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Triacylglycerols and body fat mass are possible independent predictors of C3 in apparently healthy young Brazilian adults

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ABSTRACT

Objective: To evaluate the association between serum concentrations of complement factor-3 (C3) with anthropometric, biochemical, and lifestyle features in healthy young adults.
Methods: From 157 young healthy adults 18 to 35 y old, anthropometric measurements and body composition, systolic and diastolic blood pressures, and lifestyle data were collected and analyzed. Blood samples were collected after a 12-h fast for the determination of glucose, triacylglycerols, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, insulin, C3, ceruloplasmin, and uric acid.
Results: Complement factor-3 correlated directly with body mass index ($r = 0.23417$, $P = 0.0032$), body fat mass (bioelectrical impedance analysis; $r = 0.33407$, $P < 0.0001$), percentage of body fat (bioelectrical impedance analysis; $r = 0.26873$, $P = 0.0007$), waist circumference ($r = 0.21266$, $P = 0.0075$), insulin ($r = 0.26152$, $P = 0.0009$), homeostasis model assessment of insulin resistance ($r = 0.24831$, $P = 0.0017$), total cholesterol ($r = 0.23335$, $P = 0.0033$), triacylglycerols ($r = 0.38435$, $P < 0.0001$), and other outcome measurements. In the multiple linear regression analysis, triacylglycerols ($r^2 = 0.1379$, $P < 0.0001$) and body fat mass (bioelectrical impedance analysis; $r^2 = 0.0621$, $P = 0.0010$) were independently associated with the C3 concentration after adjusting for age, gender, smoking status, and physical activity.
Conclusion: Complement factor-3 seems to be related to several anthropometric and biochemical measurements in healthy young adults. These results demonstrate an independent role of triacylglycerols, a component of the metabolic syndrome, and body fat mass as possible predictors of C3 concentrations. Thus, C3 can be used as an early marker for metabolic syndrome manifestations.

Introduction

Adipose tissue is an active endocrine organ that secretes several factors called *adipokines*, such as complement factor-3 (C3), which play a role in some inflammatory manifestations associated with obesity and the metabolic syndrome (MS) [1,2].

The third component of the complement system, C3, is a multifunctional protein because it plays a central role in the activation of three pathways of the complement system (classic, alternative, and lectin pathways) [3,4]. C3 is an adipokine produced primarily by the liver [5], but adipose tissue [3] and activated macrophages also produce the molecule [6]. In addition to its role in exercise and many important immune system functions, C3 behaves like an acute-phase protein and is synthesized by hepatocytes in response to interleukin-1β, which is secreted by activated macrophages [3,7] at the site of inflammation. In this context, C3 has been studied because of its expression and secretion by adipose tissue [3].

An increase in C3 concentrations is associated with increases in fasting plasma glucose, triacylglycerol concentrations [8], insulin resistance (IR) [9], body mass index (BMI), waist circumference (WC), body fat [8], and body weight gain [9]. Thus, these results suggest that C3 may be a risk factor for developing type 2 diabetes [10] and obesity [9] and a risk indicator for coronary heart disease [11]. Nevertheless, in addition to the MS

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and C-reactive protein, C3 has been helpful for identifying people with components of MS [12] and conferring their risk for cardiovascular disease [12].

Besides the association between anthropometric measurements and body composition with inflammation, several risk factors related to lifestyle have been associated with inflammation [13]. The effect of these parameters on the inflammation and the cumulative effect of several risk factors have not been sufficiently explored [14].

The aim of this study was to evaluate the associations between C3 and anthropometric measurements, body composition, and specified biochemical and lifestyle features in healthy young adults.

Materials and methods

Subjects

In this study, 157 healthy subjects 18 to 35 y old were recruited to participate (91 women and 66 men, 23.3 ± 3.5 y old, BMI 22.0 ± 2.9 kg/m^2). The initial enrollment screening included evaluations to exclude subjects with evidence of any disease related to chronic inflammation, oxidative stress, hydric unbalance, and changes in body composition, nutrient absorption, or metabolism. Other exclusion criteria were drug or nutritional treatments that affect energy balance, dietary intake, lipid profile, insulin levels, and glucose metabolism; contraceptive use up to 2 mo before participation in this study; and recent diets designed for weight loss or unstable weight in the previous 6 mo. In accordance with the principals of the Declaration of Helsinki, each participant provided a written informed consent to participate after a clear explanation of the study protocol. The study was approved by the committee of ethics in research with humans of Viçosa University, Minas Gerais State, Brazil (official reference no. 009/2006).

Anthropometric, body composition, and blood pressure assessments

Body weight was measured to the nearest 0.1 kg using an electronic microdigital scale balance (model TBF-300A, Tanita, Tokyo, Japan). Height was measured with a stadiometer (model 206, Seca, Hamburg, Germany) to the nearest 0.1 cm [15]. From those measurements, the BMI was calculated [16]. Thereafter, the BMI was calculated and used to categorize the underweight/normal weight (BMI ≤ 24.9 kg/m^2) and overweight/obesity (BMI ≥ 25.0 kg/m^2) of the volunteers according to the World Health Organization [16]. The WC, hip circumference (HC) [16], and arm circumference [17] were measured with an inelastic and flexible tape to the nearest 0.1 cm. The waist-to-hip ratio was also calculated [16]. Triceps, biceps, subscapular, and suprailiac skinfold thicknesses (STs) were measured to the nearest 1 mm using a skinfold caliper (Lange caliper, Cambridge Scientific Industries, Inc., Cambridge, MD, USA) according to a previously described protocol [18]. The sum of four STs was calculated, and the percentage of body fat (%BF) was estimated according to equations published previously [19,20]. Also, the arm fat area (AFA; square centimeters) and adipose–muscle arm index (A-MAI) [17] were calculated. Measurements of body fat mass (BFM; kilograms), fat-free mass (kilograms), and %BF were determined using a body composition analyzer (model 310, Biodynamics, Seattle, WA, USA) [21]. Systolic and diastolic blood pressures were measured by a mercury sphygmomanometer (BIC, São Paulo, Brazil) to the nearest 2 mmHg, as described previously [22].

Analyses of biological samples

Blood samples were drawn by vein puncture after a 12-h overnight fast. The ethylenediaminetetra-acetic acid plasma and serum samples were separated from whole blood by centrifugation at 2465 × *g* at 5°C for 15 min (model 5804R, Eppendorf AG, Hamburg, Germany) and were immediately stored at −80°C until the assay. Serum C3, glucose, total cholesterol, triacylglycerols, high-density lipoprotein cholesterol (HDL-C), total proteins, uric acid, and ceruloplasmin concentrations (milligrams per deciliter) were assessed by automatized colorimetric or turbidimetric assays (model BS-200, Shenzhen Mindray Bio-medical Electronics Co., Nanshan, China) with specific commercially available kits (Bioclin, Quibasa, Minas Gerais, Brazil). Low-density lipoprotein cholesterol (LDL-C) data were calculated by the Friedewald equation, as described elsewhere [23]. Then, the ratio of total cholesterol to HDL-C (atherogenic index) was calculated [24]. Plasma insulin concentrations (sensitivity 2 μU/mL) were measured by an enzyme-linked immunosorbent assay kit using a specific monoclonal antibody, 4E6, as described by the supplier (Mercodia, Uppsala, Sweden). Also, to determine sensitivity to insulin, the homeostasis model assessment of IR (HOMA-IR) was calculated [25].

Lifestyle assessments

For non-dietary covariates, a questionnaire on lifestyle habits was used to collect lifestyle information such as smoking (number of smokers and non-smokers), regularly practiced sports activities (yes or no), and pattern of physical activity (hours per week). To quantify the amount (Pattern) of physical activity, a metabolic equivalent index (MET) was calculated to denote a multiple of the resting metabolic rate for each physical activity performed during the week and weekend. These values were then converted into METs per hour [26].

Statistical methods

The Shapiro–Wilk normality test was used to determine the variable distribution. Comparisons between groups were made by a Wilcoxon–Mann–Whitney *U* test. Spearman correlation coefficients were used to screen for statistical associations between C3 concentrations (dependent variable) and the variables of interest. A multiple regression model was used to identify possible predictors of C3 using the technique of indirect elimination (backward) for the selection of variables and including the variables gender, smoking, and physical activity (qualitative variables) as indicator variables. Then, these analyses were performed for a subgroup without IR. For these analyses, IR was considered when the HOMA-IR was ≥p90 (HOMA-IR ≥ 3.79). Results are presented as median (interquartile range). Multiple regression coefficients (β) were described using 95% confidence intervals. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SAS 9.0 (SAS Institute, Cary, NC, USA).

Results

Anthropometric, biochemical data, and lifestyle features assessments

The general characteristics for age, anthropometric and biochemical data, body composition, data on systolic and diastolic blood pressures, and METs are presented in Table 1. The anthropometric data showed that BFM (bioelectrical impedance analysis [BIA]) and WC were significantly greater in women and men, respectively ($P < 0.05$; Table 2).

According to nutritional status, the total sample of volunteers consisted of 86.6% ($n = 136$) with underweight/normal weight and 13.4% ($n = 21$) with overweight or obesity according to the World Health Organization (WHO) categorization [16]. Except for age, all anthropometric and body composition measurements were significantly higher in overweight/obese volunteers ($P < 0.05$). For the sample divided by nutritional status, concentrations of C3, glucose, insulin, and uric acid, values of HOMA-IR, and the atherogenic index were higher in the overweight or obese group compared with the low-weight/normal-weight group ($P < 0.05$; data not shown).

For the data indicating the lifestyle of volunteers, 10.8% and 89.2% of the volunteers were smokers and non-smokers, respectively, ($n = 15/124$) and 71.95% and 28.05% of the volunteers participated or did not participate regularly in sports activities, respectively ($n = 100/39$). Only 88.53% of the volunteers returned the lifestyle habits questionnaire ($n = 139$).

Correlations between C3 and adiposity indicators, IR, and MS

The correlations between C3 and age, anthropometric and biochemical data, body composition, data on systolic and diastolic blood pressures, and MET are presented in Table 3.

For the total volunteer sample, the correlations between C3 and anthropometric measurements and body composition demonstrated a direct and significant association between C3 and BMI, tricipital ST, bicipital ST, subscapular ST, suprailiac ST, the sum of the four STs, %BF (ST), BFM (BIA), %BF (BIA), AFA, A-MAI, WC, and HC ($P < 0.05$). The correlation between C3 and biochemical characteristics of the volunteers demonstrated

Table 1
General, anthropometric, biochemical, and lifestyle data of volunteers ($n = 157$)

	Mean ± SD	CI	Median	IQR	*P*
C3 (mg/dL)	112.52 ± 23.56	20–171	114	102–128	<0.0012
Age (y)	23.3 ± 3.5	18–35	23	21–25	<0.0001
BMI (kg/m^2)	22.08 ± 2.99	16.7–34.9	21.5	20.3–23.3	<0.0001
Tricipital ST (mm)	18.13 ± 6.88	5.0–41.0	18.0	13.0–22.5	0.0185
Bicipital ST (mm)	7.27 ± 3.80	2.0–23.0	7.0	4.0–9.0	<0.0001
Subscapular ST (mm)	17.71 ± 7.31	7.0–43.0	15.5	12.5–20.5	<0.0001
Suprailiac ST (mm)	18.03 ± 8.95	4.5–48.0	18.03	11.0–22.5	<0.0001
Sum of 4 STs (mm)	61.15 ± 23.85	21.5–148.0	57.0	42.3–75.0	<0.0001
%BF (ST)	25.33 ± 7.50	8.1–42.3	26.5	19.0–31.2	0.0021
BFM (kg) (BIA)	14.79 ± 5.18	4.9–41.1	13.80	11.1–17.4	<0.0001
FFM (kg) (BIA)	47.57 ± 10.12	20.6–72.0	45.30	39.5–55.9	0.0003
%BF (BIA)	23.57 ± 6.48	9.4–38.3	23.50	18.7–28.25	0.1336
AC (cm)	28.13 ± 3.71	21.0–39.5	27.50	25.5–30.0	0.0006
AFA (cm^2)	22.75 ± 9.15	5.93–60.6	22.2	15.97–27.4	<0.0001
A-MAI	0.62 ± 0.31	0.12–1.48	0.58	0.38–0.81	0.0003
WC (cm)	78.0 ± 8.51	60.0–100.0	77.0	71.5–83.0	<0.0001
HC (cm)	95.47 ± 6.72	69.0–114.5	95.5	91.0–100.0	0.2554
WHR	0.81 ± 0.06	0.65–1.01	0.82	0.78–0.85	0.3008
Systolic blood pressure (mmHg)	109.4 ± 0.93	90–140	110	100–120	<0.0001
Diastolic blood pressure (mmHg)	73.3 ± 0.66	60–90	70	70–80	<0.0001
Glucose (mg/dL)	90.31 ± 6.26	72–106	90	87–94	0.31
Insulin (μU/mL)	10.10 ± 5.63	0.49–45.46	9.50	6.55–12.95	<0.001
HOMA-IR index	2.26 ± 1.27	0.11–9.08	2.14	1.43–2.86	<0.001
Total cholesterol (mg/dL)	159.66 ± 31.22	100–268	157	140–177	<0.0001
Triacylglycerols (mg/dL)	100.15 ± 44.62	34–373	91	69–121	<0.0001
HDL-C (mg/dL)*	46 ± 10,46	21–71	45	38–53	<0.003
LDL-C (mg/dL)	95.68 ± 26.93	41.8–181.6	91.20	78.4–109.6	<0.001
Atherogenic index*	3.56 ± 0.80	2.21–8.24	3.47	3.06–4.0	<0.0001
Total proteins (mg/dL)	6.74 ± 0.38	5.9–7.8	6.8	6.5–7.0	0.2097
Uric acid (mg/dL)	3.56 ± 1.06	1.1–6.5	3.4	2.8–4.2	0.1370
Ceruloplasmin (mg/dL)	37.21 ± 8.36	21.4–56.2	34.8	31.1–43.1	<0.001
MET†	76.93 ± 23.60	31.3–197.7	72.25	60.80–88.60	<0.0001

AC, arm circumference; AFA, arm fat area; A-MAI, adipose–muscle arm index; %BF, percentage of body fat; BFM, body fat mass; BIA, bioelectrical impedance analysis; BMI, body mass index; C3, complement factor-3; CI, confidence interval; FFM, fat-free mass; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range (quartiles 1–3); LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent index; ST, skinfold thickness; WC, waist circumference; WHR, waist-to-hip ratio
Test of normality: Shapiro-Wilk, $P < 0.05$
* For 150 volunteers.
† For 139 volunteers.

a direct and significant association between C3 and insulin, HOMA-IR, total cholesterol, triacylglycerols, the atherogenic index, and ceruloplasmin ($P < 0.05$). There was no correlation between C3 and age and blood pressure ($P > 0.05$).

For the subgroup without IR, the correlations between C3 and anthropometric measurements and body composition demonstrated a direct and significant association between C3 and tricipital ST, bicipital ST, subscapular ST, suprailiac ST, the sum of the four STs, %BF (ST), BFM (BIA), %BF (BIA), AFA, A-MAI, WC, and HC ($P < 0.05$). The correlation between C3 and the biochemical characteristics of the volunteers demonstrated a direct and significant association between C3 and insulin, HOMA-IR, total cholesterol, triacylglycerols, LDL-C, the atherogenic index, and ceruloplasmin ($P < 0.05$). There was no correlation between C3 and age and blood pressure ($P > 0.05$).

Possible predictive factors for C3 concentrations

Based on the relation between the biochemical and anthropometric data, body composition and lifestyle indicators with C3, we performed a multiple linear regression analysis to assess the possible predictors of C3 concentrations.

For the total volunteer sample, with regard to the biochemical data, the model that best explained the variation (20.48%) in C3 concentrations was defined by the following regression equation: C3 = 85.20 + 0.13 × triacylglycerols − 0.41 × HDL-C + 0.87 × ceruloplasmin ($P < 0.05$; Table 4, model 1). When we inserted the indicator variables gender, smoking, and physical activity, the model explained 13.79% of the C3 concentrations and was defined by the following regression equation: C3 = 92.49 + 0.19 × triacylglycerols ($P < 0.05$; Table 4, model 2; Fig. 1). In this model, there were no significant effects of gender, smoking, or physical activity (PA) ($P > 0.05$).

For anthropometric data and body composition, blood pressure, and MET, the model that best explained the variation (6.21%) in C3 concentration was defined by the following regression equation: C3 = 94.98 + 1.18 × BFM determined by BIA ($P < 0.05$; Table 4, model 3). When we inserted the indicator variables gender, smoking, and physical activity, the model remained unchanged ($P < 0.05$; Table 4, model 3; Fig. 2), and there were no significant effects of gender, smoking, or PA ($P > 0.05$). All models were adjusted for age.

For the subgroup without IR, with respect to the biochemical data, the model that best explained the variation (18.71%) in C3 concentrations was defined by the following regression equation: C3 = 58.68 + 0.16 × triacylglycerols + 3.81 × uric acid + 0.61 × ceruloplasmin ($P < 0.05$; Table 5, model 1). When we inserted the indicator variables gender, smoking, and physical activity, the model explained 14.23% of the C3 concentrations and was defined by the following regression equation: C3 = 88.64 + 0.22 × triacylglycerols ($P < 0.05$; Table 5, model 2). In this model, there were no significant effects of gender, smoking, or PA ($P > 0.05$).

For anthropometric data and body composition, blood pressure, and MET, the model that best explained the variation

Table 2
General, anthropometric, biochemical, and lifestyle data of volunteers by gender ($n = 157$)

	Women ($n = 91$)		Men ($n = 66$)		P
	Median	IQR	Median	IQR	
C3 (mg/dL)	115	101–131	110	102–123	0.2433
Age (y)	23	21–25	22	20–25	0.0309
BMI (kg/m^2)	21.1	19.7–23.0	22.45	21.0–24.4	<0.0016
Tricipital ST (mm)	21.0	17.0–24.5	14.25	10.0–17.5	<0.0001
Bicipital ST (mm)	8.0	6.5–11.0	4.0	3.0–6.0	<0.0001
Subscapular ST (mm)	16.7	13.0–22.0	15.0	11.0–18.5	0.0606
Suprailiac ST (mm)	20.0	15.0–24.0	12.0	9.0–17.0	<0.0001
Sum of 4 STs (mm)	66.0	52.0–80.5	46.7	35.0–57.0	<0.0001
%BF (ST)	30.2	26.5–33.1	17.7	14.7–20.1	<0.0001
BFM (kg) (BIA)	15.1	12.8–17.6	12.05	9.8–15.2	<0.0001
FFM (kg) (BIA	40.7	36.9–44.0	57.8	52.3–62.2	<0.0001
%BF (BIA)	26.8	23.8–31.0	17.65	15.0–20.7	<0.0001
AC (cm)	26.5	24.5–28.0	29.7	28.0–33.0	<0.0001
AFA (cm^2)	23.59	18.31–30.09	18.57	13.9–24.6	0.0007
A-MAI	0.75	0.59–0.97	0.38	0.26–0.48	<0.0001
WC (cm)	76.5	69.5–82.5	79.25	74.5–86.0	0.0103
HC (cm)	96.0	91.5–100.0	94.5	90.0–99.0	0.1265
WHR	0.80	0.75–0.84	0.84	0.81–0.89	<0.0001
Systolic blood pressure (mmHg)	110	100–110	110	110–120	<0.0001
Diastolic blood pressure (mmHg)	70	70–80	80	70–80	0.0001
Glucose (mg/dL)	88	85–92	92	88–97	0.0002
Insulin (μU/mL)	10.16	6.88–13.27	8.64	6.06–12.04	0.2687
HOMA-IR index	2.21	1.44–2.97	1.96	1.37–2.77	0.4837
Total cholesterol (mg/dL)	165	145–187	146	134–160	<0.0001
Triacylglycerols (mg/dL)	99	79–127	81.5	67–101	0.0038
HDL-C (mg/dL)*	52	45–57	38	36–43	<0.0001
LDL-C (mg/dL)	98.2	77.8–114.4	87.7	78.4–102.8	0.0751
Atherogenic index*	3.29	2.83–3.76	3.73	3.39–4.17	<0.0001
Total proteins (mg/dL)	6.7	6.4–7.0	6.8	6.6–7.0	0.0290
Uric acid (mg/dL)	3.0	2.4–3.5	4.15	3.6–4.8	<0.0001
Ceruloplasmin (mg/dL)	41	33.0–48.5	31.6	29.2–34.4	<0.0001
MET†	76	60.3–89.7	71.7	62.3–84.3	0.1572

AC, arm circumference; AFA, arm fat area; A-MAI, adipose–muscle arm index; %BF, percentage of body fat; BFM, body fat mass; BIA, bioelectrical impedance analysis; BMI, body mass index; C3, complement factor-3; FFM, fat-free mass; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range (quartiles 1–3); LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent index; ST, skinfold thickness; WC, waist circumference; WHR, waist-to-hip ratio
For differences between groups: Wilcoxon–Mann–Whitney U test, $P < 0.05$
* For 85 women and 65 men.
† For 78 women and 61 men.

(5.11%) in C3 concentration was defined by the following regression equation: C3 = 95.70 + 1.05 × BFM determined by BIA ($P < 0.05$; Table 5, model 3). When we inserted the indicator variables gender, smoking, and physical activity, the model remained unchanged ($P < 0.05$; Table 5, model 3), and there were no significant effects of gender, smoking, or PA ($P > 0.05$). All models were adjusted for age.

Discussion

In this study, after the correlation analysis, there was a direct and significant association of C3 with BMI (all volunteers) and adiposity measurements, including the tricipital, bicipital, subscapular, and suprailiac STs, the sum of the four STs, %BF (ST), BFM (BIA), %BF (BIA), AFA, A-MAI, WC, and HC ($P < 0.05$). Nevertheless, when the analysis was performed by multiple linear regression to assess the possible predictors of C3 concentrations, for the total volunteer sample, BFM (BIA) explained 6.21% of the variation in C3 concentration. For this sample of volunteers, for each 1-kg increase in BFM (BIA), the C3 concentrations increased 1.18 mg/dL ($P < 0.05$). For the subgroup without IR, BFM (BIA) explained 5.11% of the variation in C3 concentration. For this subgroup of volunteers, for each 1-kg increase in BFM (BIA), C3 concentrations increased 1.05 mg/dL ($P < 0.05$). Nevertheless, BFM (BIA) explained variations in C3 regardless of gender, smoking, and physical activity. In this context, and in accordance with our results, inflammation can occur in individuals independently of BMI but dependently and proportionally of BFM. This fact demonstrates that volunteers classified as being underweight/normal weight [16] can also present excesses of BFM and IR.

Complement factor-3 is a protein of liver inflammation [5], but activated macrophages and adipose tissue also synthesize C3 [3,6], thus explaining the relation between C3 and adiposity. In addition, the fragment of C3 (C3adesArg), also known as *acylation-stimulating protein* (ASP), is a hormone produced by adipocytes as a result of the interaction of C3, factor B, and adipsin (factor D) [27]. ASP stimulates glucose transport through the membrane and the synthesis of triacylglycerols in adipocytes, an action independent and additional to that exerted by insulin [28]. Thus, C3 and ASP are increased in individuals with excess adiposity and with obesity [27], confirming the C3 results found in this study.

The relation between adiposity, particularly central obesity (visceral), and the increased production of hepatic inflammatory proteins has been described by some researchers [29,30]. The expansion of adipose tissue leads to adipocyte hypertrophy and hyperplasia, and these large adipocytes decrease the local supply of oxygen, leading to autonomous cell hypoxia with the resultant activation of cellular stress pathways (oxidative or

Table 3
Correlations between complement factor-3 and general, anthropometric, biochemical, and lifestyle data of volunteers

	Total volunteers ($n = 157$)		Subgroup without IR* ($n = 139$)	
	r	P	r	P
Age (y)	0.00770	0.9237	0.00391	0.9636
BMI (kg/m^2)	0.23417	0.0032	0.16546	0.0516
Tricipital ST (mm)	0.27422	0.0005	0.24573	0.0035
Bicipital ST (mm)	0.23631	0.0029	0.19630	0.0206
Subscapular ST (mm)	0.28093	0.0004	0.23860	0.0047
Suprailiac ST (mm)	0.25729	0.0011	0.22538	0.0076
Sum of 4 STs (mm)	0.31070	<0.0001	0.27020	0.0013
%BF (ST)	0.26380	0.0008	0.23968	0.0045
BFM (kg) (BIA)	0.33407	<0.0001	0.29898	0.0003
FFM (kg) (BIA	0.05912	0.4620	0.02860	0.7382
%BF (BIA)	0.26873	0.0007	0.28288	0.0007
AC (cm)	0.10047	0.2106	0.5464	0.5229
AFA (cm^2)	0.28191	0.0003	0.24103	0.0043
A-MAI	0.26581	0.0008	0.24769	0.0033
WC (cm)	0.21266	0.0075	0.19529	0.0212
HC (cm)	0.25132	0.0015	0.22443	0.0079
WHR	0.07694	0.3381	0.07507	0.3798
Systolic blood pressure (mmHg)	0.07993	0.3197	0.03493	0.6831
Diastolic blood pressure (mmHg)	0.2969	0.7120	0.00689	0.9358
Glucose (mg/dL)	−0.01891	0.8141	−0.05154	0.5468
Insulin (μU/mL)	0.26152	0.0009	0.19175	0.0237
HOMA-IR index	0.24831	0.0017	0.17403	0.0405
Total cholesterol (mg/dL)	0.23335	0.0033	0.27754	0.0009
Triacylglycerols (mg/dL)	0.38435	<0.0001	0.35954	<0.0001
HDL-C (mg/dL)†	0.01804	0.8266	0.06555	0.4552
LDL-C (mg/dL)	0.12770	0.1110	0.17879	0.0352
Atherogenic index†	0.19622	0.0161	0.17229	0.0482
Total proteins (mg/dL)	0.14448	0.0710	0.16650	0.0501
Uric acid (mg/dL)	0.10596	0.1866	0.8308	0.3309
Ceruloplasmin (mg/dL)	0.37139	<0.0001	0.38127	<0.0001
MET‡	−0.02036	0.8126	−0.02771	0.7629

AC, arm circumference; AFA, arm fat area; A-MAI, adipose–muscle arm index; % BF, percentage of body fat; BFM, body fat mass; BIA, bioelectrical impedance analysis; BMI, body mass index; FFM, fat-free mass; HC, hip circumference; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent index; ST, skinfold thickness; WC, waist circumference; WHR, waist-to-hip ratio
Test of correlation: Spearman, $P < 0.05$
* IR was considered when the HOMA-IR was ≥p90 (HOMA-IR ≥ 3.79).
For total volunteers: † $n = 150$, ‡ $n = 139$.
For the subgroup without IR: † $n = 132$, ‡ $n = 121$.

Table 4
Multiple linear regression models to predict possible complement factor-3 concentrations ($n = 157$)

	Coefficient β ± SD	P
Model 1*		
Intercept	85.20 ± 8.79	<0.0001
Triacylglycerols (mg/dL)	0.13 ± 0.03	0.0007
HDL-C (mg/dL)	−0.41 ± 0.18	0.0300
Ceruloplasmin (mg/dL)	0.87 ± 0.25	0.0006
Model 2†		
Intercept	92.49 ± 4.30	<0.0001
Triacylglycerols (mg/dL)	0.19 ± 0.03	<0.0001
Model 3‡		
Intercept	94.98 ± 5.51	<0.0001
BFM (kg) (BIA)	1.18 ± 0.35	0.0010

BFM, body fat mass; BIA, bioelectrical impedance analysis; HDL-C, high-density lipoprotein cholesterol
* Variables included were age, glucose, insulin, homeostasis model assessment of insulin resistance index, total cholesterol, triacylglycerols, HDL-C, total proteins, uric acid, and ceruloplasmin ($r^2 = 0.2048$, $P < 0.0001$).
† Adjusted for the variables gender, smoking, and physical activity. Variables included were age, glucose, insulin, homeostasis model assessment of insulin resistance index, total cholesterol, triacylglycerols, HDL-C, total proteins, uric acid and ceruloplasmin ($r^2 = 0.1379$, $P < 0.0001$).
‡ With and without adjustment for the variables gender, smoking, and physical activity. Variables included were age, body mass index; tricipital, bicipital, subscapular, and suprailiac skinfold thicknesses; fat-free mass (BIA); BFM (BIA), percentage of body fat (BIA); arm circumference, waist circumference; hip circumference; systolic blood pressure; diastolic blood pressure; and the metabolic equivalent index ($r^2 = 0.0621$, $P = 0.0010$).

inflammatory). This inflammation leads to an autonomous cell (autocrine effect) and the release of cytokines and other proinflammatory signals. Like resistin, leptin and adiponectin, which are secreted by adipocytes, adipokines may also affect inflammation and IR. As part of the chronic inflammatory process and with low intensity, locally secreted chemokines attract proinflammatory macrophages to adipose tissue that form a crown-shaped structure around the large, dead, and/or infirm adipocytes. Then, these macrophages stimulate the release of cytokines that have yet to activate inflammatory pathways in adipocytes and other tissues, such as hepatocytes (autocrine and paracrine effect), aggravated inflammation, and IR [29,30]. Thus, hepatic inflammation can occur in individuals with an excess of adiposity because the activation of inflammatory pathways may be the result of steatosis and/or increased airway responses to stress in hepatocytes. This can result in the inflammation of autonomous hepatocytes (autocrine effect). The Kupffer cells (liver cells resembling macrophages) may also become activated, thus stimulating the local release of cytokines that further aggravate hepatic inflammation and IR. In addition, caloric surplus and an excess of adiposity are frequently accompanied by increased concentrations of circulating and tissue free fatty acids, and these can directly activate proinflammatory responses in vascular endothelial cells, adipocytes, and myeloid cells [29,30]. The result of these physiologic events induced by an excess of adiposity is the development of systemic inflammation [30]. Thus, it seems that the process is bidirectional and additive; C3 is produced by fat tissue, and its concentration can increase because of a larger supply of BF; C3 is still a liver protein of systemic inflammation, and its concentration can increase with greater inflammation independent of hepatocytes (steatosis from cellular hypoxia generated by an excess of BFM).

In regard to the biochemical data, there was a direct and significant association of C3 with total cholesterol, triacylglycerols, LDL-C (subgroup without IR), the atherogenic index, insulin, HOMA-IR, and ceruloplasmin ($P < 0.05$). When we applied multiple linear regression analysis to assess the possible predictors of C3, for the total volunteer sample, triacylglycerols, HDL-C, and ceruloplasmin explained 20.48% of the variation in C3 concentration ($P < 0.05$). For this sample of volunteers, for each increase of 1.0 mg/dL in triacylglycerols and 1.0 mg/dL in ceruloplasmin, there were 0.13- and 0.87-mg/dL increases in C3

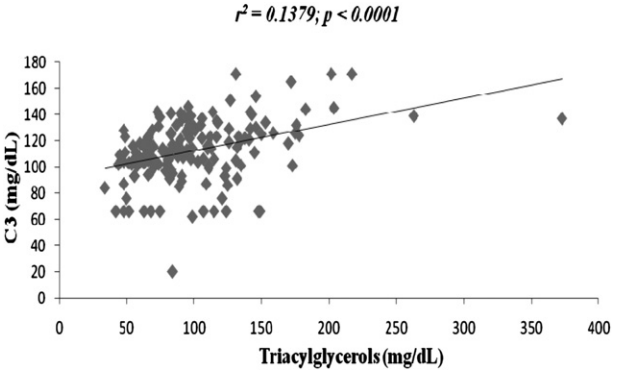

Fig. 1. Independent association between triacylglycerol concentration and C3 ($n = 157$). C3, complement factor-3.

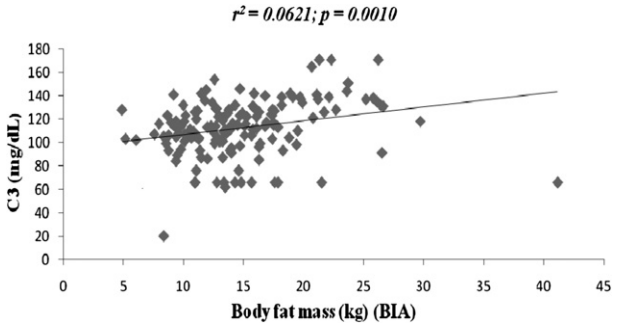

Fig. 2. Independent association between body fat mass and C3 (n = 157). BIA, bioelectrical impedance analysis; C3, complement factor-3.

concentrations, respectively. Similarly, each 1.0-mg/dL decrease in HDL-C correlated with a 0.41-mg/dL increase in C3 concentration ($P < 0.05$). For the subgroup without IR, triacylglycerols, uric acid, and ceruloplasmin explained 18.71% of the variation in C3 concentration ($P < 0.05$). For this sample of volunteers, for each increase of 1.0 mg/dL in triacylglycerols, 1.0 mg/dL in uric acid, and 1.0 mg/dL in ceruloplasmin, there were increases of 0.16, 3.81, and 0.61 mg/dL in C3 concentrations, respectively ($P < 0.05$). Nevertheless, triacylglycerols explained the variations in C3 regardless of gender, smoking, and physical activity.

Studies have confirmed that C3 is correlated with fasting insulin [31], IR [32], and total cholesterol [31,33]. However, C3 concentrations are higher in obese non-diabetics and type 2 diabetics than in healthy men, whereas C3 concentrations are similar between obese non-diabetics and type 2 diabetics and between type 1 diabetics and lean, healthy young men. In the same study, C3 concentrations did not differ between healthy men and young adults [34]. It is noteworthy that in this study there was no correlation between C3 and age, and all models of multiple linear regression analysis remained unchanged when adjusted for age. Although the age of the volunteers was restricted to young adults (18–35 y), this result is consistent with a study that showed that C3 was not modified by age [34].

The positive correlations between C3 concentrations and triacylglycerols is consistent with others studies [11,12]. The relation between C3 and biochemical measurements can be explained by the products of metabolism, i.e., ASP is a hormone also produced by adipocytes as a result of the interaction of C3, factor B, and adipsin (factor D) [27]. The events stimulate glucose transport through membranes and increase the synthesis of triacylglycerols in adipocytes, an action independent and additional to that exerted by insulin [28]. Just as for C3, ASP concentrations are increased in individuals with excess adiposity and obesity, diabetes, and cardiovascular disease [27]. Although the receptors and signaling mechanisms mediated by ASP are still unknown, there is evidence that ASP is involved in the activation of glucose transport and the stimulation of its absorption. As a result, ASP increases the re-esterification of fatty acids, the efficiency of triacylglycerol synthesis in adipocytes through the paracrine and autocrine effects, and the activation of diacylglycerol acyl-transferase and inhibits hormone-sensitive lipase activity and lipolysis [27,35]. The result of the action of ASP is a rapid postprandial lipid clearance. Therefore, the C3/ASP system has been proposed to be a regulator of fatty acid metabolism in adipose tissue [36]. Nonetheless, it is noteworthy that C3 is an inflammatory biomarker; thus, the direct association between C3 and ceruloplasmin (a glycoprotein family member of inflammation-sensitive proteins) [37,38] in the present study is expected.

Thus, inflammation assessed by C3 appears to be modulated by anthropometry and body composition and by biochemical factors, and MS components seem to be good indicators of subclinical inflammation. These results are confirmed by the scientific literature. One study demonstrated that C3 was independently associated with measurements of adiposity and triacylglycerols [11]. In our study, in a model with all covariates added, measurements of adiposity (BFM) and triacylglycerols also were independent possible predictors of C3 concentrations. In fact, C3 is partly secreted by adipocytes [6] and its fragment, ASP, is a potent simulator of glucose transport and triacylglycerol synthesis [27,35].

Other investigators have shown that a high C3 concentration is associated with cardiovascular diseases [39], WC, and high triacylglycerol concentrations in the fasting and postprandial states, proposing that it is a coronary risk factor [11], a risk factor for the development of diabetes [10] and obesity [34], and a useful marker for identifying people with MS [12].

These results are clinically important because the association between C3 and the development of atherosclerosis (myocardial infarction) can occur from the following mechanisms: 1) vulnerable plaques rich in macrophages and lipid may represent a source of the direct or indirect production of C3, which can thus be a marker for the presence of these plaques; 2) some C3 fragments and other cytokines [31,40] are associated with MS and a pro-atherogenic state, including IR and C3 (with other cytokines), and may play a causal role; and 3) these mechanisms can coexist in a circular process in which vulnerable plaques producing cytokines and C3 can trigger MS, which then promotes the growth of existing plaques and the formation of new plaques [40].

Our study had certain limitations. Because the nature of this study was cross-sectional, we cannot prove that the reported associations are causal because residual confounding may have affected the observed associations, although we controlled for

Table 5
Multiple linear regression models to predict possible complement factor-3 concentrations in volunteers without insulin resistance* (n = 139)

	Coefficient β ± SD	P
Model 1†		
Intercept	58.68 ± 12.03	<0.0001
Triacylglycerols (mg/dL)	0.16 ± 0.05	0.0026
Uric acid (mg/dL)	3.81 ± 1.82	0.0383
Ceruloplasmin (mg/dL)	0.61 ± 0.26	0.0196
Model 2‡		
Intercept	88.64 ± 4.99	<0.0001
Triacylglycerols (mg/dL)	0.22 ± 0.04	<0.0001
Model 3§		
Intercept	95.70 ± 5.88	<0.0001
BFM (kg) (BIA)	1.05 ± 0.38	0.0074

BFM, body fat mass; BIA, bioelectrical impedance analysis

* Insulin resistance was considered when the homeostasis model assessment of insulin resistance index was ≥p90 (homeostasis model assessment of insulin resistance index ≥3.79).

† Variables included were age, glucose, insulin, the homeostasis model assessment of insulin resistance index, total cholesterol, triacylglycerols, high-density lipoprotein cholesterol, total proteins, uric acid, and ceruloplasmin ($r^2 = 0.1871$, $P < 0.0001$).

‡ Adjusted for the variables gender, smoking, and physical activity. Variables included were age, glucose, insulin, the homeostasis model assessment of insulin resistance index, total cholesterol, triacylglycerols, high-density lipoprotein cholesterol, total proteins, uric acid, and ceruloplasmin ($r^2 = 0.1423$, $P < 0.0001$).

§ With and without adjustment for the variables gender, smoking, and physical activity. Variables included were age, body mass index; tricipital, bicipital, subscapular, and suprailiac skinfold thicknesses; fat-free mass (BIA); BFM (BIA); percentage of body fat (BIA); arm circumference; waist circumference; hip circumference; systolic blood pressure; diastolic blood pressure; and the metabolic equivalent index ($r^2 = 0.0511$, $P = 0.0074$).

potential covariates. However, we controlled for the more important known factors that affect inflammation. Although the sample size was adequate from the standpoint of the initial association discovery, further replication in independent and larger samples would be convenient for a future translational application at a population level.

In summary, the present study analyzed the association between C3 and anthropometric measurements and body composition, biochemical parameters, and lifestyle in apparently healthy young adults. It demonstrated also that C3 correlated with measurements of adiposity and that BFM was a possible predictive of C3 concentrations. C3 was correlated with the following biochemical measurements: insulin, total cholesterol, triacylglycerols, ceruloplasmin, HOMA-IR, and the atherogenic index. Moreover, the possible predictors of C3 were triacylglycerols and ceruloplasmin. Nevertheless, we demonstrated an independent role for triacylglycerols, a component of MS, and BFM as possible predictors of C3 in young healthy adults.

Conclusion

This study contributes to a better understanding of the associations between C3 and the risk factors for the occurrence of MS at an early stage. Triacylglycerols and BFM were possible independent predictors of C3 in apparently healthy young Brazilian adults.

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