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Triglyceride-independent associations between circulating levels of apolipoprotein C-III and biomarkers of inflammation

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Abstract

Backgrounds and aims Preclinical studies suggest that a triglyceride (TG)-independent proinflammatory action of apolipoprotein C-III (apoCIII) exists. We aimed to investigate the relationship between circulating apoCIII levels and subclinical inflammation markers across different cohorts with distinctive inflammatory patterns: patients with metabolic disorders (MDs), patients with rheumatoid arthritis (RA), and controls. Specifically, we assessed the associations of apoCIII with acute inflammation biomarkers (e.g., high sensitivity C-reactive protein (hsCRP)) and novel systemic inflammation biomarkers (e.g., glycosylated proteins: Glyc-A, Glyc-B, Glyc-F), aiming to understand the role of apoCIII beyond its traditional function in TG metabolism.

Methods This cross-sectional study involved 1242 participants: 906 patients with MD (metabolic syndrome, type 2 diabetes (T2DM) and/or obesity), 192 patients with RA, and 144 controls. ApoCIII and hsCRP levels were measured via immunoturbidimetric assays, and glycosylated proteins were quantified via 1 H-NMR spectroscopy. Correlation and multivariate linear regression analyses were conducted.

Results ApoCIII levels were significantly and positively associated with Glyc-A, Glyc-B, and Glyc-F levels across all cohorts. Most of these associations remained significant in the MD group after adjusting for TG levels. Conversely, negative associations were detected between apoCIII and hsCRP patients with MD and RA, which were maintained after including TG in the models.

Conclusion In patients with MD and RA, circulating apoCIII levels were positively associated with glycoproteins and negatively with hsCRP, in a TG-independent manner. Our results suggest that apoCIII is associated with the low-grade inflammatory profile represented by glycoproteins, independent of triglyceride levels. Additionally, we observed a negative association with hsCRP, which, while seemingly paradoxical, has been reported in previous studies.

Keywords Apolipoprotein-CIII, Inflammation, C-reactive protein, Glycoproteins, Metabolic disorders

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Introduction

Apolipoprotein C-III (apoCIII) is an 8.8 kDa glycoprotein primarily produced by the liver, with the intestine also contributing to a lesser extent [1]. In circulation most of apoCIII is bound to VLDL, chylomicrons and HDL, and can be exchanged between them [2]. It is a multifunctional protein involved in lipid metabolism, glucose homeostasis, and inflammation, all of which contribute to a worsened cardiometabolic profile. In terms of lipid metabolism, apoCIII is primarily involved in triglyceride (TG) metabolism, reducing the uptake of TG-rich lipoproteins by liver receptors and inhibiting the activity of lipoprotein lipase and hepatic lipase, though its inhibitory effects in vivo remain unclear [3–5]. With respect to glucose homeostasis, elevated glucose levels induce apoCIII transcription, linking hyperglycaemia, hypertriglyceridemia, and cardiovascular (CV) disease [6]. Regarding inflammation, apoCIII stimulates the adhesion of blood monocytes to endothelial cells and induces the production of inflammatory mediators [7]. Moreover, LDL particles containing apoCIII exhibit stronger binding to endothelial proteoglycans, leading to increased retention in the arterial wall. This promotes a proinflammatory response and exacerbates plaque instability [8]. Therefore, it is plausible that apoCIII plays a role in inflammation and CV risk independent of TG. However, whether circulating apoCIII levels associate with inflammation in a TG-independent manner remains under investigation.

Inflammation is an adaptive response to cellular and tissue injury characterised by increased blood flow, capillary dilation, leukocyte infiltration, and the production of chemical mediators to eliminate toxic agents and repair damaged tissues [9]. Subclinical inflammation is an inflammatory state without obvious clinical symptoms [10], which can be quantified with various circulating biomarkers in plasma, among which C-reactive protein (hsCRP) is one of the better recognised. hsCRP is a highly conserved plasma protein believed to have protective functions due to its rapid concentration changes during infection processes and is considered an acute inflammation biomarker, as its levels decrease exponentially once the stimuli cease [11, 12]. Nevertheless, some plasma biomarkers, in addition to being elevated during acute inflammatory processes, are also elevated in low-grade systemic inflammatory states. In this context, glycoproteins are a group of proteins with carbohydrate groups covalently bound to their structure, making them well-suited for detection by proton nuclear magnetic resonance (1 H-NMR) [13, 14]. These markers have been shown to reflect systemic inflammation in various diseases of different origins, such as rheumatoid arthritis (RA), hypertension, type 2 diabetes mellitus (T2DM), cancer and HIV [15–19], highlighting their ability to quantify subclinical inflammation. While both hsCRP and glycoproteins are biomarkers of subclinical inflammation, they capture different aspects of this condition and exhibit variability in stability and individual response [20].

In the present work, we explored the relationship between circulating apoCIII levels and inflammation status, which was assessed via measurements of hsCRP and three groups of glycosylated proteins: glycoproteins A, B and F (Glyc-A, Glyc-B, and Glyc-F) and analysed to what extent it may be independent of TG. This analysis was conducted across three cohorts: controls, patients with RA, and patients with metabolic disorders (MDs: dyslipidaemia, T2DM, obesity). These cohorts offer distinct perspectives on inflammation: one characterised by an autoimmune disease resulting in a strong and chronic subclinical inflammatory state, another driven by metabolic alterations (metabolic syndrome, T2DM, or obesity) marked by a shared pattern of insulin resistance and low-grade inflammation, and a healthy group free from significant inflammatory or metabolic abnormalities. This approach allowed us to explore the role of apoCIII across different inflammatory states independent of TG, allowing us to derive generalizable conclusions. Additionally, we conducted subgroup analyses on patients with T2DM and hypertriglyceridemia from the MD cohort due to the association between apoCIII levels, glucose metabolism and TG. These results may help reinforce the notion in population studies that circulating apoCIII exhibits intrinsic inflammatory properties independent of its effects on TG.

Materials and methods

Study design and patients

A cross-sectional study was conducted involving three different cohorts. First, the RA cohort, consisting of 192 patients, aged between 18 and 80 years, who were consecutively attending the University Hospital Sant Joan de Reus. All patients included had a confirmed RA diagnosis based on their medical history, clinical examination, laboratory results and imaging, meeting the classification criteria established by the American College of Rheumatology (ACR) in 1987 [21]. Patients were excluded if they were under 18 or over 80 years of age, had acute intercurrent illnesses, were diagnosed with T1DM or T2DM, or if their disease diagnosis was subsequently revised. Second, 906 subjects with MDs were included, comprising individuals with T2DM, obesity or metabolic syndrome as defined by the ATPIII (the presence of 3 or more metabolic syndrome components), all of whom shared an insulin resistance profile [22]. Participants for this cohort were drawn from two sources: the Di@bet.es study and the METBANC cohort. The Di@bet.es study is a national Spanish study that examined the prevalence of diabetes in the general population and has been extensively

described in previous studies [23]. The METBANC cohort consisted of patients who attended the Vascular Medicine and Metabolism Unit at the University Hospital Sant Joan de Reus, presenting cardiometabolic disorders such as obesity, T2DM, metabolic syndrome, and hypertension. For both sources, participants were excluded if they were younger than 18 or older than 80 years or had chronic liver, kidney, or lung disease, T1DM, cancer, RA, or any other debilitating condition. Given the importance of apoCIII in insulin resistance and TG metabolism, subgroup analyses were conducted within the MD cohort, focusing on the 622 participants with T2DM and on the 393 patients with hypertriglyceridemia. Finally, 144 controls were included. These individuals did not have any chronic diseases, such as T2DM, hypertension, RA, neoplasia, or chronic kidney disease, among others. The control subjects were selected from the Di@bet.es study (participants who neither developed diabetes nor presented with any other metabolic disorder or RA) and from the staff of the University Hospital Sant Joan de Reus.

This study was approved by the respective ethics committees as follows: individuals from Hospital Universitari Sant Joan de Reus (CEIm), 11-04-28/4proj5, 222/2020, C.0002036; Di@bet.es subjects, the Ethics and Clinical Investigation Committee of Carlos Haya Hospital [23]. The investigation was conducted in accordance with our institution's guidelines and the Helsinki Declaration, and all the participants gave written informed consent.

Clinical and standard biochemical analysis

Anamnesis and anthropometric data—such as sex, age, clinical history, and medication—were recorded for each subject. BMI was calculated from weight and height measurements (kg/m^2). A blood sample was collected from each subject after overnight fasting, with aliquots prepared and immediately stored at -80 °C in the respective biobanks. Analytical determinations were performed in serum via enzymatic (TG, LDL-C, HDL-C, VLDL-C) and conventional methods. ApoCIII, apoB, and high-sensitivity CRP (hsCRP) levels were measured via immunoturbidimetric assays (Randox (Spain), Byosistems (UK), Spinreact (Spain)) adapted for a SPIN230 autoanalyzer (Spinreact, SA, Spain).

Glycoprotein measurement

The different glycoproteins were measured via 1 H-NMR, which revealed signals between 2.15 and 1.90 ppm. These included Glyc-A, Glyc-B and Glyc-F, which were quantified as previously described [13]. Briefly, 200 µl of serum were diluted with 50 µl of deuterated water and 300 µl of 50 mM phosphate buffer solution (PBS) at pH 7.4. 1 H-NMR spectra were recorded at 305.95 K on a Bruker Avance III 600 spectrometer operating at a proton frequency of 600.20 MHz (14.1 T). For each function, the total area, which is proportional to the concentration, was determined. Glyc-A reflects the concentration of α-neuraminic acid, whereas Glyc-B reflects the concentration of N-acetilglucosamine and N-acetylgalactosamine. Glyc-F reflects the concentration of the acetyl groups mentioned previously that are not bound to any protein (free fraction) [18]. The coefficients of variation for the glycoproteins were below 3% in intra-assay experiments.

Statistical analysis

For descriptive analyses, the means and standard deviations were calculated for normally distributed variables, whereas the medians and interquartile ranges (IQRs) were calculated for nonnormally distributed variables. Percentages with the corresponding number of cases were provided for noncontinuous variables. To compare the different groups, t tests and one-way ANOVAs were computed for normal variables, whereas Mann–Whitney U tests and Kruskal–Wallis tests were used for nonnormal variables. Correlations were evaluated via Pearson's index for normal variables and Spearman's correlation coefficient for nonnormal variables. To describe the associations of the different markers of inflammation with apoCIII levels, multivariate linear regressions were computed, adjusting for age, sex, BMI, apoB, glucose, lipid-lowering therapies, antihypertensive therapies and hypoglycaemic therapies. Models were adjusted for apoB, considering the possibility that lipoproteins significantly contribute to the Glyc-A signal, likely originating from the glycans attached to apoB [24]. Models including patients with RA also included RA treatment as a confounder. To study the independence of the associations with the concentration of TG, additional models were constructed to adjust for TG concentrations as a confounder. To evaluate the adequacy of the sample size for the multivariable linear regression model, we computed the effect size based on the proportion of variance explained by the predictors in the model, using Cohen's formula. A power analysis was then performed using the *pwr* package, setting the power to 80% and the significance level to $p<0.05$. The analysis confirmed that the sample size in each cohort was adequate to detect the statistically significant associations of interest, accounting for the included covariates (Supplementary Table 1). Subgroup analysis was performed with patients with T2DM and hypertriglyceridemia in the MD group. ApoCIII, Glyc-A, Glyc-B, Glyc-F, hsCRP, apoB, and TG were log-transformed before being introduced into the models, as their distributions did not meet the assumption of normality. Statistical significance was considered when p-values were <0.05. To improve the robustness of the presented results, p-values are adjusted for multiple

Table 1 General characteristics of the three cohorts: patients with MD, patients with RA, and healthy control participants.

	Metabolic disorders (*n* = 906)	Rheumatoid Arthritis (*n* = 192)	Controls (*n* = 144)	*P* - value
Age (years, IQR)	60 (50–68) #, ×	57 (49–65) †	47 (40–55)	< 0.001
Sex (women, %)	472, 52% #,×	125, 65%	102, 71%	< 0.001
BMI (kg/m^2, IQR)	30.7 (28.0–34.0) #, ×	26.6 (23.2–30.6) †	23.6 (21.8–25.5)	< 0.001
Hypertension (yes, %)	599, 66% #, ×	103, 54% †	0, 0%	0.003
T2DM (yes, %)	622, 69% #,×	0, 0%	0, 0%	< 0.001
Total cholesterol (mg/dL, IQR)	203.4 (177.9–233.6) ×	201.0 (182.0–226.0)	194.1 (170.9–220.0)	0.02
LDL-C (mg/dL, IQR)	114.9 (92.8–137.7)	114.0 (99.0–134.2)	109.5 (88.3–132.0)	0.07
ApoB (mg/dL, IQR)	94.5 (83.9–108.6) ×	94 (83–111) †	89.9 (76.8–105.3)	0.001
HDL-C (mg/dL, IQR)	41.4 (28.7–51.8) #,×	66 (54–76.2) †	60.5 (48.6–73.3)	< 0.001
TG (mg/dL, IQR)	139.1 (99.2–203.3) #, ×	89.5 (66.7–119) †	69.5 (52.26–89.3)	< 0.001
Glucose 88.5 (82–97)	112 (97.7–139) #, ×	88.5 (82–97)	88 (83–94)	< 0.001
ApoCIII (mg/dL, IQR)	10.5 (8.2–14.0) #, ×	8.2 (5.8–10.9)	7.6 (5.8–10.4)	< 0.001
CRP (mg/L, IQR)	2.5 (1.3–4.3) #, ×	4.5 (2–9.3) †	0.9 (0.5–1.6)	< 0.001
Glyc-A (µmol/L)	783.5 (674.3–923) #, ×	642.7 (575.6–734.4) †	578.9 (513.2–633.6)	< 0.001
Glyc-B (µmol/L)	294.5 (246.4–349.3) #,×	325.2 (297.1–363.2) †	272.9 (243.9–301.7)	< 0.001
Glyc-F (µmol/L)	284.1 (244.6–331.2) #, ×	184.3 (167–204.6)	186.8 (164.9–227.1)	< 0.001
Lipid-lowering medication (yes, %)	269, 30% #, ×	27, 14% †	6, 4%	< 0.001
Hypotensive medication (yes, %)	410, 45.2% #, ×	56, 29% †	1, 0.7%	< 0.001
Glucose-lowering therapies(yes, %)	263, 29% #, ×	0, 0%	2, 1%	< 0.001
RA medication (yes, %)	0, 0% #	187, 97% †	0, 0%	< 0.001

Differences across the three groups were evaluated using the Kruskal-Wallis test. Post-hoc pairwise comparisons were conducted using the Wilcoxon rank sum test, with p-values adjusted using the Holm method. The following symbols denote significant differences ($p < 0.05$): #: Between patients with MD and those with RA. ×: Between patients with MD and healthy control participants. †: Between patients with RA and healthy control participants. BMI, body mass index; T2DM, type 2 diabetes mellitus; ApoB, apolipoprotein B; TG, triglycerides; hsCRP, high-sensitivity C-reactive protein; Glyc-A, glycoprotein A; Glyc-B, glycoprotein B; Glyc-F, glycoprotein F.

comparisons using the Benjamini and Hochberg method for False Discovery Rate (FDR). Analyses were performed in RStudio version 4.0.5.

Results

General characteristics of the cohorts

The general characteristics of the three cohorts, patients with MD, patients with RA, and controls, are presented in Table 1. The age ranges were similar for the MD and RA patients, whereas the control group was younger. Compared with the other groups, the MD group had a lower proportion of women. BMI, as well as the prevalence of T2DM and hypertension, was significantly greater in patients with MD than in the other groups. In terms of lipid values, although there were some statistically significant differences in the lipid profile, the most notable differences were in TG and HDL-C levels, where the MD group presented increased TG levels and decreased HDL-C levels compared with the other groups. Additionally, apoCIII concentrations were elevated in patients with MD, with no significant differences between RA patients and controls. Among the inflammatory parameters, the hsCRP levels were highest in the RA group, with the MD group showing the next highest levels. In terms of glycoprotein parameters, the MD group presented elevated Glyc-A levels, followed by the RA patients and controls. In contrast, the RA patients presented the highest Glyc-B values. For Glyc-F, patients with MD and controls presented similar levels, whereas RA patients presented decreased levels compared with those in the other cohorts. The general characteristics of the subgroup of patients with T2DM and hypertriglyceridemia are shown in Supplementary Tables 2, and we observed that these subgroups had the highest apoCIII, Glyc-A, and Glyc-F levels.

Correlations between apoCIII levels and markers of inflammation status

We then studied the correlation between apoCIII and inflammation markers (hsCRP, Glyc-A, Glyc-B, and Glyc-F). The Spearman correlation plots between apoCIII levels and the different biomarkers in each cohort are shown in Fig. 1. With respect to hsCRP, we did not observe any significant correlation between this biomarker and apoCIII levels in any of the three cohorts studied (patients with MD: ρ = -0.05, $p = 0.13$; patients with RA: ρ = -0.06, $p = 0.43$; controls: ρ = 0.08, $p = 0.37$) (Fig. 1A). However, significant correlations were observed between apoCIII and glycoprotein levels in all three cohorts. Specifically, Glyc-A levels were significantly correlated with apoCIII levels in all groups (patients with MD: ρ = 0.69, $p < 0.001$; patients with RA: ρ = 0.41, $p < 0.001$; controls: ρ = 0.29, $p < 0.001$) (Fig. 1B). A similar pattern was observed with Glyc-B levels, where significant correlations with apoCIII

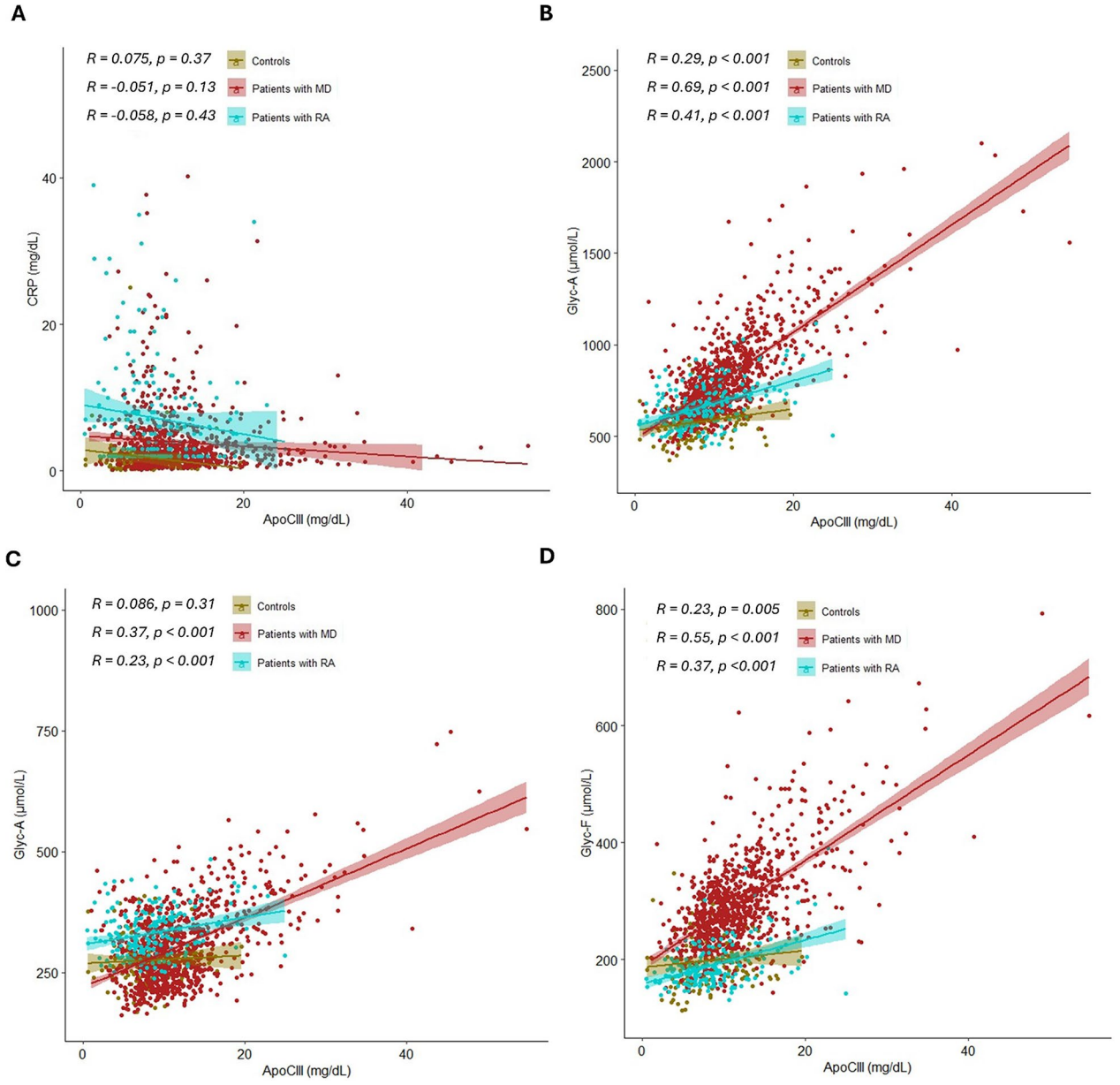

Fig. 1 Spearman correlations of ApoCIII levels with CRP, Glyc-A, Glyc-B and Glyc-F levels in the three different cohorts. A = apoCIII–CRP, B = apoCIII–Glyc-A, C = apoCIII–Glyc-B, D = apoCIII–Glyc-F

RA = rheumatoid arthritis; MD = metabolic disorder; CRP = high-sensitivity C–reactive protein; Glyc-A = glycoprotein A; Glyc-B = glycoprotein B; Glyc-F = glycoprotein F

levels were found in patients with MD and RA (patients with MD: ρ=0.37, $p<0.001$; patients with RA: ρ=0.23, $p<0.001$; controls: ρ=0.09, $p=0.31$) (Fig. 1C). Finally, all groups showed a significant correlation between apoCIII and Glyc-F levels (patients with MD: ρ=0.55, $p<0.001$; patients with RA: ρ=0.37, $p<0.001$; controls: ρ=0.23, $p=0.005$) (Fig. 1D). Furthermore, the different correlations of hsCRP levels with Glyc-A, Glyc-B, and Glyc-F levels are shown in Supplementary Fig. 1. In this regard, we observed significant positive correlations between hsCRP and Glyc-A levels in the different cohorts (patients with MD: ρ=0.11, $p=0.002$; patients with RA: ρ=0.31, $p<0.001$; controls: ρ=0.26, $p=0.001$). hsCRP and Glyc-B levels were strongly associated with each other in patients with RA (ρ=0.40, $p<0.001$), with modest correlations in the other groups (patients with MD: ρ=0.12, $p<0.001$; controls: ρ=0.22, $p=0.12$). Finally, hsCRP and Glyc-F levels were moderately correlated with each other in patients with MD (patients with MD: ρ=0.1, $p=0.004$), but no correlation was detected in RA patients (patients with RA: ρ=0.06, $p=0.43$, controls: ρ=0.05, $p=0.52$).

Multivariate linear models assessing the associations between apoCIII levels and markers of inflammation status

Multivariate linear models were adjusted to assess the impact of apoCIII levels on the levels of the included inflammatory markers (hsCRP, Glyc-A, Glyc-B, Glyc-F). These models were initially adjusted for age, sex, BMI, apoB, glucose, lipid-lowering therapies, anti-hypertensive therapies and hypoglycaemic therapies, and subsequently for TG concentrations to assess the independence of the proinflammatory effects of apoCIII from those of circulating TG concentrations. Models including patients with RA also included RA treatment as a confounder. The summaries of the models are shown in Fig. 2. We observed that all groups tended to show a negative association between apoCIII and hsCRP levels, which was statistically significant in the group of patients with metabolic disorders and the group of patients with RA after TG concentration was included as a confounder (patients with MD: $\beta = -0.52$, $p<0.001$; patients with RA: $\beta = -0.44$, $p=0.001$; controls: $\beta = -0.34$, $p=0.37$). Different associations were observed between apoCIII and the different glycoproteins across all three cohorts. Specifically, apoCIII levels were associated with Glyc-A levels in patients with MD and RA, but not in controls (patients with MD: $\beta=0.32$, $p<0.001$; patients with RA: $\beta=0.09$, $p<0.001$; controls: $\beta=0.044$, $p=0.37$). Regarding Glyc-B, apoCIII levels were significantly associated with Glyc-B levels in the metabolic group of patients (patients with MD: $\beta=0.14$, $p<0.001$; patients with RA: $\beta=0.03$, $p=0.13$; controls: $\beta = -0.02$, $p=0.81$). Finally, apoCIII levels also exhibited significant associations with Glyc-F levels across

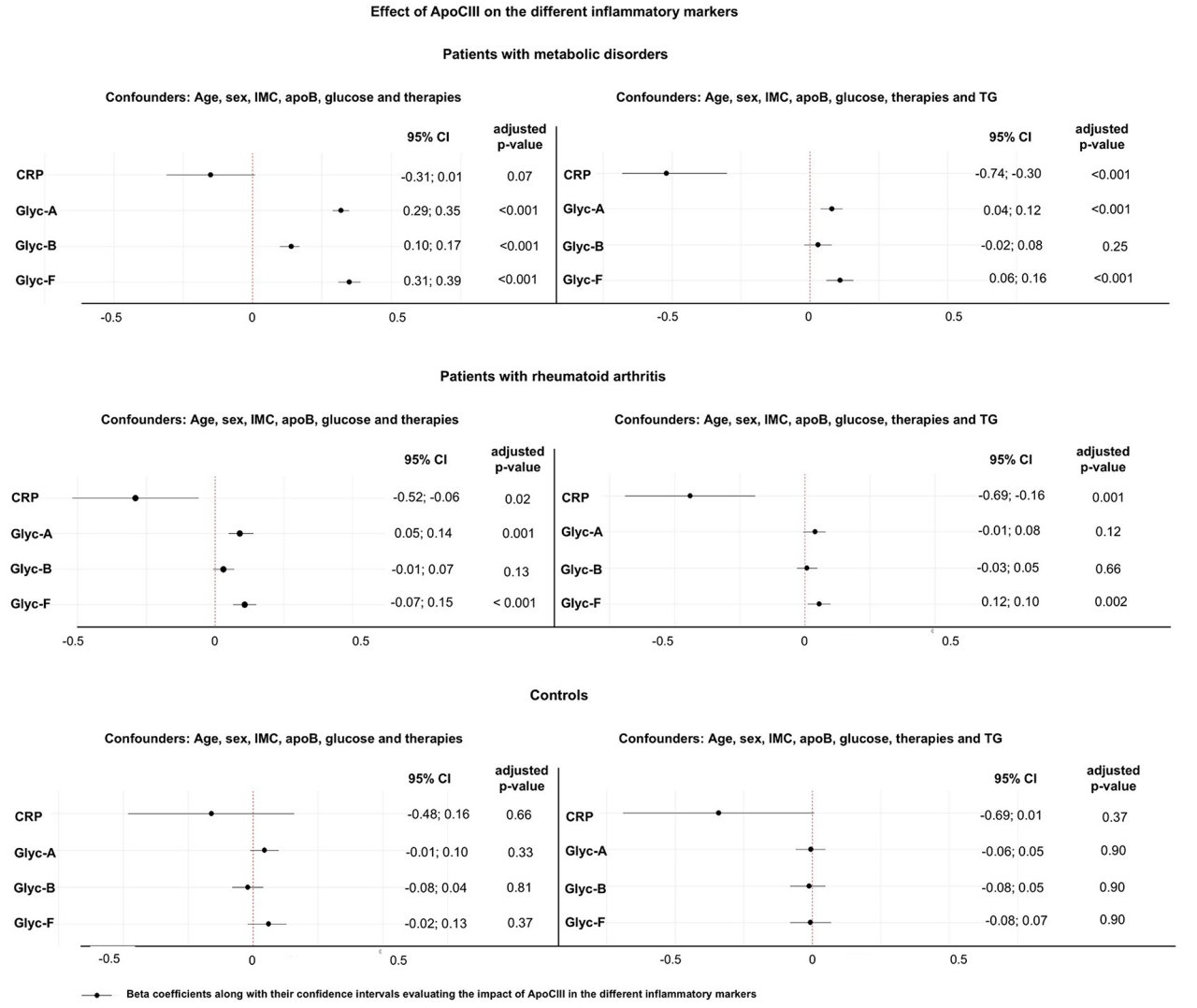

Fig. 2 Multivariate linear regressions evaluating the associations of ApoCIII levels with CRP, Glyc-A, Glyc-B and Glyc-F levels in patients with MD, patients with RA and healthy control participants. P-value is adjusted by the Benjamini & Hochberg method RA = rheumatoid arthritis; MD = metabolic disorder; CRP = C-reactive protein; Glyc-A = glycoprotein A; Glyc-B = glycoprotein B; Glyc-F = glycoprotein F; CI = confidence interval

patients with metabolic disorders and patients with RA (patients with MD: β=0.35, $p<0.001$; patients with RA: β=0.11, $p<0.001$; controls: β=0.06, $p=0.37$). When TG levels were included as a confounder, the associations between apoCIII and Glyc-A and Glyc-F remained significant in the metabolic group of patients, and the association between apoCIII and Glyc-F remained significant in the group of patients with RA. Although a trend was observed in the association between apoCIII and Glyc-A after adjusting for TG in patients with RA, this and other associations lost statistical significance (Fig. 2).

Associations in the RA group remained consistent regardless of disease activity level (DAS28). In contrast, among MD patients, the severity of associations intensified with the number of metabolic syndrome components: patients with three or more components exhibited stronger associations between apoCIII and glycoproteins than those with one or two. Detailed analyses are provided in Supplementary Tables 3 and 4.

Subgroup analysis I: apoCIII levels in patients with diabetes

The previous analyses were also performed in the group of T2DM patients within the group of patients with MD ($n=622$). ApoCIII was negatively associated with hsCRP after the addition of TG as a confounder in the model (β = -0.36, $p=0.01$). On the other hand, an increase in apoCIII levels was strongly associated with increased Glyc-A (β=0.31, $p<0.001$), Glyc-B (β=0.25, $p<0.001$), and Glyc-F (β=0.34, $p<0.001$) levels. Associations with Glyc-A and Glyc-B associations remained statistically significant even after TG levels were included in the models (Fig. 3).

Subgroup analysis II: apoCIII levels in patients with hypertriglyceridemia

Analyses were also conducted in patients with hypertriglyceridemia within the MD group ($n=393$). In this subgroup, apoCIII showed no association with hsCRP. However, higher levels of apoCIII were strongly associated with Glyc-A (β=0.35, $p<0.001$), Glyc-B (β=0.30, $p<0.001$), and Glyc-F (β=0.39, $p<0.001$). These associations remained significant even after adjusting for TG in the models (Fig. 4).

Discussion

In this study, we examined the relationships between circulating apoCIII and inflammation markers (hsCRP, Glyc-A, Glyc-B, and Glyc-F) in patients with MD characterised by insulin resistance, patients with RA, and controls, while assessing their independence from TG. ApoCIII was positively associated with Glyc-A, Glyc-B, and Glyc-F in MD and RA patients, but not in controls. These associations remained significant after adjusting for TG in the MD group (Glyc-A and Glyc-F) and the RA group (Glyc-F). Also, a trend was observed between apoCIII and Glyc-A in the RA cohort. Analyses in patients with T2DM and hypertriglyceridemia revealed strong associations between apoCIII and glycoproteins, even after adjusting for TG. In contrast, apoCIII levels were negatively associated with hsCRP in both MD (and T2DM) and RA patients, independent of TG levels. These findings suggest that apoCIII may exert an independent inflammatory role, as the associations remained significant in inflammatory conditions even when TG were included in the models. This suggests that, while TG may partly mediate the relationship, apoCIII also possesses intrinsic inflammatory properties.

ApoCIII is known for its role in inflammation via regulation of TG metabolism: it increases the production of TG-rich lipoproteins, enhances VLDL synthesis and secretion, and potentially inhibits their hydrolysis by lipoprotein lipase and hepatic lipase, as seen in in vitro studies [3, 25, 26]. ApoCIII also modulates inflammation through alternative pathways. In vitro, it triggers inflammasome activation in human monocytes and increases VCAM-1 expression in non-activated endothelial cells, promoting monocyte recruitment to the subendothelial

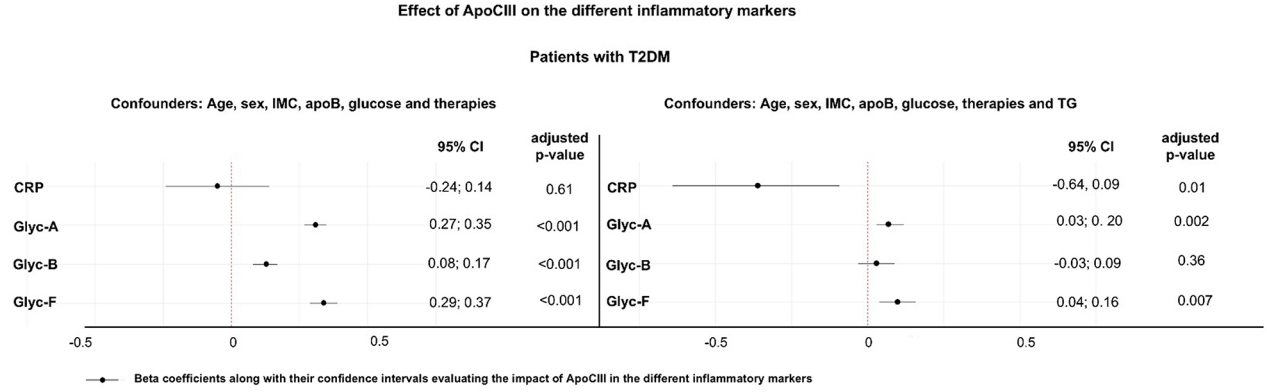

Fig. 3 Multivariate linear regressions evaluating the associations of ApoCIII levels with CRP, Glyc-A, Glyc-B and Glyc-F levels in patients the subgroup of patients with T2DM. P-value is adjusted by the Benjamini & Hochberg method
CRP = C-reactive protein; Glyc-A = glycoprotein A; Glyc-B = glycoprotein B; Glyc-F = glycoprotein F

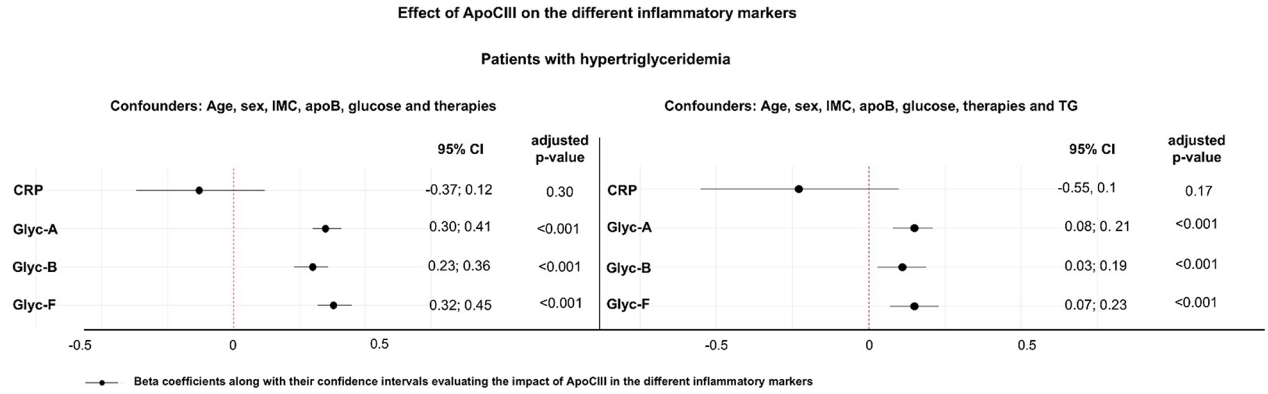

Fig. 4 Multivariate linear regressions evaluating the associations of ApoCIII levels with CRP, Glyc-A, Glyc-B and Glyc-F levels in patients the subgroup of patients with hypertriglyceridemia. P-value is adjusted by the Benjamini & Hochberg method
CRP = C-reactive protein; Glyc-A = glycoprotein A; Glyc-B = glycoprotein B; Glyc-F = glycoprotein F

space and contributing to atherosclerosis [7, 25]. ApoCIII also increases LDL particle affinity for arterial wall proteoglycans, altering their lipid composition and surface fluidity, which enhances proteoglycan binding [1, 27]. However, limited evidence exists on how circulating apoCIII levels are linked to inflammation in population studies. To investigate this property, we selected three cohorts with distinct inflammatory profiles: one driven by RA, characterised by a strong chronic inflammatory state mediated by cytokines, autoantibodies and activated immune cells; another consisting of MD patients with insulin resistance, where altered hormone production and dysregulated lipid metabolism amply a low-grade inflammatory state; and a control group free from any inflammatory or metabolic conditions.

ApoCIII is dysregulated in patients with metabolic syndrome and T2DM, contributing significantly to inflammation, insulin resistance and coronary artery disease [28–31]. High apoCIII levels can induce skeletal muscle stress and enhance the expression of inflammatory mediators, which impair insulin signalling. Elevated plasma apoCIII levels also contribute to hepatic lipid accumulation, leading to hepatic insulin resistance and diabetes [6]. Conversely, reductions in apoCIII levels are protective against inflammation and insulin resistance [32]. Consistent with this, we observed a direct association between circulating apoCIII and the low-grade systemic inflammatory state reflected by glycoproteins in patients with MD, in a TG-independent manner. In this regard, glycoproteins, previously linked to inflammation in conditions such as T2DM, RA and cancer [15, 19, 33], are recognised markers of subclinical inflammation with lower interindividual variability compared to hsCRP. This difference may stem from glycoproteins representing a diverse group of molecules, whereas hsCRP reflects the concentration of a single protein [24].

These associations were particularly evident in T2DM patients, where apoCIII is linked to β-cell dysfunction, driven by progressive inflammation and β-cell apoptosis in the pancreatic islets [34]. While insulin resistance stimulates apoCIII production, elevated apoCIII levels exacerbate insulin resistance. This interplay highlights the role of apoCIII beyond TG metabolism, worsening the inflammatory state in these patients [35]. Associations were also strong in hypertriglyceridemic patients, where elevated apoCIII, linked to TG levels, exacerbates endothelial dysfunction, impairs insulin signalling, and amplifies systemic inflammation. This highlights apoCIII not only as a key driver of lipid metabolism dysregulation but also as a driver of systemic inflammation [36, 37].

In patients with RA, circulating apoCIII levels were associated with Glyc-F in a TG-independent manner. A trend for Glyc-A was also noted, potentially reaching significance with a larger sample size. Elevated TG levels during acute inflammatory episodes in RA, driven by increased VLDL production, reduced clearance, and decreased HDL-C levels, may partly explain these associations, mirroring those in hypertriglyceridemic patients [38]. Notably, Glyc-F, though its exact role remains unclear, reflects acetylated free radicals and indicates a distinct inflammatory pathway uniquely captured by apoCIII in RA patients [33]. No associations were observed in controls, likely due to their low level of subclinical inflammation, which apoCIII failed to capture even without including TG in the models.

Negative associations between apoCIII and hsCRP were observed in MD and RA patients, even after adjusting for TG. Similarly, Hsu et al. (2023) reported that *APOC3* loss-of-function variants were linked to modestly increased serum hsCRP levels, contrary to the expected reduction [39]. Mokkala et al. (2020) also observed that VLDL particles were positively associated with Glyc-A but inversely associated with hsCRP [20]. However, evidence remains inconclusive, as some studies have reported no significant associations between apoCIII and hsCRP [40, 41], while others have observed modest

correlations, often without adjusting for TG [29, 42]. The robustness of our statistical analyses, adjusted for multiple confounders and supported by a sample size of over 900 individuals in the MD group, suggests that this association is likely convincing. Even in controls, a negative trend between apoCIII and hsCRP was noted. Nevertheless, the mechanisms underlying this relationship remain unclear and warrant further investigation. Interestingly, apoCIII exists in several proteoforms (apoCIII0a, apoCIII0b, apoCIII1, and apoCIII2). We observed distinct associations between inflammation and the relative proportions of these proteoforms, some of which were opposite to those observed with total apoCIII [43]. These findings were specific to proteoform ratios (e.g., apoCIII2/apoCIII1), while associations with total apoCIII consistently showed a positive direction. As this study focused on the total apoCIII pool, future research should explore the relationship between individual proteoforms and inflammation biomarkers to uncover proteoform-specific differences across the different cohorts.

Despite the significant association between apoCIII levels and the different glycoprotein markers and hsCRP, this study has several limitations. Owing to its cross-sectional design, we cannot infer causality from the observed associations. Therefore, mechanistic studies are needed, specifically those involving the suppression of apoCIII, to evaluate how this impacts the concentration of the different glycoproteins. However, the robustness of the methods used to measure the variables and the relatively large sample size, especially among patients with MD, strengthened our conclusions. Another limitation is that, while we observed differential associations between apoCIII and glycoprotein levels compared with hsCRP levels, we do not understand the pathways through which apoCIII acts. Therefore, further in vitro studies are warranted. Nevertheless, the consistency of our associations among the studied cohorts makes our results reliable.

In summary, this study highlights the association between circulating apoCIII and inflammatory activity, independent of TG. After adjusting for multiple confounders, consistent associations were observed in patients with autoimmune and metabolic inflammatory disorders. Additionally, we identified a compelling inverse relationship between apoCIII and hsCRP, suggesting an area for further exploration. These findings support a physiological role of apoCIII, not only in regulating TG metabolism but also inflammatory processes. This positions apoCIII as a potential target for managing inflammatory-related disorders and cardiovascular risk prevention strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12933-024-02553-z.

Supplementary Material 1

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Author contributions

DLL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Roles/Writing - original draft, Writing - review & editing PR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Roles/Writing - original draft, Writing - review & editing SP: Funding acquisition, Investigation, Methodology MG: Funding acquisition, Investigation, Supervision, and Writing - review & editing JG: Funding acquisition, Investigation, Supervision, and Writing - review & editing RR: Investigation, Methodology YE: Investigation, Methodology LM: Funding acquisition, Project administration, Resources, Writing– review & editing DI: Funding acquisition, Project administration, Resources, Writing– review & editing JCV: Conceptualization, Funding acquisition, Investigation, Project administration, Roles/Writing - original draft, Writing - review & editing JR: Conceptualization, Funding acquisition, Investigation, Project administration, Roles/Writing - original draft, Writing - review & editing.

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Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

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