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Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis.

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TITLE PAGE

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Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis.

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ABSTRACT

OBJECTIVES: To describe the prevalence of insulin resistance (IR) in patients with established rheumatoid arthritis (RA) and to analyze the contribution of cumulative inflammatory burden and other factors to its development.

METHODS. Design and participants: Observational cross-sectional study of 89 patients with RA and 80 controls matched for age, sex, and body mass index. We excluded subjects with diabetes. **Primary and secondary outcome measures:** IR was evaluated using the homeostasis model assessment for insulin resistance and beta-cell function and the quantitative insulin sensitivity check index. Other variables included the cumulative 28-joint disease activity score (DAS28-CRP), body composition, and cytokines. We constructed 2 logistic regression models to identify factors associated with IR in patients with RA.

RESULTS: The prevalence of IR was similar in both cases and controls. Inflammatory activity was controlled appropriately in patients during follow-up (mean DAS28, 3.1 [0.8]). The presence of IR in patients with RA was associated with obesity (OR [95% CI], 6.01 [1.9-8.7]), higher cumulative DAS28-CRP values during follow-up(OR [95% CI], 2.8 [1.3-6.0]), and higher IL-1βlevels (OR [95% CI], 1.6 [1.1 -2.4]).The second model showed that the risk of IR increased by 10% for each kilogram of excess body fat.

CONCLUSION: In patients with well-controlled established RA, IR is associated mainly with poorer control of inflammation from diagnosis and with obesity, specifically total fat mass.

Word Count: 2919.

KEYWORDS

Rheumatoid Arthritis, Insulin resistance, Inflammation, Obesity.

Strengths and Limitations

- It is important to clarify the factors associated with insulin resistance (IR) in rheumatoid arthritis because IR has been confirmed as a risk factor for cardiovascular diseases.
- The main factors associated with IR are obesity (specifically total fat mass) and disease activity (specifically increased levels of IL-1β and cumulative inflammatory burden).
- Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent synovitis, bone erosion, and functional disability. It is associated with premature death(1-3)and multiple morbidities (1-2), mainly because the cardiovascular risk of RA patients is similar to that of patients with type 2 diabetes mellitus (DM) (3).Accelerated atherosclerosis in patients with RA is due to the presence of traditional and nontraditional cardiovascular risk factors, including systemic inflammation, drugs, and IR (4-5).

In the general population, IR has been confirmed as a risk factor for cardiovascular diseases, DM, and metabolic syndrome (6). Its main determinant is obesity, although it has also been associated with older age, hypertension, and a sedentary lifestyle. Abundant data suggest a connection between IR and chronic inflammation (7-8). Adipose tissue produces proinflammatory cytokines and adipokines, including TNF-α, which reduce sensitivity to insulin and contribute to endothelial dysfunction (9). The relationship between adipokines, proinflammatory cytokines, and IR (10) is unclear in RA, although it could play a key role in the pathogenesis of accelerated atherosclerosis associated with chronic inflammatory states. Various studies have been specifically designed to investigate IR in RA (5,11-14). While most confirm an association between IR and inflammation, other factors continue to play a stronger role, such as abdominal obesity with sarcopenia, sedentary lifestyle, and drugs (15).

Almost all studies that examine the relationship between IR and RA were carried out in patients with chronic RA and numerous comorbid conditions associated with cardiovascular risk factors in case series not controlled for body mass index (BMI).Furthermore, as these studies have a cross-sectional design, they only take into account values for inflammation recorded at a particular point in time. We previously

studied a group of untreated patients with recent-onset RA and a control group matched for age, sex, and BMI. The patients were followed for 6 months (16). The results showed that IR was not present at diagnosis and did not appear after 6 months of treatment if the disease was well controlled. However, our results also showed that patients with higher fat mass and a longer diagnostic and therapeutic delay had the worst IR data. Based on these findings, the hypothesis of the present study was that IR in patients with RA, as with other determinants of disease, can be prevented in the long term with tight control of inflammation from onset.

The objectives of the present study were as follows: 1. to compare the prevalence of IR in an inception cohort of patients with RA and an equivalent group of healthy controls; and 2. to analyze the effect of IR on the cumulative inflammatory burden over at least 5 years, together with other possible factors that contribute to IR.

METHODS

We carried out an observational cross-sectional study of patients with RA. The study was performed at Instituto de Investigación Biomédica de Málaga (IBIMA) by the Department of Rheumatology of Hospital Regional Universitario de Málaga (HRUM), Malaga, Spain and approved by the Ethics Committee of HRUM (4/2016, PI17). All subjects signed an informed consent document.

Patients

We consecutively included patients from the RA inception cohort at HRUM. All patients had been diagnosed and treated during the first 12 months since onset of their disease. The inclusion criteria were as follows: RA according to the 2010 classification criteria of the American College of Rheumatology/European League Against

Rheumatism (17); diagnosis made between 2007 and 2011; age>16 years; and prospective follow-up with at least 2 annual DAS28 determinations. Patients were recruited between September 2016 and May 2018.We excluded patients with any inflammatory, rheumatic, or autoimmune disease other than RA (except for secondary Sjögren syndrome), a diagnosis of DM or impaired glucose tolerance (American Diabetes Association 2010 criteria)(18), active infection, pregnancy, current or previous treatment with oral antidiabetic agents or insulin, and new treatments or changes in dose during the 3 months preceding the date of inclusion.

The control group comprised randomly selected healthy volunteers from the same geographic area. All volunteers fulfilled all of the inclusion criteria and none of the exclusion criteria. The controls were matched with the cases for age, sex, race, and BMI.

Patient and Public Involvement

We consecutively included patients from the RA inception cohort at HRUM. The control group comprised randomly selected healthy volunteers from the same geographic area. The patients have been informed of the blood test and the results of the study.

Protocol

Cohort patients are followed and treated according to a pre-established protocol and managed with treat-to-target strategies following clinical practice guidelines for RA in Spain (GUIPCAR 2017) (19).All subjects were interviewed and examined by a rheumatologist on the study index date. Samples were collected between 9:00 and 10:00 AM after 12-16 hours of fasting. In order to detect impaired glucose tolerance, subjects with baseline blood glucose levels <126 mg/dl underwent an oral glucose tolerance test (OGTT)

Variables and definitions

The outcome measures were IR and insulin sensitivity. IR was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) (11) and defined as a HOMA-IR score>2.29, based on the 90th percentile for healthy persons (20) and using the homeostasis model assessment for β-cell function (HOMA-β)(11). Sensitivity to insulin was estimated using the quantitative insulin sensitivity check index (QUICKI),with a threshold value of 0.337 (μU*mmol/ml*l)(21).

On the index date, we recorded epidemiological variables, comorbidities, traditional cardiovascular risk factors, diet, physical activity, anthropometric data, and BMI.

Arterial hypertension was defined as an arterial pressure ≥140/90 mmHg or current treatment with antihypertensive medication(22). Glucose and metabolic disorders and DM were diagnosed based on the recommendations of the American Diabetes Association 2010 (23). Dyslipidemia and metabolic syndrome were defined in accordance with the guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) (24). Levels of total cholesterol, triglycerides, HDL, and LDL were evaluated using enzymatic methods(25). Levels of oxidized anti-LDL and serum insulin were determined using enzyme-linked chemiluminescence assay.

Physical activity was measured using the International Physical Activity Questionnaire, taking into account physical activity (low, <600 metabolic equivalents of task [METs]; moderate, 600-1500 METs; and high>1500 METs)(26, 27). Adherence to a Mediterranean diet was evaluated using a validated questionnaire. Adherence was defined as a score of ≥ 9out of 14(28).

Anthropometric data included BMI (kg/m^2) and percentage of obese patients(29), waist circumference (cm), hip circumference (cm), and the waist-hip index(30). Body composition was measured using dual-energy x-ray absorptiometry (DXA; GE Lunar

Prodigy enCORE^{TM} 2006) and included total mass (kg), fat mass (g), lean mass (g), and lean mass and android and gynoid fat mass. The fat mass index (FMI) was defined as fat mass (kg)/height squared (m^2) and fat-free mass index (FFMI) as fat-free mass (kg)/height squared (m^2). The values of fat mass and fat-free mass were obtained using DXA(31).

Clinical data comprised rheumatoid factor, which was positive if>20 IU/ml, and anticitrullinated protein antibody, which was positive if>10 IU/ml. Cumulative inflammatory burden was assessed using DAS28 score with C-reactive protein (PCR) level (range 0-9.4) (32) recorded at each visit throughout follow-up. High activity was defined as DAS28-CRP>5.1, moderate activity as 3.2-5.1, low activity as 2.6-3.2, and remission as ≤2.6.We also took into account severity variables such as the presence of erosions and the mean Health Assessment Questionnaire (HAQ) score throughout the course of the disease(33). Treatment was with synthetic disease-modifying anti-inflammatory drugs (DMARDs) and biological DMARDs. The laboratory values measured in all patients were as follows: serum high-sensitivity CRP, tumor necrosis factor alfa (TNF-α), interleukin (IL)6, IL-1ß, adiponectin, resistin, leptin, and insulin-like growth factor (IGF) 1. The laboratory kits used and their reference values are shown in the supplementary material.

Statistical analysis

Variables are expressed as mean (SD), median (IQR), or number (%).Comparisons between groups were performed using the χ^2, *t*, or Mann-Whitney test depending on the normality of the distribution. Binary logistic regression analysis was performed (dependent variable: IR measured using HOMA-IR). Multicollinearity of independent variables was verified using the Pearson correlation coefficient (r>0.4).Sample size was calculated assuming a prevalence of IR in RA of 51% and considering as relevant a 30% difference with respect to controls. With a 2-sidedαerror of 0.05 and a β error of 0.20,the

necessary sample size would be 77 subjects in each group (34).Sample size was increased by 10% to account for possible losses. The analysis was performed using R Commander 2.3-0.

RESULTS

The initial study population comprised 100 patients and 100 controls. However, only 89 patients with RA and 80 healthy controls fulfilled the inclusion criteria and none of the exclusion criteria. The study flow chart is shown in Figure 1.

Epidemiological and anthropometric characteristics and comorbidities

Table 1 shows the baseline characteristics of patients and controls. Mean age was slightly over 56 years, and most subjects were women (75%).Patients with RA more frequently had a family history of cardiovascular disease than controls, and 10% more were ex smokers.

Clinical and analytical variables associated with RA

Autoantibodies were only detected in patients, except for 1 subject in the control group, who was positive for rheumatoid factor, with a low titer and no other data indicating inflammatory disease. On the index date, most patients were in remission or had low arthritis-related inflammatory activity and had maintained an average DAS28-CRP<3.2 throughout follow-up (Table 1). All patients had received synthetic DMARDs, and almost 40% received a biologic alone or in combination with other agents.

While the rate of adherence to a Mediterranean diet was similar in both groups (62.9% vs 57.5%; p=0.472), healthy subjects more often engaged in physical activity than patients(median [IQR], 612.0 [313.5-1089.0] METS vs 339.0 [198.0-792.0] METS; p=0.005).

Both patients and controls generally had similar baseline characteristics with respect to carbohydrate metabolism (i.e., resistance and sensitivity to insulin, glycemia, and baseline insulinemia). While patients had slightly higher blood sugar levels after OGTT, the difference was not clinically relevant. As for lipids, patients with RA had slightly lower levels of total cholesterol and LDL-cholesterol than controls.

Inflammatory cytokines and adipokines

Disease was controlled in most patients with RA. However, RA patients had higher levels of proinflammatory cytokines (i.e., TNF-α, IL-6, and IL-1β) than controls. Similarly, IGF-1 levels tended to be higher in patients. The only adipokine that was elevated in patients was adiponectin. Resistin and leptin remained similar in both groups.

Characteristics of patients with RA and IR

Of the 89 patients with RA, 25(28.1%)had a HOMA-IR ≥2.29. Table 3 shows the characteristics of patients with RA with and without IR. As we can see, the epidemiological characteristics of patients with IR were similar to those of the others, although clinical control of their disease was worse on the index date (DAS28-CRP >3.2; 68% vs 28%; p=0.001) and throughout follow-up (mean DAS28-CRP, 3.5 [0.7] vs. 2.9 [0.7]; p=0.001). It is also important to note that patients with IR had higher values for BMI, weight, and body composition and were more often obese. They also had a higher percentage of fat and a higher waist-hip index. However, no differences were found between the groups with respect to the delay in diagnosis of RA, duration of symptoms, use of DMARDs (synthetic and biologics), or antibodies.

With respect to inflammatory cytokines and adipokines, concentrations of IL-1β and leptin were clearly higher in patients with IR. No differences were found between the groups in concentrations of TNF-αand IL-6 or in levels of resistin and adiponectin.

Factors associated with insulin resistance in rheumatoid arthritis

Table 4 shows the 2 best multivariate models. In the first, obesity, mean DAS28, and IL-1ß were significantly associated with IR; these factors account for 35% of variability in the presence of IR (R^2=0.352). In the second model, fat mass, mean DAS28, and IL-1ßwere associated with IR; these factors accounted for 40% of the variability in IR (R^2=0.404).

DISCUSSION

Given that RA is currently considered as potent a cardiovascular risk factor as type 2 DM, some risk equations take it into account (35). Although cardiovascular risk may be mediated in part by IR (9, 17-25), our study did not reveal a higher prevalence of IR in patients with RA, because disease was well controlled in patients with a cumulative mean DAS28 <3.2 from onset. This finding is consistent with data from other authors, who observed a reduction in RI associated with control of the inflammatory activity induced by methotrexate and anti-TNF αagents (36). However, these results contrast with those published elsewhere, owing to methodological differences, especially in the measurement of inflammation and matching of controls (5, 14). Our study, in contrast, was based on inflammation data obtained from patients with established RA followed prospectively since onset. We also included controls matched for age, sex, race, and BMI, and the amount of exercise and adherence to the Mediterranean diet were taken into account.

While no differences were found between cases and controls, our results support an association between IR and chronic inflammation, as confirmed elsewhere (14, 16, 36, 37).Our group studied baseline IR in patients with untreated early-onset RA and found an association between IR and time with untreated symptoms and fat mass percentage

(16). Both these aspects are important in the tight and early control of arthritis. In fact, when we analyze the determinants of IR in patients with RA, we again find a higher risk in patients with poor control of their disease during its course, higher levels of IL-1β, and more pronounced obesity. While the cytokines TNF-α, IL-6, and IL-1β are abundant in patients with active RA and reflect inflammatory status, the first two may not play as important a role in the present study, because the vast majority of patients treated with biologics received anti-TNF-αagents or tocilizumab. However, in addition to treatment, other aspects associated with IL-1β may have an effect. Although the role of IL-1β has recently been questioned(38), it has been thought to play a role in the pathogenesis of type 2 DM (39) The authors indicate that high blood sugar levels could induce more marked production of IL-1β and TNF-α in macrophages and that this in turn could lead to a greater rate of apoptosis of β cells that would eventually lead to impairment of pancreatic function (39). In this sense, there have been reports of cases of diabetic patients with RA whose arthritis has remitted and whose metabolism has been controlled with IL-1β blockers (40). Furthermore, there have been reports of diabetic patients without RA in whom HbA1c levels improve and secretion of C-peptide increases, while the proinsulin/insulin ratio is decreased or secretion of insulin is increased(41).

Our results showed that patients and controls did not differ overall in BMI or in body composition. While this observation can be explained in part by matching for BMI, disease control with biologic DMARDs is associated with recovery of total appendicular lean mass, with no changes in fat distribution(42).Nevertheless, despite treat-to-target strategies, as applied in our study, patients with RA experience a relative loss of muscle mass and an increase in adiposity (43), which, in our study, was more evident in those with poorer control of inflammation and more pronounced IR. Obesity

is more frequent in patients with RA and is closely associated with IR (44, 45), probably owing to physical inactivity, sarcopenia, and therapy with corticosteroids. Obese patients with RA in the present study had a 6-fold greater risk of IR than the other patients; this probability was mediated mainly by fat mass, since the risk increased by 10%for each kilogram of excess body fat. These data support those found in other studies, which highlight the fact that inflammation and obesity are closely linked, because adipose tissue produces TNF-αand IL-6 (16, 45).

In addition to proinflammatory cytokines, adipokines produced by fatty tissue may affect glucose homeostasis, appetite, and the inflammatory response (46). We found that adiponectin levels were higher in patients than in controls. While results were sometimes contradictory, it seems that adiponectin could increase in patients with RA as a means of offsetting the proinflammatory effects of high levels of leptin or of TNF-αand IL-6(46). This increase may also be a result of the effect of treatment with DMARDs(47).

Leptin levels (systemic, local, or both) have been reported to increase in various inflammatory diseases(46). However, we only observed an increase in patients with RA and IR. Consistent with some studies, this could be because these patients had a greater BMI and a higher grade of chronic inflammation (48).

Furthermore, our data show an increase in LDL oxidase and a decrease in IGF-1 that tended toward significance in patients with RA compared with healthy controls. The same finding was observed in other studies in which these parameters were associated with increased cardiovascular risk in RA (49, 50).

Our study is limited by the cross-sectional evaluation of IR between patients with RA and healthy subjects. However, the study patients are from a prospective RA inception

cohort in which we longitudinally collected all inflammation-related variables analyzed using a predesigned protocol. Consequently, no data were missing, and our results are consistent. A strength of the study is the association we established between IR in patients with RA and cumulative inflammatory activity during the course of the disease. In addition, the control group was matched with the cases not only for age and sex, but also for BMI. Furthermore, while most studies report the association between IR and obesity in patients with RA, we performed an exhaustive analysis of body composition using DXA in order to establish the relationship with more specific anthropometric parameters.

In conclusion, our results show that the main factors associated with IR are obesity, specifically total fat mass, and disease activity, specifically levels of IL-1β and cumulative inflammatory burden, measured based on average DAS28-CRP levels throughout follow-up. Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis. However, controlled prospective studies must continue to be performed in order to better observe the possible causal relationship between clinical and metabolic factors and IR and atherosclerosis in RA.

REFERENCES

1. Goodson N, Marks J, Lunt M, Symmons D. Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s. Ann Rheum Dis. 2005;64(11):1595-601.

2. Espino-Lorenzo P, Manrique-Arija S, Urena I, Jimenez-Nunez FG, Lopez-Lasanta M, Romero-Barco CM, et al. Baseline comorbidities in patients with

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3. Peters MJ, van Halm VP, Voskuyl AE, Smulders YM, Boers M, Lems WF, et al. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis and rheumatism. 2009;61(11):1571-9.

4. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003;108(24):2957-63.

5. La Montagna G, Cacciapuoti F, Buono R, Manzella D, Mennillo GA, Arciello A, et al. Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis. Diab Vasc Dis Res. 2007;4(2):130-5.

6. Grundy SM. What is the contribution of obesity to the metabolic syndrome? Endocrinology and metabolism clinics of North America. 2004;33(2):267-82.

7. Park K, Steffes M, Lee DH, Himes JH, Jacobs DR, Jr. Association of inflammation with worsening HOMA-insulin resistance. Diabetologia. 2009;52(11):2337-44.

8. Meng YX, Ford ES, Li C, Quarshie A, Al-Mahmoud AM, Giles W, et al. Association of C-reactive protein with surrogate measures of insulin resistance among nondiabetic US from National Health and Nutrition Examination Survey 1999-2002. Clinical chemistry. 2007;53(12):2152-9.

9. Li G, Barrett EJ, Barrett MO, Cao W, Liu Z. Tumor necrosis factor-alpha induces insulin resistance in endothelial cells via a p38 mitogen-activated protein kinase-dependent pathway. Endocrinology. 2007;148(7):3356-63.

10. El-Hini SH, Mohamed FI, Hassan AA, Ali F, Mahmoud A, Ibraheem HM. Visfatin and adiponectin as novel markers for evaluation of metabolic disturbance in recently diagnosed rheumatoid arthritis patients. Rheumatology international. 2013;33(9):2283-9.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

12. Dessein PH, Stanwix AE, Joffe BI. Cardiovascular risk in rheumatoid arthritis versus osteoarthritis: acute phase response related decreased insulin sensitivity and high-density lipoprotein cholesterol as well as clustering of metabolic syndrome features in rheumatoid arthritis. Arthritis research. 2002;4(5):R5.

13. Rosenvinge A, Krogh-Madsen R, Baslund B, Pedersen BK. Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNFalpha therapy. Scand J Rheumatol. 2007; 36:91-6.

14. Shahin D, Eltoraby E, Mesbah A, Houssen M. Insulin resistance in early untreated rheumatoid arthritis patients. Clin Biochem. 2010;43(7-8):661-5.

15. Castaneda S, Martin-Martinez MA, Gonzalez-Juanatey C, Llorca J, Garcia-Yebenes MJ, Perez-Vicente S, et al. Cardiovascular morbidity and associated risk factors in Spanish patients with chronic inflammatory rheumatic diseases attending rheumatology clinics: Baseline data of the CARMA Project. Semin Arthritis Rheum. 2015;44(6):618-26.

16. Manrique-Arija S, Urena I, Valdivielso P, Rioja J, Jimenez-Nunez FG, Irigoyen MV, et al. Insulin resistance and levels of adipokines in patients with untreated early rheumatoid arthritis. Clin Rheumatol. 2016;35(1):43-53.

17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

18. Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. Diabetes Care. 1998;21(2):310-4.

19. Sociedad Española de Reumatología. GUIPCAR actualización 2011: Actualizaciónde la guía de práctica clínica para el manejo de la artritis reumatoide en ESPAÑA. [[monografía en internet]]. Madrid: Sociedad Española de Reumatología; 2007 [updated [30 diaciembre 2011]].

20. Rojo-Martinez G, Esteva I, de Adana SR, Catala M, Merelo MJ, Tinahones F, et al. Patterns of insulin resistance in the general population of southeast Spain. Diabetes Res Clin Pract. 2004;65(3):247-56.

21. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402-10.

22. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. ESH/ESC 2007 Guidelines for the management of arterial hypertension. Rev Esp Cardiol.2007; 60:68e1-94.

23. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 (Suppl 1):S62-9.

24. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001: 285:2486-97.

25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

26. Guidelines for Data Processing andAnalysis of the International Physical Activity Questionnaire (IPAQ). 2009.

27. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.

28. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279-90.

29. WHO. Obesity Preventing and managing the global epidemic. Geneva: WHO; 1997.

30. Anonymous World Health Organization Expert Committee. Physical status: the use and interpretation of anthropometry. . WHO; Genova: Technical Report Series; 1995.

31. Taylor AE, Kuper H, Varma RD, Wells JC, Bell JD, K VR, et al. Validation of dual energy X-ray absorptiometry measures of abdominal fat by comparison with magnetic resonance imaging in an Indian population. PLoS One. 2012;7(12):e51042.

32. Iwamoto N, Kawakami A, Fujikawa K, Aramaki T, Kawashiri SY, Tamai M, et al. Prediction of DAS28-ESR remission at 6 months by baseline variables in patients with rheumatoid arthritis treated with etanercept in Japanese population. Mod Rheumatol. 2009;19(5):488-92.

33. Moyano S, Scolnik M, Vergara F, Garcia MV, Sabelli MR, Rosa JE, et al. Evaluation of Learned Helplessness, Perceived Self-efficacy, and Functional Capacity in Patients With Fibromyalgia and Rheumatoid Arthritis. J Clin Rheumatol. 2018.

34. Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, Shintani A, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis. 2008;196(2):756-63.

35. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. Bmj. 2017;357:j2099.

36. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2006;24(1):83-6.

37. Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, De Pergola G. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. Int J Obes Relat Metab Disord. 2001;25(10):1416-20.

38. Everett BM, Donath MY, Pradhan AD, Thuren T, Pais P, Nicolau JC, et al. Anti-Inflammatory Therapy With Canakinumab for the Prevention and Management of Diabetes. J Am Coll Cardiol. 2018;71(21):2392-401.

39. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2):98-107.

40. Ruscitti P, Cipriani P, Cantarini L, Liakouli V, Vitale A, Carubbi F, et al. Efficacy of inhibition of IL-1 in patients with rheumatoid arthritis and type 2 diabetes mellitus: two case reports and review of the literature. J Med Case Rep. 2015;9:123.

41. van Poppel PC, van Asseldonk EJ, Holst JJ, Vilsboll T, Netea MG, Tack CJ. The interleukin-1 receptor antagonist anakinra improves first-phase insulin secretion and insulinogenic index in subjects with impaired glucose tolerance. Diabetes Obes Metab. 2014;16(12):1269-73.

42. Tournadre A, Pereira B, Dutheil F, Giraud C, Courteix D, Sapin V, et al. Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. J Cachexia Sarcopenia Muscle. 2017;8(4):639-46.

43. Lemmey AB, Wilkinson TJ, Clayton RJ, Sheikh F, Whale J, Jones HS, et al. Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients. Rheumatology (Oxford). 2016;55(10):1736-45.

44. Muller R, Kull M, Lember M, Polluste K, Valner A, Kallikorm R. Insulin Resistance in Early Rheumatoid Arthritis Is Associated with Low Appendicular Lean Mass. Biomed Res Int. 2017;2017:9584720.

45. Castillo-Hernandez J, Maldonado-Cervantes MI, Reyes JP, Patino-Marin N, Maldonado-Cervantes E, Solorzano-Rodriguez C, et al. Obesity is the main determinant of insulin resistance more than the circulating pro-inflammatory cytokines levels in rheumatoid arthritis patients. Rev Bras Reumatol Engl Ed. 2017;57(4):320-9.

46. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gomez-Reino JJ, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis. 2006;65(9):1198-201.

47. Cansu B, Cansu DU, Kasifoglu T, Gulbas Z, Korkmaz C. Disease-modifying antirheumatic drugs increase serum adiponectin levels in patients with rheumatoid arthritis. J Clin Rheumatol. 2011;17(1):14-7.

48. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis. 2005;64(8):1195-8.

49. Erlandsson MC, Lyngfelt L, Aberg ND, Wasen C, Espino RA, Silfversward ST, et al. Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis. BMC Med. 2019;17(1):141.

50. Nowak B, Madej M, Luczak A, Malecki R, Wiland P. Disease Activity, Oxidized-LDL Fraction and Anti-Oxidized LDL Antibodies Influence Cardiovascular Risk in Rheumatoid Arthritis. Adv Clin Exp Med. 2016;25(1):43-50.

Author contributions statement

SMA: She was a contributor in including patients. A major contributor in writing the manuscript. She was a contributor in analyzing and interpreting the patient data. **NMV:** She was a contributor in writing the manuscript. She was a major contributor in analyzing and interpreting the patient data. **IUG:** She was a major contributor in including patients. **JR:** She was a major contributor in performing laboratory determination. He was a contributor in interpreting laboratory data. **PV:** He was a contributor in interpreting the patient data. A contributor in writing the manuscript. **LGM:** She was a major contributor in including controls. **SAS:** She was a major contributor in including controls. **FGJN:** She was a contributor in including patients. **BOM:** She was a contributor in performing laboratory determination. He was a contributor in interpreting laboratory data. **AFN:** A contributor in writing the manuscript. He was a contributor in analyzing and interpreting the patient data. All authors read and approved the final manuscript

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Tables

Table 1. Baseline characteristics of cases and controls

Variables	RA patients n=89	Controls n=80	p value
Epidemiological characteristics			
Age, years, mean (SD)	56.6 (10.9)	56.4 (10.9)	0.902
Female sex, n (%)	67 (75.3)	67 (83.8)	0.189
Smoking status			0.117
Never smoked, n (%)	42 (47.2)	49 (61.3)	
Exsmoker, n (%)	22 (24.7)	11 (13.8)	
Smoker, n (%)	25 (28.1)	20 (25.0)	
Clinical-laboratory characteristics			
Time since onset of RA, months, median (IQR)	98.0 (78.5-123.5)	-	-
Diagnostic delay, months, median (IQR)	10.9 (5.4 - 25.6)	-	-
Rheumatoid factor>10U/mL, n (%)	73 (82.0)	1 (1.3)	<0.001
ACPA >20U/mL, n (%)	67 (75.3)	0 (0.0)	<0.001
C-reactive protein (mg/dl), median (IQR)	2.9 (2.9-3.4)		
Erythrocyte sedimentation rate (mm/h), median (IQR)	11 (6.2-14)	11.5 (7.8-21.3)	0.088

Nº of swollen joints (0-28), median (IQR)	0 (0-1)	ND	ND
Nº of painful joints (0-28), median (IQR)	1 (0-2)	ND	ND
VAS (1-100 mm), median (IQR)	30 (20-50)	ND	ND
DAS28PCR on the index date, mean (SD)	2.83 (1.1)	ND	ND
Remission-low activity, n (%)	63 (71.0)	ND	ND
Moderate-high activity, n (%)	26 (29.0)	ND	ND
Mean DAS28PCR during follow-up, mean (SD)	3.11 (0.8)	ND	ND
Remission-low activity, n (%)	56 (63.0)	ND	ND
Moderate-high activity, n (%)	33 (37.0)	ND	ND
Mean HAQ during follow-up, median (IQR)	0.750 (0.0-1.1)	ND	ND
Synthetic DMARDs, n (%)	78 (87.6)	0 (0.0)	<0.001
Biologic DMARDs, n (%)	35 (39.3)	0 (0.0)	<0.001
Combined DMARDs, n (%)	25 (28.1)	0 (0.0)	<0.001
Methotrexate, n (%)	21 (84.0)		
Leflunomide, n (%)	3 (12.0)		
Sulfasalazine, n (%)	1 (4.0)		

RA, rheumatoid arthritis; ACPA, Anticitrullinated protein antibodies; DAS28,28-joint Disease Activity Score; HAQ, Health Assessment Questionnaire; ND,no data; DMARD, disease-modifying antirheumatic drug.

Table 2. Anthropometric and metabolic characteristics, inflammatory cytokines, and adipokines.

Variables	**RA patients n=89**	**Controls n=80**	**p value**

Anthropometric characteristics			
BMI (kg/m^2), mean (SD)	28.2 (5.0)	27.3 (4.9)	0.266
Waist circumference, (cm), mean (SD)	90.8 (11.5)	89.3 (11.3)	0.419
Hip circumference (cm), mean (SD)	103.1 (7.3)	100.5 (9.9)	0.081
Waist-hip index, mean (SD)	0.88 (0.0)	0.89 (0.0)	0.517
Body composition by DXA, mean (SD)			
Total fat mass (kg), mean (SD)	29.2(9.7)	28.5 (9.7)	0.671
FMI (kg/m^2), mean (SD)	11.3 (4.0)	11.1 (4.0)	0.144
Total lean mass (kg), mean (SD)	40.8 (8.9)	39.0 (8.6)	0.197
FFMI (kg/m^2), mean (SD)	15.6 (2.4)	15.0 (2.4)	0.726
Total mass (kg), mean (SD)	71.8 (15.5)	69.9 (14.4)	0.415
Android fat mass (kg), mean (SD)	2.5 (1.1)	2.4 (1.1)	0.671
Gynoid fat mass (kg), mean (SD)	5.1 (1.6)	5.0 (1.5)	0.785
Android lean mass (kg), mean (SD)	2.9 (0.7)	2.8 (0.7)	0.433
Gynoid lean mass (kg), mean (SD)	5.8 (1.2)	5.7 (1.2)	0.639
Metabolic characteristics			
Total cholesterol, median (IQR)	194.0 (170.5-223.6)	209.0 (188.0-238.0)	0.031
LDL cholesterol (mg/dl), median (IQR)	110.0 (95.9-137.5)	133.0 (106.0-151.0)	0.004
HDL cholesterol (mg/dl), median (IQR)	58.0 (51.0-66.0)	59.0 (51.0-72.0)	0.377
Triglycerides (mg/dl), median (IQR)	87.0 (69.0-131.0)	90.0 (66.0- 120.0)	0.102
LDL OX, median (IQR)	2.6 (0.8-5.6)	1.0 (0.3-2.8)	0.114
Baseline glycemia (mg/dl), median (IQR)	78.0 (73.0-84.5)	80.0 (72.0-87.0)	0.327
Glycemia after OGTT (mg/dl), mean (SD)	110.0 (28.9)	100.5(26.6)	0.030
Insulinemia µU/ml, median (IQR)	9.3 (6.0-12.4)	8.6 (6.0. 12.6)	0.904
HOMA-IR, median (IQR)	1.7 (1.02-2.3)	1.7 (1.15-2.7)	0.385
HOMA-IR ≥2.29, n (%)	25 (28.1)	24(30.0)	0.785

HOMA-β, median (IQR)	38.9 (22.7-53.3)	34.4 (24.0-54.9)	0.545
QUICKI, median (IQR)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.211
QUICKI ≤0.33, n (%)	25.0 (28.1)	24.0 (30.0)	0.785
Inflammatory cytokines and adipokines			
IL-6 (pg/ml), median (IQR)	11.0 (5.4-19.0)	4.31 (3.08-6.67)	<0.001
IL-1ß (pg/ml), median (IQR)	4.33 (4.2-4.5)	2.74 (2.64-3.50)	<0.001
TNF - α (pg/ml), median (IQR)	5.8 (3.7-24.7)	3.6 (3.0-4.7)	<0.001
IGF-1(pg/ml), median (IQR)	172.8 (104.7-238.9)	130.6 (49.8-252.47)	0.079
Adiponectin (ng/ml), median (IQR)	11399.5 (7771.1-14971.5)	8581.4 (6524.1-12688.9)	0.014
Resistin (ng/ml), median (IQR)	7.2 (5.5-9.3)	7.4 (5.7-9.7)	0.510
Leptin (ng/ml), median (IQR)	16.9 (9.1-36.9)	22.2 (9.6-38.8)	0.432

RA, rheumatoid arthritis; NCEP ATIII, National Cholesterol Education Program Adult Treatment Panel III; BMI, body mass index; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX , Oxidized low-density lipoprotein; OGTT, oral glucose tolerance test; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-ß, Homeostatic Model Assessment for ß-Cell Function; QUICKI, Quantitative Insulin Sensitivity Check Index; IL-6, interleukin 6; TNF-α, tumor necrosis factor α;IL-1ß, interleukin 1 ß;IGF-1, insulin-like growth factor-1.

Table3. Characteristics of patients with and without IR measured using HOMA-IR.

Variables	**RA HOMA-IR≥2.29 n=25**	**RA HOMA-IR<2.29 n=64**	**p value**
Age, years, mean (SD)	58.2 (8.3)	56.0 (11.7)	0.329
Female sex, n (%)	17 (68.0)	50 (78.1)	0.320
Clinical characteristics			

Time since onset of RA, months, median (IQR)	95.8 (81.8-111.6)	98.1 (77.5-125.5)	0.697
Diagnostic delay (months), median (IQR)	15.5 (4.6-27.4)	10.3 (5.4-23.7)	0.629
Arterial hypertension, n (%)	7 (28.0)	16 (25.0)	0.771
Dyslipidemia, n (%)	8 (32.0)	13 (20.3)	0.243
Rheumatoid factor, n (%)	20 (80.0)	53 (82.8)	0.756
ACPA, n (%)	17 (68.0)	49 (76.0)	0.407
DAS28PCR>3.2 on the index date, n (%)	17 (68.0)	18 (28.1)	**0.001**
Mean DAS28PCR during follow-up, mean (SD)	3.5 (0.7)	2.9 (0.7)	**0.001**
Mean HAQ during follow-up, mean (SD)	0.90 (0.6)	0.76 (0.8)	0.505
Synthetic DMARD, n (%)	22 (88.0)	49 (76.6)	0.227
Biologic DMARD, n (%)	10 (40.0)	19 (29.7)	0.351
Combined DMARD, n (%)	7 (28.0)	18 (28.1)	0.991
Anthropometric characteristics			
Weight, mean (SD)	82.6 (13.1)	69.5 (12.5)	**<0.001**
BMI, mean (SD)	31.9 (5.4)	26.8 (4.0)	**<0.001**
Normal weight, n (%)	1 (4.0)	27 (42.2)	
Overweight, n (%)	9 (36.0)	22 (34.4)	
Obesity, n (%)	15 (60.0)	15 (23.4)	
Waist circumference, mean (SD)	97.5 (8.9)	88.1 (11.4)	**0.001**
Hip circumference, mean (SD)	106.6 (7.0)	101.7 (7.0)	**0.005**
Waist-hip index, mean (SD)	0.91(0.1)	0.86 (0.1)	**0.022**
Body composition measured by DEXA			
Total fat mass (kg), mean (SD)	35.5 (2.2)	26.7 (7.8)	**0.001**
FMI (kg/m^2), mean (SD)	13.8 (4.7)	10.4 (3.2)	**<0.001**
Total lean mass (kg), mean (SD)	43.8 (7.7)	39.7 (9.1)	**0.051**
FFMI (kg/m^2), mean (SD)	16.8 (2.4)	15.1 (2.2)	**0.006**
Lean mass percentage,	0.53 (0.1)	0.58 (0.1)	0.051

mean (SD)			
Total mass (kg), mean (SD)	81.9(12.6)	67.9 (14.7)	**<0.001**
Android fat mass (kg), mean (SD)	34.6(0.7)	22.3 (9.1)	**<0.001**
Gynoid fat mass (kg), mean (SD)	59.5 (1.0)	47.9 (1.3)	**0.003**
Android lean mass (kg), mean (SD)	32.0 (0.5)	28.2 (0.7)	**0.014**
Gynoid lean mass (kg), mean (SD)	64.0 (1.0)	55.0 (1.2)	**0.003**
LDL OX, median (IQR)	2.7 (0.7-7.4)	2.5 (0.7-5.5)	0.868
Inflammatory cytokines and adipokines			
IL6 (pg/ml), median (IQR)	11.1 (7.3-20.8)	10.5 (4.9-19.8)	0.495
IL-1β (pg/ml), median (IQR)	4.9 (4.1-4.4)	4.3 (4.0 -4.5)	0.007
TNF-α (pg/ml), median (IQR)	5.1 (3.3-27.0)	5.8 (3.7-23.5)	0.827
IGF-1(pg/ml), mean (SD)	168.5 (93.8)	187.7 (106.6)	0.433
Adiponectin (ng/ml), median (IQR)	9271.8 (7602.0-12407.0)	12273.1 (8218.5-15677.1)	0.210
Resistin (ng/ml), median (IQR)	8.3 (5.6-9.9)	7.1 (5.5. - 9.0)	0.626
Leptin (ng/ml), median (IQR)	33.7 (18.0-55.2)	13.9 (7.9-26.1)	0.001

RA, rheumatoid arthritis; ACPA, Anticitrullinated protein antibodies; DAS28,28-joint Disease Activity Score; HAQ, Health Assessment Questionnaire; ND,no data; DMARD, disease-modifying antirheumatic drug; BMI, body mass index; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-densitylipoprotein; LDL-OX , Oxidized low-density lipoprotein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha;IL-1ß, interleukin 1ß;IGF-1, insulin-likegrowth factor-1.

Table 4. Multivariate models for RA patients with HOMA-IR >2,29 (dependent variable)

Multivariate model 1 using obesity covariate

Predictor	β	OR	95% CI	p value

Obesity (IMC>30)	1.795	6.01	1.94-8.66	p=0.002
Mean DAS28PCR	1.021	2.77	1.29-5.99	p=0.009
Il-1β (pg/ml)	0.464	1.59	1.06-2.38	p=0.024

R^2 =0,352

Independent variables: Age, sex, obesity (BMI >30), mean 28-joint Disease Activity Index (DAS28), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

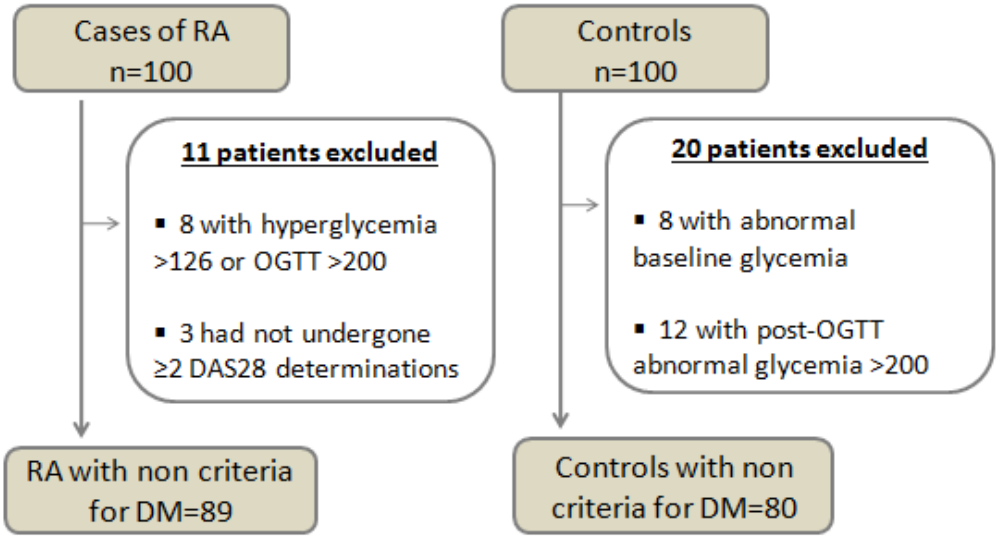
Multivariate model 2 using total fat mass covariate

Predictor	β	OR	95% CI	p value
Total Fat mass (kg)	0.123	1.10	1.05-1.22	p=0.002
Mean DAS28PCR	0.955	2.60	1.19-5.69	p=0.017
Il-1β (pg/ml)	0.456	1.57	1.06-2.34	p=0.023

R^2 =0,404

Independent variables: Age, sex, fat mass, lean mass, mean 28-joint Disease Activity Index (DAS28), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

Figure 1. Patients and controls´ flow chart



RA, Rheumatoid arthritis. OGTT, oral glucose tolerance test; DAS28, 28-joint Disease Activity Score. DM, Diabetes Mellitus.

Table S1. Laboratory tests.

Test	Method/Reference range
High-sensitivity C-reactive protein	Standard nephelometry (reference range, 0-6 mg/l
TNF-α	Automated immunoassay (Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA; reference range, 0-8.1 pg/ml)
IL-6	Enzyme-linked chemiluminescent assay (QuantiGlo®; normal range, 0-5.84 pg/ml).
IL-1ß	Enzyme-linked chemiluminescent assay (QuantiGlo®) pg/ml
Adiponectin	Enzyme-linked chemiluminescent assay (Mediagnost®) (mean [SD] reference value, 11.5 [5.9] ng/ml)
Resistin	Enzyme-linked chemiluminescent assay (Mediagnost®) (median [IQR] reference value, 7.2 [5.4-8.5] ng/ml)
Leptin	Enzyme-linked chemiluminescent assay (Mediagnost®).

	(according to the manufacturer, normal values lie between the 5th and 95th percentiles after adjusting for sex and BMI ng/ml)
IGF-1	Enzyme-linked chemiluminescent assay (Quantikine®)(pg/ml)
Serum insulin	Enzyme-amplified chemiluminescent assay (Immulite ONE®) (**µ**U/ml)
LDL-Ox	Enzyme-amplifiedchemiluminescent assay (Immulite ONE®)(U/l)

TNF-α, tumor necrosis factor alpha; IL, interleukin; IGF-1,insulin-like growth factor 1;LDL,low-densitylipoprotein; ELISA, enzyme-linked immunosorbent assay. LDL-Ox, Oxidized low-density lipoprotein

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TITLE PAGE

TITLE

Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis: cross-sectional study

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ABSTRACT

OBJECTIVES: To describe the prevalence of insulin resistance (IR) in patients with established rheumatoid arthritis (RA) and to analyze the contribution of cumulative inflammatory burden and other factors to its development.

METHODS. Design and participants: Observational cross-sectional study of 89 patients with RA and 80 controls matched for age, sex, and body mass index. We excluded subjects with diabetes. **Primary and secondary outcome measures:** IR was evaluated using the homeostasis model assessment for insulin resistance and beta-cell function and the quantitative insulin sensitivity check index. Other variables included the cumulative 28-joint disease activity score (DAS28-CRP), body composition, and cytokines. We constructed 2 logistic regression models to identify factors associated with IR in patients with RA.

RESULTS: The prevalence of IR was similar in both cases and controls. Inflammatory activity was controlled appropriately in patients during follow-up (mean DAS28, 3.1 [0.8]). The presence of IR in patients with RA was associated with obesity (OR [95% CI], 6.01 [1.9-8.7]), higher cumulative DAS28-CRP values during follow-up(OR [95% CI], 2.8 [1.3-6.0]), and higher IL-1βlevels (OR [95% CI], 1.6 [1.1 -2.4]).The second model showed that the risk of IR increased by 10% for each kilogram of excess body fat.

CONCLUSION: In patients with well-controlled established RA, IR is associated mainly with poorer control of inflammation from diagnosis and with obesity, specifically total fat mass.

Word Count: 2919.

KEYWORDS

Rheumatoid Arthritis, Insulin resistance, Inflammation, Obesity.

Strengths and Limitations

- It is important to clarify the factors associated with insulin resistance (IR) in rheumatoid arthritis because IR has been confirmed as a risk factor for cardiovascular diseases.
- The main factors associated with IR are obesity (specifically total fat mass) and disease activity (specifically increased levels of IL-1β and cumulative inflammatory burden).
- Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent synovitis, bone erosion, and functional disability. It is associated with premature death (1-3) and multiple morbidities (1-2), mainly because the cardiovascular risk of RA patients is similar to that of patients with type 2 diabetes mellitus (DM) (3). Accelerated atherosclerosis in patients with RA is due to the presence of traditional and nontraditional cardiovascular risk factors, including systemic inflammation, drugs, and IR (4-5).

In the general population, IR has been confirmed as a risk factor for cardiovascular diseases, DM, and metabolic syndrome (6). Its main determinant is obesity, although it has also been associated with older age, hypertension, and a sedentary lifestyle. Abundant data suggest a connection between IR and chronic inflammation (7-8). Adipose tissue produces proinflammatory cytokines and adipokines, including TNF-α, which reduce sensitivity to insulin and contribute to endothelial dysfunction (9). The relationship between adipokines, proinflammatory cytokines, and IR (10) is unclear in RA, although it could play a key role in the pathogenesis of accelerated atherosclerosis associated with chronic inflammatory states. Various studies have been specifically designed to investigate IR in RA (5,11-14). While most confirm an association between IR and inflammation, other factors continue to play a stronger role, such as abdominal obesity with sarcopenia, sedentary lifestyle, and drugs (15).

Almost all studies that examine the relationship between IR and RA were carried out in patients with chronic RA and numerous comorbid conditions associated with cardiovascular risk factors in case series not controlled for body mass index (BMI). Furthermore, as these studies have a cross-sectional design, they only take into account values for inflammation recorded at a particular point in time. We previously studied a

group of untreated patients with recent-onset RA and a control group matched for age, sex, and BMI. The patients were followed for 6 months (16). The results showed that IR was not present at diagnosis and did not appear after 6 months of treatment if the disease was well controlled. However, our results also showed that patients with higher fat mass and a longer diagnostic and therapeutic delay had the worst IR data. Based on these findings, the hypothesis of the present study was that IR in patients with RA, as with other determinants of disease, can be prevented in the long term with tight control of inflammation from onset.

The objectives of the present study were as follows: 1. to compare the prevalence of IR in an inception cohort of patients with RA and an equivalent group of healthy controls; and 2. to analyze the effect of IR on the cumulative inflammatory burden over at least 5 years, together with other possible factors that contribute to IR.

METHODS

We carried out an observational cross-sectional study of patients with RA. The study was performed at Instituto de Investigación Biomédica de Málaga (IBIMA) by the Department of Rheumatology of Hospital Regional Universitario de Málaga (HRUM), Malaga, Spain and approved by the Ethics Committee of HRUM. All subjects signed an informed consent document.

Patients

We consecutively included patients from the RA inception cohort at HRUM. All patients had been diagnosed and treated during the first 12 months since onset of their disease. The inclusion criteria were as follows: RA according to the 2010 classification criteria of the American College of Rheumatology/European League Against

Rheumatism (17); diagnosis made between 2007 and 2011; age>16 years; and prospective follow-up with at least 2 annual DAS28 determinations. Patients were recruited between September 2016 and May 2018.We excluded patients with any inflammatory, rheumatic, or autoimmune disease other than RA (except for secondary Sjögren syndrome), a diagnosis of DM or impaired glucose tolerance (American Diabetes Association 2010 criteria) (18), active infection, pregnancy, current or previous treatment with oral antidiabetic agents or insulin, and new treatments or changes in dose during the 3 months preceding the date of inclusion.

The control group was made up of healthy controls selected from among those who attended a health center in the same geographic area. All controls fulfilled all of the inclusion criteria and none of the exclusion criteria. The controls were matched with the cases for age, sex, race, and BMI. According to BMI, each control was taken from the same group of the WHO classification for each RA patient (normal range:19–24.9 kg/m2; overweight :25–29.9 kg/m2 and obesity: ≥ 30 kg/m2).

Patient and Public Involvement

Patients and the public did not contribute to study design, execution, and participants were not involved in publishing the results. The results of the study will be provided to patients upon request and the conclusions will be reported in publications and meetings.

También se debe agradecer a los asesores de pacientes en la declaración de contribución / agradecimientos.

Protocol

Cohort patients are followed and treated according to a pre-established protocol and managed with treat-to-target strategies following clinical practice guidelines for RA in Spain (GUIPCAR 2017) (19). All subjects were interviewed and examined by a rheumatologist on the study index date. Samples were collected between 9:00 and 10:00 AM after 12-16 hours of fasting. In order to detect impaired glucose tolerance, subjects with baseline blood glucose levels <126 mg/dl underwent an oral glucose tolerance test (OGTT)

Variables and definitions

The outcome measures were IR and insulin sensitivity. IR was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) (11) and defined as a HOMA-IR score>2.29, based on the 90th percentile for healthy persons (20) and using the homeostasis model assessment for β-cell function (HOMA-β) (11). Sensitivity to insulin was estimated using the quantitative insulin sensitivity check index (QUICKI), with a threshold value of 0.337 (μU*mmol/ml*l) (21).

On the index date, we recorded epidemiological variables, comorbidities, traditional cardiovascular risk factors, diet, physical activity, anthropometric data, and BMI.

Arterial hypertension was defined as an arterial pressure ≥140/90 mmHg or current treatment with antihypertensive medication (22). Glucose and metabolic disorders and DM were diagnosed based on the recommendations of the American Diabetes Association 2010 (23). Dyslipidemia and metabolic syndrome were defined in accordance with the guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) (24). Levels of total cholesterol, triglycerides, HDL,

and LDL were evaluated using enzymatic methods (25). Levels of oxidized anti-LDL and serum insulin were determined using enzyme-linked chemiluminescence assay.

Physical activity was measured using the International Physical Activity Questionnaire, taking into account physical activity (low, <600 metabolic equivalents of task [METs]; moderate, 600-1500 METs; and high>1500 METs) (26, 27). Sedentary lifestyle was considered less than 600 METs.

Adherence to a Mediterranean diet was evaluated using a validated questionnaire. Adherence was defined as a score of ≥ 9 out of 14 (28).

Anthropometric data included BMI (kg/m^2) and percentage of obese patients (29), waist circumference (cm), hip circumference (cm), and the waist-hip index (30). Body composition was measured using dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy enCORE^{TM} 2006) and included total mass (kg), fat mass (g), lean mass (g), and lean mass and android and gynoid fat mass. The fat mass index (FMI) was defined as fat mass (kg)/height squared (m^2) and fat-free mass index (FFMI) as fat-free mass (kg)/height squared (m^2). The values of fat mass and fat-free mass were obtained using DXA (31).

Clinical data comprised rheumatoid factor, which was positive if>20 IU/ml, and anticitrullinated protein antibody, which was positive if>10 IU/ml. Cumulative inflammatory burden was assessed using DAS28 score with C-reactive protein (PCR) level (range 0-9.4) (32) recorded at each visit throughout follow-up. High activity was defined as DAS28-CRP>5.1, moderate activity as 3.2-5.1, low activity as 2.6-3.2, and remission as ≤2.6. We also took into account severity variables such as the presence of erosions and the mean Health Assessment Questionnaire (HAQ) score throughout the course of the disease (33). Treatment was with synthetic disease-modifying anti-inflammatory drugs (DMARDs) and biological DMARDs. The laboratory values

measured in all patients were as follows: serum high-sensitivity CRP, tumor necrosis factor alfa (TNF-α), interleukin (IL) 6, IL-1ß, adiponectin, resistin, leptin, and insulin-like growth factor (IGF) 1. The laboratory kits used and their reference values are shown in the supplementary material.

Statistical analysis

Variables are expressed as mean (SD), median (IQR), or number (%). Comparisons between groups were performed using the χ^2, *t*, or Mann-Whitney test depending on the normality of the distribution. Binary logistic regression analysis was performed (dependent variable: IR measured using HOMA-IR). Multicollinearity of independent variables was verified using the Pearson correlation coefficient (r>0.4). Sample size was calculated assuming a prevalence of IR in RA of 51% and considering as relevant a 30% difference with respect to controls. With a 2-sidedαerror of 0.05 and a β error of 0.20, the necessary sample size would be 77 subjects in each group (34). Sample size was increased by 10% to account for possible losses. The analysis was performed using R Commander 2.3-0.

RESULTS

The initial study population comprised 100 patients and 100 controls. However, only 89 patients with RA and 80 healthy controls fulfilled the inclusion criteria and none of the exclusion criteria. The study flow chart is shown in Figure 1.

Epidemiological and anthropometric characteristics and comorbidities

Table 1 shows the baseline characteristics of patients and controls. Mean age was slightly over 56 years, and most subjects were women (75%). Patients with RA more frequently had a family history of cardiovascular disease than controls, and 10% more were ex-smokers.

Clinical and analytical variables associated with RA

Autoantibodies were only detected in patients, except for 1 subject in the control group, who was positive for rheumatoid factor, with a low titer and no other data indicating inflammatory disease. On the index date, most patients were in remission or had low arthritis-related inflammatory activity and had maintained an average DAS28-CRP<3.2 throughout follow-up (Table 1). All patients had received DMARDs. A total of 78/89 RA-patients (87.6%) were using synthetic DMARDs and 35/89 RA-patients (39.3%) were using biologic DMARDs and 14% were using glucocorticoids.

While the rate of adherence to a Mediterranean diet was similar in both groups (62.9% vs 57.5%; p=0.472), healthy subjects more often engaged in physical activity than patients (median [IQR], 612.0 [313.5-1089.0] METS vs 339.0 [198.0-792.0] METS; p=0.005).

Both patients and controls generally had similar baseline characteristics with respect to carbohydrate metabolism (i.e., resistance and sensitivity to insulin, glycemia, and baseline insulinemia). While patients had slightly higher blood sugar levels after OGTT, the difference was not clinically relevant. As for lipids, patients with RA had slightly lower levels of total cholesterol and LDL-cholesterol than controls (table 2).

Inflammatory cytokines and adipokines

Disease was controlled in most patients with RA. However, RA patients had higher levels of proinflammatory cytokines (i.e., TNF-α, IL-6, and IL-1β) than controls. Similarly, IGF-1 levels tended to be higher in patients. The only adipokine that was elevated in patients was adiponectin. Resistin and leptin remained similar in both groups (table 2).

Characteristics of patients with RA and IR

Of the 89 patients with RA, 25(28.1%) had a HOMA-IR ≥2.29. Table 3 shows the characteristics of patients with RA with and without IR. As we can see, the

epidemiological characteristics of patients with IR were similar to those of the others, although clinical control of their disease was worse on the index date (DAS28-CRP >3.2; 68% vs 28%; p=0.001) and throughout follow-up (mean DAS28-CRP, 3.5 [0.7] vs. 2.9 [0.7]; p=0.001). It is also important to note that patients with IR had higher values for BMI, weight, and body composition and were more often obese. They also had a higher percentage of fat and a higher waist-hip index. However, no differences were found between the groups with respect to the delay in diagnosis of RA, duration of symptoms, antibodies and synthetic DMARDs agents and biologic DMARD agents or glucocorticoids (Table 3).

There was no association between mediterranean diet in patients with and without IR (52.9% vs 65.3%; p = 0.472). Likewise, the physical activity was similar in both groups (median [IQR], 445.5 [165.0-800.0] METS vs 330.0 [198.0-700.0] METS; p=0.834).

With respect to inflammatory cytokines and adipokines, concentrations of IL-1β and leptin were clearly higher in patients with IR. No differences were found between the groups in concentrations of TNF-αand IL-6 or in levels of resistin and adiponectin.

Factors associated with insulin resistance in rheumatoid arthritis

Table 4 shows the 2 best multivariate models. In the first, obesity, mean DAS28, and IL-1ß were significantly associated with IR; these factors account for 35% of variability in the presence of IR (R^2=0.352). In the second model, fat mass, mean DAS28, and IL-1ßwere associated with IR; these factors accounted for 40% of the variability in IR (R^2=0.404).

DISCUSSION

Among RA patients, there is a high prevalence of comorbidities and cardiovascular risk factors as we can see in the International COMORA study (35). Given that RA is

currently considered as potent a cardiovascular risk factor as type 2 DM, some risk equations take it into account (36). Although cardiovascular risk may be mediated in part by IR (9, 17-25), our study did not reveal a higher prevalence of IR in patients with RA, because disease was well controlled in patients with a cumulative mean DAS28 <3.2 from onset. This finding is consistent with data from other authors who observed a reduction in RI associated with control of the inflammatory activity induced by methotrexate and anti-TNF α agents (37). However, these results contrast with those published elsewhere, owing to methodological differences, especially in the measurement of inflammation and matching of controls (5, 14). Our study, in contrast, was based on inflammation data obtained from patients with established RA followed prospectively since onset. We also included controls matched for age, sex, race, and BMI, and the amount of exercise and adherence to the Mediterranean diet were taken into account.

While no differences were found between cases and controls, our results support an association between IR and chronic inflammation, as confirmed elsewhere (14, 16, 37, 38, 39, 40). Our group studied baseline IR in patients with untreated early-onset RA and found an association between IR and time with untreated symptoms and fat mass percentage (16). Both these aspects are important in the tight and early control of arthritis. In fact, when we analyze the determinants of IR in patients with RA, we again find a higher risk in patients with poor control of their disease during its course, higher levels of IL-1β, and more pronounced obesity. While the cytokines TNF-α, IL-6, and IL-1β are abundant in patients with active RA and reflect inflammatory status and their involvement in the insulin resistance has been suggested (41), the first two may not play as important a role in the present study, because the vast majority of patients treated with biologics received anti-TNF-α agents or tocilizumab. However, in addition to

treatment, other aspects associated with IL-1β may have an effect. Although the role of IL-1β has recently been questioned (42), it has been thought to play a role in the pathogenesis of type 2 DM (43) These authors indicate that high blood sugar levels could induce more marked production of IL-1β and TNF-α in macrophages and that this in turn could lead to a greater rate of apoptosis of β cells that would eventually lead to impairment of pancreatic function (43). In this sense, there are studies, where a greater production of IL-1β has been observed in patients affected by RA and type 2 DM through the activation of the NLRP3 inflammasome, it may suggest a potential therapy directed at IL-1β in these patients (44, 45). In relation to this, there have been reports of cases of diabetic patients with RA whose arthritis has remitted and whose metabolism has been controlled with IL-1β blockers (46). Furthermore, in this context, a specifically designed clinical trial has been recently published, investigating IL-1 inhibition as a "bidirectional" therapy in patients with RA and type 2 DM**.** In this study, Ruscitti et al, (47) observed an apparent benefit of IL-1 inhibition in participants with RA and type 2 DM, reaching the therapeutic targets of both diseases. suggesting the concept that IL-1 inhibition may be considered a targeted treatment for RA and type 2 DM.

On the other hand, although in our study no differences were found between RA patients with or without IR regarding the use of DMARDs (synthetic and biological), in other work were observed that the probabilities of using bDMARD decrease by 11% for each additional chronic morbid condition (48). This may be due to high number of chronic conditions of these patients compared to our cohort.

Our results showed that patients and controls did not differ overall in BMI or in body composition. While this observation can be explained in part by matching for BMI, disease control with biologic DMARDs is associated with recovery of total appendicular lean mass, with no changes in fat distribution (49).Nevertheless, despite

treat-to-target strategies, as applied in our study, patients with RA experience a relative loss of muscle mass and an increase in adiposity (50), which, in our study, was more evident in those with poorer control of inflammation and more pronounced IR. Obesity is more frequent in patients with RA and is closely associated with IR (51, 52), probably owing to physical inactivity, sarcopenia, and therapy with corticosteroids. Obese patients with RA in the present study had a 6-fold greater risk of IR than the other patients; this probability was mediated mainly by fat mass, since the risk increased by 10% for each kilogram of excess body fat. These data support those found in other studies, which highlight the fact that inflammation and obesity are closely linked, because adipose tissue produces TNF-α and IL-6 (16, 52).

In addition to proinflammatory cytokines, adipokines produced by fatty tissue may affect glucose homeostasis, appetite, and the inflammatory response (53). We found that adiponectin levels were higher in patients than in controls. While results were sometimes contradictory, it seems that adiponectin could increase in patients with RA as a means of offsetting the proinflammatory effects of high levels of leptin or of TNF-αand IL-6 (53). This increase may also be a result of the effect of treatment with DMARDs (54).

Leptin levels (systemic, local, or both) have been reported to increase in various inflammatory diseases (53). However, we only observed an increase in patients with RA and IR. Consistent with some studies, this could be because these patients had a greater BMI and a higher grade of chronic inflammation (55).

Furthermore, our data show an increase in LDL oxidase and a decrease in IGF-1 that tended toward significance in patients with RA compared with healthy controls. The same finding was observed in other studies in which these parameters were associated with increased cardiovascular risk in RA (56, 57).

Our study is limited mainly due to the cross-sectional evaluation of IR between patients with RA and healthy subjects. However, these patients came from a prospective RA inception cohort in which we longitudinally collected all inflammation-related variables analysed using a predesigned protocol. Consequently, no data were missing, and our results are consistent. On the other hand, the use of HOMA-IR instead of the hyperinsulinemic euglycemic clamp method may seem as a limitation, but indirect methods such as HOMA and QUICKI have been validated for use. They are reliable indices and clamp substitutes for measuring IR in epidemiological studies, clinical trials, and clinical practice. Among the strengths, we performed OGTT, the only technique that allow to recognize the presence of impaired glucose tolerance. In addition, another strength is that the control group was matched with the cases not only for age and sex, but also for BMI.

In conclusion, our results show that the main factors associated with IR are obesity, specifically total fat mass, and disease activity, specifically levels of IL-1β and cumulative inflammatory burden, measured based on average DAS28-CRP levels throughout follow-up. Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis. However, controlled prospective studies must continue to be performed in order to better observe the possible causal relationship between clinical and metabolic factors and IR and atherosclerosis in RA.

Figure 1. Patients and controls´ flow chart

No additional data available.

Author contributions statement

SMA contributed including patients, writing the manuscript and contributed analysing and interpreting the patient data. NMV contributed writing the manuscript, and analysing and interpreting the patient data. IUG was a major contributor in including patients. JR was a major contributor in performing laboratory determination, and contributed interpreting laboratory data. PV contributed was a contributor in interpreting the patient data and writing the manuscript. LGM was a major contributor in including controls. SAS was a major contributor in including controls. FGJN contributed including patients. BOM: contributed performing laboratory determination and interpreting laboratory data. AFN contributed writing the manuscript, and analysing and interpreting the patient data. All authors read and approved the final manuscript.

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Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information.

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REFERENCES

1. Goodson N, Marks J, Lunt M, Symmons D. Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s. Ann Rheum Dis. 2005;64(11):1595-601.

2. Espino-Lorenzo P, Manrique-Arija S, Urena I, Jimenez-Nunez FG, Lopez-Lasanta M, Romero-Barco CM, et al. Baseline comorbidities in patients with rheumatoid arthritis who have been prescribed biological therapy: a case control study. Reumatologia clinica. 2013;9(1):18-23.

3. Peters MJ, van Halm VP, Voskuyl AE, Smulders YM, Boers M, Lems WF, et al. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis and rheumatism. 2009;61(11):1571-9.

4. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003;108(24):2957-63.

5. La Montagna G, Cacciapuoti F, Buono R, Manzella D, Mennillo GA, Arciello A, et al. Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis. Diab Vasc Dis Res. 2007;4(2):130-5.

6. Grundy SM. What is the contribution of obesity to the metabolic syndrome? Endocrinology and metabolism clinics of North America. 2004;33(2):267-82.

7. Park K, Steffes M, Lee DH, Himes JH, Jacobs DR, Jr. Association of inflammation with worsening HOMA-insulin resistance. Diabetologia. 2009;52(11):2337-44.

8. Meng YX, Ford ES, Li C, Quarshie A, Al-Mahmoud AM, Giles W, et al. Association of C-reactive protein with surrogate measures of insulin resistance among nondiabetic US from National Health and Nutrition Examination Survey 1999-2002. Clinical chemistry. 2007;53(12):2152-9.

9. Li G, Barrett EJ, Barrett MO, Cao W, Liu Z. Tumor necrosis factor-alpha induces insulin resistance in endothelial cells via a p38 mitogen-activated protein kinase-dependent pathway. Endocrinology. 2007;148(7):3356-63.

10. El-Hini SH, Mohamed FI, Hassan AA, Ali F, Mahmoud A, Ibraheem HM. Visfatin and adiponectin as novel markers for evaluation of metabolic disturbance in recently diagnosed rheumatoid arthritis patients. Rheumatology international. 2013;33(9):2283-9.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

12. Dessein PH, Stanwix AE, Joffe BI. Cardiovascular risk in rheumatoid arthritis versus osteoarthritis: acute phase response related decreased insulin sensitivity and high-density lipoprotein cholesterol as well as clustering of metabolic syndrome features in rheumatoid arthritis. Arthritis research. 2002;4(5):R5.

13. Rosenvinge A, Krogh-Madsen R, Baslund B, Pedersen BK. Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNFalpha therapy. Scand J Rheumatol. 2007; 36:91-6.

14. Shahin D, Eltoraby E, Mesbah A, Houssen M. Insulin resistance in early untreated rheumatoid arthritis patients. Clin Biochem. 2010;43(7-8):661-5.

15. Castaneda S, Martin-Martinez MA, Gonzalez-Juanatey C, Llorca J, Garcia-Yebenes MJ, Perez-Vicente S, et al. Cardiovascular morbidity and associated risk factors in Spanish patients with chronic inflammatory rheumatic diseases attending rheumatology clinics: Baseline data of the CARMA Project. Semin Arthritis Rheum. 2015;44(6):618-26.

16. Manrique-Arija S, Urena I, Valdivielso P, Rioja J, Jimenez-Nunez FG, Irigoyen MV, et al. Insulin resistance and levels of adipokines in patients with untreated early rheumatoid arthritis. Clin Rheumatol. 2016;35(1):43-53.

17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

18. Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. Diabetes Care. 1998;21(2):310-4.

19. Sociedad Española de Reumatología. GUIPCAR actualización 2011: Actualizaciónde la guía de práctica clínica para el manejo de la artritis reumatoide en ESPAÑA. [[monografía en internet]]. Madrid: Sociedad Española de Reumatología; 2007 [updated [30 diaciembre 2011]].

20. Rojo-Martinez G, Esteva I, de Adana SR, Catala M, Merelo MJ, Tinahones F, et al. Patterns of insulin resistance in the general population of southeast Spain. Diabetes Res Clin Pract. 2004;65(3):247-56.

21. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402-10.

22. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. ESH/ESC 2007 Guidelines for the management of arterial hypertension. Rev Esp Cardiol.2007; 60:68e1-94.

23. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 (Suppl 1):S62-9.

24. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001: 285:2486-97.

25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

26. Guidelines for Data Processing andAnalysis of the International Physical Activity Questionnaire (IPAQ). 2009.

27. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.

28. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279-90.

29. WHO. Obesity Preventing and managing the global epidemic. Geneva: WHO; 1997.

30. Anonymous World Health Organization Expert Committee. Physical status: the use and interpretation of anthropometry. . WHO; Genova: Technical Report Series; 1995.

31. Taylor AE, Kuper H, Varma RD, Wells JC, Bell JD, K VR, et al. Validation of dual energy X-ray absorptiometry measures of abdominal fat by comparison with magnetic resonance imaging in an Indian population. PLoS One. 2012;7(12):e51042.

32. Iwamoto N, Kawakami A, Fujikawa K, Aramaki T, Kawashiri SY, Tamai M, et al. Prediction of DAS28-ESR remission at 6 months by baseline variables in patients with rheumatoid arthritis treated with etanercept in Japanese population. Mod Rheumatol. 2009;19(5):488-92.

33. Moyano S, Scolnik M, Vergara F, Garcia MV, Sabelli MR, Rosa JE, et al. Evaluation of Learned Helplessness, Perceived Self-efficacy, and Functional Capacity in Patients With Fibromyalgia and Rheumatoid Arthritis. J Clin Rheumatol. 2018.

34. Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, Shintani A, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis. 2008;196(2):756-63.

35. Dougados M, Soubrier M, Antunez A, Balint P, Balsa A, Buch MH, et al. Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). Ann Rheum Dis. 2014;73(1):62-8.

36. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. Bmj. 2017;357:j2099.

37. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2006;24(1):83-6.

38. Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, De Pergola G. C-reactive protein is independently associated with total body fat, central fat,

and insulin resistance in adult women. Int J Obes Relat Metab Disord. 2001;25(10):1416-20.

39. Crepaldi G, Scirè CA, Carrara G, Sakellariou G, Caporali R, Hmamouchi I, et al. Cardiovascular Comorbidities Relate More than Others with Disease Activity in Rheumatoid Arthritis. PLoS One. 2016;11(1):e0146991.

40. Ruscitti P, Ursini F, Cipriani P, Liakouli V, Carubbi F, Berardicurti O, et al. Poor clinical response in rheumatoid arthritis is the main risk factor for diabetes development in the short-term: A 1-year, single-centre, longitudinal study. PLoS One. 2017;12(7):e0181203.

41. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol. 2009;5(6):305-11.

42. Everett BM, Donath MY, Pradhan AD, Thuren T, Pais P, Nicolau JC, et al. Anti-Inflammatory Therapy With Canakinumab for the Prevention and Management of Diabetes. J Am Coll Cardiol. 2018;71(21):2392-401.

43. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2):98-107.

44. Fève B, Bastard JP.The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nature reviews Endocrinology. 2009;5(6).

45. Ruscitti P, Cipriani P, Di Benedetto P, Liakouli V, Berardicurti O, Carubbi F, et al. Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1β via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3(NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. Clin Exp Immunol. 2015;182(1):35-44.

46. Ruscitti P, Cipriani P, Cantarini L, Liakouli V, Vitale A, Carubbi F, et al. Efficacy of inhibition of IL-1 in patients with rheumatoid arthritis and type 2 diabetes mellitus: two case reports and review of the literature. J Med Case Rep. 2015;9:123.

47. Ruscitti P, Masedu F, Alvaro S, Airò P, Battafarano N, Cantarini L, et al. Anti-interleukin-1 treatment in patients with rheumatoid arthritis and type 2 diabetes

(TRACK): A multicentre, open-label, randomised controlled trial. PLoS Med. 2019;16(9):e1002901.

48. Radner H, Yoshida K, Hmamouchi I, Dougados M, Smolen JS, Solomon DH. Treatment Patterns of Multimorbid Patients with Rheumatoid Arthritis: Results from an International Cross-sectional Study. J Rheumatol. 2015;42(7):1099-104.

49. Tournadre A, Pereira B, Dutheil F, Giraud C, Courteix D, Sapin V, et al. Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. J Cachexia Sarcopenia Muscle. 2017;8(4):639-46.

50. Lemmey AB, Wilkinson TJ, Clayton RJ, Sheikh F, Whale J, Jones HS, et al. Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients. Rheumatology (Oxford). 2016;55(10):1736-45.

51. Muller R, Kull M, Lember M, Polluste K, Valner A, Kallikorm R. Insulin Resistance in Early Rheumatoid Arthritis Is Associated with Low Appendicular Lean Mass. Biomed Res Int. 2017;2017:9584720.

52. Castillo-Hernandez J, Maldonado-Cervantes MI, Reyes JP, Patino-Marin N, Maldonado-Cervantes E, Solorzano-Rodriguez C, et al. Obesity is the main determinant of insulin resistance more than the circulating pro-inflammatory cytokines levels in rheumatoid arthritis patients. Rev Bras Reumatol Engl Ed. 2017;57(4):320-9.

53. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gomez-Reino JJ, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis. 2006;65(9):1198-201.

54. Cansu B, Cansu DU, Kasifoglu T, Gulbas Z, Korkmaz C. Disease-modifying antirheumatic drugs increase serum adiponectin levels in patients with rheumatoid arthritis. J Clin Rheumatol. 2011;17(1):14-7.

55. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis. 2005;64(8):1195-8.

56. Erlandsson MC, Lyngfelt L, Aberg ND, Wasen C, Espino RA, Silfversward ST, et al. Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis. BMC Med. 2019;17(1):141.

57. Nowak B, Madej M, Luczak A, Malecki R, Wiland P. Disease Activity, Oxidized-LDL Fraction and Anti-Oxidized LDL Antibodies Influence Cardiovascular Risk in Rheumatoid Arthritis. Adv Clin Exp Med. 2016;25(1):43-50.

Tables

Table 1. Baseline characteristics of cases and controls

Variables	RA patients n=89	Controls n=80	p value
Epidemiological characteristics			
Age, years, mean (SD)	56.6 (10.9)	56.4 (10.9)	0.902
Female sex, n (%)	67 (75.3)	67 (83.8)	0.189
Smoking status			0.117
Never smoked, n (%)	42 (47.2)	49 (61.3)	
Ex-smoker, n (%)	22 (24.7)	11 (13.8)	
Smoker, n (%)	25 (28.1)	20 (25.0)	
Clinical-laboratory characteristics			
Time since onset of RA, months, median (IQR)	98.0 (78.5-123.5)	-	-
Diagnostic delay, months, median (IQR)	10.9 (5.4 - 25.6)	-	-
Rheumatoid factor>10U/mL, n (%)	73 (82.0)	1 (1.3)	<0.001
ACPA >20U/mL, n (%)	67 (75.3)	0 (0.0)	<0.001
C-reactive protein (mg/dl), median (IQR)	2.9 (2.9-3.4)		
Erythrocyte sedimentation rate (mm/h), median (IQR)	11 (6.2-14)	11.5 (7.8-21.3)	0.088

Nº of swollen joints (0-28), median (IQR)	0 (0-1)	ND	ND
Nº of painful joints (0-28), median (IQR)	1 (0-2)	ND	ND
VAS patient global (1-100 mm), median (IQR)	30 (20-50)	ND	ND
DAS28PCR on the index date, mean (SD)	2.83 (1.1)	ND	ND
Remission-low activity, n (%)	63 (71.0)	ND	ND
Moderate-high activity, n (%)	26 (29.0)	ND	ND
Mean DAS28PCR during follow-up, mean (SD)	3.11 (0.8)	ND	ND
Remission-low activity, n (%)	56 (63.0)	ND	ND
Moderate-high activity, n (%)	33 (37.0)	ND	ND
Mean HAQ during follow-up, median (IQR)	0.750 (0.0-1.1)	ND	ND
Synthetic DMARDs, n (%)	78 (87.6)	0 (0.0)	<0.001
Biologic DMARDs, n (%)	35 (39.3)	0 (0.0)	<0.001
Combined DMARDs, n (%)	25 (28.1)	0 (0.0)	<0.001
Methotrexate, n (%)	21 (84.0)		
Leflunomide, n (%)	3 (12.0)		
Sulfasalazine, n (%)	1 (4.0)		

RA, rheumatoid arthritis; ACPA, Anticitrullinated protein antibodies; DAS28,28-joint Disease Activity Score; HAQ, Health Assessment Questionnaire; ND,no data; DMARD, disease-modifying antirheumatic drug.

Table 2. Anthropometric and metabolic characteristics, inflammatory cytokines, and adipokines.

Variables	RA patients n=89	Controls n=80	p value
Anthropometric characteristics			
BMI (kg/m^2), mean (SD)	28.2 (5.0)	27.3 (4.9)	0.266
Waist circumference, (cm), mean (SD)	90.8 (11.5)	89.3 (11.3)	0.419
Hip circumference (cm), mean (SD)	103.1 (7.3)	100.5 (9.9)	0.081
Waist-hip index, mean (SD)	0.88 (0.0)	0.89 (0.0)	0.517
Body composition by DXA, mean (SD)			
Total fat mass (kg), mean (SD)	29.2(9.7)	28.5 (9.7)	0.671
FMI (kg/m^2), mean (SD)	11.3 (4.0)	11.1 (4.0)	0.144
Total lean mass (kg), mean (SD)	40.8 (8.9)	39.0 (8.6)	0.197
FFMI (kg/m^2), mean (SD)	15.6 (2.4)	15.0 (2.4)	0.726
Total mass (kg), mean (SD)	71.8 (15.5)	69.9 (14.4)	0.415
Android fat mass (kg), mean (SD)	2.5 (1.1)	2.4 (1.1)	0.671
Gynoid fat mass (kg), mean (SD)	5.1 (1.6)	5.0 (1.5)	0.785
Android lean mass (kg), mean (SD)	2.9 (0.7)	2.8 (0.7)	0.433
Gynoid lean mass (kg), mean (SD)	5.8 (1.2)	5.7 (1.2)	0.639
Metabolic characteristics			
Total cholesterol, median (IQR)	194.0 (170.5-223.6)	209.0 (188.0-238.0)	0.031
LDL cholesterol (mg/dl), median (IQR)	110.0 (95.9-137.5)	133.0 (106.0-151.0)	0.004
HDL cholesterol (mg/dl), median (IQR)	58.0 (51.0-66.0)	59.0 (51.0-72.0)	0.377
Triglycerides (mg/dl), median (IQR)	87.0 (69.0-131.0)	90.0 (66.0- 120.0)	0.102
LDL OX, median (IQR)	2.6 (0.8-5.6)	1.0 (0.3-2.8)	0.114
Baseline glycemia (mg/dl), median (IQR)	78.0 (73.0-84.5)	80.0 (72.0-87.0)	0.327
Glycemia after OGTT (mg/dl), mean (SD)	110.0 (28.9)	100.5(26.6)	0.030
Insulinemia µU/ml, median (IQR)	9.3 (6.0-12.4)	8.6 (6.0. 12.6)	0.904

HOMA-IR, median (IQR)	1.7 (1.02-2.3)	1.7 (1.15-2.7)	0.385
HOMA-IR ≥2.29, n (%)	25 (28.1)	24(30.0)	0.785
HOMA-β, median (IQR)	38.9 (22.7-53.3)	34.4 (24.0-54.9)	0.545
QUICKI, median (IQR)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.211
QUICKI ≤0.33, n (%)	25.0 (28.1)	24.0 (30.0)	0.785
Inflammatory cytokines and adipokines			
IL-6 (pg/ml), median (IQR)	11.0 (5.4-19.0)	4.31 (3.08-6.67)	<0.001
IL-1ß (pg/ml), median (IQR)	4.33 (4.2-4.5)	2.74 (2.64-3.50)	<0.001
TNF - α (pg/ml), median (IQR)	5.8 (3.7-24.7)	3.6 (3.0-4.7)	<0.001
IGF-1(pg/ml), median (IQR)	172.8 (104.7-238.9)	130.6 (49.8-252.47)	0.079
Adiponectin (ng/ml), median (IQR)	11399.5 (7771.1-14971.5)	8581.4 (6524.1-12688.9)	0.014
Resistin (ng/ml), median (IQR)	7.2 (5.5-9.3)	7.4 (5.7-9.7)	0.510
Leptin (ng/ml), median (IQR)	16.9 (9.1-36.9)	22.2 (9.6-38.8)	0.432

RA, rheumatoid arthritis; NCEP ATIII, National Cholesterol Education Program Adult Treatment Panel III; BMI, body mass index; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX , Oxidized low-density lipoprotein; OGTT, oral glucose tolerance test; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-ß, Homeostatic Model Assessment for ß-Cell Function; QUICKI, Quantitative Insulin Sensitivity Check Index; IL-6, interleukin 6; TNF-α, tumor necrosis factor α;IL-1ß, interleukin 1 ß;IGF-1, insulin-like growth factor-1.

Table3. Characteristics of patients with and without IR measured using HOMA-IR.

Variables	**RA HOMA-IR≥2.29 n=25**	**RA HOMA-IR<2.29 n=64**	**p value**
Age, years, mean (SD)	58.2 (8.3)	56.0 (11.7)	0.329
Female sex, n (%)	17 (68.0)	50 (78.1)	0.320
Clinical characteristics			

Time since onset of RA, months, median (IQR)	95.8 (81.8-111.6)	98.1 (77.5-125.5)	0.697
Diagnostic delay (months), median (IQR)	15.5 (4.6-27.4)	10.3 (5.4-23.7)	0.629
Arterial hypertension, n (%)	7 (28.0)	16 (25.0)	0.771
Dyslipidemia, n (%)	8 (32.0)	13 (20.3)	0.243
Rheumatoid factor, n (%)	20 (80.0)	53 (82.8)	0.756
ACPA, n (%)	17 (68.0)	49 (76.0)	0.407
DAS28PCR>3.2 on the index date, n (%)	17 (68.0)	18 (28.1)	**0.001**
Mean DAS28PCR during follow-up, mean (SD)	3.5 (0.7)	2.9 (0.7)	**0.001**
Mean HAQ during follow-up, mean (SD)	0.90 (0.6)	0.76 (0.8)	0.505
Synthetic DMARD, n (%)	22 (88.0)	49 (76.6)	0.227
Biologic DMARD, n (%)	10 (40.0)	19 (29.7)	0.351
Combined DMARD, n (%)	7 (28.0)	18 (28.1)	0.991
Anthropometric characteristics			
Weight, mean (SD)	82.6 (13.1)	69.5 (12.5)	**<0.001**
BMI, mean (SD)	31.9 (5.4)	26.8 (4.0)	**<0.001**
Normal weight, n (%)	1 (4.0)	27 (42.2)	
Overweight, n (%)	9 (36.0)	22 (34.4)	
Obesity, n (%)	15 (60.0)	15 (23.4)	
Waist circumference, mean (SD)	97.5 (8.9)	88.1 (11.4)	**0.001**
Hip circumference, mean (SD)	106.6 (7.0)	101.7 (7.0)	**0.005**
Waist-hip index, mean (SD)	0.91(0.1)	0.86 (0.1)	**0.022**
Body composition measured by DEXA			
Total fat mass (kg), mean (SD)	35.5 (2.2)	26.7 (7.8)	**0.001**
FMI (kg/m^2), mean (SD)	13.8 (4.7)	10.4 (3.2)	**<0.001**
Total lean mass (kg), mean (SD)	43.8 (7.7)	39.7 (9.1)	**0.051**
FFMI (kg/m^2), mean (SD)	16.8 (2.4)	15.1 (2.2)	**0.006**
Lean mass percentage,	0.53 (0.1)	0.58 (0.1)	0.051

mean (SD)			
Total mass (kg), mean (SD)	81.9(12.6)	67.9 (14.7)	**<0.001**
Android fat mass (kg), mean (SD)	34.6(0.7)	22.3 (9.1)	**<0.001**
Gynoid fat mass (kg), mean (SD)	59.5 (1.0)	47.9 (1.3)	**0.003**
Android lean mass (kg), mean (SD)	32.0 (0.5)	28.2 (0.7)	**0.014**
Gynoid lean mass (kg), mean (SD)	64.0 (1.0)	55.0 (1.2)	**0.003**
LDL OX, median (IQR)	2.7 (0.7-7.4)	2.5 (0.7-5.5)	0.868
Inflammatory cytokines and adipokines			
IL6 (pg/ml), median (IQR)	11.1 (7.3-20.8)	10.5 (4.9-19.8)	0.495
IL-1β (pg/ml), median (IQR)	4.9 (4.1-4.4)	4.3 (4.0 -4.5)	0.007
TNF-α (pg/ml), median (IQR)	5.1 (3.3-27.0)	5.8 (3.7-23.5)	0.827
IGF-1(pg/ml), mean (SD)	168.5 (93.8)	187.7 (106.6)	0.433
Adiponectin (ng/ml), median (IQR)	9271.8 (7602.0-12407.0)	12273.1 (8218.5-15677.1)	0.210
Resistin (ng/ml), median (IQR)	8.3 (5.6-9.9)	7.1 (5.5. - 9.0)	0.626
Leptin (ng/ml), median (IQR)	33.7 (18.0-55.2)	13.9 (7.9-26.1)	0.001

RA, rheumatoid arthritis; ACPA, Anticitrullinated protein antibodies; DAS28,28-joint Disease Activity Score; HAQ, Health Assessment Questionnaire; ND,no data; DMARD, disease-modifying antirheumatic drug; BMI, body mass index; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-densitylipoprotein; LDL-OX , Oxidized low-density lipoprotein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha;IL-1ß, interleukin 1ß;IGF-1, insulin-likegrowth factor-1.

Table 4. Multivariate models for RA patients with HOMA-IR >2,29 (dependent variable)

Multivariate model 1 using obesity covariate

Predictor	β	OR	95% CI	p value

Obesity (BMI>30)	1.795	6.01	1.94-8.66	p=0.002
Mean DAS28 CRP	1.021	2.77	1.29-5.99	p=0.009
Il-1β (pg/ml)	0.464	1.59	1.06-2.38	p=0.024

R^2 =0,352

Independent variables: Age, sex, obesity (BMI >30), mean 28-joint Disease Activity Index with C-reactive protein (DAS28 CRP), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

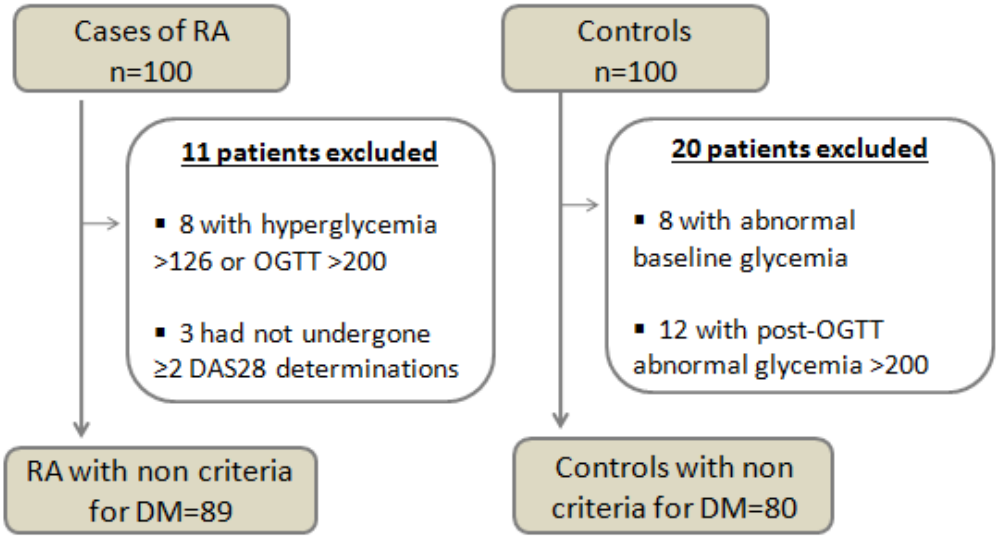
Multivariate model 2 using total fat mass covariate

Predictor	β	OR	95% CI	p value
Total Fat mass (kg)	0.123	1.10	1.05-1.22	p=0.002
Mean DAS28PCR	0.955	2.60	1.19-5.69	p=0.017
Il-1β (pg/ml)	0.456	1.57	1.06-2.34	p=0.023

R^2 =0,404

Independent variables: Age, sex, fat mass, lean mass, mean 28-joint Disease Activity Index (DAS28), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

Figure 1. Patients and controls´ flow chart



RA, Rheumatoid arthritis. OGTT, oral glucose tolerance test; DAS28, 28-joint Disease Activity Score. DM, Diabetes Mellitus.

Table S1. Laboratory tests.

Test	Method/Reference range
High-sensitivity C-reactive protein	Standard nephelometry (reference range, 0-6 mg/l
TNF-α	Automated immunoassay (Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA; reference range, 0-8.1 pg/ml)
IL-6	Enzyme-linked chemiluminescent assay (QuantiGlo®; normal range, 0-5.84 pg/ml).
IL-1ß	Enzyme-linked chemiluminescent assay (QuantiGlo®) pg/ml
Adiponectin	Enzyme-linked chemiluminescent assay (Mediagnost®) (mean [SD] reference value, 11.5 [5.9] ng/ml)
Resistin	Enzyme-linked chemiluminescent assay (Mediagnost®) (median [IQR] reference value, 7.2 [5.4-8.5] ng/ml)
Leptin	Enzyme-linked chemiluminescent assay (Mediagnost®).

	(according to the manufacturer, normal values lie between the 5th and 95th percentiles after adjusting for sex and BMI ng/ml)
IGF-1	Enzyme-linked chemiluminescent assay (Quantikine®)(pg/ml)
Serum insulin	Enzyme-amplified chemiluminescent assay (Immulite ONE®) (**µ**U/ml)
LDL-Ox	Enzyme-amplifiedchemiluminescent assay (Immulite ONE®)(U/l)

TNF-α, tumor necrosis factor alpha; IL, interleukin; IGF-1,insulin-like growth factor 1;LDL,low-densitylipoprotein; ELISA, enzyme-linked immunosorbent assay. LDL-Ox, Oxidized low-density lipoprotein

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8-9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	10-11
Bias	9	Describe any efforts to address potential sources of bias	11-12
Study size	10	Explain how the study size was arrived at	11-12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11-12
		(*b*) Describe any methods used to examine subgroups and interactions	11-12
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12 and figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-14
		(b) Indicate number of participants with missing data for each variable of interest	12 and figure 1

Outcome data	15*	Report numbers of outcome events or summary measures	12-13 and tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14 and table 4
		(*b*) Report category boundaries when continuous variables were categorized	12-14 and tables
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	26

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis: cross-sectional study

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TITLE PAGE

TITLE

Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis: cross-sectional study

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ABSTRACT

OBJECTIVES: To describe the prevalence of insulin resistance (IR) in patients with established rheumatoid arthritis (RA) and to analyze the contribution of cumulative inflammatory burden and other factors to its development.

METHODS:

Design Observational cross-sectional study.

P**articipants:** Patients with RA and controls matched for age, sex, and body mass index. We excluded patients with diabetes.

Settings: Patients from an RA inception cohort at Hospital Regional Universitario de Málaga (HRUM), Spain were recruited between September 2016 and May 2018.

Primary and secondary outcome measures: IR was evaluated using the homeostasis model assessment for IR and beta-cell function and the quantitative insulin sensitivity check index. Other variables included the cumulative 28-joint disease activity score (DAS28-CRP), body composition, and cytokines. Two logistic regression models were constructed to identify factors associated with IR in patients with RA.

RESULTS: Eighty-nine patients with RA and 80 controls were included. The prevalence of IR was similar in both cases and controls. Inflammatory activity was controlled appropriately in patients during follow-up (mean DAS28, 3.1 [0.8]). The presence of IR in patients with RA was associated with obesity (OR [95% CI], 6.01 [1.9-8.7]), higher cumulative DAS28-CRP values during follow-up (OR [95% CI], 2.8 [1.3-6.0]), and higher IL-1β levels (OR [95% CI], 1.6 [1.1 -2.4]). The second model showed that the risk of IR increased by 10% for each kilogram of excess body fat.

CONCLUSION: In patients with well-controlled, established RA, IR is associated mainly with poorer control of inflammation from diagnosis and with obesity, specifically total fat mass.

Word Count: 3314

KEYWORDS

Rheumatoid arthritis, Insulin resistance, Inflammation, Obesity.

Strengths and Limitations

- It is important to clarify the factors associated with insulin resistance (IR) in rheumatoid arthritis, because IR has been confirmed as a risk factor for cardiovascular diseases.
- The main factors associated with IR are obesity (specifically total fat mass) and disease activity (specifically increased levels of IL-1β and cumulative inflammatory burden).
- Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent synovitis, bone erosion, and functional disability. It is associated with premature death (1-3) and multiple morbidities (1-2), mainly because the cardiovascular risk of RA patients is similar to that of patients with type 2 diabetes mellitus (DM) (3). Accelerated atherosclerosis in patients with RA is due to the presence of traditional and nontraditional cardiovascular risk factors, including systemic inflammation, drugs, and IR (4-5).

In the general population, IR has been confirmed as a risk factor for cardiovascular diseases, DM, and metabolic syndrome (6). Its main determinant is obesity, although it has also been associated with older age, hypertension, and a sedentary lifestyle. Abundant data suggest a connection between IR and chronic inflammation (7-8). Adipose tissue produces proinflammatory cytokines and adipokines, including TNF-α, which reduce sensitivity to insulin and contribute to endothelial dysfunction (9). The relationship between adipokines, proinflammatory cytokines, and IR (10) is unclear in RA, although it could play a key role in the pathogenesis of accelerated atherosclerosis associated with chronic inflammatory states. Various studies have been specifically designed to investigate IR in RA (5,11-14). While most confirm an association between IR and inflammation, other factors continue to play a stronger role, such as abdominal obesity with sarcopenia, sedentary lifestyle, and drugs (15).

Almost all studies that examine the relationship between IR and RA were carried out in patients with chronic RA and numerous comorbid conditions associated with cardiovascular risk factors in case series not controlled for body mass index (BMI). Furthermore, as these studies have a cross-sectional design, they only take into account values for inflammation recorded at a particular point in time. We previously studied a

group of untreated patients with recent-onset RA and a control group matched for age, sex, and BMI. The patients were followed for 6 months (16). The results showed that IR was not present at diagnosis and did not appear after 6 months of treatment if the disease was well controlled. However, our results also showed that patients with higher fat mass and a longer diagnostic and therapeutic delay had the worst IR data. Based on these findings, the hypothesis of the present study was that IR in patients with RA, as with other determinants of disease, can be prevented in the long term with tight control of inflammation from onset.

The objectives of the present study were as follows: 1. To compare the prevalence of IR in an inception cohort of patients with RA and an equivalent group of healthy controls; and 2. To analyze the effect of IR on the cumulative inflammatory burden over at least 5 years, together with other possible factors that contribute to IR.

METHODS

We carried out an observational cross-sectional study of patients with RA. The study was performed at Instituto de Investigación Biomédica de Málaga (IBIMA) by the Department of Rheumatology of Hospital Regional Universitario de Málaga (HRUM), Malaga, Spain and approved by the Ethics Committee of HRUM. All participants signed an informed consent document.

Patients

We consecutively included patients from the RA inception cohort at HRUM. All patients had been diagnosed and treated during the first 12 months since onset of their disease. The inclusion criteria were as follows: RA according to the 2010 classification criteria of the American College of Rheumatology/European League Against

Rheumatism (17); diagnosis made between 2007 and 2011; age >16 years; and prospective follow-up with at least 2 annual DAS28 determinations. Patients were recruited between September 2016 and May 2018. We excluded patients with any inflammatory, rheumatic, or autoimmune disease other than RA (except for secondary Sjögren syndrome), a diagnosis of DM or impaired glucose tolerance (American Diabetes Association 2010 criteria) (18), active infection, pregnancy, current or previous treatment with oral antidiabetic agents or insulin, and new treatments or changes in dose during the 3 months preceding the date of inclusion.

The control group was made up of healthy controls selected from among those who attended a health center in the same geographic area. All controls fulfilled all of the inclusion criteria and none of the exclusion criteria. The controls were matched with the cases for age, sex, race, and BMI. According to BMI, each control was taken from the same group of the WHO classification for each RA patient (normal range:19–24.9 kg/m^2; overweight :25–29.9 kg/m^2 and obesity: $\geq$ 30 kg/m^2).

Patient and public involvement

Neither patients nor the public contributed to the study design or performance, and participants were not involved in the publication of the results. The results of the study will be provided to patients upon request, and the conclusions will be reported in publications and meetings.

Protocol

Cohort patients are followed and treated according to a pre-established protocol and managed with treat-to-target strategies following clinical practice guidelines for RA in Spain (GUIPCAR 2017) (19). All participants were interviewed and examined by a

rheumatologist on the study index date. Samples were collected between 9:00 and 10:00 AM after 12-16 hours of fasting. In order to detect impaired glucose tolerance, subjects with baseline blood glucose levels <126 mg/dl underwent an oral glucose tolerance test (OGTT).

Variables and definitions

The outcome measures were IR and insulin sensitivity. IR was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) (11) and defined as a HOMA-IR score >2.29, based on the 90th percentile for healthy persons (20) and using the homeostasis model assessment of β-cell function (HOMA-β) (11). Sensitivity to insulin was estimated using the quantitative insulin sensitivity check index (QUICKI), with a threshold value of 0.337 (μU*mmol/ml*l) (21).

On the index date, we recorded epidemiological variables, comorbidities, traditional cardiovascular risk factors, diet, physical activity, anthropometric data, and BMI.

Arterial hypertension was defined as an arterial pressure ≥140/90 mmHg or current treatment with antihypertensive medication (22). Glucose and metabolic disorders and DM were diagnosed based on the recommendations of the American Diabetes Association 2010 (23). Dyslipidemia and metabolic syndrome were defined in accordance with the guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) (24). Levels of total cholesterol, triglycerides, HDL, and LDL were evaluated using enzymatic methods (25). Levels of oxidized anti-LDL and serum insulin were determined using enzyme-linked chemiluminescence assay.

Physical activity was measured using the International Physical Activity Questionnaire, taking into account physical activity (low, <600 metabolic equivalents of task [METs]; moderate, 600-1500 METs; and high>1500 METs) (26, 27). Sedentary lifestyle was considered less than 600 METs.

Adherence to a Mediterranean diet was evaluated using a validated questionnaire. Adherence was defined as a score of ≥9 out of 14 (28).

Anthropometric data included BMI (kg/m^2) and percentage of obese patients (29), waist circumference (cm), hip circumference (cm), and the waist-hip index (30). Body composition was measured using dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy enCORE^{TM} 2006) and included total mass (kg), fat mass (g), lean mass (g), and lean mass and android and gynoid fat mass. The fat mass index (FMI) was defined as fat mass (kg)/height squared (m^2) and fat-free mass index (FFMI) as fat-free mass (kg)/height squared (m^2). The values of fat mass and fat-free mass were obtained using DXA (31).

Clinical data comprised rheumatoid factor, which was positive if >20 IU/ml, and anticitrullinated protein antibody, which was positive if >10 IU/ml. Cumulative inflammatory burden was assessed using DAS28 score with C-reactive protein (CRP) level (range 0-9.4) (32) recorded at each visit throughout follow-up. High activity was defined as DAS28-CRP >5.1, moderate activity as 3.2-5.1, low activity as 2.6-3.2, and remission as ≤2.6. We also took into account severity variables such as the presence of erosions and the mean Health Assessment Questionnaire (HAQ) score throughout the course of the disease (33). Treatment was with synthetic disease-modifying anti-inflammatory drugs (DMARDs) and biological DMARDs. The laboratory values measured in all patients were as follows: serum high-sensitivity CRP, tumor necrosis factor alfa (TNF-α), interleukin (IL) 6, IL-1ß, adiponectin, resistin, leptin, and insulin-

like growth factor (IGF) 1. The laboratory kits used and their reference values are shown in the supplementary material.

Statistical analysis

Variables are expressed as mean (SD), median (IQR), or number (%). Comparisons between groups were performed using the χ^2, *t*, or Mann-Whitney test depending on the normality of the distribution. Binary logistic regression analysis was performed (dependent variable: IR measured using HOMA-IR). Multicollinearity of independent variables was verified using the Pearson correlation coefficient (r>0.4). Sample size was calculated assuming a prevalence of IR in RA of 51% and considering as relevant a 30% difference with respect to controls. With a 2-sided α error of 0.05 and a β error of 0.20, the necessary sample size would be 77 participants per group (34). Sample size was increased by 10% to account for possible losses. The analysis was performed using R Commander 2.3-0.

RESULTS

The initial study population comprised 100 patients and 100 controls. However, only 89 patients with RA and 80 healthy controls fulfilled the inclusion criteria and none of the exclusion criteria. The study flow chart is shown in Figure 1.

Epidemiological and anthropometric characteristics and comorbidities

Table 1 shows the baseline characteristics of patients and controls. Mean age was slightly over 56 years, and most patients were women (75%). Patients with RA more frequently had a family history of cardiovascular disease than controls, and 10% more were ex-smokers.

Clinical and analytical variables associated with RA

Autoantibodies were only detected in patients, except for 1 participant in the control group, who was positive for rheumatoid factor, with a low titer and no other data indicating inflammatory disease. On the index date, most patients were in remission or had low arthritis-related inflammatory activity and had maintained an average DAS28-CRP <3.2 throughout follow-up (Table 1). All patients had received DMARDs. A total of 78/89 RA patients (87.6%) were using synthetic DMARDs, 35/89 (39.3%) were using biologic DMARDs, and 12/89 (13.5%) were using corticosteroids.

While the rate of adherence to a Mediterranean diet was similar in both groups (62.9% vs 57.5%; p=0.472), healthy participants more often engaged in physical activity than patients (median [IQR], 612.0 [313.5-1089.0] METS vs 339.0 [198.0-792.0] METS; p=0.005).

Both patients and controls generally had similar baseline characteristics with respect to carbohydrate metabolism (i.e., resistance and sensitivity to insulin, glycemia, and baseline insulinemia). While patients had slightly higher blood sugar levels after OGTT, the difference was not clinically relevant. As for lipids, patients with RA had slightly lower levels of total cholesterol and LDL-cholesterol than controls (table 2).

Inflammatory cytokines and adipokines

Disease was controlled in most patients with RA. However, RA patients had higher levels of proinflammatory cytokines (i.e., TNF-α, IL-6, and IL-1β) than controls. Similarly, IGF-1 levels tended to be higher in patients. The only adipokine that was elevated in patients was adiponectin. Resistin and leptin remained similar in both groups (table 2).

Characteristics of patients with RA and IR

Of the 89 patients with RA, 25 (28.1%) had a HOMA-IR ≥2.29. Table 3 shows the characteristics of patients with RA with and without IR. As we can see, the

epidemiological characteristics of patients with IR were similar to those of the others, although clinical control of their disease was worse on the index date (DAS28-CRP >3.2; 68% vs 28%; p=0.001) and throughout follow-up (mean DAS28-CRP, 3.5 [0.7] vs. 2.9 [0.7]; p=0.001). It is also important to note that patients with IR had higher values for BMI, weight, and body composition and were more often obese. They also had a higher percentage of fat and a higher waist-hip index. However, no differences were found between the groups with respect to the delay in diagnosis of RA, duration of symptoms, antibodies, and synthetic and biologic DMARD or corticosteroids (Table 3). There was no association between Mediterranean diet in patients with and without IR (52.9% vs 65.3%; p = 0.472). Likewise, physical activity was similar in both groups (median [IQR], 445.5 [165.0-800.0] METS vs 330.0 [198.0-700.0] METS; p=0.834).

With respect to inflammatory cytokines and adipokines, concentrations of IL-1β and leptin were clearly higher in patients with IR. No differences were found between the groups in concentrations of TNF-α and IL-6 or in levels of resistin and adiponectin.

Factors associated with insulin resistance in RA

Table 4 shows the 2 best multivariate models. In the first, obesity, mean DAS28, and IL-1ß were significantly associated with IR; these factors accounted for 35% of variability in the presence of IR (R^2=0.352). In the second model, fat mass, mean DAS28, and IL-1ß were associated with IR; these factors accounted for 40% of the variability in IR (R^2=0.404).

DISCUSSION

Among RA patients, there is a high prevalence of comorbidities and cardiovascular risk factors, as shown in the International COMORA study (35). Given that RA is currently considered as potent a cardiovascular risk factor as type 2 DM, some risk equations take

it into account (36). Although cardiovascular risk may be mediated in part by IR (9, 17-25), our study did not reveal a higher prevalence of IR in patients with RA, because the disease was well controlled in patients with a cumulative mean DAS28 <3.2 from onset. This finding is consistent with data from other authors who observed a reduction in IR associated with control of the inflammatory activity induced by methotrexate and anti-TNFα agents (37). However, these results contrast with those published elsewhere, owing to methodological differences, especially in the measurement of inflammation and matching of controls (5, 14). Our study, in contrast, was based on inflammation data obtained from patients with established RA followed prospectively since onset. We also included controls matched for age, sex, race, and BMI, and the amount of exercise and adherence to the Mediterranean diet were taken into account.

While no differences were found between cases and controls, our results support an association between IR and chronic inflammation, as confirmed elsewhere (14, 16, 37, 38, 39, 40). Our group studied baseline IR in patients with untreated early-onset RA and found an association between IR and time with untreated symptoms and fat mass percentage (16). Both these aspects are important for tight and early control of arthritis. In fact, when we analyze the determinants of IR in patients with RA, we again find a higher risk in patients with poor control of their disease during its course, higher levels of IL-1β, and more pronounced obesity. While the cytokines TNF-α, IL-6, and IL-1β are abundant in patients with active RA and reflect inflammatory status and their involvement in IR has been suggested (41), the first 2 may not play as important a role in the present study, because the vast majority of patients treated with biologics received anti-TNF-α agents or tocilizumab. However, in addition to treatment, other aspects associated with IL-1β may have an effect. Although the role of IL-1β has recently been questioned (42), it has been thought to play a role in the pathogenesis of

type 2 DM (43). These authors indicate that high blood sugar levels could induce more marked production of IL-1β and TNF-α in macrophages and that this in turn could lead to a greater rate of apoptosis of β cells that would eventually lead to impairment of pancreatic function (43). In this sense, some studies report greater production of IL-1β in patients affected by RA and type 2 DM as a result of activation of the NLRP3 inflammasome, thus suggesting a role for therapy targeting IL-1β in these patients (44, 45). Furthermore, there have been reports of cases of diabetic patients with RA whose arthritis has remitted and whose metabolism has been controlled with IL-1β blockers (46). In addition, in this context, Ruscitti et al (47) recently performed a specifically designed clinical trial to investigate inhibition of IL-1 as "bidirectional" therapy in patients with RA and type 2 DM. The authors observed an apparent benefit of this approach in participants with RA and type 2 DM. The fact that the therapeutic targets were reached in both diseases suggests that inhibition of IL-1 may be considered a therapeutic target for RA and type 2 DM.

On the other hand, while we found no differences between RA patients with or without IR regarding the use of DMARDs (synthetic and biological), some authors observed that the probabilities of using bDMARDs decrease by 11% for each additional chronic morbid condition (48), possibly as a result of the higher number of chronic conditions in these patients than in our cohort.

Our results showed that patients and controls did not differ overall in BMI or in body composition. While this observation can be explained in part by matching for BMI, disease control with biologic DMARDs is associated with recovery of total appendicular lean mass, with no changes in fat distribution (49). Nevertheless, despite treat-to-target strategies, as applied in our study, patients with RA experience a relative loss of muscle mass and an increase in adiposity (50), which we found to be more

evident in those with poorer control of inflammation and more pronounced IR. Obesity is more frequent in patients with RA and is closely associated with IR (51, 52), probably owing to physical inactivity, sarcopenia, and therapy with corticosteroids. Obese patients with RA in the present study had a 6-fold greater risk of IR than the other patients; this probability was mediated mainly by fat mass, since the risk increased by 10% for each kilogram of excess body fat. These data support those found in other studies, which highlight the fact that inflammation and obesity are closely linked, because adipose tissue produces TNF-α and IL-6 (16, 52).

In addition to proinflammatory cytokines, adipokines produced by fatty tissue may affect glucose homeostasis, appetite, and the inflammatory response (53). We found that adiponectin levels were higher in patients than in controls. While results were sometimes contradictory, it seems that adiponectin could increase in patients with RA as a means of offsetting the proinflammatory effects of high levels of leptin or of TNF-α and IL-6 (53). This increase may also be a result of the effect of treatment with DMARDs (54).

Leptin levels (systemic, local, or both) have been reported to increase in various inflammatory diseases (53). However, we only observed an increase in patients with RA and IR. Consistent with some studies, this could be because these patients had a greater BMI and a higher grade of chronic inflammation (55).

Furthermore, our data show an increase in LDL oxidase and a decrease in IGF-1 that tended toward significance in patients with RA compared with healthy controls. The same finding was observed in other studies in which these parameters were associated with increased cardiovascular risk in RA (56, 57).

Our study is limited mainly by the cross-sectional evaluation of IR between patients with RA and healthy participants. However, these patients came from a prospective RA inception cohort in which we longitudinally collected all inflammation-related variables analyzed using a predesigned protocol. Consequently, no data were missing, and our results are consistent. On the other hand, while the use of HOMA-IR instead of the hyperinsulinemic-euglycemic clamp method may seem to be a limitation, indirect methods such as HOMA and QUICKI have been validated for use. They are reliable indices and can replace the clamp method for measuring IR in epidemiological studies, clinical trials, and clinical practice. Among the strengths, we performed OGTT, the only technique that makes it possible to recognize the presence of impaired glucose tolerance. Another strength is that the control group was matched with the cases not only for age and sex, but also for BMI.

In conclusion, our results show that the main factors associated with IR are obesity, specifically total fat mass, and disease activity, specifically levels of IL-1β and cumulative inflammatory burden, measured based on average DAS28-CRP levels throughout follow-up. Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis. However, controlled prospective studies must continue to be performed in order to better observe the possible causal relationship between clinical and metabolic factors and IR and atherosclerosis in RA.

Figure 1. Patients and controls flow chart

Data available.

All data relevant to the study are included in the article or uploaded as supplementary information.

Author contributions statement

SMA included patients, wrote the manuscript, and analyzed and interpreted patient data. NMV wrote the manuscript and analyzed and interpreted patient data. IUG included patients. JR performed laboratory determinations and interpreted laboratory data. PV interpreted patient data and wrote the manuscript. LGM included controls. SAS included controls. FGJN included patients. BOM performed laboratory determinations and interpreted laboratory data. AFN wrote the manuscript and analyzed and interpreted patient data. All authors read and approved the final manuscript

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REFERENCES

1. Goodson N, Marks J, Lunt M, Symmons D. Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s. Ann Rheum Dis. 2005;64(11):1595-601.

2. Espino-Lorenzo P, Manrique-Arija S, Urena I, Jimenez-Nunez FG, Lopez-Lasanta M, Romero-Barco CM, et al. Baseline comorbidities in patients with

rheumatoid arthritis who have been prescribed biological therapy: a case control study. Reumatologia clinica. 2013;9(1):18-23.

3. Peters MJ, van Halm VP, Voskuyl AE, Smulders YM, Boers M, Lems WF, et al. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis and rheumatism. 2009;61(11):1571-9.

4. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003;108(24):2957-63.

5. La Montagna G, Cacciapuoti F, Buono R, Manzella D, Mennillo GA, Arciello A, et al. Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis. Diab Vasc Dis Res. 2007;4(2):130-5.

6. Grundy SM. What is the contribution of obesity to the metabolic syndrome? Endocrinology and metabolism clinics of North America. 2004;33(2):267-82.

7. Park K, Steffes M, Lee DH, Himes JH, Jacobs DR, Jr. Association of inflammation with worsening HOMA-insulin resistance. Diabetologia. 2009;52(11):2337-44.

8. Meng YX, Ford ES, Li C, Quarshie A, Al-Mahmoud AM, Giles W, et al. Association of C-reactive protein with surrogate measures of insulin resistance among nondiabetic US from National Health and Nutrition Examination Survey 1999-2002. Clinical chemistry. 2007;53(12):2152-9.

9. Li G, Barrett EJ, Barrett MO, Cao W, Liu Z. Tumor necrosis factor-alpha induces insulin resistance in endothelial cells via a p38 mitogen-activated protein kinase-dependent pathway. Endocrinology. 2007;148(7):3356-63.

10. El-Hini SH, Mohamed FI, Hassan AA, Ali F, Mahmoud A, Ibraheem HM. Visfatin and adiponectin as novel markers for evaluation of metabolic disturbance in recently diagnosed rheumatoid arthritis patients. Rheumatology international. 2013;33(9):2283-9.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

12. Dessein PH, Stanwix AE, Joffe BI. Cardiovascular risk in rheumatoid arthritis versus osteoarthritis: acute phase response related decreased insulin sensitivity and high-density lipoprotein cholesterol as well as clustering of metabolic syndrome features in rheumatoid arthritis. Arthritis research. 2002;4(5):R5.

13. Rosenvinge A, Krogh-Madsen R, Baslund B, Pedersen BK. Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNFalpha therapy. Scand J Rheumatol. 2007; 36:91-6.

14. Shahin D, Eltoraby E, Mesbah A, Houssen M. Insulin resistance in early untreated rheumatoid arthritis patients. Clin Biochem. 2010;43(7-8):661-5.

15. Castaneda S, Martin-Martinez MA, Gonzalez-Juanatey C, Llorca J, Garcia-Yebenes MJ, Perez-Vicente S, et al. Cardiovascular morbidity and associated risk factors in Spanish patients with chronic inflammatory rheumatic diseases attending rheumatology clinics: Baseline data of the CARMA Project. Semin Arthritis Rheum. 2015;44(6):618-26.

16. Manrique-Arija S, Urena I, Valdivielso P, Rioja J, Jimenez-Nunez FG, Irigoyen MV, et al. Insulin resistance and levels of adipokines in patients with untreated early rheumatoid arthritis. Clin Rheumatol. 2016;35(1):43-53.

17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

18. Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. Diabetes Care. 1998;21(2):310-4.

19. Sociedad Española de Reumatología. GUIPCAR actualización 2011: Actualizaciónde la guía de práctica clínica para el manejo de la artritis reumatoide en ESPAÑA. [[monografía en internet]]. Madrid: Sociedad Española de Reumatología; 2007 [updated [30 diaciembre 2011]].

20. Rojo-Martinez G, Esteva I, de Adana SR, Catala M, Merelo MJ, Tinahones F, et al. Patterns of insulin resistance in the general population of southeast Spain. Diabetes Res Clin Pract. 2004;65(3):247-56.

21. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402-10.

22. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. ESH/ESC 2007 Guidelines for the management of arterial hypertension. Rev Esp Cardiol.2007; 60:68e1-94.

23. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 (Suppl 1):S62-9.

24. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001: 285:2486-97.

25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

26. Guidelines for Data Processing andAnalysis of the International Physical Activity Questionnaire (IPAQ). 2009.

27. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.

28. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279-90.

29. WHO. Obesity Preventing and managing the global epidemic. Geneva: WHO; 1997.

30. Anonymous World Health Organization Expert Committee. Physical status: the use and interpretation of anthropometry. . WHO; Genova: Technical Report Series; 1995.

31. Taylor AE, Kuper H, Varma RD, Wells JC, Bell JD, K VR, et al. Validation of dual energy X-ray absorptiometry measures of abdominal fat by comparison with magnetic resonance imaging in an Indian population. PLoS One. 2012;7(12):e51042.

32. Iwamoto N, Kawakami A, Fujikawa K, Aramaki T, Kawashiri SY, Tamai M, et al. Prediction of DAS28-ESR remission at 6 months by baseline variables in patients with rheumatoid arthritis treated with etanercept in Japanese population. Mod Rheumatol. 2009;19(5):488-92.

33. Moyano S, Scolnik M, Vergara F, Garcia MV, Sabelli MR, Rosa JE, et al. Evaluation of Learned Helplessness, Perceived Self-efficacy, and Functional Capacity in Patients With Fibromyalgia and Rheumatoid Arthritis. J Clin Rheumatol. 2018.

34. Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, Shintani A, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis. 2008;196(2):756-63.

35. Dougados M, Soubrier M, Antunez A, Balint P, Balsa A, Buch MH, et al. Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). Ann Rheum Dis. 2014;73(1):62-8.

36. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. Bmj. 2017;357:j2099.

37. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2006;24(1):83-6.

38. Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, De Pergola G. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. Int J Obes Relat Metab Disord. 2001;25(10):1416-20.

39. Crepaldi G, Scirè CA, Carrara G, Sakellariou G, Caporali R, Hmamouchi I, et al. Cardiovascular Comorbidities Relate More than Others with Disease Activity in Rheumatoid Arthritis. PLoS One. 2016;11(1):e0146991.

40. Ruscitti P, Ursini F, Cipriani P, Liakouli V, Carubbi F, Berardicurti O, et al. Poor clinical response in rheumatoid arthritis is the main risk factor for diabetes

development in the short-term: A 1-year, single-centre, longitudinal study. PLoS One. 2017;12(7):e0181203.

41. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol. 2009;5(6):305-11.

42. Everett BM, Donath MY, Pradhan AD, Thuren T, Pais P, Nicolau JC, et al. Anti-Inflammatory Therapy With Canakinumab for the Prevention and Management of Diabetes. J Am Coll Cardiol. 2018;71(21):2392-401.

43. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2):98-107.

44. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nature reviews Endocrinology. 2009;5(6).

45. Ruscitti P, Cipriani P, Di Benedetto P, Liakouli V, Berardicurti O, Carubbi F, et al. Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1β via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3(NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. Clin Exp Immunol. 2015;182(1):35-44.

46. Ruscitti P, Cipriani P, Cantarini L, Liakouli V, Vitale A, Carubbi F, et al. Efficacy of inhibition of IL-1 in patients with rheumatoid arthritis and type 2 diabetes mellitus: two case reports and review of the literature. J Med Case Rep. 2015;9:123.

47. Ruscitti P, Masedu F, Alvaro S, Airò P, Battafarano N, Cantarini L, et al. Anti-interleukin-1 treatment in patients with rheumatoid arthritis and type 2 diabetes (TRACK): A multicentre, open-label, randomised controlled trial. PLoS Med. 2019;16(9):e1002901.

48. Radner H, Yoshida K, Hmamouchi I, Dougados M, Smolen JS, Solomon DH. Treatment Patterns of Multimorbid Patients with Rheumatoid Arthritis: Results from an International Cross-sectional Study. J Rheumatol. 2015;42(7):1099-104.

49. Tournadre A, Pereira B, Dutheil F, Giraud C, Courteix D, Sapin V, et al. Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. J Cachexia Sarcopenia Muscle. 2017;8(4):639-46.

50. Lemmey AB, Wilkinson TJ, Clayton RJ, Sheikh F, Whale J, Jones HS, et al. Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients. Rheumatology (Oxford). 2016;55(10):1736-45.

51. Muller R, Kull M, Lember M, Polluste K, Valner A, Kallikorm R. Insulin Resistance in Early Rheumatoid Arthritis Is Associated with Low Appendicular Lean Mass. Biomed Res Int. 2017;2017:9584720.

52. Castillo-Hernandez J, Maldonado-Cervantes MI, Reyes JP, Patino-Marin N, Maldonado-Cervantes E, Solorzano-Rodriguez C, et al. Obesity is the main determinant of insulin resistance more than the circulating pro-inflammatory cytokines levels in rheumatoid arthritis patients. Rev Bras Reumatol Engl Ed. 2017;57(4):320-9.

53. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gomez-Reino JJ, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis. 2006;65(9):1198-201.

54. Cansu B, Cansu DU, Kasifoglu T, Gulbas Z, Korkmaz C. Disease-modifying antirheumatic drugs increase serum adiponectin levels in patients with rheumatoid arthritis. J Clin Rheumatol. 2011;17(1):14-7.

55. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis. 2005;64(8):1195-8.

56. Erlandsson MC, Lyngfelt L, Aberg ND, Wasen C, Espino RA, Silfversward ST, et al. Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis. BMC Med. 2019;17(1):141.

57. Nowak B, Madej M, Luczak A, Malecki R, Wiland P. Disease Activity, Oxidized-LDL Fraction and Anti-Oxidized LDL Antibodies Influence Cardiovascular Risk in Rheumatoid Arthritis. Adv Clin Exp Med. 2016;25(1):43-50.

Tables

Table 1. Baseline characteristics of cases and controls

Variables	RA patients n=89	Controls n=80	p value
Epidemiological characteristics			
Age, years, mean (SD)	56.6 (10.9)	56.4 (10.9)	0.902
Female sex, n (%)	67 (75.3)	67 (83.8)	0.189
Smoking status			0.117
Never smoked, n (%)	42 (47.2)	49 (61.3)	
Ex-smoker, n (%)	22 (24.7)	11 (13.8)	
Smoker, n (%)	25 (28.1)	20 (25.0)	
Clinical-laboratory characteristics			
Time since onset of RA, months, median (IQR)	98.0 (78.5-123.5)	-	-
Diagnostic delay, months, median (IQR)	10.9 (5.4 - 25.6)	-	-
Rheumatoid factor >10 U/mL, n (%)	73 (82.0)	1 (1.3)	<0.001
ACPA >20 U/mL, n (%)	67 (75.3)	0 (0.0)	<0.001
C-reactive protein (mg/dl), median (IQR)	2.9 (2.9-3.4)		
Erythrocyte sedimentation rate (mm/h), median (IQR)	11 (6.2-14)	11.5 (7.8-21.3)	0.088
No. of swollen joints (0-28), median (IQR)	0 (0-1)	ND	ND
No. of painful joints (0-28), median (IQR)	1 (0-2)	ND	ND
VAS patient global (1-100 mm), median (IQR)	30 (20-50)	ND	ND
DAS28-CRP on the index date, mean (SD)	2.83 (1.1)	ND	ND
Remission-low activity, n (%)	63 (71.0)	ND	ND
Moderate-high activity, n (%)	26 (29.0)	ND	ND

Mean DAS28-CRP during follow-up, mean (SD)	3.11 (0.8)	ND	ND
Remission-low activity, n (%)	56 (63.0)	ND	ND
Moderate-high activity, n (%)	33 (37.0)	ND	ND
Mean HAQ during follow-up, median (IQR)	0.750 (0.0-1.1)	ND	ND
Synthetic DMARDs, n (%)	78 (87.6)	0 (0.0)	<0.001
Biologic DMARDs, n (%)	35 (39.3)	0 (0.0)	<0.001
Combined DMARDs, n (%)	25 (28.1)	0 (0.0)	<0.001
Methotrexate, n (%)	21 (84.0)		
Leflunomide, n (%)	3 (12.0)		
Sulfasalazine, n (%)	1 (4.0)		

RA, rheumatoid arthritis; ACPA, Anticitrullinated protein antibodies; DAS28, 28-joint Disease Activity Score; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; ND, no data; DMARD, disease-modifying antirheumatic drug.

Table 2. Anthropometric and metabolic characteristics, inflammatory cytokines, and adipokines.

Variables	**RA patients n=89**	**Controls n=80**	**p value**
Anthropometric characteristics			
BMI (kg/m^2), mean (SD)	28.2 (5.0)	27.3 (4.9)	0.266
Waist circumference, (cm), mean (SD)	90.8 (11.5)	89.3 (11.3)	0.419
Hip circumference (cm), mean (SD)	103.1 (7.3)	100.5 (9.9)	0.081
Waist-hip index, mean (SD)	0.88 (0.0)	0.89 (0.0)	0.517
Body composition by DXA, mean (SD)			

Total fat mass (kg), mean (SD)	29.2(9.7)	28.5 (9.7)	0.671
FMI (kg/m^2), mean (SD)	11.3 (4.0)	11.1 (4.0)	0.144
Total lean mass (kg), mean (SD)	40.8 (8.9)	39.0 (8.6)	0.197
FFMI (kg/m^2), mean (SD)	15.6 (2.4)	15.0 (2.4)	0.726
Total mass (kg), mean (SD)	71.8 (15.5)	69.9 (14.4)	0.415
Android fat mass (kg), mean (SD)	2.5 (1.1)	2.4 (1.1)	0.671
Gynoid fat mass (kg), mean (SD)	5.1 (1.6)	5.0 (1.5)	0.785
Android lean mass (kg), mean (SD)	2.9 (0.7)	2.8 (0.7)	0.433
Gynoid lean mass (kg), mean (SD)	5.8 (1.2)	5.7 (1.2)	0.639
Metabolic characteristics			
Total cholesterol, median (IQR)	194.0 (170.5-223.6)	209.0 (188.0-238.0)	0.031
LDL cholesterol (mg/dl), median (IQR)	110.0 (95.9-137.5)	133.0 (106.0-151.0)	0.004
HDL cholesterol (mg/dl), median (IQR)	58.0 (51.0-66.0)	59.0 (51.0-72.0)	0.377
Triglycerides (mg/dl), median (IQR)	87.0 (69.0-131.0)	90.0 (66.0- 120.0)	0.102
LDL OX, median (IQR)	2.6 (0.8-5.6)	1.0 (0.3-2.8)	0.114
Baseline glycemia (mg/dl), median (IQR)	78.0 (73.0-84.5)	80.0 (72.0-87.0)	0.327
Glycemia after OGTT (mg/dl), mean (SD)	110.0 (28.9)	100.5(26.6)	0.030
Insulinemia (µU/ml), median (IQR)	9.3 (6.0-12.4)	8.6 (6.0. 12.6)	0.904
HOMA-IR, median (IQR)	1.7 (1.02-2.3)	1.7 (1.15-2.7)	0.385
HOMA-IR ≥2.29, n (%)	25 (28.1)	24(30.0)	0.785
HOMA-β, median (IQR)	38.9 (22.7-53.3)	34.4 (24.0-54.9)	0.545
QUICKI, median (IQR)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.211
QUICKI ≤0.33, n (%)	25.0 (28.1)	24.0 (30.0)	0.785
Inflammatory cytokines and adipokines			
IL-6 (pg/ml), median (IQR)	11.0 (5.4-19.0)	4.31 (3.08-6.67)	<0.001
IL-1ß (pg/ml), median (IQR)	4.33 (4.2-4.5)	2.74 (2.64-3.50)	<0.001
TNF - α (pg/ml), median (IQR)	5.8 (3.7-24.7)	3.6 (3.0-4.7)	<0.001

IGF-1(pg/ml), median (IQR)	172.8 (104.7-238.9)	130.6 (49.8-252.47)	0.079
Adiponectin (ng/ml), median (IQR)	11399.5 (7771.1-14971.5)	8581.4 (6524.1-12688.9)	0.014
Resistin (ng/ml), median (IQR)	7.2 (5.5-9.3)	7.4 (5.7-9.7)	0.510
Leptin (ng/ml), median (IQR)	16.9 (9.1-36.9)	22.2 (9.6-38.8)	0.432

RA, rheumatoid arthritis; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX, oxidized low-density lipoprotein; OGTT, oral glucose tolerance test; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-ß, Homeostatic Model Assessment for ß-Cell Function; QUICKI, Quantitative Insulin Sensitivity Check Index; IL-6, interleukin 6; TNF-α, tumor necrosis factor α;IL-1ß, interleukin 1 ß;IGF-1, insulin-like growth factor-1.

Table 3. Characteristics of patients with and without IR measured using HOMA-IR.

Variables	**RA HOMA-IR≥2.29 n=25**	**RA HOMA-IR<2.29 n=64**	**p value**
Age, years, mean (SD)	58.2 (8.3)	56.0 (11.7)	0.329
Female sex, n (%)	17 (68.0)	50 (78.1)	0.320
Clinical characteristics			
Time since onset of RA, months, median (IQR)	95.8 (81.8-111.6)	98.1 (77.5-125.5)	0.697
Diagnostic delay (months), median (IQR)	15.5 (4.6-27.4)	10.3 (5.4-23.7)	0.629
Arterial hypertension, n (%)	7 (28.0)	16 (25.0)	0.771
Dyslipidemia, n (%)	8 (32.0)	13 (20.3)	0.243
Rheumatoid factor, n (%)	20 (80.0)	53 (82.8)	0.756
ACPA, n (%)	17 (68.0)	49 (76.0)	0.407

DAS28-CRP >3.2 on the index date, n (%)	17 (68.0)	18 (28.1)	**0.001**
Mean DAS28-CRP during follow-up, mean (SD)	3.5 (0.7)	2.9 (0.7)	**0.001**
Mean HAQ during follow-up, mean (SD)	0.90 (0.6)	0.76 (0.8)	0.505
Synthetic DMARD, n (%)	22 (88.0)	49 (76.6)	0.227
Biologic DMARD, n (%)	10 (40.0)	19 (29.7)	0.351
Combined DMARD, n (%)	7 (28.0)	18 (28.1)	0.991
Anthropometric characteristics			
Weight, mean (SD)	82.6 (13.1)	69.5 (12.5)	**<0.001**
BMI, mean (SD)	31.9 (5.4)	26.8 (4.0)	**<0.001**
Normal weight, n (%)	1 (4.0)	27 (42.2)	
Overweight, n (%)	9 (36.0)	22 (34.4)	
Obesity, n (%)	15 (60.0)	15 (23.4)	
Waist circumference, mean (SD)	97.5 (8.9)	88.1 (11.4)	**0.001**
Hip circumference, mean (SD)	106.6 (7.0)	101.7 (7.0)	**0.005**
Waist-hip index, mean (SD)	0.91(0.1)	0.86 (0.1)	**0.022**
Body composition measured by DXA			
Total fat mass (kg), mean (SD)	35.5 (2.2)	26.7 (7.8)	**0.001**
FMI (kg/m^2), mean (SD)	13.8 (4.7)	10.4 (3.2)	**<0.001**
Total lean mass (kg), mean (SD)	43.8 (7.7)	39.7 (9.1)	**0.051**
FFMI (kg/m^2), mean (SD)	16.8 (2.4)	15.1 (2.2)	**0.006**
Lean mass percentage, mean (SD)	0.53 (0.1)	0.58 (0.1)	0.051
Total mass (kg), mean (SD)	81.9(12.6)	67.9 (14.7)	**<0.001**
Android fat mass (kg), mean (SD)	34.6(0.7)	22.3 (9.1)	**<0.001**
Gynoid fat mass (kg), mean (SD)	59.5 (1.0)	47.9 (1.3)	**0.003**
Android lean mass (kg), mean (SD)	32.0 (0.5)	28.2 (0.7)	**0.014**
Gynoid lean mass (kg), mean (SD)	64.0 (1.0)	55.0 (1.2)	**0.003**
LDL OX, median (IQR)	2.7 (0.7-7.4)	2.5 (0.7-5.5)	0.868

Inflammatory cytokines and adipokines			
IL6 (pg/ml), median (IQR)	11.1 (7.3-20.8)	10.5 (4.9-19.8)	0.495
IL-1β (pg/ml), median (IQR)	4.9 (4.1-4.4)	4.3 (4.0 -4.5)	0.007
TNF-α (pg/ml), median (IQR)	5.1 (3.3-27.0)	5.8 (3.7-23.5)	0.827
IGF-1(pg/ml), mean (SD)	168.5 (93.8)	187.7 (106.6)	0.433
Adiponectin (ng/ml), median (IQR)	9271.8 (7602.0-12407.0)	12273.1 (8218.5-15677.1)	0.210
Resistin (ng/ml), median (IQR)	8.3 (5.6-9.9)	7.1 (5.5. - 9.0)	0.626
Leptin (ng/ml), median (IQR)	33.7 (18.0-55.2)	13.9 (7.9-26.1)	0.001

RA, rheumatoid arthritis; ACPA, anticitrullinated protein antibodies; DAS28, 28-joint Disease Activity Score; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; ND, no data; DMARD, disease-modifying antirheumatic drug; DXA, dual-energy x-ray absorptiometry; BMI, body mass index; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX, oxidized low-density lipoprotein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; IL-1ß, interleukin 1ß; IGF-1, insulin-like growth factor-1.

Table 4. Multivariate models for RA patients with HOMA-IR >2.29 (dependent variable)

Multivariate model 1 using obesity covariate

Predictor	β	OR	95% CI	p value
Obesity (BMI>30)	1.795	6.01	1.94-8.66	p=0.002
Mean DAS28-CRP	1.021	2.77	1.29-5.99	p=0.009
Il-1β (pg/ml)	0.464	1.59	1.06-2.38	p=0.024

R^2 =0.352

Independent variables: Age, sex, obesity (BMI >30), mean 28-joint Disease Activity Index with C-reactive protein (DAS28 CRP), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

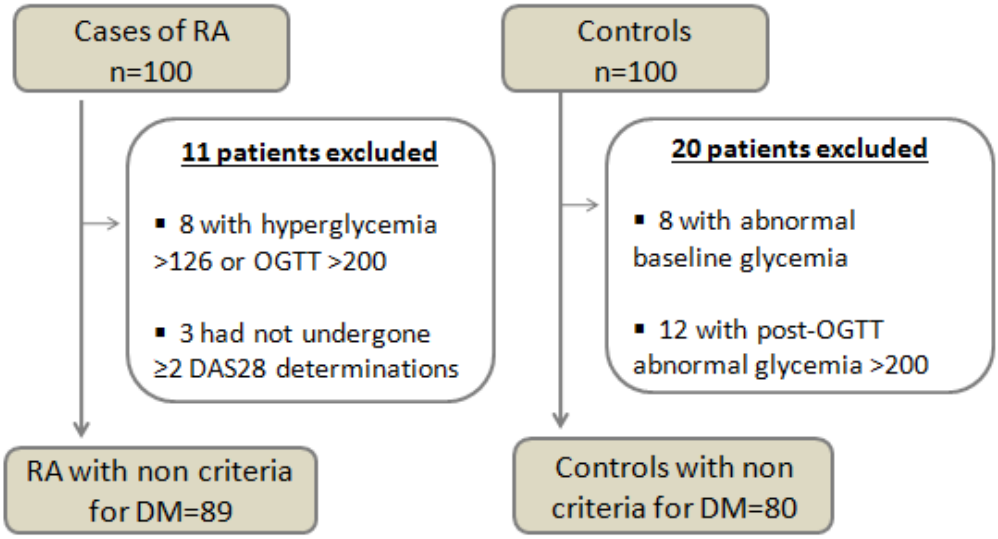
Multivariate model 2 using total fat mass covariate

Predictor	β	OR	95% CI	p value
Total fat mass (kg)	0.123	1.10	1.05-1.22	p=0.002
Mean DAS28-CRP	0.955	2.60	1.19-5.69	p=0.017
Il-1β (pg/ml)	0.456	1.57	1.06-2.34	p=0.023

R^2 =0.404

Independent variables: Age, sex, fat mass, lean mass, mean 28-joint Disease Activity Index (DAS28), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

Figure 1. Patients and controls´ flow chart



RA, Rheumatoid arthritis. OGTT, oral glucose tolerance test; DAS28, 28-joint Disease Activity Score. DM, Diabetes Mellitus.

Table S1. Laboratory tests.

Test	Method/Reference range
High-sensitivity C-reactive protein	Standard nephelometry (reference range, 0-6 mg/l
TNF-α	Automated immunoassay (Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA; reference range, 0-8.1 pg/ml)
IL-6	Enzyme-linked chemiluminescent assay (QuantiGlo®; normal range, 0-5.84 pg/ml).
IL-1ß	Enzyme-linked chemiluminescent assay (QuantiGlo®) pg/ml
Adiponectin	Enzyme-linked chemiluminescent assay (Mediagnost®) (mean [SD] reference value, 11.5 [5.9] ng/ml)
Resistin	Enzyme-linked chemiluminescent assay (Mediagnost®) (median [IQR] reference value, 7.2 [5.4-8.5] ng/ml)
Leptin	Enzyme-linked chemiluminescent assay (Mediagnost®).

	(according to the manufacturer, normal values lie between the 5th and 95th percentiles after adjusting for sex and BMI ng/ml)
IGF-1	Enzyme-linked chemiluminescent assay (Quantikine®)(pg/ml)
Serum insulin	Enzyme-amplified chemiluminescent assay (Immulite ONE®) (**µ**U/ml)
LDL-Ox	Enzyme-amplifiedchemiluminescent assay (Immulite ONE®)(U/l)

TNF-α, tumor necrosis factor alpha; IL, interleukin; IGF-1,insulin-like growth factor 1;LDL,low-densitylipoprotein; ELISA, enzyme-linked immunosorbent assay. LDL-Ox, Oxidized low-density lipoprotein

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8-9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	10-11
Bias	9	Describe any efforts to address potential sources of bias	11-12
Study size	10	Explain how the study size was arrived at	11-12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11-12
		(*b*) Describe any methods used to examine subgroups and interactions	11-12
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12 and figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-14
		(b) Indicate number of participants with missing data for each variable of interest	12 and figure 1

Outcome data	15*	Report numbers of outcome events or summary measures	12-13 and tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14 and table 4
		(*b*) Report category boundaries when continuous variables were categorized	12-14 and tables
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	26

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis: cross-sectional study

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TITLE PAGE

TITLE

Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis: cross-sectional study

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ABSTRACT

OBJECTIVES: To describe the prevalence of insulin resistance (IR) in patients with established rheumatoid arthritis (RA) and to analyze the contribution of cumulative inflammatory burden and other factors to its development.

METHODS:

Design Observational cross-sectional study.

P**articipants:** Patients with RA and controls matched for age, sex, and body mass index. We excluded patients with diabetes.

Settings: Patients from an RA inception cohort at Hospital Regional Universitario de Málaga (HRUM), Spain were recruited between September 2016 and May 2018.

Primary and secondary outcome measures: IR was evaluated using the homeostasis model assessment for IR and beta-cell function and the quantitative insulin sensitivity check index. Other variables included the cumulative 28-joint disease activity score (DAS28-CRP), body composition, and cytokines. Two logistic regression models were constructed to identify factors associated with IR in patients with RA.

RESULTS: Eighty-nine patients with RA and 80 controls were included. The prevalence of IR was similar in both cases and controls. Inflammatory activity was controlled appropriately in patients during follow-up (mean DAS28, 3.1 [0.8]). The presence of IR in patients with RA was associated with obesity (OR [95% CI], 6.01 [1.9-8.7]), higher cumulative DAS28-CRP values during follow-up (OR [95% CI], 2.8 [1.3-6.0]), and higher IL-1β levels (OR [95% CI], 1.6 [1.1 -2.4]). The second model showed that the risk of IR increased by 10% for each kilogram of excess body fat.

CONCLUSION: In patients with well-controlled, established RA, IR is associated mainly with poorer control of inflammation from diagnosis and with obesity, specifically total fat mass.

Word Count: 3314

KEYWORDS

Rheumatoid arthritis, Insulin resistance, Inflammation, Obesity.

Strengths and Limitations

- We studied the role of cumulative inflammatory burden since the onset of disease in a cohort of patients with rheumatoid arthritis.
- The main factors associated with insulin resistance are obesity (specifically total fat mass) and cumulative disease activity (specifically increased levels of IL-1β).
- Our study is limited by its cross-sectional evaluation of insulin resistance in patients and healthy participants.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent synovitis, bone erosion, and functional disability. It is associated with premature death (1-3) and multiple morbidities (1-2), mainly because the cardiovascular risk of RA patients is similar to that of patients with type 2 diabetes mellitus (DM) (3). Accelerated atherosclerosis in patients with RA is due to the presence of traditional and nontraditional cardiovascular risk factors, including systemic inflammation, drugs, and IR (4-5).

In the general population, IR has been confirmed as a risk factor for cardiovascular diseases, DM, and metabolic syndrome (6). Its main determinant is obesity, although it has also been associated with older age, hypertension, and a sedentary lifestyle. Abundant data suggest a connection between IR and chronic inflammation (7-8). Adipose tissue produces proinflammatory cytokines and adipokines, including TNF-α, which reduce sensitivity to insulin and contribute to endothelial dysfunction (9). The relationship between adipokines, proinflammatory cytokines, and IR (10) is unclear in RA, although it could play a key role in the pathogenesis of accelerated atherosclerosis associated with chronic inflammatory states. Various studies have been specifically designed to investigate IR in RA (5,11-14). While most confirm an association between IR and inflammation, other factors continue to play a stronger role, such as abdominal obesity with sarcopenia, sedentary lifestyle, and drugs (15).

Almost all studies that examine the relationship between IR and RA were carried out in patients with chronic RA and numerous comorbid conditions associated with cardiovascular risk factors in case series not controlled for body mass index (BMI). Furthermore, as these studies have a cross-sectional design, they only take into account values for inflammation recorded at a particular point in time. We previously studied a

group of untreated patients with recent-onset RA and a control group matched for age, sex, and BMI. The patients were followed for 6 months (16). The results showed that IR was not present at diagnosis and did not appear after 6 months of treatment if the disease was well controlled. However, our results also showed that patients with higher fat mass and a longer diagnostic and therapeutic delay had the worst IR data. Based on these findings, the hypothesis of the present study was that IR in patients with RA, as with other determinants of disease, can be prevented in the long term with tight control of inflammation from onset.

The objectives of the present study were as follows: 1. To compare the prevalence of IR in an inception cohort of patients with RA and an equivalent group of healthy controls; and 2. To analyze the effect of IR on the cumulative inflammatory burden over at least 5 years, together with other possible factors that contribute to IR.

METHODS

We carried out an observational cross-sectional study of patients with RA. The study was performed at Instituto de Investigación Biomédica de Málaga (IBIMA) by the Department of Rheumatology of Hospital Regional Universitario de Málaga (HRUM), Malaga, Spain and approved by the Ethics Committee of HRUM. All participants signed an informed consent document.

Patients

We consecutively included patients from the RA inception cohort at HRUM. All patients had been diagnosed and treated during the first 12 months since onset of their disease. The inclusion criteria were as follows: RA according to the 2010 classification criteria of the American College of Rheumatology/European League Against

Rheumatism (17); diagnosis made between 2007 and 2011; age >16 years; and prospective follow-up with at least 2 annual DAS28 determinations. Patients were recruited between September 2016 and May 2018. We excluded patients with any inflammatory, rheumatic, or autoimmune disease other than RA (except for secondary Sjögren syndrome), a diagnosis of DM or impaired glucose tolerance (American Diabetes Association 2010 criteria) (18), active infection, pregnancy, current or previous treatment with oral antidiabetic agents or insulin, and new treatments or changes in dose during the 3 months preceding the date of inclusion.

The control group was made up of healthy controls selected from among those who attended a health center in the same geographic area. All controls fulfilled all of the inclusion criteria and none of the exclusion criteria. The controls were matched with the cases for age, sex, race, and BMI. According to BMI, each control was taken from the same group of the WHO classification for each RA patient (normal range:19–24.9 kg/m^2; overweight :25–29.9 kg/m^2 and obesity: $\geq$ 30 kg/m^2).

Patient and public involvement

Neither patients nor the public contributed to the study design or performance, and participants were not involved in the publication of the results. The results of the study will be provided to patients upon request, and the conclusions will be reported in publications and meetings.

Protocol

Cohort patients are followed and treated according to a pre-established protocol and managed with treat-to-target strategies following clinical practice guidelines for RA in Spain (GUIPCAR 2017) (19). All participants were interviewed and examined by a

rheumatologist on the study index date. Samples were collected between 9:00 and 10:00 AM after 12-16 hours of fasting. In order to detect impaired glucose tolerance, subjects with baseline blood glucose levels <126 mg/dl underwent an oral glucose tolerance test (OGTT).

Variables and definitions

The outcome measures were IR and insulin sensitivity. IR was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) (11) and defined as a HOMA-IR score >2.29, based on the 90th percentile for healthy persons (20) and using the homeostasis model assessment of β-cell function (HOMA-β) (11). Sensitivity to insulin was estimated using the quantitative insulin sensitivity check index (QUICKI), with a threshold value of 0.337 (μU*mmol/ml*l) (21).

On the index date, we recorded epidemiological variables, comorbidities, traditional cardiovascular risk factors, diet, physical activity, anthropometric data, and BMI.

Arterial hypertension was defined as an arterial pressure ≥140/90 mmHg or current treatment with antihypertensive medication (22). Glucose and metabolic disorders and DM were diagnosed based on the recommendations of the American Diabetes Association 2010 (23). Dyslipidemia and metabolic syndrome were defined in accordance with the guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) (24). Levels of total cholesterol, triglycerides, HDL, and LDL were evaluated using enzymatic methods (25). Levels of oxidized anti-LDL and serum insulin were determined using enzyme-linked chemiluminescence assay.

Physical activity was measured using the International Physical Activity Questionnaire, taking into account physical activity (low, <600 metabolic equivalents of task [METs]; moderate, 600-1500 METs; and high>1500 METs) (26, 27). Sedentary lifestyle was considered less than 600 METs.

Adherence to a Mediterranean diet was evaluated using a validated questionnaire. Adherence was defined as a score of ≥9 out of 14 (28).

Anthropometric data included BMI (kg/m^2) and percentage of obese patients (29), waist circumference (cm), hip circumference (cm), and the waist-hip index (30). Body composition was measured using dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy enCORE^{TM} 2006) and included total mass (kg), fat mass (g), lean mass (g), and lean mass and android and gynoid fat mass. The fat mass index (FMI) was defined as fat mass (kg)/height squared (m^2) and fat-free mass index (FFMI) as fat-free mass (kg)/height squared (m^2). The values of fat mass and fat-free mass were obtained using DXA (31).

Clinical data comprised rheumatoid factor, which was positive if >20 IU/ml, and anticitrullinated protein antibody, which was positive if >10 IU/ml. Cumulative inflammatory burden was assessed using DAS28 score with C-reactive protein (CRP) level (range 0-9.4) (32) recorded at each visit throughout follow-up. High activity was defined as DAS28-CRP >5.1, moderate activity as 3.2-5.1, low activity as 2.6-3.2, and remission as ≤2.6. We also took into account severity variables such as the presence of erosions and the mean Health Assessment Questionnaire (HAQ) score throughout the course of the disease (33). Treatment was with synthetic disease-modifying anti-inflammatory drugs (DMARDs) and biological DMARDs. The laboratory values measured in all patients were as follows: serum high-sensitivity CRP, tumor necrosis factor alfa (TNF-α), interleukin (IL) 6, IL-1ß, adiponectin, resistin, leptin, and insulin-

like growth factor (IGF) 1. The laboratory kits used and their reference values are shown in the supplementary material.

Statistical analysis

Variables are expressed as mean (SD), median (IQR), or number (%). Comparisons between groups were performed using the χ^2, *t*, or Mann-Whitney test depending on the normality of the distribution. Binary logistic regression analysis was performed (dependent variable: IR measured using HOMA-IR). Multicollinearity of independent variables was verified using the Pearson correlation coefficient (r>0.4). Sample size was calculated assuming a prevalence of IR in RA of 51% and considering as relevant a 30% difference with respect to controls. With a 2-sided α error of 0.05 and a β error of 0.20, the necessary sample size would be 77 participants per group (34). Sample size was increased by 10% to account for possible losses. The analysis was performed using R Commander 2.3-0.

RESULTS

The initial study population comprised 100 patients and 100 controls. However, only 89 patients with RA and 80 healthy controls fulfilled the inclusion criteria and none of the exclusion criteria. The study flow chart is shown in Figure 1.

Epidemiological and anthropometric characteristics and comorbidities

Table 1 shows the baseline characteristics of patients and controls. Mean age was slightly over 56 years, and most patients were women (75%). Patients with RA more frequently had a family history of cardiovascular disease than controls, and 10% more were ex-smokers.

Clinical and analytical variables associated with RA

Autoantibodies were only detected in patients, except for 1 participant in the control group, who was positive for rheumatoid factor, with a low titer and no other data indicating inflammatory disease. On the index date, most patients were in remission or had low arthritis-related inflammatory activity and had maintained an average DAS28-CRP <3.2 throughout follow-up (Table 1). All patients had received DMARDs. A total of 78/89 RA patients (87.6%) were using synthetic DMARDs, 35/89 (39.3%) were using biologic DMARDs, and 12/89 (13.5%) were using corticosteroids.

While the rate of adherence to a Mediterranean diet was similar in both groups (62.9% vs 57.5%; p=0.472), healthy participants more often engaged in physical activity than patients (median [IQR], 612.0 [313.5-1089.0] METS vs 339.0 [198.0-792.0] METS; p=0.005).

Both patients and controls generally had similar baseline characteristics with respect to carbohydrate metabolism (i.e., resistance and sensitivity to insulin, glycemia, and baseline insulinemia). While patients had slightly higher blood sugar levels after OGTT, the difference was not clinically relevant. As for lipids, patients with RA had slightly lower levels of total cholesterol and LDL-cholesterol than controls (table 2).

Inflammatory cytokines and adipokines

Disease was controlled in most patients with RA. However, RA patients had higher levels of proinflammatory cytokines (i.e., TNF-α, IL-6, and IL-1β) than controls. Similarly, IGF-1 levels tended to be higher in patients. The only adipokine that was elevated in patients was adiponectin. Resistin and leptin remained similar in both groups (table 2).

Characteristics of patients with RA and IR

Of the 89 patients with RA, 25 (28.1%) had a HOMA-IR ≥2.29. Table 3 shows the characteristics of patients with RA with and without IR. As we can see, the

epidemiological characteristics of patients with IR were similar to those of the others, although clinical control of their disease was worse on the index date (DAS28-CRP >3.2; 68% vs 28%; p=0.001) and throughout follow-up (mean DAS28-CRP, 3.5 [0.7] vs. 2.9 [0.7]; p=0.001). It is also important to note that patients with IR had higher values for BMI, weight, and body composition and were more often obese. They also had a higher percentage of fat and a higher waist-hip index. However, no differences were found between the groups with respect to the delay in diagnosis of RA, duration of symptoms, antibodies, and synthetic and biologic DMARD or corticosteroids (Table 3). There was no association between Mediterranean diet in patients with and without IR (52.9% vs 65.3%; p = 0.472). Likewise, physical activity was similar in both groups (median [IQR], 445.5 [165.0-800.0] METS vs 330.0 [198.0-700.0] METS; p=0.834).

With respect to inflammatory cytokines and adipokines, concentrations of IL-1β and leptin were clearly higher in patients with IR. No differences were found between the groups in concentrations of TNF-α and IL-6 or in levels of resistin and adiponectin.

Factors associated with insulin resistance in RA

Table 4 shows the 2 best multivariate models. In the first, obesity, mean DAS28, and IL-1ß were significantly associated with IR; these factors accounted for 35% of variability in the presence of IR (R^2=0.352). In the second model, fat mass, mean DAS28, and IL-1ß were associated with IR; these factors accounted for 40% of the variability in IR (R^2=0.404).

DISCUSSION

Among RA patients, there is a high prevalence of comorbidities and cardiovascular risk factors, as shown in the International COMORA study (35). Given that RA is currently considered as potent a cardiovascular risk factor as type 2 DM, some risk equations take

it into account (36). Although cardiovascular risk may be mediated in part by IR (9, 17-25), our study did not reveal a higher prevalence of IR in patients with RA, because the disease was well controlled in patients with a cumulative mean DAS28 <3.2 from onset. This finding is consistent with data from other authors who observed a reduction in IR associated with control of the inflammatory activity induced by methotrexate and anti-TNFα agents (37). However, these results contrast with those published elsewhere, owing to methodological differences, especially in the measurement of inflammation and matching of controls (5, 14). Our study, in contrast, was based on inflammation data obtained from patients with established RA followed prospectively since onset. We also included controls matched for age, sex, race, and BMI, and the amount of exercise and adherence to the Mediterranean diet were taken into account.

While no differences were found between cases and controls, our results support an association between IR and chronic inflammation, as confirmed elsewhere (14, 16, 37, 38, 39, 40). Our group studied baseline IR in patients with untreated early-onset RA and found an association between IR and time with untreated symptoms and fat mass percentage (16). Both these aspects are important for tight and early control of arthritis. In fact, when we analyze the determinants of IR in patients with RA, we again find a higher risk in patients with poor control of their disease during its course, higher levels of IL-1β, and more pronounced obesity. While the cytokines TNF-α, IL-6, and IL-1β are abundant in patients with active RA and reflect inflammatory status and their involvement in IR has been suggested (41), the first 2 may not play as important a role in the present study, because the vast majority of patients treated with biologics received anti-TNF-α agents or tocilizumab. However, in addition to treatment, other aspects associated with IL-1β may have an effect. Although the role of IL-1β has recently been questioned (42), it has been thought to play a role in the pathogenesis of

type 2 DM (43). These authors indicate that high blood sugar levels could induce more marked production of IL-1β and TNF-α in macrophages and that this in turn could lead to a greater rate of apoptosis of β cells that would eventually lead to impairment of pancreatic function (43). In this sense, some studies report greater production of IL-1β in patients affected by RA and type 2 DM as a result of activation of the NLRP3 inflammasome, thus suggesting a role for therapy targeting IL-1β in these patients (44, 45). Furthermore, there have been reports of cases of diabetic patients with RA whose arthritis has remitted and whose metabolism has been controlled with IL-1β blockers (46). In addition, in this context, Ruscitti et al (47) recently performed a specifically designed clinical trial to investigate inhibition of IL-1 as "bidirectional" therapy in patients with RA and type 2 DM. The authors observed an apparent benefit of this approach in participants with RA and type 2 DM. The fact that the therapeutic targets were reached in both diseases suggests that inhibition of IL-1 may be considered a therapeutic target for RA and type 2 DM.

On the other hand, while we found no differences between RA patients with or without IR regarding the use of DMARDs (synthetic and biological), some authors observed that the probabilities of using bDMARDs decrease by 11% for each additional chronic morbid condition (48), possibly as a result of the higher number of chronic conditions in these patients than in our cohort.

Our results showed that patients and controls did not differ overall in BMI or in body composition. While this observation can be explained in part by matching for BMI, disease control with biologic DMARDs is associated with recovery of total appendicular lean mass, with no changes in fat distribution (49). Nevertheless, despite treat-to-target strategies, as applied in our study, patients with RA experience a relative loss of muscle mass and an increase in adiposity (50), which we found to be more

evident in those with poorer control of inflammation and more pronounced IR. Obesity is more frequent in patients with RA and is closely associated with IR (51, 52), probably owing to physical inactivity, sarcopenia, and therapy with corticosteroids. Obese patients with RA in the present study had a 6-fold greater risk of IR than the other patients; this probability was mediated mainly by fat mass, since the risk increased by 10% for each kilogram of excess body fat. These data support those found in other studies, which highlight the fact that inflammation and obesity are closely linked, because adipose tissue produces TNF-α and IL-6 (16, 52).

In addition to proinflammatory cytokines, adipokines produced by fatty tissue may affect glucose homeostasis, appetite, and the inflammatory response (53). We found that adiponectin levels were higher in patients than in controls. While results were sometimes contradictory, it seems that adiponectin could increase in patients with RA as a means of offsetting the proinflammatory effects of high levels of leptin or of TNF-α and IL-6 (53). This increase may also be a result of the effect of treatment with DMARDs (54).

Leptin levels (systemic, local, or both) have been reported to increase in various inflammatory diseases (53). However, we only observed an increase in patients with RA and IR. Consistent with some studies, this could be because these patients had a greater BMI and a higher grade of chronic inflammation (55).

Furthermore, our data show an increase in LDL oxidase and a decrease in IGF-1 that tended toward significance in patients with RA compared with healthy controls. The same finding was observed in other studies in which these parameters were associated with increased cardiovascular risk in RA (56, 57).

Our study is limited mainly by the cross-sectional evaluation of IR between patients with RA and healthy participants. However, these patients came from a prospective RA inception cohort in which we longitudinally collected all inflammation-related variables analyzed using a predesigned protocol. Consequently, no data were missing, and our results are consistent. On the other hand, while the use of HOMA-IR instead of the hyperinsulinemic-euglycemic clamp method may seem to be a limitation, indirect methods such as HOMA and QUICKI have been validated for use. They are reliable indices and can replace the clamp method for measuring IR in epidemiological studies, clinical trials, and clinical practice. Among the strengths, we performed OGTT, the only technique that makes it possible to recognize the presence of impaired glucose tolerance. Another strength is that the control group was matched with the cases not only for age and sex, but also for BMI.

In conclusion, our results show that the main factors associated with IR are obesity, specifically total fat mass, and disease activity, specifically levels of IL-1β and cumulative inflammatory burden, measured based on average DAS28-CRP levels throughout follow-up. Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis. However, controlled prospective studies must continue to be performed in order to better observe the possible causal relationship between clinical and metabolic factors and IR and atherosclerosis in RA.

Figure 1. Patients and controls flow chart

Data available.

All data relevant to the study are included in the article or uploaded as supplementary information.

Author contributions statement

SMA included patients, wrote the manuscript, and analyzed and interpreted patient data. NMV wrote the manuscript and analyzed and interpreted patient data. IUG included patients. JR performed laboratory determinations and interpreted laboratory data. PV interpreted patient data and wrote the manuscript. LGM included controls. SAS included controls. FGJN included patients. BOM performed laboratory determinations and interpreted laboratory data. AFN wrote the manuscript and analyzed and interpreted patient data. All authors read and approved the final manuscript

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REFERENCES

1. Goodson N, Marks J, Lunt M, Symmons D. Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s. Ann Rheum Dis. 2005;64(11):1595-601.

2. Espino-Lorenzo P, Manrique-Arija S, Urena I, Jimenez-Nunez FG, Lopez-Lasanta M, Romero-Barco CM, et al. Baseline comorbidities in patients with

rheumatoid arthritis who have been prescribed biological therapy: a case control study. Reumatologia clinica. 2013;9(1):18-23.

3. Peters MJ, van Halm VP, Voskuyl AE, Smulders YM, Boers M, Lems WF, et al. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis and rheumatism. 2009;61(11):1571-9.

4. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003;108(24):2957-63.

5. La Montagna G, Cacciapuoti F, Buono R, Manzella D, Mennillo GA, Arciello A, et al. Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis. Diab Vasc Dis Res. 2007;4(2):130-5.

6. Grundy SM. What is the contribution of obesity to the metabolic syndrome? Endocrinology and metabolism clinics of North America. 2004;33(2):267-82.

7. Park K, Steffes M, Lee DH, Himes JH, Jacobs DR, Jr. Association of inflammation with worsening HOMA-insulin resistance. Diabetologia. 2009;52(11):2337-44.

8. Meng YX, Ford ES, Li C, Quarshie A, Al-Mahmoud AM, Giles W, et al. Association of C-reactive protein with surrogate measures of insulin resistance among nondiabetic US from National Health and Nutrition Examination Survey 1999-2002. Clinical chemistry. 2007;53(12):2152-9.

9. Li G, Barrett EJ, Barrett MO, Cao W, Liu Z. Tumor necrosis factor-alpha induces insulin resistance in endothelial cells via a p38 mitogen-activated protein kinase-dependent pathway. Endocrinology. 2007;148(7):3356-63.

10. El-Hini SH, Mohamed FI, Hassan AA, Ali F, Mahmoud A, Ibraheem HM. Visfatin and adiponectin as novel markers for evaluation of metabolic disturbance in recently diagnosed rheumatoid arthritis patients. Rheumatology international. 2013;33(9):2283-9.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

12. Dessein PH, Stanwix AE, Joffe BI. Cardiovascular risk in rheumatoid arthritis versus osteoarthritis: acute phase response related decreased insulin sensitivity and high-density lipoprotein cholesterol as well as clustering of metabolic syndrome features in rheumatoid arthritis. Arthritis research. 2002;4(5):R5.

13. Rosenvinge A, Krogh-Madsen R, Baslund B, Pedersen BK. Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNFalpha therapy. Scand J Rheumatol. 2007; 36:91-6.

14. Shahin D, Eltoraby E, Mesbah A, Houssen M. Insulin resistance in early untreated rheumatoid arthritis patients. Clin Biochem. 2010;43(7-8):661-5.

15. Castaneda S, Martin-Martinez MA, Gonzalez-Juanatey C, Llorca J, Garcia-Yebenes MJ, Perez-Vicente S, et al. Cardiovascular morbidity and associated risk factors in Spanish patients with chronic inflammatory rheumatic diseases attending rheumatology clinics: Baseline data of the CARMA Project. Semin Arthritis Rheum. 2015;44(6):618-26.

16. Manrique-Arija S, Urena I, Valdivielso P, Rioja J, Jimenez-Nunez FG, Irigoyen MV, et al. Insulin resistance and levels of adipokines in patients with untreated early rheumatoid arthritis. Clin Rheumatol. 2016;35(1):43-53.

17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

18. Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. Diabetes Care. 1998;21(2):310-4.

19. Sociedad Española de Reumatología. GUIPCAR actualización 2011: Actualizaciónde la guía de práctica clínica para el manejo de la artritis reumatoide en ESPAÑA. [[monografía en internet]]. Madrid: Sociedad Española de Reumatología; 2007 [updated [30 diaciembre 2011]].

20. Rojo-Martinez G, Esteva I, de Adana SR, Catala M, Merelo MJ, Tinahones F, et al. Patterns of insulin resistance in the general population of southeast Spain. Diabetes Res Clin Pract. 2004;65(3):247-56.

21. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402-10.

22. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. ESH/ESC 2007 Guidelines for the management of arterial hypertension. Rev Esp Cardiol.2007; 60:68e1-94.

23. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 (Suppl 1):S62-9.

24. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001: 285:2486-97.

25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

26. Guidelines for Data Processing andAnalysis of the International Physical Activity Questionnaire (IPAQ). 2009.

27. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.

28. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279-90.

29. WHO. Obesity Preventing and managing the global epidemic. Geneva: WHO; 1997.

30. Anonymous World Health Organization Expert Committee. Physical status: the use and interpretation of anthropometry. . WHO; Genova: Technical Report Series; 1995.

31. Taylor AE, Kuper H, Varma RD, Wells JC, Bell JD, K VR, et al. Validation of dual energy X-ray absorptiometry measures of abdominal fat by comparison with magnetic resonance imaging in an Indian population. PLoS One. 2012;7(12):e51042.

32. Iwamoto N, Kawakami A, Fujikawa K, Aramaki T, Kawashiri SY, Tamai M, et al. Prediction of DAS28-ESR remission at 6 months by baseline variables in patients with rheumatoid arthritis treated with etanercept in Japanese population. Mod Rheumatol. 2009;19(5):488-92.

33. Moyano S, Scolnik M, Vergara F, Garcia MV, Sabelli MR, Rosa JE, et al. Evaluation of Learned Helplessness, Perceived Self-efficacy, and Functional Capacity in Patients With Fibromyalgia and Rheumatoid Arthritis. J Clin Rheumatol. 2018.

34. Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, Shintani A, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis. 2008;196(2):756-63.

35. Dougados M, Soubrier M, Antunez A, Balint P, Balsa A, Buch MH, et al. Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). Ann Rheum Dis. 2014;73(1):62-8.

36. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. Bmj. 2017;357:j2099.

37. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2006;24(1):83-6.

38. Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, De Pergola G. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. Int J Obes Relat Metab Disord. 2001;25(10):1416-20.

39. Crepaldi G, Scirè CA, Carrara G, Sakellariou G, Caporali R, Hmamouchi I, et al. Cardiovascular Comorbidities Relate More than Others with Disease Activity in Rheumatoid Arthritis. PLoS One. 2016;11(1):e0146991.

40. Ruscitti P, Ursini F, Cipriani P, Liakouli V, Carubbi F, Berardicurti O, et al. Poor clinical response in rheumatoid arthritis is the main risk factor for diabetes

development in the short-term: A 1-year, single-centre, longitudinal study. PLoS One. 2017;12(7):e0181203.

41. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol. 2009;5(6):305-11.

42. Everett BM, Donath MY, Pradhan AD, Thuren T, Pais P, Nicolau JC, et al. Anti-Inflammatory Therapy With Canakinumab for the Prevention and Management of Diabetes. J Am Coll Cardiol. 2018;71(21):2392-401.

43. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2):98-107.

44. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nature reviews Endocrinology. 2009;5(6).

45. Ruscitti P, Cipriani P, Di Benedetto P, Liakouli V, Berardicurti O, Carubbi F, et al. Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1β via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3(NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. Clin Exp Immunol. 2015;182(1):35-44.

46. Ruscitti P, Cipriani P, Cantarini L, Liakouli V, Vitale A, Carubbi F, et al. Efficacy of inhibition of IL-1 in patients with rheumatoid arthritis and type 2 diabetes mellitus: two case reports and review of the literature. J Med Case Rep. 2015;9:123.

47. Ruscitti P, Masedu F, Alvaro S, Airò P, Battafarano N, Cantarini L, et al. Anti-interleukin-1 treatment in patients with rheumatoid arthritis and type 2 diabetes (TRACK): A multicentre, open-label, randomised controlled trial. PLoS Med. 2019;16(9):e1002901.

48. Radner H, Yoshida K, Hmamouchi I, Dougados M, Smolen JS, Solomon DH. Treatment Patterns of Multimorbid Patients with Rheumatoid Arthritis: Results from an International Cross-sectional Study. J Rheumatol. 2015;42(7):1099-104.

49. Tournadre A, Pereira B, Dutheil F, Giraud C, Courteix D, Sapin V, et al. Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. J Cachexia Sarcopenia Muscle. 2017;8(4):639-46.

50. Lemmey AB, Wilkinson TJ, Clayton RJ, Sheikh F, Whale J, Jones HS, et al. Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients. Rheumatology (Oxford). 2016;55(10):1736-45.

51. Muller R, Kull M, Lember M, Polluste K, Valner A, Kallikorm R. Insulin Resistance in Early Rheumatoid Arthritis Is Associated with Low Appendicular Lean Mass. Biomed Res Int. 2017;2017:9584720.

52. Castillo-Hernandez J, Maldonado-Cervantes MI, Reyes JP, Patino-Marin N, Maldonado-Cervantes E, Solorzano-Rodriguez C, et al. Obesity is the main determinant of insulin resistance more than the circulating pro-inflammatory cytokines levels in rheumatoid arthritis patients. Rev Bras Reumatol Engl Ed. 2017;57(4):320-9.

53. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gomez-Reino JJ, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis. 2006;65(9):1198-201.

54. Cansu B, Cansu DU, Kasifoglu T, Gulbas Z, Korkmaz C. Disease-modifying antirheumatic drugs increase serum adiponectin levels in patients with rheumatoid arthritis. J Clin Rheumatol. 2011;17(1):14-7.

55. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis. 2005;64(8):1195-8.

56. Erlandsson MC, Lyngfelt L, Aberg ND, Wasen C, Espino RA, Silfversward ST, et al. Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis. BMC Med. 2019;17(1):141.

57. Nowak B, Madej M, Luczak A, Malecki R, Wiland P. Disease Activity, Oxidized-LDL Fraction and Anti-Oxidized LDL Antibodies Influence Cardiovascular Risk in Rheumatoid Arthritis. Adv Clin Exp Med. 2016;25(1):43-50.

Tables

Table 1. Baseline characteristics of cases and controls

Variables	RA patients n=89	Controls n=80	p value
Epidemiological characteristics			
Age, years, mean (SD)	56.6 (10.9)	56.4 (10.9)	0.902
Female sex, n (%)	67 (75.3)	67 (83.8)	0.189
Smoking status			0.117
Never smoked, n (%)	42 (47.2)	49 (61.3)	
Ex-smoker, n (%)	22 (24.7)	11 (13.8)	
Smoker, n (%)	25 (28.1)	20 (25.0)	
Clinical-laboratory characteristics			
Time since onset of RA, months, median (IQR)	98.0 (78.5-123.5)	-	-
Diagnostic delay, months, median (IQR)	10.9 (5.4 - 25.6)	-	-
Rheumatoid factor >10 U/mL, n (%)	73 (82.0)	1 (1.3)	<0.001
ACPA >20 U/mL, n (%)	67 (75.3)	0 (0.0)	<0.001
C-reactive protein (mg/dl), median (IQR)	2.9 (2.9-3.4)		
Erythrocyte sedimentation rate (mm/h), median (IQR)	11 (6.2-14)	11.5 (7.8-21.3)	0.088
No. of swollen joints (0-28), median (IQR)	0 (0-1)	ND	ND
No. of painful joints (0-28), median (IQR)	1 (0-2)	ND	ND
VAS patient global (1-100 mm), median (IQR)	30 (20-50)	ND	ND
DAS28-CRP on the index date, mean (SD)	2.83 (1.1)	ND	ND
Remission-low activity, n (%)	63 (71.0)	ND	ND
Moderate-high activity, n (%)	26 (29.0)	ND	ND

Mean DAS28-CRP during follow-up, mean (SD)	3.11 (0.8)	ND	ND
Remission-low activity, n (%)	56 (63.0)	ND	ND
Moderate-high activity, n (%)	33 (37.0)	ND	ND
Mean HAQ during follow-up, median (IQR)	0.750 (0.0-1.1)	ND	ND
Synthetic DMARDs, n (%)	78 (87.6)	0 (0.0)	<0.001
Biologic DMARDs, n (%)	35 (39.3)	0 (0.0)	<0.001
Combined DMARDs, n (%)	25 (28.1)	0 (0.0)	<0.001
Methotrexate, n (%)	21 (84.0)		
Leflunomide, n (%)	3 (12.0)		
Sulfasalazine, n (%)	1 (4.0)		

RA, rheumatoid arthritis; ACPA, Anticitrullinated protein antibodies; DAS28, 28-joint Disease Activity Score; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; ND, no data; DMARD, disease-modifying antirheumatic drug.

Table 2. Anthropometric and metabolic characteristics, inflammatory cytokines, and adipokines.

Variables	**RA patients n=89**	**Controls n=80**	**p value**
Anthropometric characteristics			
BMI (kg/m^2), mean (SD)	28.2 (5.0)	27.3 (4.9)	0.266
Waist circumference, (cm), mean (SD)	90.8 (11.5)	89.3 (11.3)	0.419
Hip circumference (cm), mean (SD)	103.1 (7.3)	100.5 (9.9)	0.081
Waist-hip index, mean (SD)	0.88 (0.0)	0.89 (0.0)	0.517
Body composition by DXA, mean (SD)			

Total fat mass (kg), mean (SD)	29.2(9.7)	28.5 (9.7)	0.671
FMI (kg/m^2), mean (SD)	11.3 (4.0)	11.1 (4.0)	0.144
Total lean mass (kg), mean (SD)	40.8 (8.9)	39.0 (8.6)	0.197
FFMI (kg/m^2), mean (SD)	15.6 (2.4)	15.0 (2.4)	0.726
Total mass (kg), mean (SD)	71.8 (15.5)	69.9 (14.4)	0.415
Android fat mass (kg), mean (SD)	2.5 (1.1)	2.4 (1.1)	0.671
Gynoid fat mass (kg), mean (SD)	5.1 (1.6)	5.0 (1.5)	0.785
Android lean mass (kg), mean (SD)	2.9 (0.7)	2.8 (0.7)	0.433
Gynoid lean mass (kg), mean (SD)	5.8 (1.2)	5.7 (1.2)	0.639
Metabolic characteristics			
Total cholesterol, median (IQR)	194.0 (170.5-223.6)	209.0 (188.0-238.0)	0.031
LDL cholesterol (mg/dl), median (IQR)	110.0 (95.9-137.5)	133.0 (106.0-151.0)	0.004
HDL cholesterol (mg/dl), median (IQR)	58.0 (51.0-66.0)	59.0 (51.0-72.0)	0.377
Triglycerides (mg/dl), median (IQR)	87.0 (69.0-131.0)	90.0 (66.0- 120.0)	0.102
LDL OX, median (IQR)	2.6 (0.8-5.6)	1.0 (0.3-2.8)	0.114
Baseline glycemia (mg/dl), median (IQR)	78.0 (73.0-84.5)	80.0 (72.0-87.0)	0.327
Glycemia after OGTT (mg/dl), mean (SD)	110.0 (28.9)	100.5(26.6)	0.030
Insulinemia (µU/ml), median (IQR)	9.3 (6.0-12.4)	8.6 (6.0. 12.6)	0.904
HOMA-IR, median (IQR)	1.7 (1.02-2.3)	1.7 (1.15-2.7)	0.385
HOMA-IR ≥2.29, n (%)	25 (28.1)	24(30.0)	0.785
HOMA-β, median (IQR)	38.9 (22.7-53.3)	34.4 (24.0-54.9)	0.545
QUICKI, median (IQR)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.211
QUICKI ≤0.33, n (%)	25.0 (28.1)	24.0 (30.0)	0.785
Inflammatory cytokines and adipokines			
IL-6 (pg/ml), median (IQR)	11.0 (5.4-19.0)	4.31 (3.08-6.67)	<0.001
IL-1ß (pg/ml), median (IQR)	4.33 (4.2-4.5)	2.74 (2.64-3.50)	<0.001
TNF - α (pg/ml), median (IQR)	5.8 (3.7-24.7)	3.6 (3.0-4.7)	<0.001

IGF-1(pg/ml), median (IQR)	172.8 (104.7-238.9)	130.6 (49.8-252.47)	0.079
Adiponectin (ng/ml), median (IQR)	11399.5 (7771.1-14971.5)	8581.4 (6524.1-12688.9)	0.014
Resistin (ng/ml), median (IQR)	7.2 (5.5-9.3)	7.4 (5.7-9.7)	0.510
Leptin (ng/ml), median (IQR)	16.9 (9.1-36.9)	22.2 (9.6-38.8)	0.432

RA, rheumatoid arthritis; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX, oxidized low-density lipoprotein; OGTT, oral glucose tolerance test; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-ß, Homeostatic Model Assessment for ß-Cell Function; QUICKI, Quantitative Insulin Sensitivity Check Index; IL-6, interleukin 6; TNF-α, tumor necrosis factor α;IL-1ß, interleukin 1 ß;IGF-1, insulin-like growth factor-1.

Table 3. Characteristics of patients with and without IR measured using HOMA-IR.

Variables	**RA HOMA-IR≥2.29 n=25**	**RA HOMA-IR<2.29 n=64**	**p value**
Age, years, mean (SD)	58.2 (8.3)	56.0 (11.7)	0.329
Female sex, n (%)	17 (68.0)	50 (78.1)	0.320
Clinical characteristics			
Time since onset of RA, months, median (IQR)	95.8 (81.8-111.6)	98.1 (77.5-125.5)	0.697
Diagnostic delay (months), median (IQR)	15.5 (4.6-27.4)	10.3 (5.4-23.7)	0.629
Arterial hypertension, n (%)	7 (28.0)	16 (25.0)	0.771
Dyslipidemia, n (%)	8 (32.0)	13 (20.3)	0.243
Rheumatoid factor, n (%)	20 (80.0)	53 (82.8)	0.756
ACPA, n (%)	17 (68.0)	49 (76.0)	0.407

DAS28-CRP >3.2 on the index date, n (%)	17 (68.0)	18 (28.1)	**0.001**
Mean DAS28-CRP during follow-up, mean (SD)	3.5 (0.7)	2.9 (0.7)	**0.001**
Mean HAQ during follow-up, mean (SD)	0.90 (0.6)	0.76 (0.8)	0.505
Synthetic DMARD, n (%)	22 (88.0)	49 (76.6)	0.227
Biologic DMARD, n (%)	10 (40.0)	19 (29.7)	0.351
Combined DMARD, n (%)	7 (28.0)	18 (28.1)	0.991
Anthropometric characteristics			
Weight, mean (SD)	82.6 (13.1)	69.5 (12.5)	**<0.001**
BMI, mean (SD)	31.9 (5.4)	26.8 (4.0)	**<0.001**
Normal weight, n (%)	1 (4.0)	27 (42.2)	
Overweight, n (%)	9 (36.0)	22 (34.4)	
Obesity, n (%)	15 (60.0)	15 (23.4)	
Waist circumference, mean (SD)	97.5 (8.9)	88.1 (11.4)	**0.001**
Hip circumference, mean (SD)	106.6 (7.0)	101.7 (7.0)	**0.005**
Waist-hip index, mean (SD)	0.91(0.1)	0.86 (0.1)	**0.022**
Body composition measured by DXA			
Total fat mass (kg), mean (SD)	35.5 (2.2)	26.7 (7.8)	**0.001**
FMI (kg/m^2), mean (SD)	13.8 (4.7)	10.4 (3.2)	**<0.001**
Total lean mass (kg), mean (SD)	43.8 (7.7)	39.7 (9.1)	**0.051**
FFMI (kg/m^2), mean (SD)	16.8 (2.4)	15.1 (2.2)	**0.006**
Lean mass percentage, mean (SD)	0.53 (0.1)	0.58 (0.1)	0.051
Total mass (kg), mean (SD)	81.9(12.6)	67.9 (14.7)	**<0.001**
Android fat mass (kg), mean (SD)	34.6(0.7)	22.3 (9.1)	**<0.001**
Gynoid fat mass (kg), mean (SD)	59.5 (1.0)	47.9 (1.3)	**0.003**
Android lean mass (kg), mean (SD)	32.0 (0.5)	28.2 (0.7)	**0.014**
Gynoid lean mass (kg), mean (SD)	64.0 (1.0)	55.0 (1.2)	**0.003**
LDL OX, median (IQR)	2.7 (0.7-7.4)	2.5 (0.7-5.5)	0.868

Inflammatory cytokines and adipokines			
IL6 (pg/ml), median (IQR)	11.1 (7.3-20.8)	10.5 (4.9-19.8)	0.495
IL-1β (pg/ml), median (IQR)	4.9 (4.1-4.4)	4.3 (4.0 -4.5)	0.007
TNF-α (pg/ml), median (IQR)	5.1 (3.3-27.0)	5.8 (3.7-23.5)	0.827
IGF-1(pg/ml), mean (SD)	168.5 (93.8)	187.7 (106.6)	0.433
Adiponectin (ng/ml), median (IQR)	9271.8 (7602.0-12407.0)	12273.1 (8218.5-15677.1)	0.210
Resistin (ng/ml), median (IQR)	8.3 (5.6-9.9)	7.1 (5.5. - 9.0)	0.626
Leptin (ng/ml), median (IQR)	33.7 (18.0-55.2)	13.9 (7.9-26.1)	0.001

RA, rheumatoid arthritis; ACPA, anticitrullinated protein antibodies; DAS28, 28-joint Disease Activity Score; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; ND, no data; DMARD, disease-modifying antirheumatic drug; DXA, dual-energy x-ray absorptiometry; BMI, body mass index; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX, oxidized low-density lipoprotein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; IL-1ß, interleukin 1ß; IGF-1, insulin-like growth factor-1.

Table 4. Multivariate models for RA patients with HOMA-IR >2.29 (dependent variable)

Multivariate model 1 using obesity covariate

Predictor	β	OR	95% CI	p value
Obesity (BMI>30)	1.795	6.01	1.94-8.66	p=0.002
Mean DAS28-CRP	1.021	2.77	1.29-5.99	p=0.009
Il-1β (pg/ml)	0.464	1.59	1.06-2.38	p=0.024

R^2 =0.352

Independent variables: Age, sex, obesity (BMI >30), mean 28-joint Disease Activity Index with C-reactive protein (DAS28 CRP), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

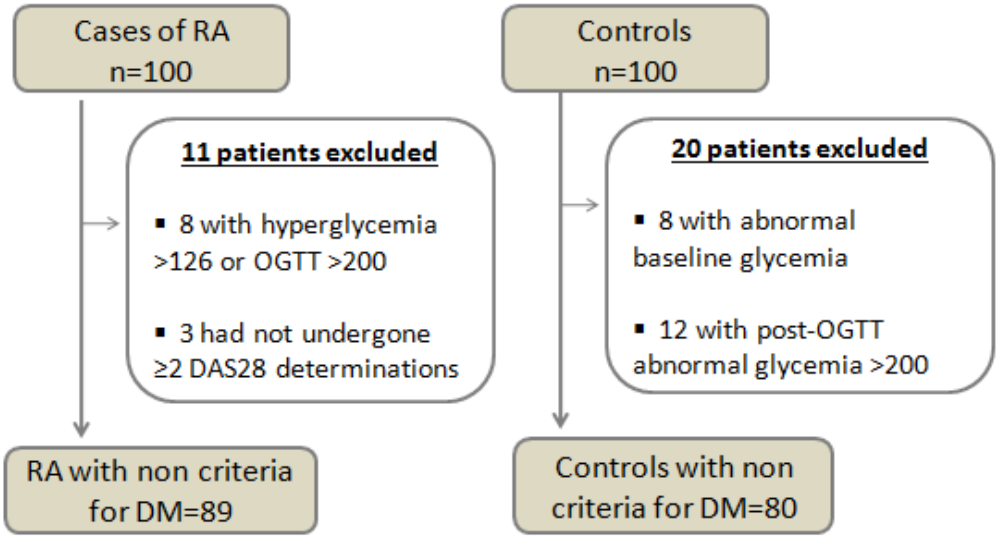
Multivariate model 2 using total fat mass covariate

Predictor	β	OR	95% CI	p value
Total fat mass (kg)	0.123	1.10	1.05-1.22	p=0.002
Mean DAS28-CRP	0.955	2.60	1.19-5.69	p=0.017
Il-1β (pg/ml)	0.456	1.57	1.06-2.34	p=0.023

R^2 =0.404

Independent variables: Age, sex, fat mass, lean mass, mean 28-joint Disease Activity Index (DAS28), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

Figure 1. Patients and controls´ flow chart



RA, Rheumatoid arthritis. OGTT, oral glucose tolerance test; DAS28, 28-joint Disease Activity Score. DM, Diabetes Mellitus.

Table S1. Laboratory tests.

Test	Method/Reference range
High-sensitivity C-reactive protein	Standard nephelometry (reference range, 0-6 mg/l
TNF-α	Automated immunoassay (Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA; reference range, 0-8.1 pg/ml)
IL-6	Enzyme-linked chemiluminescent assay (QuantiGlo®; normal range, 0-5.84 pg/ml).
IL-1ß	Enzyme-linked chemiluminescent assay (QuantiGlo®) pg/ml
Adiponectin	Enzyme-linked chemiluminescent assay (Mediagnost®) (mean [SD] reference value, 11.5 [5.9] ng/ml)
Resistin	Enzyme-linked chemiluminescent assay (Mediagnost®) (median [IQR] reference value, 7.2 [5.4-8.5] ng/ml)
Leptin	Enzyme-linked chemiluminescent assay (Mediagnost®).

	(according to the manufacturer, normal values lie between the 5th and 95th percentiles after adjusting for sex and BMI ng/ml)
IGF-1	Enzyme-linked chemiluminescent assay (Quantikine®)(pg/ml)
Serum insulin	Enzyme-amplified chemiluminescent assay (Immulite ONE®) (**µ**U/ml)
LDL-Ox	Enzyme-amplifiedchemiluminescent assay (Immulite ONE®)(U/l)

TNF-α, tumor necrosis factor alpha; IL, interleukin; IGF-1,insulin-like growth factor 1;LDL,low-densitylipoprotein; ELISA, enzyme-linked immunosorbent assay. LDL-Ox, Oxidized low-density lipoprotein

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8-9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	10-11
Bias	9	Describe any efforts to address potential sources of bias	11-12
Study size	10	Explain how the study size was arrived at	11-12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11-12
		(*b*) Describe any methods used to examine subgroups and interactions	11-12
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12 and figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-14
		(b) Indicate number of participants with missing data for each variable of interest	12 and figure 1

Outcome data	15*	Report numbers of outcome events or summary measures	12-13 and tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14 and table 4
		(*b*) Report category boundaries when continuous variables were categorized	12-14 and tables
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	26

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis: cross-sectional study

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TITLE PAGE

TITLE

Cumulative inflammatory burden and obesity as determinants of insulin resistance in patients with established rheumatoid arthritis: cross-sectional study

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ABSTRACT

OBJECTIVES: To describe the prevalence of insulin resistance (IR) in patients with established rheumatoid arthritis (RA) and to analyze the contribution of cumulative inflammatory burden and other factors to its development.

METHODS:

Design Observational cross-sectional study.

P**articipants:** Patients with RA and controls matched for age, sex, and body mass index. We excluded patients with diabetes.

Settings: Patients from an RA inception cohort at Hospital Regional Universitario de Málaga (HRUM), Spain were recruited between September 2016 and May 2018.

Primary and secondary outcome measures: IR was evaluated using the homeostasis model assessment for IR and beta-cell function and the quantitative insulin sensitivity check index. Other variables included the cumulative 28-joint disease activity score (DAS28-CRP), body composition, and cytokines. Two logistic regression models were constructed to identify factors associated with IR in patients with RA.

RESULTS: Eighty-nine patients with RA and 80 controls were included. The prevalence of IR was similar in both cases and controls. Inflammatory activity was controlled appropriately in patients during follow-up (mean DAS28, 3.1 [0.8]). The presence of IR in patients with RA was associated with obesity (OR [95% CI], 6.01 [1.9-8.7]), higher cumulative DAS28-CRP values during follow-up (OR [95% CI], 2.8 [1.3-6.0]), and higher IL-1β levels (OR [95% CI], 1.6 [1.1 -2.4]). The second model showed that the risk of IR increased by 10% for each kilogram of excess body fat.

CONCLUSION: In patients with well-controlled, established RA, IR is associated mainly with poorer control of inflammation from diagnosis and with obesity, specifically total fat mass.

Word Count: 3314

KEYWORDS

Rheumatoid arthritis, Insulin resistance, Inflammation, Obesity.

Strengths and Limitations

- The control group in this study was matched with the cases not only by age and sex, but also by BMI, not generally controlled for elsewhere.
- Widely validated questionnaires, indices, and measurement parameters were used to minimize measurement biases.
- The cross-sectional evaluation of IR estimates the association between variables but does not guarantee causality of the event of interest.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent synovitis, bone erosion, and functional disability. It is associated with premature death (1-3) and multiple morbidities (1-2), mainly because the cardiovascular risk of RA patients is similar to that of patients with type 2 diabetes mellitus (DM) (3). Accelerated atherosclerosis in patients with RA is due to the presence of traditional and nontraditional cardiovascular risk factors, including systemic inflammation, drugs, and IR (4-5).

In the general population, IR has been confirmed as a risk factor for cardiovascular diseases, DM, and metabolic syndrome (6). Its main determinant is obesity, although it has also been associated with older age, hypertension, and a sedentary lifestyle. Abundant data suggest a connection between IR and chronic inflammation (7-8). Adipose tissue produces proinflammatory cytokines and adipokines, including TNF-α, which reduce sensitivity to insulin and contribute to endothelial dysfunction (9). The relationship between adipokines, proinflammatory cytokines, and IR (10) is unclear in RA, although it could play a key role in the pathogenesis of accelerated atherosclerosis associated with chronic inflammatory states. Various studies have been specifically designed to investigate IR in RA (5,11-14). While most confirm an association between IR and inflammation, other factors continue to play a stronger role, such as abdominal obesity with sarcopenia, sedentary lifestyle, and drugs (15).

Almost all studies that examine the relationship between IR and RA were carried out in patients with chronic RA and numerous comorbid conditions associated with cardiovascular risk factors in case series not controlled for body mass index (BMI). Furthermore, as these studies have a cross-sectional design, they only take into account values for inflammation recorded at a particular point in time. We previously studied a

group of untreated patients with recent-onset RA and a control group matched for age, sex, and BMI. The patients were followed for 6 months (16). The results showed that IR was not present at diagnosis and did not appear after 6 months of treatment if the disease was well controlled. However, our results also showed that patients with higher fat mass and a longer diagnostic and therapeutic delay had the worst IR data. Based on these findings, the hypothesis of the present study was that IR in patients with RA, as with other determinants of disease, can be prevented in the long term with tight control of inflammation from onset.

The objectives of the present study were as follows: 1. To compare the prevalence of IR in an inception cohort of patients with RA and an equivalent group of healthy controls; and 2. To analyze the effect of IR on the cumulative inflammatory burden over at least 5 years, together with other possible factors that contribute to IR.

METHODS

We carried out an observational cross-sectional study of patients with RA. The study was performed at Instituto de Investigación Biomédica de Málaga (IBIMA) by the Department of Rheumatology of Hospital Regional Universitario de Málaga (HRUM), Malaga, Spain and approved by the Ethics Committee of HRUM. All participants signed an informed consent document.

Patients

We consecutively included patients from the RA inception cohort at HRUM. All patients had been diagnosed and treated during the first 12 months since onset of their disease. The inclusion criteria were as follows: RA according to the 2010 classification criteria of the American College of Rheumatology/European League Against

Rheumatism (17); diagnosis made between 2007 and 2011; age >16 years; and prospective follow-up with at least 2 annual DAS28 determinations. Patients were recruited between September 2016 and May 2018. We excluded patients with any inflammatory, rheumatic, or autoimmune disease other than RA (except for secondary Sjögren syndrome), a diagnosis of DM or impaired glucose tolerance (American Diabetes Association 2010 criteria) (18), active infection, pregnancy, current or previous treatment with oral antidiabetic agents or insulin, and new treatments or changes in dose during the 3 months preceding the date of inclusion.

The control group was made up of healthy controls selected from among those who attended a health center in the same geographic area. All controls fulfilled all of the inclusion criteria and none of the exclusion criteria. The controls were matched with the cases for age, sex, race, and BMI. According to BMI, each control was taken from the same group of the WHO classification for each RA patient (normal range:19–24.9 kg/m^2; overweight :25–29.9 kg/m^2 and obesity: $\geq$ 30 kg/m^2).

Patient and public involvement

Neither patients nor the public contributed to the study design or performance, and participants were not involved in the publication of the results. The results of the study will be provided to patients upon request, and the conclusions will be reported in publications and meetings.

Protocol

Cohort patients are followed and treated according to a pre-established protocol and managed with treat-to-target strategies following clinical practice guidelines for RA in Spain (GUIPCAR 2017) (19). All participants were interviewed and examined by a

rheumatologist on the study index date. Samples were collected between 9:00 and 10:00 AM after 12-16 hours of fasting. In order to detect impaired glucose tolerance, subjects with baseline blood glucose levels <126 mg/dl underwent an oral glucose tolerance test (OGTT).

Variables and definitions

The outcome measures were IR and insulin sensitivity. IR was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) (11) and defined as a HOMA-IR score >2.29, based on the 90th percentile for healthy persons (20) and using the homeostasis model assessment of β-cell function (HOMA-β) (11). Sensitivity to insulin was estimated using the quantitative insulin sensitivity check index (QUICKI), with a threshold value of 0.337 (μU*mmol/ml*l) (21).

On the index date, we recorded epidemiological variables, comorbidities, traditional cardiovascular risk factors, diet, physical activity, anthropometric data, and BMI.

Arterial hypertension was defined as an arterial pressure ≥140/90 mmHg or current treatment with antihypertensive medication (22). Glucose and metabolic disorders and DM were diagnosed based on the recommendations of the American Diabetes Association 2010 (23). Dyslipidemia and metabolic syndrome were defined in accordance with the guidelines of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) (24). Levels of total cholesterol, triglycerides, HDL, and LDL were evaluated using enzymatic methods (25). Levels of oxidized anti-LDL and serum insulin were determined using enzyme-linked chemiluminescence assay.

Physical activity was measured using the International Physical Activity Questionnaire, taking into account physical activity (low, <600 metabolic equivalents of task [METs]; moderate, 600-1500 METs; and high>1500 METs) (26, 27). Sedentary lifestyle was considered less than 600 METs.

Adherence to a Mediterranean diet was evaluated using a validated questionnaire. Adherence was defined as a score of ≥9 out of 14 (28).

Anthropometric data included BMI (kg/m^2) and percentage of obese patients (29), waist circumference (cm), hip circumference (cm), and the waist-hip index (30). Body composition was measured using dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy enCORE^{TM} 2006) and included total mass (kg), fat mass (g), lean mass (g), and lean mass and android and gynoid fat mass. The fat mass index (FMI) was defined as fat mass (kg)/height squared (m^2) and fat-free mass index (FFMI) as fat-free mass (kg)/height squared (m^2). The values of fat mass and fat-free mass were obtained using DXA (31).

Clinical data comprised rheumatoid factor, which was positive if >20 IU/ml, and anticitrullinated protein antibody, which was positive if >10 IU/ml. Cumulative inflammatory burden was assessed using DAS28 score with C-reactive protein (CRP) level (range 0-9.4) (32) recorded at each visit throughout follow-up. High activity was defined as DAS28-CRP >5.1, moderate activity as 3.2-5.1, low activity as 2.6-3.2, and remission as ≤2.6. We also took into account severity variables such as the presence of erosions and the mean Health Assessment Questionnaire (HAQ) score throughout the course of the disease (33). Treatment was with synthetic disease-modifying anti-inflammatory drugs (DMARDs) and biological DMARDs. The laboratory values measured in all patients were as follows: serum high-sensitivity CRP, tumor necrosis factor alfa (TNF-α), interleukin (IL) 6, IL-1ß, adiponectin, resistin, leptin, and insulin-

like growth factor (IGF) 1. The laboratory kits used and their reference values are shown in the supplementary material.

Statistical analysis

Variables are expressed as mean (SD), median (IQR), or number (%). Comparisons between groups were performed using the χ^2, *t*, or Mann-Whitney test depending on the normality of the distribution. Binary logistic regression analysis was performed (dependent variable: IR measured using HOMA-IR). Multicollinearity of independent variables was verified using the Pearson correlation coefficient (r>0.4). Sample size was calculated assuming a prevalence of IR in RA of 51% and considering as relevant a 30% difference with respect to controls. With a 2-sided α error of 0.05 and a β error of 0.20, the necessary sample size would be 77 participants per group (34). Sample size was increased by 10% to account for possible losses. The analysis was performed using R Commander 2.3-0.

RESULTS

The initial study population comprised 100 patients and 100 controls. However, only 89 patients with RA and 80 healthy controls fulfilled the inclusion criteria and none of the exclusion criteria. The study flow chart is shown in Figure 1.

Epidemiological and anthropometric characteristics and comorbidities

Table 1 shows the baseline characteristics of patients and controls. Mean age was slightly over 56 years, and most patients were women (75%). Patients with RA more frequently had a family history of cardiovascular disease than controls, and 10% more were ex-smokers.

Clinical and analytical variables associated with RA

Autoantibodies were only detected in patients, except for 1 participant in the control group, who was positive for rheumatoid factor, with a low titer and no other data indicating inflammatory disease. On the index date, most patients were in remission or had low arthritis-related inflammatory activity and had maintained an average DAS28-CRP <3.2 throughout follow-up (Table 1). All patients had received DMARDs. A total of 78/89 RA patients (87.6%) were using synthetic DMARDs, 35/89 (39.3%) were using biologic DMARDs, and 12/89 (13.5%) were using corticosteroids.

While the rate of adherence to a Mediterranean diet was similar in both groups (62.9% vs 57.5%; p=0.472), healthy participants more often engaged in physical activity than patients (median [IQR], 612.0 [313.5-1089.0] METS vs 339.0 [198.0-792.0] METS; p=0.005).

Both patients and controls generally had similar baseline characteristics with respect to carbohydrate metabolism (i.e., resistance and sensitivity to insulin, glycemia, and baseline insulinemia). While patients had slightly higher blood sugar levels after OGTT, the difference was not clinically relevant. As for lipids, patients with RA had slightly lower levels of total cholesterol and LDL-cholesterol than controls (table 2).

Inflammatory cytokines and adipokines

Disease was controlled in most patients with RA. However, RA patients had higher levels of proinflammatory cytokines (i.e., TNF-α, IL-6, and IL-1β) than controls. Similarly, IGF-1 levels tended to be higher in patients. The only adipokine that was elevated in patients was adiponectin. Resistin and leptin remained similar in both groups (table 2).

Characteristics of patients with RA and IR

Of the 89 patients with RA, 25 (28.1%) had a HOMA-IR ≥2.29. Table 3 shows the characteristics of patients with RA with and without IR. As we can see, the

epidemiological characteristics of patients with IR were similar to those of the others, although clinical control of their disease was worse on the index date (DAS28-CRP >3.2; 68% vs 28%; p=0.001) and throughout follow-up (mean DAS28-CRP, 3.5 [0.7] vs. 2.9 [0.7]; p=0.001). It is also important to note that patients with IR had higher values for BMI, weight, and body composition and were more often obese. They also had a higher percentage of fat and a higher waist-hip index. However, no differences were found between the groups with respect to the delay in diagnosis of RA, duration of symptoms, antibodies, and synthetic and biologic DMARD or corticosteroids (Table 3). There was no association between Mediterranean diet in patients with and without IR (52.9% vs 65.3%; p = 0.472). Likewise, physical activity was similar in both groups (median [IQR], 445.5 [165.0-800.0] METS vs 330.0 [198.0-700.0] METS; p=0.834).

With respect to inflammatory cytokines and adipokines, concentrations of IL-1β and leptin were clearly higher in patients with IR. No differences were found between the groups in concentrations of TNF-α and IL-6 or in levels of resistin and adiponectin.

Factors associated with insulin resistance in RA

Table 4 shows the 2 best multivariate models. In the first, obesity, mean DAS28, and IL-1ß were significantly associated with IR; these factors accounted for 35% of variability in the presence of IR (R^2=0.352). In the second model, fat mass, mean DAS28, and IL-1ß were associated with IR; these factors accounted for 40% of the variability in IR (R^2=0.404).

DISCUSSION

Among RA patients, there is a high prevalence of comorbidities and cardiovascular risk factors, as shown in the International COMORA study (35). Given that RA is currently considered as potent a cardiovascular risk factor as type 2 DM, some risk equations take

it into account (36). Although cardiovascular risk may be mediated in part by IR (9, 17-25), our study did not reveal a higher prevalence of IR in patients with RA, because the disease was well controlled in patients with a cumulative mean DAS28 <3.2 from onset. This finding is consistent with data from other authors who observed a reduction in IR associated with control of the inflammatory activity induced by methotrexate and anti-TNFα agents (37). However, these results contrast with those published elsewhere, owing to methodological differences, especially in the measurement of inflammation and matching of controls (5, 14). Our study, in contrast, was based on inflammation data obtained from patients with established RA followed prospectively since onset. We also included controls matched for age, sex, race, and BMI, and the amount of exercise and adherence to the Mediterranean diet were taken into account.

While no differences were found between cases and controls, our results support an association between IR and chronic inflammation, as confirmed elsewhere (14, 16, 37, 38, 39, 40). Our group studied baseline IR in patients with untreated early-onset RA and found an association between IR and time with untreated symptoms and fat mass percentage (16). Both these aspects are important for tight and early control of arthritis. In fact, when we analyze the determinants of IR in patients with RA, we again find a higher risk in patients with poor control of their disease during its course, higher levels of IL-1β, and more pronounced obesity. While the cytokines TNF-α, IL-6, and IL-1β are abundant in patients with active RA and reflect inflammatory status and their involvement in IR has been suggested (41), the first 2 may not play as important a role in the present study, because the vast majority of patients treated with biologics received anti-TNF-α agents or tocilizumab. However, in addition to treatment, other aspects associated with IL-1β may have an effect. Although the role of IL-1β has recently been questioned (42), it has been thought to play a role in the pathogenesis of

type 2 DM (43). These authors indicate that high blood sugar levels could induce more marked production of IL-1β and TNF-α in macrophages and that this in turn could lead to a greater rate of apoptosis of β cells that would eventually lead to impairment of pancreatic function (43). In this sense, some studies report greater production of IL-1β in patients affected by RA and type 2 DM as a result of activation of the NLRP3 inflammasome, thus suggesting a role for therapy targeting IL-1β in these patients (44, 45). Furthermore, there have been reports of cases of diabetic patients with RA whose arthritis has remitted and whose metabolism has been controlled with IL-1β blockers (46). In addition, in this context, Ruscitti et al (47) recently performed a specifically designed clinical trial to investigate inhibition of IL-1 as "bidirectional" therapy in patients with RA and type 2 DM. The authors observed an apparent benefit of this approach in participants with RA and type 2 DM. The fact that the therapeutic targets were reached in both diseases suggests that inhibition of IL-1 may be considered a therapeutic target for RA and type 2 DM.

On the other hand, while we found no differences between RA patients with or without IR regarding the use of DMARDs (synthetic and biological), some authors observed that the probabilities of using bDMARDs decrease by 11% for each additional chronic morbid condition (48), possibly as a result of the higher number of chronic conditions in these patients than in our cohort.

Our results showed that patients and controls did not differ overall in BMI or in body composition. While this observation can be explained in part by matching for BMI, disease control with biologic DMARDs is associated with recovery of total appendicular lean mass, with no changes in fat distribution (49). Nevertheless, despite treat-to-target strategies, as applied in our study, patients with RA experience a relative loss of muscle mass and an increase in adiposity (50), which we found to be more

evident in those with poorer control of inflammation and more pronounced IR. Obesity is more frequent in patients with RA and is closely associated with IR (51, 52), probably owing to physical inactivity, sarcopenia, and therapy with corticosteroids. Obese patients with RA in the present study had a 6-fold greater risk of IR than the other patients; this probability was mediated mainly by fat mass, since the risk increased by 10% for each kilogram of excess body fat. These data support those found in other studies, which highlight the fact that inflammation and obesity are closely linked, because adipose tissue produces TNF-α and IL-6 (16, 52).

In addition to proinflammatory cytokines, adipokines produced by fatty tissue may affect glucose homeostasis, appetite, and the inflammatory response (53). We found that adiponectin levels were higher in patients than in controls. While results were sometimes contradictory, it seems that adiponectin could increase in patients with RA as a means of offsetting the proinflammatory effects of high levels of leptin or of TNF-α and IL-6 (53). This increase may also be a result of the effect of treatment with DMARDs (54).

Leptin levels (systemic, local, or both) have been reported to increase in various inflammatory diseases (53). However, we only observed an increase in patients with RA and IR. Consistent with some studies, this could be because these patients had a greater BMI and a higher grade of chronic inflammation (55).

Furthermore, our data show an increase in LDL oxidase and a decrease in IGF-1 that tended toward significance in patients with RA compared with healthy controls. The same finding was observed in other studies in which these parameters were associated with increased cardiovascular risk in RA (56, 57).

Our study is limited mainly by the cross-sectional evaluation of IR between patients with RA and healthy participants. However, these patients came from a prospective RA inception cohort in which we longitudinally collected all inflammation-related variables analyzed using a predesigned protocol. Consequently, no data were missing, and our results are consistent. On the other hand, while the use of HOMA-IR instead of the hyperinsulinemic-euglycemic clamp method may seem to be a limitation, indirect methods such as HOMA and QUICKI have been validated for use. They are reliable indices and can replace the clamp method for measuring IR in epidemiological studies, clinical trials, and clinical practice. Among the strengths, we performed OGTT, the only technique that makes it possible to recognize the presence of impaired glucose tolerance. Another strength is that the control group was matched with the cases not only for age and sex, but also for BMI.

In conclusion, our results show that the main factors associated with IR are obesity, specifically total fat mass, and disease activity, specifically levels of IL-1β and cumulative inflammatory burden, measured based on average DAS28-CRP levels throughout follow-up. Therefore, early treatment and good control of inflammatory activity and weight are essential for reducing the risk of IR and accelerated atherosclerosis. However, controlled prospective studies must continue to be performed in order to better observe the possible causal relationship between clinical and metabolic factors and IR and atherosclerosis in RA.

Figure 1. Patients and controls flow chart

Data available.

No additional data available.

Author contributions statement

SMA included patients, wrote the manuscript, and analyzed and interpreted patient data. NMV wrote the manuscript and analyzed and interpreted patient data. IUG included patients. JR performed laboratory determinations and interpreted laboratory data. PV interpreted patient data and wrote the manuscript. LGM included controls. SAS included controls. FGJN included patients. BOM performed laboratory determinations and interpreted laboratory data. AFN wrote the manuscript and analyzed and interpreted patient data. All authors read and approved the final manuscript

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REFERENCES

1. Goodson N, Marks J, Lunt M, Symmons D. Cardiovascular admissions and mortality in an inception cohort of patients with rheumatoid arthritis with onset in the 1980s and 1990s. Ann Rheum Dis. 2005;64(11):1595-601.

2. Espino-Lorenzo P, Manrique-Arija S, Urena I, Jimenez-Nunez FG, Lopez-Lasanta M, Romero-Barco CM, et al. Baseline comorbidities in patients with rheumatoid arthritis who have been prescribed biological therapy: a case control study. Reumatologia clinica. 2013;9(1):18-23.

3. Peters MJ, van Halm VP, Voskuyl AE, Smulders YM, Boers M, Lems WF, et al. Does rheumatoid arthritis equal diabetes mellitus as an independent risk factor for cardiovascular disease? A prospective study. Arthritis and rheumatism. 2009;61(11):1571-9.

4. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation. 2003;108(24):2957-63.

5. La Montagna G, Cacciapuoti F, Buono R, Manzella D, Mennillo GA, Arciello A, et al. Insulin resistance is an independent risk factor for atherosclerosis in rheumatoid arthritis. Diab Vasc Dis Res. 2007;4(2):130-5.

6. Grundy SM. What is the contribution of obesity to the metabolic syndrome? Endocrinology and metabolism clinics of North America. 2004;33(2):267-82.

7. Park K, Steffes M, Lee DH, Himes JH, Jacobs DR, Jr. Association of inflammation with worsening HOMA-insulin resistance. Diabetologia. 2009;52(11):2337-44.

8. Meng YX, Ford ES, Li C, Quarshie A, Al-Mahmoud AM, Giles W, et al. Association of C-reactive protein with surrogate measures of insulin resistance among nondiabetic US from National Health and Nutrition Examination Survey 1999-2002. Clinical chemistry. 2007;53(12):2152-9.

9. Li G, Barrett EJ, Barrett MO, Cao W, Liu Z. Tumor necrosis factor-alpha induces insulin resistance in endothelial cells via a p38 mitogen-activated protein kinase-dependent pathway. Endocrinology. 2007;148(7):3356-63.

10. El-Hini SH, Mohamed FI, Hassan AA, Ali F, Mahmoud A, Ibraheem HM. Visfatin and adiponectin as novel markers for evaluation of metabolic disturbance in recently diagnosed rheumatoid arthritis patients. Rheumatology international. 2013;33(9):2283-9.

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

12. Dessein PH, Stanwix AE, Joffe BI. Cardiovascular risk in rheumatoid arthritis versus osteoarthritis: acute phase response related decreased insulin sensitivity and

high-density lipoprotein cholesterol as well as clustering of metabolic syndrome features in rheumatoid arthritis. Arthritis research. 2002;4(5):R5.

13. Rosenvinge A, Krogh-Madsen R, Baslund B, Pedersen BK. Insulin resistance in patients with rheumatoid arthritis: effect of anti-TNFalpha therapy. Scand J Rheumatol. 2007; 36:91-6.

14. Shahin D, Eltoraby E, Mesbah A, Houssen M. Insulin resistance in early untreated rheumatoid arthritis patients. Clin Biochem. 2010;43(7-8):661-5.

15. Castaneda S, Martin-Martinez MA, Gonzalez-Juanatey C, Llorca J, Garcia-Yebenes MJ, Perez-Vicente S, et al. Cardiovascular morbidity and associated risk factors in Spanish patients with chronic inflammatory rheumatic diseases attending rheumatology clinics: Baseline data of the CARMA Project. Semin Arthritis Rheum. 2015;44(6):618-26.

16. Manrique-Arija S, Urena I, Valdivielso P, Rioja J, Jimenez-Nunez FG, Irigoyen MV, et al. Insulin resistance and levels of adipokines in patients with untreated early rheumatoid arthritis. Clin Rheumatol. 2016;35(1):43-53.

17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum. 2010;62(9):2569-81.

18. Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. Diabetes Care. 1998;21(2):310-4.

19. Sociedad Española de Reumatología. GUIPCAR actualización 2011: Actualizaciónde la guía de práctica clínica para el manejo de la artritis reumatoide en ESPAÑA. [[monografía en internet]]. Madrid: Sociedad Española de Reumatología; 2007 [updated [30 diaciembre 2011]].

20. Rojo-Martinez G, Esteva I, de Adana SR, Catala M, Merelo MJ, Tinahones F, et al. Patterns of insulin resistance in the general population of southeast Spain. Diabetes Res Clin Pract. 2004;65(3):247-56.

21. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402-10.

22. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. ESH/ESC 2007 Guidelines for the management of arterial hypertension. Rev Esp Cardiol.2007; 60:68e1-94.

23. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 (Suppl 1):S62-9.

24. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 2001: 285:2486-97.

25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

26. Guidelines for Data Processing andAnalysis of the International Physical Activity Questionnaire (IPAQ). 2009.

27. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.

28. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279-90.

29. WHO. Obesity Preventing and managing the global epidemic. Geneva: WHO; 1997.

30. Anonymous World Health Organization Expert Committee. Physical status: the use and interpretation of anthropometry. . WHO; Genova: Technical Report Series; 1995.

31. Taylor AE, Kuper H, Varma RD, Wells JC, Bell JD, K VR, et al. Validation of dual energy X-ray absorptiometry measures of abdominal fat by comparison with magnetic resonance imaging in an Indian population. PLoS One. 2012;7(12):e51042.

32. Iwamoto N, Kawakami A, Fujikawa K, Aramaki T, Kawashiri SY, Tamai M, et al. Prediction of DAS28-ESR remission at 6 months by baseline variables in patients with rheumatoid arthritis treated with etanercept in Japanese population. Mod Rheumatol. 2009;19(5):488-92.

33. Moyano S, Scolnik M, Vergara F, Garcia MV, Sabelli MR, Rosa JE, et al. Evaluation of Learned Helplessness, Perceived Self-efficacy, and Functional Capacity in Patients With Fibromyalgia and Rheumatoid Arthritis. J Clin Rheumatol. 2018.

34. Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, Shintani A, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis. 2008;196(2):756-63.

35. Dougados M, Soubrier M, Antunez A, Balint P, Balsa A, Buch MH, et al. Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). Ann Rheum Dis. 2014;73(1):62-8.

36. Hippisley-Cox J, Coupland C, Brindle P. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. Bmj. 2017;357:j2099.

37. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. Clin Exp Rheumatol. 2006;24(1):83-6.

38. Pannacciulli N, Cantatore FP, Minenna A, Bellacicco M, Giorgino R, De Pergola G. C-reactive protein is independently associated with total body fat, central fat, and insulin resistance in adult women. Int J Obes Relat Metab Disord. 2001;25(10):1416-20.

39. Crepaldi G, Scirè CA, Carrara G, Sakellariou G, Caporali R, Hmamouchi I, et al. Cardiovascular Comorbidities Relate More than Others with Disease Activity in Rheumatoid Arthritis. PLoS One. 2016;11(1):e0146991.

40. Ruscitti P, Ursini F, Cipriani P, Liakouli V, Carubbi F, Berardicurti O, et al. Poor clinical response in rheumatoid arthritis is the main risk factor for diabetes

development in the short-term: A 1-year, single-centre, longitudinal study. PLoS One. 2017;12(7):e0181203.

41. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol. 2009;5(6):305-11.

42. Everett BM, Donath MY, Pradhan AD, Thuren T, Pais P, Nicolau JC, et al. Anti-Inflammatory Therapy With Canakinumab for the Prevention and Management of Diabetes. J Am Coll Cardiol. 2018;71(21):2392-401.

43. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2):98-107.

44. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nature reviews Endocrinology. 2009;5(6).

45. Ruscitti P, Cipriani P, Di Benedetto P, Liakouli V, Berardicurti O, Carubbi F, et al. Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1β via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3(NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. Clin Exp Immunol. 2015;182(1):35-44.

46. Ruscitti P, Cipriani P, Cantarini L, Liakouli V, Vitale A, Carubbi F, et al. Efficacy of inhibition of IL-1 in patients with rheumatoid arthritis and type 2 diabetes mellitus: two case reports and review of the literature. J Med Case Rep. 2015;9:123.

47. Ruscitti P, Masedu F, Alvaro S, Airò P, Battafarano N, Cantarini L, et al. Anti-interleukin-1 treatment in patients with rheumatoid arthritis and type 2 diabetes (TRACK): A multicentre, open-label, randomised controlled trial. PLoS Med. 2019;16(9):e1002901.

48. Radner H, Yoshida K, Hmamouchi I, Dougados M, Smolen JS, Solomon DH. Treatment Patterns of Multimorbid Patients with Rheumatoid Arthritis: Results from an International Cross-sectional Study. J Rheumatol. 2015;42(7):1099-104.

49. Tournadre A, Pereira B, Dutheil F, Giraud C, Courteix D, Sapin V, et al. Changes in body composition and metabolic profile during interleukin 6 inhibition in rheumatoid arthritis. J Cachexia Sarcopenia Muscle. 2017;8(4):639-46.

50. Lemmey AB, Wilkinson TJ, Clayton RJ, Sheikh F, Whale J, Jones HS, et al. Tight control of disease activity fails to improve body composition or physical function in rheumatoid arthritis patients. Rheumatology (Oxford). 2016;55(10):1736-45.

51. Muller R, Kull M, Lember M, Polluste K, Valner A, Kallikorm R. Insulin Resistance in Early Rheumatoid Arthritis Is Associated with Low Appendicular Lean Mass. Biomed Res Int. 2017;2017:9584720.

52. Castillo-Hernandez J, Maldonado-Cervantes MI, Reyes JP, Patino-Marin N, Maldonado-Cervantes E, Solorzano-Rodriguez C, et al. Obesity is the main determinant of insulin resistance more than the circulating pro-inflammatory cytokines levels in rheumatoid arthritis patients. Rev Bras Reumatol Engl Ed. 2017;57(4):320-9.

53. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gomez-Reino JJ, et al. Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann Rheum Dis. 2006;65(9):1198-201.

54. Cansu B, Cansu DU, Kasifoglu T, Gulbas Z, Korkmaz C. Disease-modifying antirheumatic drugs increase serum adiponectin levels in patients with rheumatoid arthritis. J Clin Rheumatol. 2011;17(1):14-7.

55. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P, van der Meer JW. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. Ann Rheum Dis. 2005;64(8):1195-8.

56. Erlandsson MC, Lyngfelt L, Aberg ND, Wasen C, Espino RA, Silfversward ST, et al. Low serum IGF1 is associated with hypertension and predicts early cardiovascular events in women with rheumatoid arthritis. BMC Med. 2019;17(1):141.

57. Nowak B, Madej M, Luczak A, Malecki R, Wiland P. Disease Activity, Oxidized-LDL Fraction and Anti-Oxidized LDL Antibodies Influence Cardiovascular Risk in Rheumatoid Arthritis. Adv Clin Exp Med. 2016;25(1):43-50.

Tables

Table 1. Baseline characteristics of cases and controls

Variables	RA patients n=89	Controls n=80	p value
Epidemiological characteristics			
Age, years, mean (SD)	56.6 (10.9)	56.4 (10.9)	0.902
Female sex, n (%)	67 (75.3)	67 (83.8)	0.189
Smoking status			0.117
Never smoked, n (%)	42 (47.2)	49 (61.3)	
Ex-smoker, n (%)	22 (24.7)	11 (13.8)	
Smoker, n (%)	25 (28.1)	20 (25.0)	
Clinical-laboratory characteristics			
Time since onset of RA, months, median (IQR)	98.0 (78.5-123.5)	-	-
Diagnostic delay, months, median (IQR)	10.9 (5.4 - 25.6)	-	-
Rheumatoid factor >10 U/mL, n (%)	73 (82.0)	1 (1.3)	<0.001
ACPA >20 U/mL, n (%)	67 (75.3)	0 (0.0)	<0.001
C-reactive protein (mg/dl), median (IQR)	2.9 (2.9-3.4)		
Erythrocyte sedimentation rate (mm/h), median (IQR)	11 (6.2-14)	11.5 (7.8-21.3)	0.088
No. of swollen joints (0-28), median (IQR)	0 (0-1)	ND	ND
No. of painful joints (0-28), median (IQR)	1 (0-2)	ND	ND
VAS patient global (1-100 mm), median (IQR)	30 (20-50)	ND	ND
DAS28-CRP on the index date, mean (SD)	2.83 (1.1)	ND	ND
Remission-low activity, n (%)	63 (71.0)	ND	ND
Moderate-high activity, n (%)	26 (29.0)	ND	ND

Mean DAS28-CRP during follow-up, mean (SD)	3.11 (0.8)	ND	ND
Remission-low activity, n (%)	56 (63.0)	ND	ND
Moderate-high activity, n (%)	33 (37.0)	ND	ND
Mean HAQ during follow-up, median (IQR)	0.750 (0.0-1.1)	ND	ND
Synthetic DMARDs, n (%)	78 (87.6)	0 (0.0)	<0.001
Biologic DMARDs, n (%)	35 (39.3)	0 (0.0)	<0.001
Combined DMARDs, n (%)	25 (28.1)	0 (0.0)	<0.001
Methotrexate, n (%)	21 (84.0)		
Leflunomide, n (%)	3 (12.0)		
Sulfasalazine, n (%)	1 (4.0)		

RA, rheumatoid arthritis; ACPA, Anticitrullinated protein antibodies; DAS28, 28-joint Disease Activity Score; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; ND, no data; DMARD, disease-modifying antirheumatic drug.

Table 2. Anthropometric and metabolic characteristics, inflammatory cytokines, and adipokines.

Variables	**RA patients n=89**	**Controls n=80**	**p value**
Anthropometric characteristics			
BMI (kg/m^2), mean (SD)	28.2 (5.0)	27.3 (4.9)	0.266
Waist circumference, (cm), mean (SD)	90.8 (11.5)	89.3 (11.3)	0.419
Hip circumference (cm), mean (SD)	103.1 (7.3)	100.5 (9.9)	0.081
Waist-hip index, mean (SD)	0.88 (0.0)	0.89 (0.0)	0.517
Body composition by DXA, mean (SD)			

Total fat mass (kg), mean (SD)	29.2(9.7)	28.5 (9.7)	0.671
FMI (kg/m^2), mean (SD)	11.3 (4.0)	11.1 (4.0)	0.144
Total lean mass (kg), mean (SD)	40.8 (8.9)	39.0 (8.6)	0.197
FFMI (kg/m^2), mean (SD)	15.6 (2.4)	15.0 (2.4)	0.726
Total mass (kg), mean (SD)	71.8 (15.5)	69.9 (14.4)	0.415
Android fat mass (kg), mean (SD)	2.5 (1.1)	2.4 (1.1)	0.671
Gynoid fat mass (kg), mean (SD)	5.1 (1.6)	5.0 (1.5)	0.785
Android lean mass (kg), mean (SD)	2.9 (0.7)	2.8 (0.7)	0.433
Gynoid lean mass (kg), mean (SD)	5.8 (1.2)	5.7 (1.2)	0.639
Metabolic characteristics			
Total cholesterol, median (IQR)	194.0 (170.5-223.6)	209.0 (188.0-238.0)	0.031
LDL cholesterol (mg/dl), median (IQR)	110.0 (95.9-137.5)	133.0 (106.0-151.0)	0.004
HDL cholesterol (mg/dl), median (IQR)	58.0 (51.0-66.0)	59.0 (51.0-72.0)	0.377
Triglycerides (mg/dl), median (IQR)	87.0 (69.0-131.0)	90.0 (66.0- 120.0)	0.102
LDL OX, median (IQR)	2.6 (0.8-5.6)	1.0 (0.3-2.8)	0.114
Baseline glycemia (mg/dl), median (IQR)	78.0 (73.0-84.5)	80.0 (72.0-87.0)	0.327
Glycemia after OGTT (mg/dl), mean (SD)	110.0 (28.9)	100.5(26.6)	0.030
Insulinemia (µU/ml), median (IQR)	9.3 (6.0-12.4)	8.6 (6.0. 12.6)	0.904
HOMA-IR, median (IQR)	1.7 (1.02-2.3)	1.7 (1.15-2.7)	0.385
HOMA-IR ≥2.29, n (%)	25 (28.1)	24(30.0)	0.785
HOMA-β, median (IQR)	38.9 (22.7-53.3)	34.4 (24.0-54.9)	0.545
QUICKI, median (IQR)	0.4 (0.3-0.4)	0.4 (0.3-0.4)	0.211
QUICKI ≤0.33, n (%)	25.0 (28.1)	24.0 (30.0)	0.785
Inflammatory cytokines and adipokines			
IL-6 (pg/ml), median (IQR)	11.0 (5.4-19.0)	4.31 (3.08-6.67)	<0.001
IL-1ß (pg/ml), median (IQR)	4.33 (4.2-4.5)	2.74 (2.64-3.50)	<0.001
TNF - α (pg/ml), median (IQR)	5.8 (3.7-24.7)	3.6 (3.0-4.7)	<0.001

IGF-1(pg/ml), median (IQR)	172.8 (104.7-238.9)	130.6 (49.8-252.47)	0.079
Adiponectin (ng/ml), median (IQR)	11399.5 (7771.1-14971.5)	8581.4 (6524.1-12688.9)	0.014
Resistin (ng/ml), median (IQR)	7.2 (5.5-9.3)	7.4 (5.7-9.7)	0.510
Leptin (ng/ml), median (IQR)	16.9 (9.1-36.9)	22.2 (9.6-38.8)	0.432

RA, rheumatoid arthritis; BMI, body mass index; DXA, dual-energy x-ray absorptiometry; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX, oxidized low-density lipoprotein; OGTT, oral glucose tolerance test; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HOMA-ß, Homeostatic Model Assessment for ß-Cell Function; QUICKI, Quantitative Insulin Sensitivity Check Index; IL-6, interleukin 6; TNF-α, tumor necrosis factor α;IL-1ß, interleukin 1 ß;IGF-1, insulin-like growth factor-1.

Table 3. Characteristics of patients with and without IR measured using HOMA-IR.

Variables	**RA HOMA-IR≥2.29 n=25**	**RA HOMA-IR<2.29 n=64**	**p value**
Age, years, mean (SD)	58.2 (8.3)	56.0 (11.7)	0.329
Female sex, n (%)	17 (68.0)	50 (78.1)	0.320
Clinical characteristics			
Time since onset of RA, months, median (IQR)	95.8 (81.8-111.6)	98.1 (77.5-125.5)	0.697
Diagnostic delay (months), median (IQR)	15.5 (4.6-27.4)	10.3 (5.4-23.7)	0.629
Arterial hypertension, n (%)	7 (28.0)	16 (25.0)	0.771
Dyslipidemia, n (%)	8 (32.0)	13 (20.3)	0.243
Rheumatoid factor, n (%)	20 (80.0)	53 (82.8)	0.756
ACPA, n (%)	17 (68.0)	49 (76.0)	0.407

DAS28-CRP >3.2 on the index date, n (%)	17 (68.0)	18 (28.1)	**0.001**
Mean DAS28-CRP during follow-up, mean (SD)	3.5 (0.7)	2.9 (0.7)	**0.001**
Mean HAQ during follow-up, mean (SD)	0.90 (0.6)	0.76 (0.8)	0.505
Synthetic DMARD, n (%)	22 (88.0)	49 (76.6)	0.227
Biologic DMARD, n (%)	10 (40.0)	19 (29.7)	0.351
Combined DMARD, n (%)	7 (28.0)	18 (28.1)	0.991
Anthropometric characteristics			
Weight, mean (SD)	82.6 (13.1)	69.5 (12.5)	**<0.001**
BMI, mean (SD)	31.9 (5.4)	26.8 (4.0)	**<0.001**
Normal weight, n (%)	1 (4.0)	27 (42.2)	
Overweight, n (%)	9 (36.0)	22 (34.4)	
Obesity, n (%)	15 (60.0)	15 (23.4)	
Waist circumference, mean (SD)	97.5 (8.9)	88.1 (11.4)	**0.001**
Hip circumference, mean (SD)	106.6 (7.0)	101.7 (7.0)	**0.005**
Waist-hip index, mean (SD)	0.91(0.1)	0.86 (0.1)	**0.022**
Body composition measured by DXA			
Total fat mass (kg), mean (SD)	35.5 (2.2)	26.7 (7.8)	**0.001**
FMI (kg/m^2), mean (SD)	13.8 (4.7)	10.4 (3.2)	**<0.001**
Total lean mass (kg), mean (SD)	43.8 (7.7)	39.7 (9.1)	**0.051**
FFMI (kg/m^2), mean (SD)	16.8 (2.4)	15.1 (2.2)	**0.006**
Lean mass percentage, mean (SD)	0.53 (0.1)	0.58 (0.1)	0.051
Total mass (kg), mean (SD)	81.9(12.6)	67.9 (14.7)	**<0.001**
Android fat mass (kg), mean (SD)	34.6(0.7)	22.3 (9.1)	**<0.001**
Gynoid fat mass (kg), mean (SD)	59.5 (1.0)	47.9 (1.3)	**0.003**
Android lean mass (kg), mean (SD)	32.0 (0.5)	28.2 (0.7)	**0.014**
Gynoid lean mass (kg), mean (SD)	64.0 (1.0)	55.0 (1.2)	**0.003**
LDL OX, median (IQR)	2.7 (0.7-7.4)	2.5 (0.7-5.5)	0.868

Inflammatory cytokines and adipokines			
IL6 (pg/ml), median (IQR)	11.1 (7.3-20.8)	10.5 (4.9-19.8)	0.495
IL-1β (pg/ml), median (IQR)	4.9 (4.1-4.4)	4.3 (4.0 -4.5)	0.007
TNF-α (pg/ml), median (IQR)	5.1 (3.3-27.0)	5.8 (3.7-23.5)	0.827
IGF-1(pg/ml), mean (SD)	168.5 (93.8)	187.7 (106.6)	0.433
Adiponectin (ng/ml), median (IQR)	9271.8 (7602.0-12407.0)	12273.1 (8218.5-15677.1)	0.210
Resistin (ng/ml), median (IQR)	8.3 (5.6-9.9)	7.1 (5.5. - 9.0)	0.626
Leptin (ng/ml), median (IQR)	33.7 (18.0-55.2)	13.9 (7.9-26.1)	0.001

RA, rheumatoid arthritis; ACPA, anticitrullinated protein antibodies; DAS28, 28-joint Disease Activity Score; CRP, C-reactive protein; HAQ, Health Assessment Questionnaire; ND, no data; DMARD, disease-modifying antirheumatic drug; DXA, dual-energy x-ray absorptiometry; BMI, body mass index; FMI, fat mass index (kg of fat/m^2); FFMI, fat-free mass index (kg of lean mass/m^2); LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDL-OX, oxidized low-density lipoprotein; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; IL-1ß, interleukin 1ß; IGF-1, insulin-like growth factor-1.

Table 4. Multivariate models for RA patients with HOMA-IR >2.29 (dependent variable)

Multivariate model 1 using obesity covariate

Predictor	β	OR	95% CI	p value
Obesity (BMI>30)	1.795	6.01	1.94-8.66	p=0.002
Mean DAS28-CRP	1.021	2.77	1.29-5.99	p=0.009
Il-1β (pg/ml)	0.464	1.59	1.06-2.38	p=0.024

R^2 =0.352

Independent variables: Age, sex, obesity (BMI >30), mean 28-joint Disease Activity Index with C-reactive protein (DAS28 CRP), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

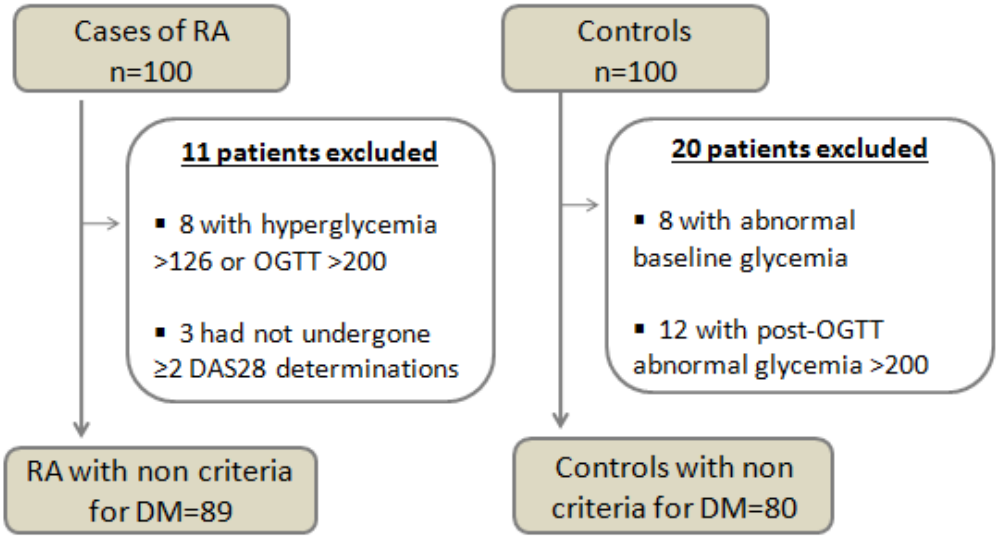
Multivariate model 2 using total fat mass covariate

Predictor	β	OR	95% CI	p value
Total fat mass (kg)	0.123	1.10	1.05-1.22	p=0.002
Mean DAS28-CRP	0.955	2.60	1.19-5.69	p=0.017
Il-1β (pg/ml)	0.456	1.57	1.06-2.34	p=0.023

R^2 =0.404

Independent variables: Age, sex, fat mass, lean mass, mean 28-joint Disease Activity Index (DAS28), diagnostic delay, sedentary lifestyle, IL-1β, and adiponectin.

Figure 1. Patients and controls´ flow chart



RA, Rheumatoid arthritis. OGTT, oral glucose tolerance test; DAS28, 28-joint Disease Activity Score. DM, Diabetes Mellitus.

Table S1. Laboratory tests.

Test	Method/Reference range
High-sensitivity C-reactive protein	Standard nephelometry (reference range, 0-6 mg/l
TNF-α	Automated immunoassay (Immulite®, Diagnostic Products Corporation, Los Angeles, CA, USA; reference range, 0-8.1 pg/ml)
IL-6	Enzyme-linked chemiluminescent assay (QuantiGlo®; normal range, 0-5.84 pg/ml).
IL-1ß	Enzyme-linked chemiluminescent assay (QuantiGlo®) pg/ml
Adiponectin	Enzyme-linked chemiluminescent assay (Mediagnost®) (mean [SD] reference value, 11.5 [5.9] ng/ml)
Resistin	Enzyme-linked chemiluminescent assay (Mediagnost®) (median [IQR] reference value, 7.2 [5.4-8.5] ng/ml)
Leptin	Enzyme-linked chemiluminescent assay (Mediagnost®).

	(according to the manufacturer, normal values lie between the 5th and 95th percentiles after adjusting for sex and BMI ng/ml)
IGF-1	Enzyme-linked chemiluminescent assay (Quantikine®)(pg/ml)
Serum insulin	Enzyme-amplified chemiluminescent assay (Immulite ONE®) (**µ**U/ml)
LDL-Ox	Enzyme-amplifiedchemiluminescent assay (Immulite ONE®)(U/l)

TNF-α, tumor necrosis factor alpha; IL, interleukin; IGF-1,insulin-like growth factor 1;LDL,low-densitylipoprotein; ELISA, enzyme-linked immunosorbent assay. LDL-Ox, Oxidized low-density lipoprotein

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	8-9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	10-11
Bias	9	Describe any efforts to address potential sources of bias	11-12
Study size	10	Explain how the study size was arrived at	11-12
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	11-12
		(*b*) Describe any methods used to examine subgroups and interactions	11-12
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	12
		(b) Give reasons for non-participation at each stage	12
		(c) Consider use of a flow diagram	12 and figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12-14
		(b) Indicate number of participants with missing data for each variable of interest	12 and figure 1

Outcome data	15*	Report numbers of outcome events or summary measures	12-13 and tables
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	14 and table 4
		(*b*) Report category boundaries when continuous variables were categorized	12-14 and tables
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	14-15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	16-18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	26

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.