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Complement component 3 (C3) as a biomarker for insulin resistance after bariatric surgery

Letícia de Oliveira Souza Bratti [a], Ícaro Andrade Rodrigues do Carmo [b], Taís Ferreira Vilela [c], Sandro Wopereis [a], Ana Carolina Rabello de Moraes [b], Beatriz Garcia Mendes Borba [b], Liliete Canes Souza [b], Fabíola Branco Filippin-Monteiro [b,*]

[a] Programa de Pós-Gaduação em Farmácia, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis, SC 88040900, Brazil
[b] Departamento de Análises Clínicas, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis, SC 88040900, Brazil
[c] Departamento de Clínica Médica, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina, Florianópolis, SC 88040900, Brazil

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ABSTRACT

Objectives: Since complement system has been recently associated with metabolic and cardiovascular diseases, and closely related to insulin resistance, we investigated the association of plasma complement factor 3 (C3) and factor 4 (C4) with insulin sensibility and weight loss after bariatric surgery.

Methods: Serum levels of C3, C4, total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol and homeostatic model assessment (HOMA-IR) measurements were assessed in morbidly obese patients before and after bariatric surgery, including a 6-month follow-up period, as well as a comparison with a lean group.

Results: Weight loss decreased body mass index (BMI), serum triacylglycerol, and increased serum HDL-cholesterol and insulin sensitivity, as expected. C3 and C4 were significantly higher in obese individuals when compared to lean subjects ($p < 0.001$). In addition, C3 and C4 positively correlated with BMI and HOMA-IR, however, only C3 were significantly decreased 6 months after surgery.

Conclusion: C3 was strongly associated with insulin sensitivity after bariatric surgery,

1. Introduction

Recent data about global obesity revealed that approximately 39% of adults were overweight in 2014, and 13% were obese. Besides, obesity has been increased exponentially in the last 30 years and it has become a public health problem due to obesity-associated comorbidities such as type 2 diabetes, cardiovascular diseases, and cancer [1].

In the treatment of morbid obesity, bariatric surgery became popular due to the difficulty in weight loss maintenance with clinical treatment. Approximately 450 thousand bariatric surgeries are performed each year worldwide. This procedure has been used as an elective intervention, promoting substantial and durable weight loss, reducing comorbidities, and improving the quality of life of these individuals. Bariatric surgery can be divided into three categories: restrictive, malabsorptive and a combination of both. The most common procedure is the Roux-en-Y gastric bypass (RYGB), a combination of restrictive and malabsorptive procedures, and the restrictive one, called sleeve gastrectomy (SG) [2] [3].

It is well-established that inflammation in obesity is characterized by a systemic, chronic and low-grade inflammation [4] that leads to an increase in pro-inflammatory proteins such as C-reactive protein (CRP) and interleukin-6 (IL-6) [5] and some components of complement system [6] [7]. The complement system is mainly associated with innate immunity. However, recent studies have shown that this system is involved in metabolic events [7] [8]. The majority of complement components are synthesized mainly by hepatocytes, but also can be produced by other cells such as macrophages and adipocytes [6] [8]. Recently, it was demonstrated that complement C3 (C3) synthesis can be up-regulated by pro-inflammatory cytokines IL-6 and interleukin-1 beta (IL-1β), while complement C4 (C4) can be influenced by gamma interferon (IFN-γ) [9].

Obese adipose tissue can contribute to the increase of pro-inflammatory cytokines leading to the hepatic production of C3 and C4 proteins. An increase of these proteins in obese individuals has already been reported [5]. In addition, C3 originates C3a-desArg lipogenic hormone, involved in the uptake of fatty acids, glucose and triacylglycerol synthesis by adipocytes [10]; it has been associated with cardiovascular risk

* Corresponding author at: Campus Universitário, Universidade Federal de Santa Catarina, Trindade, 88040900, Florianópolis, SC, Brazil.
E-mail address: fabiola.monteiro@ufsc.br (F.B. Filippin-Monteiro).

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factors, metabolic syndrome, insulin resistance as well as type 2 diabetes, high levels of CRP and increased waist circumference [7] [8]. Likewise, C3 and C4 have been associated with BMI and fat distribution, corroborating the concept that these two components are associated with metabolic syndrome and cardiovascular disease [6] [7].

Glucose metabolism is generally impaired in obese individuals since increased serum levels of IL-6 are implicated in the pro-inflammatory state that leads to peripheral insulin resistance [11]. In addition, morbidly obese individuals generally present high levels of adipokines that impair glucose homeostasis leading to the need of pharmacological treatment with oral hypoglycemic agents [12]. After bariatric surgery, however, glucose tolerance should be evaluated due to the new circumstances of weight loss and metabolic improvement. Therefore, regarding the previous association between C3, adipose tissue-related inflammation, and insulin resistance, our aim was to evaluate the level of C3 and C4 in morbidly obese individuals before and after bariatric surgery to ascertain insulin resistance–associated variables and evaluate complement proteins C3 and C4 in a follow-up after bariatric surgery.

2. Methods

2.1. Study design and data collection

Consecutive severely and morbidly obese patients (BMI ≥40 kg/m^2 or ≥35 kg/m^2 with obesity-related comorbidities) referred for Roux-en-Y gastric bypass or sleeve gastrectomy were included between August 2014 and September 2015 at Hospital Universitário from Universidade Federal de Santa Catarina (Florianópolis, Brazil). All patients met the criteria for bariatric surgery established by the National Institutes of Health for gastrointestinal surgery for obesity [3]. Lean individuals (BMI ≤25 kg/m^2) were recruited to comprise control group. All participants provided written informed consent. Gender, age, weight, and height were obtained from medical records or provided by participants during recruitment.

2.2. Serum assays

Fasting venous blood samples were collected before and six months after surgery, or on a single event for lean subjects (10 h fasting). After blood collection, serum was separated and stored into microtubes at −80 °C until analysis. C3 and C4 concentrations were determined by immunonephelometry, an assay based on the antigen-antibody reaction (BN-II, Siemens, Berlin, Germany), and serum insulin was measured by the immunochemiluminometric method (Advia Centaur XP, Siemens, Berlin, Germany). Total cholesterol, triacylglycerol, HDL-cholesterol, and glucose were measured by enzymatic methods (Dimension RXL Max, Siemens, Berlin, Germany). LDL-cholesterol was calculated by Friedewald equation and insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) with the equation HOMA-IR = [(fasting insulin in μUI/ml) × (fasting glucose in mg/dl)] / 405.

2.3. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS), version 22.0 (SPSS, Illinois, USA). To evaluate the symmetry of variables Shapiro-Wilk test was used. Group differences were assessed using Mann-Whitney *U* test, while Wilcoxon test was used to evaluate changes before and after surgery. Correlations were described using Spearman's rank correlation coefficient (r_S). For the multivariate regression model was used the Medcalc statistical software program, version 16.8.4 (Mariakerke, Belgium) $p \leq 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Clinical characteristics

Table 1 shows biochemical, anthropometric, and clinical data of obese subjects in the preoperative period and 6 months after surgery, as well as data of the lean group. Forty obese and 30 lean subjects underwent evaluation. Women were 86% and 80% in obese and lean groups, respectively. The median age of obese subjects was 43 years, and median BMI was 49.1 kg/m^2, compared to 32 years and 21 kg/m^2 of lean subjects (BMI $p < 0.0001$).

3.2. Complement system and biochemistry parameters

Overall, the lipid metabolism improved substantially six months after surgery. Baseline serum total cholesterol was 184 mg/dL vs. 168 mg/dL after surgery and LDL-cholesterol was 114 mg/dL vs. 101 mg/dL. Triacylglycerol reduced significantly (33%, $p = 0.0018$) and HDL-cholesterol increased from 38 mg/dL to 44 mg/dL ($p = 0.0275$). Improvements were also observed in fasting glucose, but more importantly in serum levels of insulin, and HOMA-IR ($p = 0.0078$). These data corroborates the reduced number of individuals who took oral hypoglycemic agents after surgery (reduction of 75%). Regarding the comparison between the obese and lean groups, a significant difference was observed in all parameters shown in Table 1.

Regarding C3 and C4 levels, differences between obese and lean individuals were observed ($p < 0.0001$). Besides, differences between pre-operative period and 6-months after surgery for C3 was observed (Fig. 1A and B) as well as a positive correlation with BMI was for both C3 ($r_s = 0.626$, $p < 0.0001$) and C4 ($r_s = 0.566$, $p < 0.0001$). However, when these tests were performed for the two groups separately, the correlations were not sustained. In the correlation between C3 and BMI in the lean group, r_s and p values were 0.039 and 0.830 and for the obese group were 0.193 and 0.094, respectively. And in the correlation between C4 and BMI the values of r_s and p were −0.088 and 0.640 and for the obese group were 0.011 and 0.929, respectively. To further clarify the association of serum complement C3 and C4 levels with weight loss and insulin sensitivity, we performed a correlation analysis between C3 or C4 and HOMA-IR and correlations between C3, or C4, and HOMA-IR were significant ($r_s = 0.550$, $p < 0.0001$; $r_s = 0.509$, $p < 0.0001$, respectively). These results are shown in Fig. 1C and D. We also performed the correlations between C3 or C4 and HOMA-IR separately for the lean and obese groups. As a result, the correlation between HOMA and C3 in the lean group did not follow the trend ($r_s = 0.040$, $p = 0.874$), whereas in the obese group the correlation was maintained ($r_s = 0.574$, $p < 0.0001$). The correlation between HOMA-IR and C4 followed the trend for both the lean group ($r_s = 0.595$, $p = 0.001$) and the obese group ($r_s = 0.520$, $p < 0.0001$). We also verified C3 and C4 levels alone in patients without insulin resistance and we observed that levels of these proteins did not decrease after bariatric surgery in these patients. In addition, we did a stepwise multivariate regression model using HOMA-IR as a dependent variable and BMI, C3, C4, total cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerol as independent variables. Multivariate analysis was significant, with r^2-adjusted of 0.271 and in this regression analysis, only C3 remained in the model as an independent variable ($r_{partial} = 0.530$; $p < 0.0001$).

4. Discussion

In the present study, we evaluated C3 and C4 proteins in patients who underwent surgery. Our results showed that serum C3 and C4 were not only significantly higher in obese patients when compared to lean subjects, but also decreased substantially 6 months after surgery. Moreover, correlations of C3 or C4 with HOMA, a model for insulin resistance evaluation, were found, revealing the close relationship between complement C3 and C4 with insulin resistance.

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