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Tocilizumab-related hypertriglyceridemia is independent of key molecules regulating lipid metabolism

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Abstract

Introduction: Tocilizumab (TCZ) treatment is associated with dyslipidaemia, including a rise in triglycerides through a mechanism poorly understood. Three molecules play key roles in the regulation of triglyceride metabolism: apolipoprotein C-III (ApoC-III), angiopoietin-like protein 4(ANGPLT4) and lipoprotein lipase (LPL). The aim of this work was to analyse whether the changes in triglycerides shown by TCZ-treated RA patients could stem from the dysregulation that can occur in these regulatory molecules.

Methods: Twenty-seven RA patients included in the TOCRIVAR study who received TCZ (8 mg/kg IV/q4w) were evaluated at baseline and at Weeks 12, 24 and 52 of treatment. ANGPTL4, ApoC-III and LPL, a complete lipid profile and RA disease activity, were analysed at baseline and at each visit. Multivariable linear mixed models were performed to study changes over time in lipids and regulatory molecules.

Results: After 24 weeks of TCZ treatment, HDL cholesterol, apolipoprotein A1 and triglycerides increased, whereas lipoprotein (a) decreased significantly from baseline values. However, 1 year after TCZ, no significant differences in lipid pattern were observed with respect to baseline. Serum ANGPTL4 and Apo-CIII levels decreased gradually over time, both being significantly lower than baseline values at Week 52. LPL concentration did not change significantly during TCZ treatment. Remarkably, the elevation of triglycerides at Week 24 maintained its statistical significance after adjusting for the changes in ApoC-III, ANGPTL4 and LPL.

Conclusion: In TCZ-treated RA patients basal serum levels of ANGPLT4 and ApoC-III, but not LPL, decreased significantly. However, the elevation of triglycerides after TCZ was not related to changes in these regulatory molecules.

KEYWORDS
rheumatoid arthritis, tocilizumab, tryglicerides

1 | INTRODUCTION

Tocilizumab (TCZ), a monoclonal antibody that neutralizes interleukin (IL)-6 used to treat rheumatoid arthritis (RA)[1] and other inflammatory diseases,[2] has been associated with changes in lipid profiles.[3] TCZ increases the levels of several lipid molecules, which appears to be mainly secondary to the inhibition of inflammation. This is believed to stem from the fact that RA activity and inflammation are associated with decreased lipid levels, while decreases in inflammation may coincide with increases in serum lipid values.[4] Accordingly, a systematic review reported that patients receiving TCZ were more likely to experience elevated triglyceride levels, and in the ratios of low-density lipoprotein (LDL) to high-density lipoprotein (HDL) cholesterol, as well as of total to HDL cholesterol levels.[5] Moreover, although compared with controls RA is associated with a greater burden of atherosclerosis and deaths from cardiovascular events[6] evidence has ruled out an increased risk of cardiovascular events associated with this altered lipid profiles following TCZ treatment in RA patients.[7]

The key molecules involved in triglyceride metabolism include lipoprotein lipase (LPL), angiopoietin-like protein 4 (ANGPTL4) and apolipoprotein C-3 (ApoC-III). LPL is the primary enzyme that hydrolyses triglycerides, thereby releasing free fatty acids for utilization by and clearance from tissues.[7] In addition, ANGPTL4 and ApoC-III are endogenous modulators that inhibit LPL and, respectively, modulate the uptake of free fatty acids in fasting and fed states,[8] and inhibit the lipolysis of triglyceride-rich lipoproteins by LPL.[9]

Hypertriglyceridemia is a significant independent cardiovascular risk factor in the general population.[10] In RA patients, increased triglyceride levels, as well as their relationships to atherosclerosis and cardiovascular events, have been reported.[11] Moreover, the axis consisting of ANGPTL4, ApoC-III and LPL has been found to be disrupted in patients with RA.[12] In this report, patients with RA showed higher serum levels of ANGPTL4 and ApoC-III, but lower circulating LPL, compared with controls. Similar results have been described in systemic lupus erythematosus patients.[13,14]

Since TCZ causes changes in serum triglycerides over time, the aim of this study was to analyse whether this effect is associated with modifications to the triglyceride metabolism pathway constituted by ANGPTL4, ApoC-III and LPL in RA patients treated with this IL-6 inhibitor.

2 | MATERIALS AND METHODS

2.1 | Study participants

TOCRIVAR (Effect of the Monoclonal Anti-IL6 Antibody—Tocilizumab—on Cardiovascular Risk in Patients with Rheumatoid Arthritis) is a 1-year prospective study that analysed the influence of TCZ on different cardiovascular risk factors in RA patients (ClinicalTrials.gov NCT017523359). Twenty-seven adult RA patients diagnosed according to the 2010 ACR/EULAR classification criteria[15] and whose disease duration was ≥6 months were recruited. Enrolment criteria included diagnosis of active moderate-to-severe RA (≥3.2 DAS28-ESR); patients with an inadequate clinical response to a stable dose of nonbiological DMARDs or failure to respond to one anti-TNF-α treatment for a period ≥8 weeks before treatment; and those patients who were receiving oral corticosteroids, whose dose should have been ≤10 mg prednisone or equivalents and remained with a stable dose for at least 1 month prior to the start of TCZ. All patients were offered open-label treatment with TCZ 8 mg/kg every 4 weeks for 12 months between January 2012 and July 2015. Open-label assessments were performed at baseline and at Weeks 12, 24 and 52. None of the patients were taking statins. Patients diagnosed with any other rheumatic disease, hepatitis C infection, active diverticulitis, latent tuberculosis (based on positive PPD or interferon-gamma release assay or suspicious chest X-ray), or who were pregnant, or lactating were excluded. For patients who stopped treatment or who had missing data at 24 or 54 weeks, analyses were performed on the intent-to-treat population using last-observation-carried-forward to impute missing data at the relevant time point. This trial was approved by an independent ethics committee and an institutional review committee of the Hospital Universitario de Canarias (Spain) and assigned the reference number FRC-TOC-2009-01 (TOCRIVAR). All participating patients signed a written informed consent.

2.2 | Data collection

The subjects completed a cardiovascular risk factor and medication use questionnaire and underwent a physical examination at every visit. Weight, height, body mass index, waist-to-hip ratio and systolic and diastolic blood pressure (measured with the participant in a supine position) were assessed under standardized conditions. Information regarding smoking status (current smoker versus nonsmoker), diabetes and hypertension was obtained from the questionnaire. At every visit, disease activity, disability and physical activity were assessed. Disease activity was measured using the Disease Activity Score in 28 joints (DAS28), with the form that addresses both erythrocyte sedimentation rate (-ESR) and C-reactive protein (-CRP),[16] the Clinical Disease Activity Index (CDAI)[17] and the Simple Disease Activity Index (SDAI).[18] Disease disability was determined using the Health Assessment Questionnaire (HAQ).[19]

Serum LPL mass was measured using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) (Biomatik). The assay sensitivity (minimum detectable concentration) for LPL was 0.58 ng/mL. Precision was estimated as an inter-assay <15%, and an intra-assay with <10% coefficients of variability. ANGPTL4 was assessed through an R&D Duoset ELISA. ANGPTL4 minimum detectable values were 1.3 ng/mL, and both inter and inter-assay coefficients of variability were <10%. For the detection of ApoC-III, an ELISA kit was used (Elabscience). No significant cross-reactivity or interference between human ApoC-III and analogues was observed with this kit. Both intra- and inter-coefficients of variability were <10% for this assay. Cholesterol, triglycerides and HDL cholesterol were measured using the enzymatic colorimetric assay. LDL cholesterol was calculated using the Friedewald formula. Apo-B is a protein involved in lipid metabolism, being the main protein constituent of very low-density lipoproteins (VLDL) and LDL, and ApoA-1 is the major protein component of HDL. For this reason, both apolipoproteins B and A1 were included in the lipid pattern molecules to be analysed.

2.3 | Statistical analysis

In a previous work of our group, patients with RA showed higher serum levels of ANGPTL4 and ApoC3, but lower circulating LPL, compared with controls.[12] These differences in serum levels were of at least a 20% between populations. Based on this, we expected to find a similarly proportional effect of TCZ over the ANGPTL4, ApoC3 and LPL axis. Accordingly, assuming an alpha level of 0.05 and a beta level of 0.20, we estimated that we would need to enrol 24 patients. Demographic and clinical characteristics in patients with RA are described as mean (standard deviation [SD]) or percentages for categorical variables. For nonnormally distributed continuous variables, data are expressed as median and interquartile ranges (IQR). To avoid any impact on the statistical analyses of data lost during the follow-up period, variations in clinical characteristics, disease activity scores, acute-phase reactants, lipid-related molecules and ANGPTL4, ApoC-III and LPL overtime were analysed using linear regression mixed models for repeated measures. Collinearity between confounders included in the multivariate models was ruled out by the calculation of variance inflation factor. None of the cofactors analysed in this study showed a significant collinearity. Correlations between changes in lipid patterns during follow-up were assessed by Spearman's Rho correlations. All the analyses used a 5% two-sided significance level and were performed using SPSS, version 25, IBM SPSS, Armonk, NY. *p*-values <.05 were considered statistically significant. Graphs were generated using GraphPad Prism version 9.3.1 for Windows, GraphPad Software, www.graphpad.com.

3 | RESULTS

3.1 | Characteristics of the participants

A total of 27 RA patients, 24 females and three males, with a mean ± SD age of 52 ± 11 years were included in this study. The demographic, disease-related characteristics and comorbidities of the participants are shown in Table 1. Regarding cardiovascular risk factors, 35% per cent of the patients had been diagnosed with hypertension, 46% were considered to have dyslipidaemia and 15% diabetes. Disease duration was 8 years (IQR 2–12); 69% were positive both for ACPA anti-citrullinated protein antibodies (ACPA) and rheumatoid factor. Patients had active disease as shown by DAS28-ESR (5.77 ± 0.88), SDAI (29 ± 10) and CDAI (27 ± 10). Twenty (77%) were taking prednisone, and 19 (70%) were taking TCZ in addition to either methotrexate or leflunomide. Only eight (30%) patients were undergoing TCZ in monotherapy. Those patients included in the trial taking prednisone, methotrexate or leflunomide maintained these medications at stable doses during the follow-up year.

3.2 | Changes in anthropometric characteristics, RA disease activity, triglycerides, lipid pattern and ANGPTL4, ApoC-III and LPL during 1 year of follow-up

BMI and waist circumference remained stable and did not reveal any differences after 1 year of treatment compared with baseline values. As expected, during follow-up acute-phase reactants and disease activity scores improved over time (Table S1).

After 12 weeks of TCZ treatment, total- and HDL cholesterols, and apolipoprotein A1 significantly increased. This was not the case with other lipid-related molecules. HDL-cholesterol elevation maintained its significance at Week 24, and the decrement in lipoprotein A became significant at this 24-week visit. Remarkably, at the end of the study (1 year after TCZ start), no significant differences were found in the entire lipid pattern including triglycerides. Triglyceride serum levels were significantly higher at Week 24 compared with baseline, but after 1 year they fell again to values similar to those at baseline (Table 2).

TABLE 1 Baseline demographic, comorbidities and disease-related characteristics of RA patients

	Patients ($n=27$)
Female, *n* (%)	24 (92)
Age, years	52 ± 11
Weight, kg	71 ± 14
Height, cm	160 ± 7
BMI, mg/cm2	28 ± 5
Waist circumference, cm	95 ± 14
Hip circumference, cm	102 ± 14
Waist/hip ratio	0.93 ± 0.05
Systolic pressure, mmHg	133 ± 23
Diastolic pressure, mmHg	84 ± 9
Comorbidities	
Hypertension, *n* (%)	9 (35)
Diabetes, *n* (%)	4 (15)
Dyslipidaemia, *n* (%)	12 (46)
Current smoker, *n* (%)	5 (19)
Antihypertension treatment, *n* (%)	9 (35)
AR-related data	
ESR, mm/h	40 (22–56)
CRP, mg/dL	8.8 (2.9–16.2)
Disease duration, years	8 (2–12)
Number of tender joints	10 (6–15)
Number of swollen joints	4 (2–7)
DAS28-ESR	5.77 ± 0.88
DAS28-CRP	5.16 ± 1.04
HAQ	1.250 (1.000–2.125)
SDAI	27 ± 10
CDAI	29 ± 10
Rheumatoid factor +, *n* (%)	18 (69)
ACPA +, *n* (%)	18 (69)
Current prednisone, *n* (%)	20 (77)
Prednisone, mg/day	6 (2–8)
Current csDMARD	18 (69)
Methotrexate, *n* (%)	14 (54)
Leflunomide, *n* (%)	5 (19)

Abbreviations: ACPA, anti-cyclic citrullinated peptide antibody; BMI, body mass index; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; csDMARD, classic synthetic DMARD; DAS28, Disease Activation Score using 28 joints; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; HAQ, Health Assessment Questionnaire; HDL, high-density lipoprotein; SDAI, Simple Disease Activity Index; TNF, tumour necrosis factor.Data represent mean ± SD or median (interquartile rate).

Concerning the ANGPTL4, ApoC-III and LPL axis, ANGPTL4 serum levels were significantly lower compared with the baseline values at Weeks 12 and 52. Similarly, ApoC-III gradually decreased at every visit, and at Week 52 was significantly lower than the baseline level. In contrast, circulating LPL did not show any differences across visits during the 1-year follow-up, although a trend to decrease over time was observed (Table 2). Variations in these three triglycerides' regulatory molecules did not correlate with changes in RA disease activity over 1 year of TCZ treatment (Figure 1).

3.3 | Multivariable linear mixed model analysis examining the differences between visits in triglyceride serum levels

After 24 weeks of TCZ treatment, triglyceride levels significantly increased (mean difference 32 [95% confidence interval CI −2–65], mg/dL, $p=.040$) in the univariable linear mixed analysis. At Week 52, however, circulating triglycerides did not differ compared with baseline levels (Table 3). When this analysis was adjusted for those lipid-related molecules that showed significant univariable differences between visits (see Table 2: total cholesterol, HDL cholesterol, apolipoprotein A1 and lipoprotein (a)), this elevation in triglyceride levels retained its significance (Model #1 as shown in Table 3: mean difference was 44 [95% CI 17–71], mg/dL, $p=.002$). Similarly, the increase in triglycerides at Week 24 was significant after adjusting for ANGPTL4, APO-CIII and LPL (as shown in Model #2 in Table 3: mean difference 37 [95% CI 3–72], mg/dL, $p=.036$). Furthermore, when the adjustment was performed by both lipid-related molecules and the ANGPTL4, APO-CIII and LPL serum levels, circulating triglyceride levels still were greater at Week 24 compared with those at baseline (Model #3 in Table 3, mean difference 50 [95% CI 17–83], mg/dL, $p=.004$).

4 | DISCUSSION

Our study is the first in the literature to evaluate the influence of systemic inhibition of IL-6 on several key regulatory molecules regulating triglyceride metabolism. According to our results, ANGPTL4 and ApoC-III, but not LPL, were downregulated after the initiation of TCZ treatment. However, the variation in triglyceride levels caused by TCZ treatment over time in RA patients was not associated with changes in any of these three molecules.

The influence of TCZ on lipids has been extensively studied, with the pattern of change in the lipid profile found in our study proving consistent with prior findings. In a previous report involving 40 patients with RA, following TCZ treatment, total- and HDL cholesterols and triglycerides were significantly elevated, although

TABLE 2 Univariate linear mixed model analysis of changes in lipid profiles and serum levels of ANGPTL4, APO-CIII and LPL over 1 year of TCZ treatment.

	Baseline	Week 12	p1	Week 24	p2	Week 52	p3	p4
Cholesterol, mg/dL	208 ± 40	221 ± 35	**0.048**	223 ± 42	0.057	196 ± 32	0.50	**0.029**
Triglycerides, mg/dL	136 ± 58	140 ± 79	0.80	166 v 93	**0.040**	130 ± 56	0.74	0.21
HDL cholesterol, mg/dL	160 ± 39	165 ± 37	**0.005**	166 ± 45	**0.003**	144 ± 33	0.21	**0.010**
LDL cholesterol, mg/dL	46 ± 14	53 ± 16	0.31	54 ± 15	0.40	49 ± 15	0.29	0.21
Apolipoprotein A1, mg/dL	167 ± 32	187 ± 31	**0.001**	182 ± 32	**0.024**	168 ± 29	0.71	**0.001**
Apolipoprotein B, mg/dL	107 ± 34	111 ± 26	0.29	114 ± 30	0.25	105 ± 32	0.67	0.62
Apo B1:ApoA1 ratio	0.67 ± 0.27	0.61 ± 0.19	0.29	0.65 ± 0.22	0.76	0.65 ± 0.30	0.65	0.55
Lipoprotein (a), mg/dL	28 (7–101)	12 (5–66)	0.15	16 (5–102)	**0.012**	13 (4–72)	0.11	0.080
LDL: HDL-cholesterol ratio	3.90 ± 1.68	3.49 ± 1.60	0.25	3.44 ± 1.79	0.26	3.29 ± 1.49	0.24	0.54
Non-HDL cholesterol, mg/dL	163 ± 40	168 ± 38	0.31	169 ± 46	0.37	147 ± 33	0.30	0.21
Atherogenic index	4.97 ± 1.72	4.54 ± 1.65	0.25	4.51 ± 1.83	0.28	4.34 ± 1.52	0.25	0.56
ANGPTL4, ng/mL	386 ± 204	317 ± 205	**0.029**	270 ± 139	0.088	257 ± 32	**0.012**	0.056
Apo-CIII, mg/dL	10.1 ± 6.7	7.0 ± 3.9	**0.036**	6.3 ± 4.4	**0.016**	6.3 ± 3.7	**0.010**	**0.030**
LPL, ng/mL	345 ± 289	329 ± 285	0.65	339 ± 288	0.66	292 ± 197	0.63	0.95

Abbreviations: ANGPTL4, angiopoietin-like protein 4; Apo-CIII, apolipoprotein C-III; HDL, High-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase.p1 refers to the comparison between Week 12 and baseline; p3 for the comparison between Week 24 and baseline; and p3 between Week 52 and baseline. P4 refers to the inter-visit differences in the linear mixed model analysis.

Numbers highlighted in bold show associations with statistical significance.

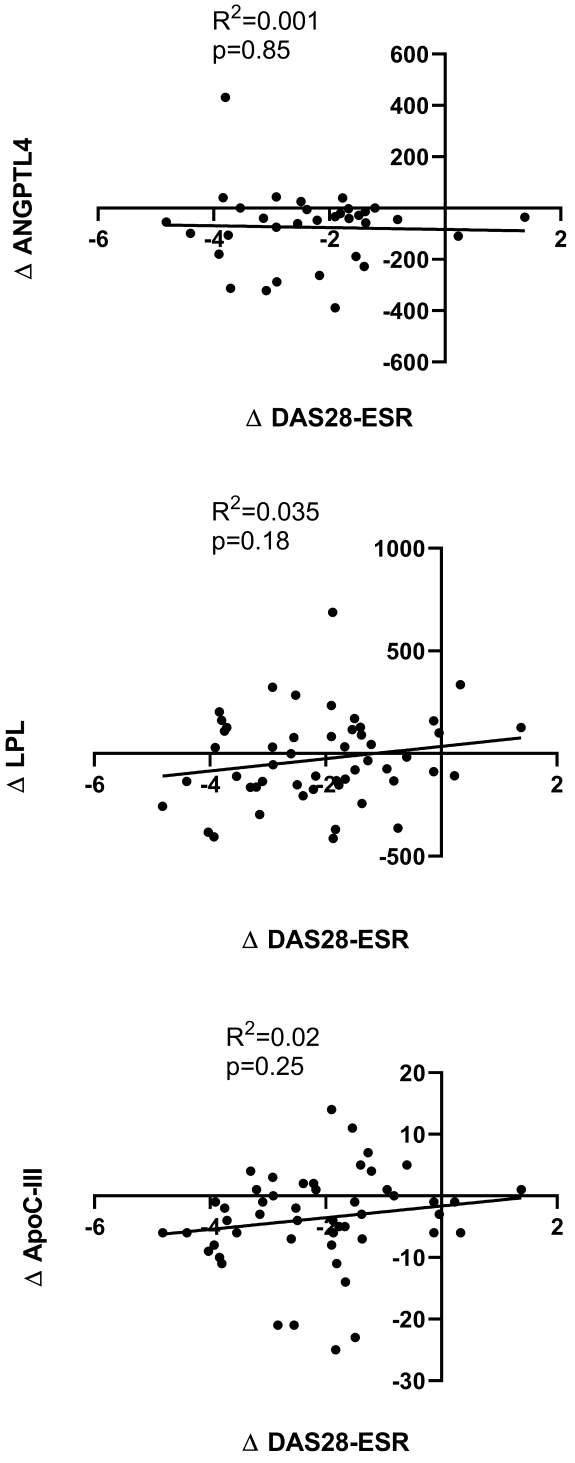

FIGURE 1 Correlation of variations of baseline values with respect to those at 12, 24 and 54 weeks of TCZ treatment. Each dots represent the difference between basal and each of those time points of disease activity (ΔDAS28-ESR), and the concentration of angiopoietin-like protein 4 (ΔANGPTL4), lipoprotein lipase (ΔLPL) and apolipoprotein C-III (ΔApo-CIII). The x-axis represents variations in DAS28-ESR, and the y-axis represents variations in each of the regulatory molecules. Correlation values and statistical significance are shown in each figure.

no significant changes in weight, body mass index, low-density lipoprotein and atherogenic index were observed.[16] In another report involving 40 naïve-biologic RA patients treated with intravenous TCZ, a statistically significant increase in total cholesterol, LDL, HDL and triglycerides was observed during the first 24 weeks, which returned close to baseline levels at 52 weeks.[17] Notably, in that report a statistically significant negative correlation between changes in lipid fractions and DAS28 or CDAI was detected. In addition, the effects of TCZ on lipids have been reported to be superior to those of tumour necrosis factor inhibitors.[18] In a previous report that examined the TOCRIVAR database, the effect of TCZ on proprotein convertase subtilisin/kexin−9 serum concentrations and cholesterol efflux capacity were studied. Long-term TCZ-treated RA patients showed an increase in cholesterol efflux capacity inversely proportional to CRP reduction, and changes in LDL cholesterol were partially explained by variations in circulating proprotein convertase subtilisin/kexin-9.[19] Overall, in that report, TCZ treatment showed a qualitatively favourable net effect in terms of the atherogenic role it plays in RA patients. In this previous TOCRIVAR work,[19] changes over time in BMI, waist circumference, trunk fat mass (assessed by dual-energy X-ray absorptiometry) and physical activity (assessed by the IPAQ questionnaire) were analysed. None of these parameters changed significantly after 1 year of TCZ treatment, so it is unlikely that the observed changes in lipid profile described in the present work can be influenced by changes in fitness or physical activity.

Lipoprotein lipase (LPL), angiopoietin-like protein 4 (ANGPTL4) and apolipoprotein C-3 (ApoC-III) play essential roles in the regulation of triglyceride levels. LPL hydrolyses triglycerides, thereby releasing free fatty acids for utilization and removal from tissues.[4] Both ANGPTL4 and ApoC-III are endogenous modulators that inhibit LPL by modulating free fatty acid uptake in both fasting and fed states,[5] thereby interfering with the lipolysis of triglyceride-rich lipoproteins.[6] In two previous studies by our group, the ANGPTL4, ApoC-III, LPL and axis has been studied in RA and systemic lupus erythematosus patients.[12,13] In 323 patients with RA and 246 age-matched controls, after multivariable analysis including cardiovascular risk factors, statin use and lipid profile changes caused by the disease itself, RA patients showed higher serum levels of ANGPTL4 and ApoC-III, but lower circulating LPL.[12] In addition, ANGPTL4 serum levels were positively and independently associated with higher carotid intima-media thickness in patients with RA after multivariable adjustment. In another study of 185 subjects diagnosed with SLE and 162 age-matched controls,

TABLE 3 Multivariable linear mixed analysis differences in triglycerides serum levels between visits

	Mean difference (95% CI), *p*							
	Univariable		**Model #1**		**Model #2**		**Model #3**	
Triglycerides, mg/dL								
Week 12	4 (−27–35)	.80	6 (−20–32)	.64	−3 (−37–31)	.87	3 (−29–34)	.87
Week 24	**32 (0.02–65)**	**.040**	**44 (17–71)**	**.002**	**37 (3–72)**	**.036**	**50 (17–83)**	**.004**
Week 52	6 (−30–41)	.74	26 (−2–54)	.065	−9 (−47–29)	.63	14 (−22–50)	.44

Abbreviations: ANGPTL4, angiopoietin-like protein 4; Apo-CIII, apolipoprotein C-III; HDL, high-density lipoprotein; LPL, lipoprotein lipase.Model #1: Adjusted for total cholesterol, HDL cholesterol, apolipoprotein A1 and lipoprotein (a).

Model #2: Adjusted for ANGPTL4, APO-CIII and LPL.

Model #3: Adjusted for Model 1 plus Model 2.

Numbers highlighted in bold show associations with statistical significance.

ApoC-III levels were found to be significantly lower, while ANGPTL4 and LPL were significantly higher in patients with SLE compared with controls.[13] Moreover, the disease damage score in SLE was significantly and independently associated with higher LPL serum levels. These two reports support the fact that the axis formed by ANGPTL4, ApoC-III and LPL may be disrupted in inflammatory diseases.

The effects of TCZ on ANGPTL4 and LPL had not been previously studied in the context of an IL-6 systemic inhibition until the present study[13] The modifications observed over time during our work on ANGPTL4, ApoC-III and LPL did not show any association with the decrease that occurred in disease activity scores in response to TCZ. To our knowledge, the influence of TCZ on ApoC-III has been studied once previously, a study that revealed Apo-CIII to have a nonsignificant tendency to increase its serum level by 11% at 12 weeks after TCZ treatment.[20] Interestingly, ApoC-III-related pathways have been associated with proteins and pathway networks involved in inflammation[21] and calcification.[22] These findings, in association with the fact that elevated ApoC-III levels, are associated with increased triglyceride levels, link this regulatory molecule to an increased risk of atherosclerotic cardiovascular disease.[23] With respect to ANGPTL4, its plasma levels are thought capable of predicting cardiovascular events, indicating the potential use of ANGPTL4 as a biomarker for coronary artery disease.[24] Conversely, higher LPL levels are linked with a beneficial lipid profile.[25] In the present work, both ApoC-III and ANGPTL4 decreased, while LPL showed no relevant changes with respect to basal levels during treatment with TZC. These finding are in line with the atherogenic effects of this anti-IL-6 compound and should be regarded as beneficial. Our findings would therefore support the previously defined concept that while TCZ increases the amounts of certain molecules in the lipid profile, this does not lead to a net increase in cardiovascular risk, at least not in RA patients.[26]

The main strength of our study is that it includes the systematic clinical and biochemical evaluation of 27 RA patients undergoing systemic IL-6 inhibition over 1 year of follow-up, using multivariable linear mixed models for analysis. However, our work has several limitations. The use of prednisone in some patients may have influenced the lipid profile. However, it should also be considered that the glucocorticoid dose was low and stable during follow-up. Therefore, we believe that its influence on our results is very little or none. We acknowledge the fact that we measured LPL serum mass and not its activity. However, while serum LPL is catalytically inactive, its mass reflects the level of systemic LPL biosynthesis and there is an excellent correlation between mass and LPL activity, as reported previously.[27] We also recognize that there are other molecules and metabolic pathways related to triglycerides that were not examined in our study. We have focused on these three molecules because of their known relationships with cardiovascular disease in the general population. We believe that our findings warrant the further study of other physiological pathways related to triglyceride metabolism in patients being treated with TCZ.

In conclusion, TCZ treatment significantly decreases ApoC-III and ANGPTL4 serum levels in patients with RA. However, the upregulation of triglycerides following TCZ treatment seems does not appear to be related to disruptions in these regulatory molecules. The exact physiological mechanism underlying this phenomenon, and the implications it may have for cardiovascular disease in RA patients deserves further study.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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REFERENCES

1. Emery P, Keystone E, Tony HP, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann Rheum Dis* [Internet]. 2008;67(11):1516-1523.
2. Stone JH, Tuckwell K, Dimonaco S, et al. Trial of tocilizumab in Giant-cell arteritis. *N Engl J Med [Internet]*. 2017;377(4):317-328.
3. Sadeq A, Elnour AA, Ramadan A, et al. Randomized clinical trials on the efficacy and safety of tocilizumab in subjects with rheumatoid arthritis: a systematic review. *Curr Rev Clin Exp Pharmacol* [Internet]. 2022;18(1):64-87.
4. Souto A, Salgado E, Maneiro JR, Mera A, Carmona L, Gõmez-Reino JJ. Lipid profile changes in patients with chronic inflammatory arthritis treated with biologic agents and tofacitinib in randomized clinical trials: a systematic review and meta-analysis. *Arthritis Rheum*. 2015;67(1):117-127.
5. Singh JA, Beg S, Lopez-Olivo MA. Tocilizumab for rheumatoid arthritis. In: Cochrane database of systematic reviews. *Cochrane Database Syst Rev*. 2011;38(1):10-20.
6. Kerola AM, Rollefstad S, Semb AG. Atherosclerotic cardiovascular disease in rheumatoid arthritis: impact of inflammation and antirheumatic treatment. *European Cardiology Review*. 2021;16:e18.
7. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. Vol. 297, American journal of physiology – endocrinology and metabolism. *Am J Physiol Endocrinol Metab*. 2009;297(2):E271-E288.
8. Köster A, Chao YB, Mosior M, et al. Transgenic angiopoietin-like (Angptl)4 overexpression and targeted disruption of Angptl4 and Angptl3: regulation of triglyceride metabolism. *Endocrinology*. 2005;146(11):4943-4950.
9. Bobik A. Editorial: apolipoprotein CIII and atherosclerosis;beyond effects on lipid metabolism. *Circulation*. 2008;118:702-704.
10. Sarwar N, Sandhu MS, Ricketts SL, et al. Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. *Lancet*. 2010;375(9726):1634-1639.
11. Crowson CS, Rollefstad S, Ikdahl E, et al. Impact of risk factors associated with cardiovascular outcomes in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2018;77(1):48-54.
12. de Armas-Rillo L, Quevedo-Abeledo JC, Hernández-Hernández V, et al. The angiopoietin-like protein 4, apolipoprotein C3, and lipoprotein lipase axis is disrupted in patients with rheumatoid arthritis. *Arthritis Res Ther [Internet]*. 2022;24(1):99.
13. Quevedo-Abeledo JC, Martín-González C, Ferrer-Moure C, et al. Key molecules of triglycerides pathway metabolism are disturbed in patients with systemic lupus erythematosus. *Front Immunol*. 2022;9:13.
14. Martín-González C, Ferrer-Moure C, Carlos Quevedo-Abeledo J, et al. Apolipoprotein C-III in patients with systemic lupus erythematosus. *Arthritis Res Ther*. 2022;24(1):104.
15. Aletaha D, Neogi T, Silman AJ, et al. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European league against rheumatism collaborative initiative. Vol. 69, annals of the rheumatic diseases. *Ann Rheum Dis*. 2010;2010:1580-1588.
16. Hoffman E, Rahat MA, Feld J, et al. Effects of tocilizumab, an anti-Interleukin-6 receptor antibody, on serum lipid and adipokine levels in patients with rheumatoid arthritis. *International Journal of Molecular Sciences*. 2019;20:4633.
17. Cacciapaglia F, Anelli MG, Rinaldi A, et al. Lipids and atherogenic indices fluctuation in rheumatoid arthritis patients on long-term tocilizumab treatment. *Mediators Inflamm [Internet]*. 2018;2018:2453265.
18. Gabay C, Mcinnes IB, Kavanaugh A, et al. Comparison of lipid and lipid-associated cardiovascular risk marker changes after treatment with tocilizumab or adalimumab in patients with rheumatoid arthritis. *Ann Rheum Dis [Internet]*. 2016;75(10):1806-1812.
19. Ferraz-Amaro I, Hernández-Hernández MV, Tejera-Segura B, et al. Effect of IL-6 receptor blockade on proprotein convertase subtilisin/Kexin Type-9 and cholesterol efflux capacity in rheumatoid arthritis patients. *Horm Metab Res*. 2019;51(3):200-209.
20. Lee JS, Chapman MJ, Piraino P, et al. Remodeling of plasma lipoproteins in patients with rheumatoid arthritis: Interleukin-6 receptor-alpha inhibition with tocilizumab. *Proteomics Clin Appl*. 2016;10(2):183-194.
21. Song JS, Wang RS, Leopold JA, Loscalzo J. Network determinants of cardiovascular calcification and repositioned drug treatments. *FASEB J [Internet]*. 2020;34(8):11087-11100.
22. Schlotter F, de Freitas RCC, Rogers MA, et al. ApoC-III is a novel inducer of calcification in human aortic valves. *J Biol Chem [Internet]*. 2021;296:100193.
23. J C, GM P, PL A, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. *N Engl J Med*. 2014 Jul 3;371(1):22-31.
24. Muendlein A, Saely CH, Leiherer A, et al. Angiopoietin-like protein 4 significantly predicts future cardiovascular events in coronary patients. *Atherosclerosis*. 2014;237(2):632-638.
25. Geldenhuys WJ, Lin L, Darvesh AS, Sadana P. Emerging strategies of targeting lipoprotein lipase for metabolic and cardiovascular diseases. *Drug Discov Today*. 2017;22(2):352-365.
26. Giles JT, Sattar N, Gabriel S, et al. Cardiovascular safety of tocilizumab versus etanercept in rheumatoid arthritis: a randomized controlled trial. *Arthritis Rheum*. 2020;72(1):31-40.
27. Hirano T, Nishioka F, Murakami T. Measurement of the serum lipoprotein lipase concentration is useful for studying

triglyceride metabolism: comparison with Postheparin plasma. *Metabolism*. 2004;53(4):526-531.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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