A double-blind, randomized, placebo-controlled study to explore the efficacy of a dietary plant-derived polysaccharide supplement in patients with rheumatoid arthritis

Azita Alavi1, Lucy Goodfellow1, Owen Fraser1, Edward Tarelli2, Martin Bland3 and John Axford1

Abstract

Objectives. There is increased interest in the potential benefits of complementary therapies, of which dietary plant-derived polysaccharides (dPPs) are an important component. We examined the impact of oral ingestion of a pre-biotic dPP supplement active compound (AC) on serum glycosylation and clinical variables associated with inflammation and general health in patients with RA.

Methods. A double-blind, placebo-controlled, parallel-group clinical trial was used. Participants were randomly assigned to receive AC (n = 33) or placebo (n = 36) for 6 months. Serum protein N-glycosylation was determined by mass spectrometry. Patient outcomes were assessed by validated clinical trial health questionnaires. The primary clinical efficacy variable was DAS-28.

Results. The groups had comparable baseline clinical characteristics. AC was well tolerated with low drop-out rates. Supplementation resulted in a 12% significant drop in the levels of the agalactosylated (G0F) glycans [8.10 (0.89) to 7.16 (0.60); P = 0.03], but had no significant overall effect on patient outcomes. The placebo-treated group showed no change in G0F but exhibited a reduction in the levels of fully digalactosylated (G2) glycans (11%; P = 0.03). Although not clinically significant, DAS scores were, however, marginally lower in the placebo group [difference = 0.63 (0.23) S.E.; 95% CI 0.17, 1.10; P = 0.009], as were two of the secondary variables.

Conclusions. Short-term dietary supplementation with AC resulted in a moderate, but significant, reduction in G0F levels, but did not result in any clinically significant improvement in disease activity when assessing the study group as a whole.

Key words: Rheumatoid arthritis, Glycosylation, Serum glycosylation, Glycomodifications, Dietary plant polysaccharides, Dietary supplements, Ambrotose.

Introduction

RA is a chronic inflammatory, autoimmune rheumatic disease, resulting in progressive joint inflammation and destruction. Although its pathogenesis has not yet been fully elucidated, RA is considered to be a complex, clinically variable disease that is influenced by a combination of genetic and environmental factors [1].

The initiation and progression of RA is a multistep process involving several intermediate stages, including key immunological and glycosylation changes [1–3]. The glycosylation changes centre on marked changes in peripheral and intra-articular total immunoglobulin G (IgG) and ACPA galactosylation [2–4], which can trigger inflammation and subsequent recruitment and targeting of immunomodulating cells within affected joints [1, 2, 5]. These and the other immunopathological changes in RA are interconnected and governed by a complex network of cellular and biochemical events which, in addition to
being under a high level of genetic control, are also influenced by multi-interdependent systems involving both endogenous and exogenous factors [1, 5–8].

The impact of exogenous factors is significant and accounts for 40–50% of the risk [1, 6]. The predisposing environmental factors include infectious agents, smoking, sex hormones and diet. The latter of which is interesting, given the current research into the link between biological activities and potential beneficial effects of dietary supplements, such as dietary plant-derived polysaccharides (dPPs) [9–16].

The possible link between diet and RA stems from the results of large epidemiological studies that indicate lower prevalence of RA in South European countries when compared with North European and North American countries [17], and suggest that the impact of dietary factors, such as omega-3 fatty acids, anti-oxidants, plant flavonoids, pre-biotics and pro-biotics, may be more influential than previously recognized [1, 16, 18–21].

Although the association with diet is open to debate and requires further more detailed studies, there is evidence to suggest a potential link between increased risk of developing inflammatory polyarthritis and lower intake of fish oil [22], dietary vitamin C as well as fibre [1, 23, 24]. There is also evidence suggesting poor nutrient status in RA patients [24, 25], indicating ingestion of significantly lower levels of fibre, as well as dietary deficiencies in pyridoxine, zinc, copper and magnesium [26]. The latter observation is interesting since there may be a synergistic link between fibre intake and bioavailability of essential minerals [13, 27].

Diet may also have an important role in the management of established RA, particularly in alleviating the symptoms of disease and reducing the risk of complications, as demonstrated by the findings of a number of randomized controlled dietary supplementation trials [18, 21, 28]. In this respect, it is interesting to note that 33–75% of RA patients believe that diet plays an important role in providing symptomatic benefit, and 20–60% of patients have used some form of dietary manipulation to alleviate their symptoms [29–33].

In view of this, and given the recent interest in the use of dPPs as pre-biotics that have the potential to manage and steer the gut microbiota towards improved human health [15, 34], we set out to probe the possible affects, if any, that oral administration of a standardized mixed dPP supplement may have on the general health and clinical status of patients with RA. In examining possible beneficial effects we also looked for the impact of these pre-biotic dPPs on serum protein \(N\)-glycosylation, changes in which may impact the immune response and, therefore, disease activity [2–5, 7, 35, 36].

For this purpose we chose a commercially available mixed dPP supplement, Ambrotose Complex, which has previously been used in human trials and has no known toxicity or side effects [37, 38]. This product was chosen because it has been shown, in vitro and in vivo, to exhibit pre-biotic [39, 40] and anti-inflammatory activities [41, 42] and, as demonstrated in a recent study by our group, affect the \(N\)-glycosylation status of serum glycoproteins in normal healthy subjects [43].

**Materials and methods**

**Study design**

The placebo-controlled, randomized, double-blind clinical trial was conducted in a cohort of RA patients recruited from the rheumatology outpatient clinic at St George’s Healthcare Trust (SGHT; London, UK). The study design was approved by the NHS Central Office for Research Ethics Committees (COREC; REC number 03.0148). Patients gave written informed consent and the study was conducted according to the principles of the Second Declaration of Helsinki.

**Determination of group size**

Power calculations based on published data for patients with RA indicated that 52 patients, with a 1 : 1 active compound (AC) : placebo randomization scheme, were required to detect a maximum delta between treatment groups as recommended by a Delphi Round 3 consensus exercise [44], with 80% power, and significance at the 5% level.

**Randomization, masking and data quality assurance**

Patients were sequentially assigned randomization numbers at the time of their recruitment into the study. The trial dietary product for each randomization number was packaged and labelled by the manufacturer (Mannatech Incorporated, Coppell, TX, USA) and stored in the outpatient SGHT Pharmacy Department, which was responsible for dispensing the products at each monthly follow-up visit. The packaging and appearance of AC and placebo were identical.

Product allocation was concealed from the patients, the investigator, the research nurse who recruited and assessed participating patients and the dispensing pharmacist. Randomization codes, held by the trial pharmacist, were identical.

**Recruitment**

Invitation letters were sent out to 159 possible participants. Those who replied (\(n = 84\)) were interviewed and informed of the trial details. Those agreeing to participate (\(n = 78\)) were assessed and, if they met inclusion criteria, were issued a written information sheet prior to signing the consent form. They underwent history review and physical examination and were issued with the relevant questionnaires.

**Inclusion/exclusion criteria**

Eligible patients were aged \(\geq 18\) years, met the ACR criteria for RA [45], had active disease at the time of enrollment and were on a stable regime of treatment for \(\geq 2\) months. Patients with quiescent disease, acute severe RA or severe concomitant disease requiring immunosuppressive or immunomodifying drugs were excluded, as were those who were pregnant, breast feeding or using herbal remedies.
Treatments

Treatments consisted of a powder to be taken orally. The AC was Ambrotose Complex, a dietary supplement approved as a source of dietary fibre by the Dietary Supplement Standard [The Public Health and Safety Company/™/American National Standards Institute (NSF/ ANSI 173) for Dietary Supplements; see supplementary data available at Rheumatology Online, for further information regarding this product].

AC is a dPP containing a standardized mixture of partially purified saccharide biopolymers, including rice starch. The main active ingredients in AC are aloe vera gel extract, arabinogalactan, gum ghatti, gum tragacanth and glucosamine [38, 39]. All the ingredients are approved food supplements and generally recognized as safe (GRAS) by the US Food and Drug Administration [FDA; Everything Added to Food in the United States (EAFUS): A Food Additive Database. http://www.foodsafety.gov/dms/eafus.html. 17 October 2008. 4 December 2008] and by EUROPA Food Safety (http://ec.europa.eu/food/food/biotechnology).

The dose, 1.3 g/day, was recorded by participants on their daily symptom diary card. Adherence was assessed using returned container weight at each monthly follow-up visit. The placebo was rice flour (prepared and supplied by Mannatech), which was identical in texture and appearance to AC.

Trial protocol

Patients were reviewed after recruitment and randomization (i.e. at baseline = Day 0) and were required to complete the trial questionnaires: (i) the SF-36 health-related quality of life: yields an eight-scale profile of functional health and well-being scores that include physical and mental component scores; (ii) Hospital Anxiety and Depression (HAD) questionnaire; (iii) HAQ: measures the ability to cope with arthritis; (iv) Beliefs about Medication Questionnaire (BMQ): assesses beliefs about medication, likelihood of side effects and has been shown to be a good predictor of adherence; (v) WHO quality of life (WHOQoL-BREF) questionnaire; and (vi) Patients’ expectations of treatment and their criteria for effectiveness at baseline.

At the same visit, a full clinical assessment was conducted by a clinician and trial research nurse. Blood and urine samples were taken and sent for full blood count, liver and kidney function tests, as well as RF and ACPA levels.

Patients were reviewed at monthly intervals for a period of 6 months after starting treatment. At each visit, diary cards were collected. The interviewing rheumatologist made a global assessment of disease activity. Blood and urine were collected for full blood count and routine biochemistry. After 6 months, all the procedures undertaken at baseline were repeated.

Medication

Patients were instructed to continue any long-term prophylactic medication (NSAIDs) unaltered throughout the 6-month trial. Patients were allowed access to paracetamol or other proprietary or prescribed simple analgesia. Use of all medication was recorded at each follow-up assessment.

Criteria for withdrawal

To minimize risk of adverse effects, plans were put into place to withdraw patients if, at any time, they or their physician believed that they had deteriorated substantially. No patients fell into this category.

Measurement of patient outcomes

The patient outcome questionnaires were collected at two time points: baseline and end of treatment period.

Measurement of clinical disease activity

Primary and secondary efficacy criteria

The primary efficacy criterion was clinical remission as assessed by changes in the DAS score from baseline (Day 0) to end of treatment (Month 6). The secondary efficacy criteria were patient global score (Pa.GS), physician global score (Ph.GS), swollen joint score (SJS), tender joint score (TJS) and changes in laboratory measures of inflammation: ESR, CRP and haemoglobin (Hb), as well as ACPA and RF.

Measurement of serum N-glycosylation changes

Sample collection and handling

Blood samples were collected at monthly intervals and the serum extracted, aliquoted and stored at −40°C until testing.

Serum glycosylation profiling

Serum glycoproteins were deglycosylated using Peptide N-Glycosidase-F (New England BioLabs Inc., Hitchin, Herts, UK). Released N-glycans were isolated by solid phase extraction [43] ready for glycan profiling by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), using a Kratos Axima curved-field reflectron (CFR) instrument (Kratos Analytical Ltd, Manchester, UK) and a 2,5-dihydroxy benzoic acid matrix.

Percentage intensity of eight main biantennary N-glycans (G0, G0F, G1, G1F, G2, G2F, A1 and A2) were analysed [2]. G0, G1 and G2 refer to the number of galactose (Gal) present (on the biantennary arms of the core pentasaccharide). Further modification due to the presence of (i) fucose (Fuc) gives rise to G0F, G1F and G2F and (ii) sialic acid (NeuAc) give rise to A1/A2 (mono-/di-sialylated) structures (supplementary figure 1, available as supplementary data at Rheumatology Online). Composition of a fully processed A2 glycan is: [NeuAc(b2–6)Gal(b1–4)GlcNAc(b1–2)Man(b1–6)[NeuAc(c2–6)Gal(b1–4)GlcNAc(b1–2)Man(b1–3)[NeuAc(c2–6)Gal(b1–4)GlcNAc(b1–2)Man(b1–3)[NeuAc(c2–6)GlcNAc (GlcNAc: N-acetylglucosamine; Man: mannose).

Statistics

All patients who met the inclusion criteria and were followed up were included in the analyses (Fig. 1). All analyses were undertaken on an intention-to-treat basis; when data at the end of the trial visit were missing...
because of earlier patient withdrawals, the last-value-carried-forward technique was used. Area under the curve analysis and analysis of variance (ANOVA) were used to compare the outcomes after 6 months. Odds ratios (ORs) (with 95% CI) were calculated to compare the effects of AC and placebo. Trends with time were analysed using analysis of covariance with subject as a factor and time (visit) as a covariate, treated as numerical. Variables with a skewed distribution (ESR, CRP and RF) were log transformed. Numerical results are expressed as median and interquartile range. Because all the comparisons to be made were planned prospectively, no correction for multiple comparisons was applied. All tests were two-tailed, and carried out using Stata 8 (Stata Corp., College Station, TX, USA), SPSS.PC version 8.0 statistical software (IBM, Feltham, Middlesex, UK). The patient outcome data were independently analysed by Dr Alison Carr (University of Nottingham, Nottingham, UK).

Results

Participant flow and follow-up
Of the 78 patients assessed, 9 were excluded after randomization. Of these, eight patients were unable to complete questionnaires due to difficulty with English (n = 5), relocation (n = 2) or failure to return for review after the trial consultation (n = 1). Sixty-nine patients (Fig. 1) were therefore randomly given AC (n = 33) or placebo (n = 36).

Patient withdrawals
Five patients given AC and three given placebo withdrew or were lost to follow-up. Reasons included allergic reactions (n = 2; one to AC, one to placebo) or complications unrelated to the study; breast cancer diagnosis (n = 1), knee surgery (n = 1), deceased (n = 1), moved away (n = 2) or unable to attend follow-up assessments (n = 1).

Adherence and adverse events
Adherence exceeded 80% [AC 84.0 (11.8%); placebo 83.4 (14.5%)]. No serious adverse effects of treatment were reported during the trial. All clinical biochemistry laboratory blood results, including the liver function tests, alanine transaminase, ALP and γ-glutamyltransferase, were unaffected and remained within the normal range. The only observed change was a moderate drop [38.69 (0.67) to 37.47 (0.75); P = 0.02] in albumin levels in the AC-treated group (see supplementary table 1s, available as supplementary data at Rheumatology Online). Patients were given the option to continue to take AC for a further 12 months. This option was accepted by >90% of the patients. No adverse events were reported.

Data
Participants had a mean age of 60 (10.5) years (range 30–79 years) and included 56 females. They reflected a spectrum of symptom and disease severity: median DAS score = 4.29 (range 1.98–6.77), median SJS = 5 (range 0–18), median TJS = 5 (range 0–21), RF levels = 106 (range 20–574 U/ml) and ACPA levels 58 (range 2–100 U/ml).

Baseline
Baseline comparisons between the treatment groups on demographic variables and all patient outcome variables as well as disease activity and RF and ACPA status confirmed that randomization had been effective (see supplementary table 2s, available as supplementary data at Rheumatology Online). The only differences noted were: (i) marginally lower, but within normal range, blood lymphocyte [1.61 (0.62) × 10⁹/l and 1.93 (0.10); P = 0.018] and eosinophil [0.13 (0.10) and 0.24 (0.03); P = 0.002] counts; and (ii) higher G0F (8.10 and 6.07; P = 0.03) and lower G2F (2.99 and 3.62; P = 0.04) glycan levels in the AC compared with the placebo group, respectively. The latter
is an important observation since RA is generally associated with high G0F and low G2F levels.

**Differences between groups over the treatment period**

Treatment effects were evaluated by calculating the change in outcome over the treatment period and comparing the changes in outcome between the two treatment groups.

**Serum N-glycosylation**

AC supplementation resulted in a 12% reduction in G0F [8.10 (0.89) to 7.16 (0.60); P = 0.035]. Placebo treatment did not result in any significant changes in G0F [6.07 (0.56) to 6.39 (0.60)], but did result in an 11% reduction in G2 levels [8.16 (0.58) to 7.23 (0.64); P = 0.03].

Comparative analysis of the glycosylation profiles of each group at the end of treatment showed that G0F levels continued to remain high in the AC group [7.16 (0.60)] when compared with the placebo [8.39 (0.67)], and that G2F levels continued to remain significantly lower (21%; P = 0.020) at the end of the treatment period in the AC group compared with the placebo. The AC group also had significantly higher levels of G1 (37%; P = 0.020) at the end of the trial when compared with placebo.

**Patient outcome**

Supplementation did not result in any detectable difference between the two groups on any of the patient outcomes measured (Table 1).

**Clinical efficacy data**

Supplementation did not result in any clinically significant changes in disease activity (Table 2). Although there were responders in both the AC and the placebo groups (48% of AC and 58% of the placebo showed a decrease in their DAS scores), analysis of the overall data showed that the placebo group exhibited lower, though not clinically significant, DAS scores (supplementary figure 2s, available as supplementary data at *Rheumatology* Online), as well as a reduction in the secondary variables, PA.GS and CRP.

**Analysis of time trends in DAS**

Analysis of changes over the treatment period revealed marginal, though not clinically significant, changes in DAS (Fig. 2). The change per visit was not significant in the AC group [decrease per visit = −0.017 (0.020); P = 0.4; 95% CI −0.057, 0.023], but was significant in the placebo group [decrease per visit = 0.073 (0.021); P = 0.001; 95% CI 0.032, 0.114].

**Discussion**

When assessing the RA study group as a whole, oral supplementation with AC was found to have limited effect on the patients’ gross serum N-glycosylation profile, their disease clinical outcome or general health status. AC supplementation did, however, result in a moderate, but significant, 12% lowering of G0F glycan levels. This decrease in the degree of hypogalactosylation is an interesting observation since the presence of high levels of G0 and G0F

**Table 1** Differences in patient outcomes between treatment groups for the change in outcome over the treatment period

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Mean change in outcome (s.d.)</th>
<th>95% CI for mean difference in change in outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMQ necessity</td>
<td></td>
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<tr>
<td>Placebo</td>
<td>0.42 (2.72)</td>
<td>−1.51, 1.45</td>
</tr>
<tr>
<td>AC</td>
<td>0.45 (3.00)</td>
<td>−1.78, 1.68</td>
</tr>
<tr>
<td>BMQ concerns</td>
<td></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>−0.10 (3.47)</td>
<td>−2.12, 1.98</td>
</tr>
<tr>
<td>AC</td>
<td>−0.17 (3.88)</td>
<td>−2.21, 1.72</td>
</tr>
<tr>
<td>BMQ overuse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>−0.64 (2.81)</td>
<td>−2.21, 0.72</td>
</tr>
<tr>
<td>AC</td>
<td>0.10 (2.86)</td>
<td>0.08, 1.68</td>
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<tr>
<td>BMQ harm</td>
<td></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>−0.35 (2.17)</td>
<td>−1.54, 1.11</td>
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<tr>
<td>AC</td>
<td>−0.14 (2.94)</td>
<td>−1.76, 0.97</td>
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<tr>
<td>HAD anxiety</td>
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<tr>
<td>Placebo</td>
<td>−0.06 (2.95)</td>
<td>−1.68, 0.19</td>
</tr>
<tr>
<td>AC</td>
<td>0.33 (3.22)</td>
<td>−1.54, 1.11</td>
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<td>HAD depression</td>
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<td>−1.68, 0.19</td>
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<tr>
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<td>−1.54, 1.11</td>
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<td>HAQ</td>
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<td></td>
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<tr>
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<td>−0.08, 0.23</td>
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<tr>
<td>AC</td>
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<td>−1.34, 1.56</td>
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<tr>
<td>SF-36 physical functioning</td>
<td></td>
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<tr>
<td>Placebo</td>
<td>4.22 (18.97)</td>
<td>−7.89, 11.99</td>
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<tr>
<td>AC</td>
<td>2.17 (20.16)</td>
<td>−4.55, 16.57</td>
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<td>SF-36 physical role</td>
<td></td>
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<td>Placebo</td>
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<td>−5.45, 16.57</td>
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<tr>
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<td>−4.58 (23.27)</td>
<td>−7.89, 11.99</td>
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<td>SF-36 pain</td>
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<tr>
<td>Placebo</td>
<td>4.28 (21.61)</td>
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<td>AC</td>
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<td>−7.89, 11.99</td>
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<tr>
<td>SF-36 social functioning</td>
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<td>−12.79, 7.47</td>
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<tr>
<td>AC</td>
<td>−13.67 (20.81)</td>
<td>−12.79, 7.47</td>
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<tr>
<td>SF-36 mental health</td>
<td></td>
<td></td>
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<td>Placebo</td>
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<td>−6.36, 9.73</td>
</tr>
<tr>
<td>AC</td>
<td>−2.00 (17.30)</td>
<td>−10.57, 15.78</td>
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<td>SF-36 emotional role</td>
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<td>−10.57, 15.78</td>
</tr>
<tr>
<td>AC</td>
<td>−4.17 (24.34)</td>
<td>−10.57, 15.78</td>
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<tr>
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<td>−6.71, 9.88</td>
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<td>0.47 (2.90)</td>
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<tr>
<td>AC</td>
<td>1.21 (5.61)</td>
<td>−3.00, 1.52</td>
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<td>WHOQol Domain 2</td>
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<td>0.81 (2.40)</td>
<td>−1.70, 1.53</td>
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<td>AC</td>
<td>0.90 (3.80)</td>
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<tr>
<td>AC</td>
<td>−0.38 (3.02)</td>
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<td>WHOQol overall score</td>
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<td>1.53 (3.86)</td>
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<tr>
<td>AC</td>
<td>1.41 (5.70)</td>
<td>−2.36, 2.59</td>
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</table>
is a well-established feature of RA, and decreased levels of these glycans (on IgG, as well as ACPA) have been shown to correlate with decreased disease activity and disease remission during pregnancy [2, 5, 7, 35-36]. In this instance, however, the decrease in G0F does not appear to have had a major impact on disease activity. This may be because despite effective randomization, as assessed by baseline demographic and clinical characteristics, the two groups were found to exhibit different glycosylation profiles. The patients in the AC group were found to have had a more pronounced RA glycosylation profile (high G0F and low G2F), at the point of recruitment, than those in the placebo group. As such, the 12% decrease, following AC supplementation, may not have been sufficient to substantially lower G0F levels, which continued to remain high throughout the study in patients in the AC group when compared with those in the placebo group.

Interactions that directly/indirectly involve glycan structures are key in governing many biological functions and form the basis for the link between disease activity and glycosylation changes in RA [2-4, 7]. Glycosylation changes in, for example, the level of IgG-Fc Gal and Fuc have been shown to alter Fc-mediated effector functions—by affecting its interaction with other immunoregulatory factors, such as mannose binding protein (MBP) and or activating/inhibitory Fc receptors—and thereby enhance or dampen inflammatory responses and subsequently affect disease pathogenesis and outcome [2, 46]. However, the exact mechanism of how the observed serum glycosylation change occurs and what its biological significance is, remains to be evaluated further. The observed decrease in G0F in the AC-treated group is comparable to the results obtained in another recent study by our group looking at the effect of AC on the serum N-glycosylation status of normal healthy individuals [43]. There we showed that supplementation with a different Ambrotose preparation (aloe vera gel extract was substituted with gel powder and wakame algae extract was added), given at increasing doses (1.3-5.2 g/day), could result in a significant, dose-dependent, overall shift in serum glycosylation [43].

The absence of a similar major shift in serum glycosylation in this study may, therefore, be a reflection of either the different formulation [10, 40], and/or the lower levels of supplement used in this study compared with that used in our previous investigation of the effect in healthy volunteers [43]. Another factor, however, that may be a major contributor to the observed overall lack of response may be the innate differences in the gut microflora or gut

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**Table 2** Comparative analysis of the differences in primary and secondary outcomes between the AC and placebo groups after adjustment for baseline measurements

<table>
<thead>
<tr>
<th>Efficacy outcome</th>
<th>Difference (test – placebo)</th>
<th>Significance P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS</td>
<td>0.63 (0.23)</td>
<td>0.009</td>
<td>0.17, 1.10</td>
</tr>
<tr>
<td>Secondary outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pa.GS</td>
<td>10.5 (4.4)</td>
<td>0.02</td>
<td>1.7, 19.4</td>
</tr>
<tr>
<td>Ph.GS</td>
<td>4.5 (3.5)</td>
<td>0.2</td>
<td>−2.4, 11.4</td>
</tr>
<tr>
<td>SJS</td>
<td>0.62 (1.07)</td>
<td>0.6</td>
<td>−1.53, 2.77</td>
</tr>
<tr>
<td>TJS</td>
<td>2.4 (2.4)</td>
<td>0.07</td>
<td>−0.2, 5.0</td>
</tr>
<tr>
<td>Hb</td>
<td>0.09 (0.17)</td>
<td>0.6</td>
<td>−0.26, 0.44</td>
</tr>
<tr>
<td>ACPA</td>
<td>20 (12)</td>
<td>0.1</td>
<td>−8, 48</td>
</tr>
</tbody>
</table>

| Ratio (test – placebo) | | | |
|------------------------| | | |
| ESRa                   | 1.20 | 0.09 | 0.97, 1.47 |
| CRPa                   | 1.54 | 0.03 | 1.05, 2.27 |
| RFa                    | 0.90 | 0.3  | 0.73, 1.11 |

*a* Refers to variables which were log transformed.

**Fig. 2** Differences in trend over time, from baseline to final measurements, in the AC- (*n* = 33; •) and the placebo- (*n* = 36; *) treated groups: Box-and-whisker plot of the DAS scores.
permeability in RA patients compared with normal healthy individuals [34, 47, 48], which could result in possible aberrant processing of these dPPs. The latter proposal is supported by evidence from recent molecular analysis of the quantity of intestinal microbes and the bacterial genera in faecal samples obtained from RA patients, the results of which indicate widespread gut microbial differences in RA compared with controls [49]. In this respect, it is interesting to note that using a dynamic model of the human gastrointestinal tract, Marzorati et al. have recently shown that AC can increase the population of *Bifidobacteria* and *Bacteroides/Prevotella* group [40], which are generally low in RA patients [47, 50].

These gut microflora differences, which may be further influenced by the genetic background and MHC status of the patients [50], as well as certain drug therapies (e.g. the anti-bacterial effects of SSZ), would not only influence the digestion of certain foods but could also cause a breakdown in the symbiotic relationship of commensal gut bacteria and the gut-associated lymphoid system (GALT), which is critical to maintaining a normal immune system [15, 16, 34, 47, 48].

As such, it is possible that the role that commensal gut bacteria play in relation to GALT may be a far more important factor in RA than previously recognized [47]. This would not only help explain why patients with RA often feel there is an association between their diet and disease status, but would also partially explain the evidence for RA-associated dietary deficiency in certain essential minerals, the bioavailability of which may be linked to fibre intake and digestion [13, 27]. It also supports the findings from experimental studies that strongly indicate that the incidence and severity of arthritis are interdependent on diet and exposure to environmental microorganisms, and altered when the animals are reared in germ-free conditions [51].

In conclusion, supplementation with AC, albeit at a dose lower than that which is currently recommended by the manufacturers and in contrast to our previous findings in healthy individuals (with another similar preparation of AC), had little affect on the overall clinical outcome when assessing the RA study group as a whole. AC supplementation did, however, result in a significant drop in the levels of hypogalactosylated, G0F, glycans, high levels of which are a well-established feature of active RA. The data from this study raise some interesting questions regarding the potential impact that differences in glycosylation status may have on the clinical outcome, and highlight the importance of conducting rigorous clinical trials to validate or refute the clinical usefulness of this or other similar supplements.

**Rheumatology key messages**

- AC supplementation did not result in any clinically significant improvement in RA disease activity.
- AC supplementation did, however, result in a significant drop in hypogalactosylated, G0F, glycans, high levels of which are a well-established feature of active RA.

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**Supplementary data**

Supplementary data are available at Rheumatology Online.

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