity during the initial phase of PPL.

**Methods:** This study assessed forty sedentary men and women with no alterations to fasting lipid profiles and waist circumferences above the normal cutoff point, measuring their CRP levels after fasting for twelve hours and three hours after ingesting 50g of fat.

**Results:** The mean CRP values after fasting and three hours after lipids intake were 0.6 mg/L (0.2 to 1.8 mg/L) and 0.4 mg/L (0.2 to 1.8 mg/L) (p=1.000) respectively.

**Conclusion:** In this study, the initial phase of PPL did not present any variations in CRP concentrations among subjects with central obesity.

**Keywords:** Inflammation; Metabolism; Lipids

Introduction

Postprandial lipemia (PPL), which reflects the body’s ability to metabolize lipids after a meal, is one of the main factors of atherosclerosis¹. PPL can be divided into three phases: phase of high triglycerides, plateau phase and phase of lipid values returning to baseline. The average duration of the three phases is six to eight hours and its peak in healthy individuals is achieved between the third and fourth hour². The first phase of PPL to the peak point is measured primarily by the elevation of triglycerides, since no variations are observed in plasma concentrations of lipoproteins²,³.

Studies indicate that during PPL, there is formation of free radicals, thus stimulating the endothelium to secrete cytokines that mediate the inflammatory response, leading to the formation of adhesion molecules. Even in healthy individuals, after meals with high fat contents, there is a significant increase in the concentration of proinflammatory cytokines (TNF-α and
IL-6) and adhesion molecules (ICAM-1 and VCAM-1)\(^5\). Consequently, this may change the plasma concentration of C-reactive protein (CRP).

CRP is regarded as one of the main biomarkers of subclinical arterial vascular inflammation. The plasma concentration of CRP rises in response to arterial attacks even before clinical cardiovascular manifestations\(^5\).

Excess adipose tissue, especially in the abdominal region, correlates positively with CRP values. This correlation occurs because adipocytes are responsible for the production of pro-inflammatory adipokines such as TNF-\(\alpha\) and IL-6. Once in the bloodstream, these adipokines stimulate hepatocytes to produce messenger RNA for the synthesis of proteins in the acute phase of inflammation such as CRP\(^6\). It is also known that in individuals with obesity, triglyceride clearance is reduced, which leads to an increase in the average time of PPL\(^6\).

The elevation of CRP starts on average six hours after lipid overload in patients with normal weight\(^7\), however, studies show that the lipid curve of overweight or obese individuals is associated with increased in magnitude and time\(^6\). This increased lipid overload response could stimulate early inflammatory response in overweight or obese individuals, since these individuals usually have high baseline CRP values, characterizing a low-intensity systemic inflammatory state\(^4,8\).

Confirming this hypothesis, Holmer-Jensen et al.\(^8\) showed inflammatory abnormalities in the initial state of PPL when they observed elevation of ICAM-1 and VCAM-1 in this population. However, the authors also investigated any variations in the CRP.

A study by Schaan et al.\(^9\) evaluated the inflammatory response at the initial stage of PPL in individuals with diabetes mellitus, because they have an increased PPL\(^10\). Unlike the findings of Holmer-Jensen et al.\(^8\) there were no abnormalities in the inflammatory state. But this time, the inflammatory marker used was CRP\(^9\). This counterpoint between the studies leaves an important gap to be filled by the scientific community: to check whether there are any rises in CRP in obese individuals in the initial phase of the PPL, since inflammatory abnormalities were found to exist with other markers.

Hence, this study aims to determine any variations in the values of CRP during the initial phase in individuals with central obesity.

**Methods**

Longitudinal prospective analytical study held at Faculdade Adventista in the city of Cachoeira, state of Bahia. The study evaluated sedentary individuals of both sexes, aged 18 to 30 years, with waist circumference >80 cm for women and >94 cm for men.

Individuals with cardiovascular disease, metabolic disease, hypothyroidism, renal parenchymal disease or diabetes; individuals in acute inflammatory or infectious state in the last month or chronic state in the last three months; individuals with a history of alcoholism or smoking, under use of lipid-lowering drugs, steroids, diuretics, beta blockers, contraceptives or those with CRP levels ≥10 mg/L were excluded from the study.

To classify an individual as sedentary, an international questionnaire of physical activity — full version — was used\(^11\). The cutoff of waist circumference was based on the 4th Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis\(^1\).

**Initial Evaluation**

The participants completed a questionnaire prepared by the authors of the study and underwent physical examination. The questionnaire collected information for general characterization of the sample and raised any potential factors of exclusion. The physical examination included measures of resting blood pressure, total body weight, height and waist circumference.

Height was measured using a professional Sanny stadiometer (São Paulo, SP - Brazil) with precision of 0.1 cm, on barefoot individuals with glutes and shoulders supported on a vertical backrest. Total body mass measured with digital scales Filizola (São Paulo, SP - Brazil) maximum capacity of 150 kg, measured by Inmetro, with a proper certificate specifying a margin of error of ± 100 g.

Waist circumference was obtained with metal inelastic Starrett tape (São Paulo, SP - Brazil), with a defined
measurement of 0.1 cm, measured at the smallest curvature between the last rib and the iliac crest without compressing the tissues\textsuperscript{12}.

Body mass index (BMI) was calculated with the measurements of weight and height, according to the Quetelet equation: \(\text{BMI} = \frac{\text{weight (kg)}}{\text{height}^2 (\text{m})}\).

**Postprandial Lipemia Testing**

To do the test, 5 mg of 12-hour fasting venous blood was collected; triglycerides, total cholesterol, low and high-density lipoprotein, CRP and blood glucose were measured. After the first sampling, the individuals ingested an industrial liquid lipid compound sold by Tecnovida (Salvador, Bahia - Brazil) containing 50 g of fat, of which 30 g is monounsaturated, 14 g is polyunsaturated and 5 g is saturated. Three hours later (time 180 minutes) triglycerides and CRP were measured again.

All participants were instructed not to perform physical exercise, not to make any changes to their diet, eating or ingesting any amount of alcohol 48 hours before the test. Sampling was conducted by a trained professional at a laboratory suitable to this type of procedure.

The values of triglycerides, total cholesterol and high-density lipoprotein were obtained by the enzymatic method. The values of low-density lipoprotein were calculated by the Friedewald equation\textsuperscript{13}. For blood glucose, the clinical chemistry system method was used. CRP was measured by the nephelometry method with serum plasma and precision of 0.1 mg/L.

The study was submitted to the Research Ethics Committee from Faculdade Adventista da Bahia, under no. CAAE 0071.0.070.000-11. The administration of fat in the study (50 g) did not infringe any ethical criteria, since the Western diet has on average 20-70 g of fat per meal\textsuperscript{14}. After completion of the intervention protocol of this study, all participants were referred for follow-up at the cardiology department of Faculdade Adventista da Bahia, where a free supervised physical activity program was offered for three months.

All participants read and signed an informed consent form that described the risks and benefits of this research. To calculate the sample size, alpha=0.05 (two-way) and beta=0.80 were set, adopting 20\% as a significant difference between the baseline sampling and a 180-minute sampling. Considering that the CRP coefficient of laboratory variation is 5\% and that a difference four times greater than the expected difference eliminates the bias of this coefficient of analytical variation, at least 32 volunteers were necessary. The sample size calculation was performed using GraphPad StatMate 2.0 for Windows.

**Statistical Analysis**

Tests of symmetry and kurtosis and the Shapiro-Wilk test were applied to assess the distribution of variables. Lipoproteins and triglycerides showed a normal distribution and are expressed as mean and standard deviation. The CRP did not present a normal distribution and its values were then expressed as median and quartile deviation. The two-way Mann-Whitney test was used to compare fasting and postprandial CRP values.

For comparisons of metabolic and anthropometric variables of the sample stratified by sex, unpaired two-way Student t test was used. Correlation analyzes were performed between the fasting CRP values and the fasting lipid profile variables, as well as CRP with BMI and waist circumference. For these correlations, the Spearman test was used. For all analyses, the significance level of \(p <0.05\) was adopted. Data were analyzed using the software Statistical Package for the Social Science, version 14.0.

**Results**

The study evaluated 40 individuals of which 7 were excluded for presenting baseline CRP values \(\geq 10 \text{mg/L}\). The general characteristics of the sample are presented in Table 1. It is important to note that the sample presents fasting lipid and blood pressure levels regarded as normal\textsuperscript{1} even though it is a sample of overweight and obese individuals.

In the comparison of variables stratified by gender there was a statistically significant difference only between waist circumference and high-density lipoprotein \((p<0.05)\). Note that although fasting triglycerides and peak PPL are greater in men, as seen...
in another study\textsuperscript{15}, no significant differences were found when comparing genders in these variables (p=0.173 and p=0.072).

Table 2 shows the correlation analyses of the overall sample between baseline CRP and fasting lipid variables and between CRP and BMI. A moderate positive correlation was found between baseline CPR and BMI $r=0.41$ (p=0.022). The other correlations were not statistically significant.

Figure 1 shows the values of fasting CRP and peak PPL, which were 0.6 mg/L (0.2-1.8 mg/L and 0.4 mg/L (0.2-1.8 mg/L), respectively. There was no statistically significant difference in the variation of CRP between the two periods (p=1.000).

Table 1
General characteristics of the sample

<table>
<thead>
<tr>
<th>Variables</th>
<th>*Men (n=6)</th>
<th>*Women (n=27)</th>
<th>*General (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25±3.8</td>
<td>23±3.7</td>
<td>24±3.8</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>28±2.1</td>
<td>28±3.5</td>
<td>28±3.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99±3.9</td>
<td>90±6.3</td>
<td>91±6.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>118±4.4</td>
<td>112±7.5</td>
<td>113±7.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78±4.4</td>
<td>76±5.5</td>
<td>77±5.4</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>80±11.8</td>
<td>81±11.6</td>
<td>80±11.3</td>
</tr>
<tr>
<td>Fasting triglycerides (mg/dL)</td>
<td>187±148.3</td>
<td>109±67.5</td>
<td>128±29.8</td>
</tr>
<tr>
<td>Triglyceride at peak PPL (mg/dL)</td>
<td>276±182.5</td>
<td>161±103.9</td>
<td>178±123</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>177±26.8</td>
<td>168±33.7</td>
<td>170±29.8</td>
</tr>
<tr>
<td>Low-density lipoprotein (mg/dL)</td>
<td>104±27.6</td>
<td>103±30.6</td>
<td>102±26.3</td>
</tr>
<tr>
<td>High-density lipoprotein (mg/dL)</td>
<td>36±3.7</td>
<td>43±7.6</td>
<td>42±7.5</td>
</tr>
</tbody>
</table>

PPL - postprandial lipemia
*Values expressed as mean and standard deviation.

Table 2
Correlation of baseline CRP with lipid variables and BMI

<table>
<thead>
<tr>
<th>Variables correlated</th>
<th>r</th>
<th>*p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-density lipoprotein and CRP</td>
<td>-0.25</td>
<td>0.161</td>
</tr>
<tr>
<td>Low-density lipoprotein and CRP</td>
<td>0.03</td>
<td>0.824</td>
</tr>
<tr>
<td>Total cholesterol and CRP</td>
<td>0.03</td>
<td>0.849</td>
</tr>
<tr>
<td>Triglycerides and CRP</td>
<td>0.22</td>
<td>0.210</td>
</tr>
<tr>
<td>Blood glucose and CRP</td>
<td>-0.11</td>
<td>0.524</td>
</tr>
<tr>
<td>Waist circumference and CRP</td>
<td>0.24</td>
<td>0.181</td>
</tr>
<tr>
<td>Body mass index and CRP</td>
<td>0.41</td>
<td>0.022#</td>
</tr>
</tbody>
</table>

CRP - C-reactive protein; BMI – body mass index
*Spearman correlation test; #Significant positive correlation
Discussion

While studies show that pro-inflammatory adipokines such as TNF-α and IL-6 increase during PPL, during this study, CRP values did not change in the initial phase of PPL in individuals with central obesity. The investigation of the mechanisms that determine atherosclerosis suggests that inflammation plays a central role in its development, progression and outcome. The inflammatory process causes structural and functional changes that lead to endothelial dysfunction and development of atherosclerotic plaques.

Inflammatory biomarkers are important markers in this process and are connected with cell activation, such as recruitment of inflammatory cells and proliferation of smooth muscle cells in the wall arterial. Although there are many inflammatory biomarkers, CRP has been the most widely studied inflammatory biomarker.

More recently, other factors have been correlated with the pathogenesis of atherosclerosis, including PPL, considered a possible early marker of metabolic abnormalities and vascular dysfunction. In the postprandial state, longer persistence of elevated lipids promotes endothelial dysfunction with increased inflammatory response, reduced availability of nitric oxide and increased oxidative stress. All these abnormalities are involved in the origin of atherosclerosis.

Although it has been evident that obesity, especially central obesity, increases the extent and magnitude of PPL, in this study it was observed that obesity did not induce, at least in its initial phase, the variation in the concentration of CRP. This finding can be explained by the fact that more significant CRP abnormalities are perceived mainly six hours after tissue aggression. If the time follow-up of this study was larger, significant changes in CRP could possibly be observed.

However, we cannot rule out the possibility that the inclusion and exclusion criteria of this study have strongly influenced the outcome. Note, for example, that in our sample, CRP showed baseline values considered of low cardiovascular risk. It was expected...
that in overweight or obese, sedentary and increased waist circumference individuals, CRP values were higher, as observed in other studies. But, possibly because it is a sample of individuals who had no factors know to have caused endothelial inflammation such as hypertension, diabetes mellitus, dislipidemias, smoking history and oral contraceptives, there was no significant variation of CRP in early PPL.

Another important aspect was the positive correlation between BMI and the fasting CRP values, as also reported in other research studies. This also demonstrates that an increase in body weight is of influence on increases CRP.

Concerning this aspect, some studies suggest that obesity is a powerful inflammation inducer. Since the adipose tissue is an important endocrine organ that secretes adipokines, the low level of chronic inflammation of obesity is characterized by increased circulating concentrations of adipokines and inflammatory cytokines. These aspects highlight the interrelation between inflammatory and metabolic pathways, which increases plasma circulating levels of biomarkers like CRP.

Therefore, further studies are required to compare populations with central obesity with and without proinflammatory modifiable factors in order to show whether central obesity has actually no influence on the variation of CRP in the initial phase of PPL.

**Conclusion**

Although evidence shows that obesity is directly correlated with high fasting CRP, in this study, there was no variation of CRP during the initial phase of PPL in individuals with central obesity.

**Potential Conflicts of Interest**

No relevant conflicts of interest.

**Sources of Funding**

This study had no external funding sources.

**Academic Association**

This study is not associated to any graduate programs.

**References**


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