EXTENDED REPORT

Influence of glucocorticoids and disease activity on total and high density lipoprotein cholesterol in patients with rheumatoid arthritis

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Background: Glucocorticoids induce hypercholesterolaemia, a cardiovascular risk factor, in patients with diseases other than rheumatoid arthritis (RA), but the data in RA are contradictory.
Objective: To determine the effects of antirheumatic treatment, including prednisolone (combination) therapy on total and high density lipoprotein (HDL) cholesterol levels in RA, taking disease activity into account.
Methods: HDL cholesterol and total cholesterol levels were determined in:(*a*) established RA (*b*) two cohorts with early active RA, (*c*) a previously conducted 56 week trial among patients with early RA comparing the value of intensive combination therapy (that included glucocorticoids) with sulfasalazine alone (COBRA trial).
Results: In established RA total cholesterol levels were only slightly raised, irrespective of disease activity. However, HDL cholesterol was significantly higher in patients in remission than in patients with active disease. In contrast, in active early RA at baseline total cholesterol was low normal: between 4.6 and 5.1 mmol/l in the different populations. The level of HDL cholesterol was highly dependent on the duration of storage. In both COBRA groups total cholesterol increased by a mean of 0.6 mmol/l. HDL cholesterol increased by more than 50% after treatment, leading to an improvement of the total cholesterol/HDL ratio (atherogenic index). This increase (and index improvement) was much more rapid in the group receiving combination treatment. A similar pattern was seen in the 2001 cohort with early RA. In all the groups with active disease HDL and total cholesterol levels correlated inversely with disease activity.
Conclusion: In established, but especially in early RA, disease activity is accompanied by atherogenic lipid levels. This dyslipidaemia can be rapidly reversed by aggressive antirheumatic treatment including glucocorticoids.

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Mortality is increased in patients with rheumatoid arthritis (RA) compared with the general population, and cardiovascular disease is the most important cause of death.[1] Theoretically, this increased cardiovascular risk in patients with RA could be caused by (1) an increased prevalence of (known) risk factors for cardiovascular disease such as dyslipidaemia, diabetes mellitus, hypertension, body mass index, physical fitness, and smoking habits; (2) RA itself by either (*a*) the underlying inflammatory process, or (*b*) decreased functional capacity; and (3) undertreatment of cardiovascular disease as a comorbid condition in patients with RA (Boers M, unpublished data).

In this investigation we focused on dyslipidaemia. Published reports are sparse and contradictory about the levels of total, high density lipoprotein (HDL) and low density lipoprotein cholesterol, and triglycerides in patients with RA, even though there is some evidence for increased Lp(a) lipoprotein levels and lowered lipid levels in patients with active disease.[1]

Glucocorticoids are commonly used in patients with RA. Although it is well known that glucocorticoids induce hypercholesterolaemia, a well known cardiovascular risk factor, in patients with diseases other than RA,[2][3] the effect of (long term) glucocorticoid administration on lipid profiles in patients with RA is uncertain. Thus far a limited number of small scale studies have dealt with this subject in patients with RA, indicating either an increase or no effect of total cholesterol levels during (long term) glucocorticoid administration.

Therefore, in stored serum samples we investigated total cholesterol and HDL cholesterol levels cross sectionally, in patients with early RA[4] and in patients with longstanding, established disease.[5] Moreover, we studied the course of lipid levels and the influence of antirheumatic treatment (including glucocorticoids), in a previously conducted, 56 week multicentre, randomised controlled trial among patients with early RA investigating the value of intensive combination therapy.[6] Finally, we determined these levels in one year follow up samples of the 2001 cohort with early RA.

PATIENTS AND METHODS

This study is the result of an incremental process: the COBRA trial samples were determined first, followed by confirmation in patients with established active RA and in patients with RA in remission, and baseline samples of a cohort with early RA collected in 1996–97. All patients fulfilled the American College of Rheumatology (ACR) 1987 criteria for RA.[7] The apparent effect of serum storage on HDL cholesterol led us to analyse "fresh" samples of patients with early RA collected in

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Abbreviations: ACR, American College of Rheumatology; DAS, disease activity score; DMARDs, disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; HDL, high density lipoprotein; RA, rheumatoid arthritis

Table 1 Baseline characteristics of study patients

	COBRA	Early arthritis clinic 1996–97	Early arthritis clinic 2001	Established disease active	Established disease remission
Number	134	26	21	56	56
Age, mean (SD)	50 (12)	62 (17)	60 (14)	60 (13)	59 (14)
No (%) male	55 (41)	9 (35)	8 (38)	9 (16)	9 (16)
Disease duration	Median 4 months (range 1.5–24)	0 months	0 months	8 (9) years	10 (9) years
ESR	55 (33)	52 (28)	43 (30)	39 (22)	15 (15)
DAS28*	5.9 (1.0)	5.2 (0.8)	5.8 (1.0)	5.2 (1.5)	≤2.6 (0.7)

Disease activity score (28 joints), at baseline ⩾5.1 is high and ⩽2.6 is remission.

2001, and these results prompted us to repeat lipid measurements of these patients after one year, in order to confirm our findings in fresh samples.

Cross sectional investigations
Patients with established RA
Serum samples of 56 patients in remission according to the ACR criteria,[8] were matched with serum samples of 56 age and sex matched patients with established RA with active disease. The patients had participated in other studies and the samples were chosen according to availability.[5]

Most patients were receiving treatment with sulfasalazine or methotrexate. None of the patients in remission and only a few patients with active disease used systemic (low dose) glucocorticoids.

Early arthritis clinic 1996–97
Serum samples of a group of 26 patients with early active RA were used. The samples collected at the first visit were selected according to availability. None of the patients had been treated with disease modifying antirheumatic drugs (DMARDs) or glucocorticoids.

Longitudinal investigations
56 Week randomised controlled trial (COBRA)
In this investigation the combination of sulfasalazine, methotrexate, and prednisolone (initially 60 mg/day, tapered in six weekly steps to 7.5 mg/day and stopped after 28 weeks) was compared with sulfasalazine alone. A total of 155 patients with early active RA (median disease duration four months) were randomly assigned to combined treatment (76) or sulfasalazine alone (79). Prednisolone and methotrexate were tapered and stopped after 28 and 40 weeks, respectively. Blood samples were taken at baseline and at weeks 16, 28, 40, and 56.

Early arthritis clinic 2001
The 2001 group comprised 21 patients with early active RA. Serum samples collected at the first visit were selected according to availability; none of the patients had been treated with DMARDs or glucocorticoids. In addition, we determined lipid levels in one year follow up samples of the group. At this time most patients had been treated with DMARDs, but only a few had received glucocorticoids.

Total cholesterol and HDL cholesterol determinations
All (non-fasting) serum samples had been stored at −20°C or lower. Total serum cholesterol (normal 5.0–6.4 mmol/l) was measured by an enzymatic method in an autoanalyser; HDL cholesterol (normal >0.9 mmol/l) was determined enzymatically with polyethylene glycol modified enzymes.

Although, the classical (chemical) reference value considers a total cholesterol value of 6.4 mmol/l as the upper limit of normal we have, for the interpretation of our results, chosen 5.0 mmol/l as the upper limit in view of the recommendations made by several national and international consensus committees.[9–11] These guidelines were made in view of the increasing evidence that "high-normal" total cholesterol serum levels already imply an increased cardiovascular risk. Likewise an HDL cholesterol level of 1.0 mmol/l instead of 0.9 mmol/l was chosen as the lower limit of normal.

Statistical analyses
All results are expressed as mean (SD). Total cholesterol and HDL cholesterol and their SD values are expressed in mmol/l. In patients with established RA a paired t test was used to analyse the differences between active disease and remission. Likewise, a paired t test was used to analyse the change between baseline and one year follow up in the 2001 group with early RA. Analysis of variance was used to tested the differences between the groups with active early and established RA.

In the COBRA trial, paired and unpaired t tests were used to analyse the changes within and between the treatment groups, respectively. Stepwise multiple regression was used to analyse the changes in lipids up to week 16, with treatment group, age, sex, disease activity score (DAS) at baseline, erythrocyte sedimentation rate (ESR) at baseline, and changes in ESR at week 16 as possible predictors. This 16 week period was chosen for our main analyses as changes in disease activity and doses of glucocorticoids were most prominent over this period. Simple regression analysis was used to test the relation between baseline lipid levels and storage duration.

RESULTS
Patients with established RA
The mean age of the patients with established active disease was 60 years, and their disease duration was 8 years (table 1). For patients in remission the mean age was 59 years and the disease duration 10 years. These patients with established disease had slightly raised levels of total cholesterol—that is, 5.5 (1.1) and 5.7 (0.8) mmol/l, respectively (table 2). However, HDL cholesterol levels were significantly lower in the patients with active disease than in those in remission, 0.94 (0.31) v

Table 2 Total cholesterol, HDL cholesterol and atherogenic index (cholesterol/HDL cholesterol) levels at baseline

	COBRA	Early arthritis clinic 1996–97	Early arthritis clinic 2001	Established disease active	Established disease remission
Total cholesterol (TC) (mmol/l, SD)	4.7 (1.1)	5.1 (1.0)	5.1 (1.0)	5.5 (1.1)	5.7 (0.8)
HDL cholesterol (mmol/l, SD)	0.28 (0.11)	0.49 (0.l3)	1.0 (0.33)	0.94 (0.31)	1.18 (0.13)
TC/HDL cholesterol ratio	16.7	10.4	5.4	6.4	5.1
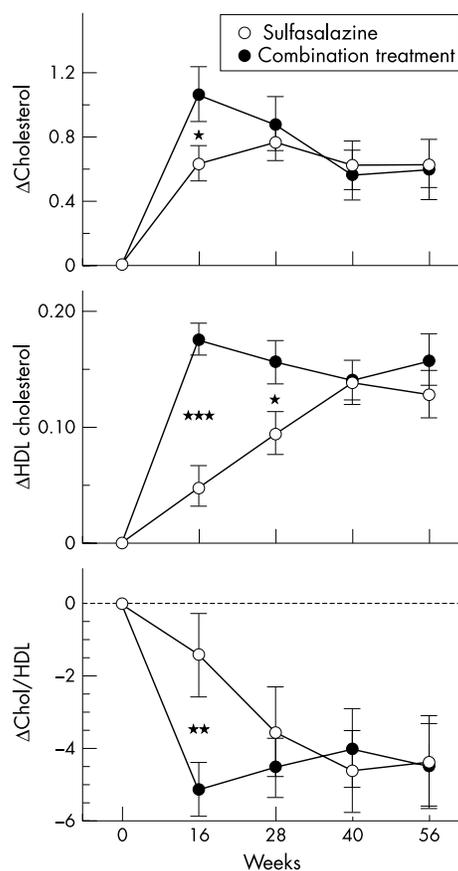
Figure 1 Changes in total cholesterol, HDL cholesterol, and the atherogenic index (chol/HDL) in the COBRA trial.

1.18 (0.13) (p<0.001), respectively. In explanatory regression analysis, within the patients with active disease total cholesterol and HDL cholesterol levels correlated inversely with disease activity, as assessed by the disease activity index 28 joint score (DAS28).[12]

Early arthritis clinic 1996–97
The mean age of the patients was 62 years (table 1). At baseline, total cholesterol was 5.1 (1.0) and HDL cholesterol 0.49 (0.13) in the 1996–97 group.

56 Week randomised controlled trial (COBRA)
The mean age of the patients was 50 years; median disease duration was four months (table 1). Disease activity improved more rapidly and effectively in the group receiving combination therapy at week 16, and this effect persisted until week 28. The clinical difference between the groups decreased and was no longer significant after prednisolone was stopped, and there were no further changes after methotrexate was stopped.[6] However, persistently lower yearly rates of radiological damage progression were observed at least four years after the trial ended in the (initial) combination group.[13]

During the trial both groups showed a strong increase from low baseline cholesterol levels (fig 1), initially more pronounced in the combination group (week 16: 1.1 v 0.6; p=0.04). Likewise, for HDL cholesterol, both groups showed a strong increase from a very low baseline level (fig 1). At week 16 the increase in HDL cholesterol was much more pronounced in the combination group (0.18 v 0.05; p<0.001); this difference was still apparent at week 28 (0.16 v 0.10; p=0.02). Likewise, at week 16 the decrease in atherogenic index was more rapid in the combination group (−5.1 v −1.4; p=0.008). Thereafter, it decreased gradually in the sulfasalazine group, but remained stable in the combination group,
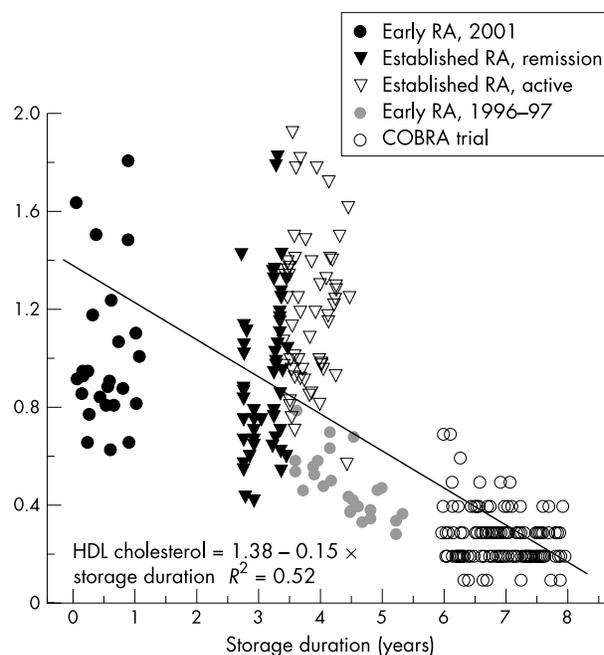
Figure 2 HDL cholesterol levels at baseline related to storage duration.

resulting in similar values at week 28 and thereafter (fig 1). Of note, the changes in lipids were mirrored by the pattern of weight gain, initially greater in the combination group, but more than 3 kg in both groups at 56 weeks (data not shown).

Regression analysis on the changes at week 16 showed that changes in total cholesterol were only predicted by changes in ESR. The change in HDL cholesterol levels at week 16 could be partially predicted by the change in ESR and DAS at baseline. However, the treatment group was a more important predictor independently of the ESR or DAS. Similar findings were found for the ratio total cholesterol/HDL cholesterol

Early arthritis clinic 2001
The mean age of the patients was 60 years (table 1) and at baseline the total cholesterol was 5.1 (1.0) mmol/l and HDL cholesterol 1.0 (0.33) mmol/l (table 2). After one year the total cholesterol had increased to 5.8 (1.4) (p< 0.005 compared with baseline) and HDL to 1.23 (0.39) (p<0.005). The atherogenic index—that is, total cholesterol/HDL cholesterol ratio, was 5.4 (2.1) at baseline and decreased to 5.0 (1.5) at the one year follow up visit (p=0.12).

Effect of storage duration on lipid levels
Overall, storage duration of baseline samples did not influence total cholesterol levels. However, the estimated decrease for HDL cholesterol was 0.15 for each storage year (fig 2).

DISCUSSION
This study shows that patients with early RA with active disease present with low concentrations of cholesterol and HDL cholesterol. Although both total and HDL cholesterol levels appear to be only moderately decreased the total cholesterol/HDL ratio is at least 5.1 at baseline. This so-called atherogenic index is an important prognostic marker for future cardiovascular disease; the desirable ratio is four or lower.[14 15] A higher index implies an increased cardiovascular risk, and lowering this ratio has been shown to decrease this risk—for example, lowering the ratio from 6.0 to 4.5 halves the five year rate of coronary artery disease in women.[16] Our longitudinal investigations indicated an improvement of the atherogenic index by antirheumatic treatment, which might result in a lower

(future) cardiovascular risk, provided that the improvement is maintained over a longer period.

In the COBRA trial the rapidity of response was faster in the group receiving combined treatment. Owing to the design of the trial, we are unable to answer the question as to whether this is the result of better disease suppression, specific to one of the components or even their combination. However, we did find a linear relationship between the DAS28 score and the atherogenic index in both early and established RA. Regression analyses of the COBRA data showed that changes in total cholesterol were best predicted by changes in ESR, but the model for changes in HDL cholesterol also contained disease activity at baseline and treatment group as additional factors. These findings suggest that changes in total cholesterol are the result of effective RA treatment, but changes in HDL are more specific to glucocorticoid (or combination) treatment.

To our surprise, and not reported before, HDL cholesterol (but not total cholesterol) levels were strongly influenced by storage duration of the samples. Hence, the absolute values of the atherogenic index in all except the latest (early arthritis clinic 2001) samples, should be interpreted cautiously, as they are too low. However, the differences between the trial groups, and the changes over the year (and their determinants) can still be studied validly.

Therefore, comparisons between the patients with established RA whose disease is active and those in remission and of both groups with the COBRA group remain valid.

It is well known that physical activity has beneficial effects on the total cholesterol/HDL cholesterol ratio. In the transverse comparisons of patients with established RA who have active disease and those in remission such an effect cannot be excluded. However, the similarity in Health Assessment Questionnaire scores makes this explanation for the lipid findings less likely. In the trial COBRA patients with RA improved much more rapidly than patients receiving sulfasalazine, and the former may well have increased their physical activity. However, it seems unlikely that this would have such major effects on blood lipids in such a short period of time.

The lipid profile may be also influenced by weight/body mass index or drugs such as antimalarial drugs or statins. In the transverse comparisons there were no significant differences in body weight. In the trial, both groups gained weight, (about 3 kg in 56 weeks, difference NS), but the COBRA group gained weight more rapidly in the first 28 weeks. Only a very few patients were receiving antimalarial drugs and/or statins. Moreover, these drugs were equally distributed between the several groups. In the COBRA trial such drugs were not used.

Altogether, the combination of cross sectional and longitudinal evidence appears strong. Our findings indicate an atherogenic lipid profile in active RA, especially in untreated patients with early RA, which improves on treatment. The concomitant changes in body weight suggest a state of rheumatoid cachexia at baseline.[17] This is supported by a recent uncontrolled investigation in 42 patients with early RA which also indicated improvement of the lipid profile in the 27 patients who responded to antirheumatic treatment.[18]

Thus far published reports are contradictory about the effect of glucocorticoids in RA, and data of properly designed studies are lacking. The controlled COBRA data indicate that glucocorticoid (combination) treatment increases total cholesterol and HDL cholesterol more quickly, with a concomitant faster improvement of the atherogenic index, than sulfasalazine alone. Whether or not this favourable influence on the cardiovascular risk is ultimately offset by adverse cardiovascular effects of long term glucocorticoid administration—for example, hypertension and insulin resistance,[19] remains to be established.

There is an increasing awareness that patients with RA have an enhanced cardiovascular risk, which appears to be, at least partly, caused by inflammation.[1] Our study shows that inflammation (disease activity) has a negative influence on the lipid profile and that effective treatment can ameliorate this.

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