

9

Quercetin reduces markers of  
oxidative stress and inflammation in  
sarcoidosis

AW Boots, M Drent, VCS de Boer, A Bast, GRMM Haenen

*Submitted*

## Abstract

### Background

Oxidative stress and low antioxidant levels are implicated in the etiology of sarcoidosis, an inflammatory disease. Quercetin is a potent dietary antioxidant that was also found to display anti-inflammatory activities. This prompted us to examine the effect of quercetin supplementation on markers of oxidative stress and inflammation in sarcoidosis.

### Methods

A double-blind intervention study has been conducted with two groups of non-smoking, un-treated sarcoidosis patients, matched for age and gender. One group was given 4x500 mg quercetin (n=12) orally in a 24-hour period, the other group was given a placebo (n=6). Plasma malondialdehyde (MDA) levels were used as a marker of oxidative damage, the ratios of TNF $\alpha$ /IL-10 and IL-8/IL-10 in the blood of the patients as pro-inflammatory markers. *Ex vivo*, an LPS challenge in blood was performed to study the potential of the supplementation to reduce an inflammatory response.

### Results

This study demonstrated that quercetin supplementation improved the antioxidant defence system, indicated by the increased total plasma antioxidant capacity. *Ex vivo* LPS-induced cytokine levels appeared to be also reduced by the supplementation. Moreover, this supplementation also reduced markers of oxidative stress and inflammation in sarcoidosis. The effects of the quercetin supplementation appeared to be more pronounced when the levels of the oxidative stress and inflammation markers were higher at baseline.

### Conclusions

This study indicates that sarcoidosis patients might benefit from the use of antioxidants such as quercetin to reduce the occurring oxidative stress as well as inflammation. Long-term use of antioxidant supplementation in sarcoidosis, using e.g. quercetin, remains to be investigated.

## Introduction

Sarcoidosis is a chronic inflammatory disease of which the exact cause still needs to be elucidated. There is growing evidence that sarcoidosis is associated with oxidative stress, i.e. an imbalance between the production of and the protection against reactive oxygen species (ROS). This is deduced from e.g. increased nuclear factor  $\kappa$ B (NF- $\kappa$ B) activation (1) and increased levels of biomarkers of oxidative damage such as exhaled ethane (2) and both 8-isoprostane (3) and oxidized proteins (4) in the bronchoalveolar lavage fluid (BALF) of sarcoidosis patients. Recently, we have found that the total antioxidant capacity of sarcoidosis patients is approximately 75% of that of matched controls (unpublished results).

Quercetin is an excellent antioxidant. Within the flavonoid family, quercetin is the most active scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This can be explained by the presence of two antioxidant pharmacophores within the molecule that have the optimal configuration for free radical scavenging (5). Recently, it has been shown that quercetin supplementation effectively increases the total plasma antioxidant capacity in healthy volunteers (6).

As an inflammatory disorder, sarcoidosis is characterized by increased levels of pro-inflammatory cytokines such as TNF $\alpha$  and IL-8 (7-10). Several studies have indicated that the flavonoid quercetin can also display anti-inflammatory effects (11-13).

Interestingly, de Boer *et al* have recently demonstrated that quercetin accumulates in the lungs of rats and pigs (14). The combination of its tissue specific distribution and potent anti-oxidative as well as anti-inflammatory capacities prompted us to examine the effect of quercetin supplementation on oxidative stress and inflammation in sarcoidosis patients. To mimic a severe inflammatory burden that might occur by incidental exposure to e.g. dust particles, cigarette smoke or other triggers, an *ex vivo* LPS challenge was applied.

## Material and Methods

### Chemicals

Quercetin and lipopolysaccharide (LPS, *E. coli* 0.26:B6) were purchased from Sigma Chemical Co. (St. Louis, USA). RPMI 1640 medium containing L-glutamine was obtained from *Gibco* (UK). Human TNF $\alpha$  (7300 pg/ml), human IL-10 (4000 pg/ml) and human IL-8 (10 ng/ml) were acquired from CLB/Sanquin (Amsterdam, the Netherlands). All other chemicals were of analytical grade.

## Methods

### *General information*

All participating patients were recruited via their own physician (MD). All participants were fully informed, both written and orally, about the aim and details of the study and have given their written informed consent.

The study was carried out at the University Hospital Maastricht, a tertiary referral for the Netherlands, after approval of the protocol by the Medical Ethics Committee of Maastricht University and the University Hospital Maastricht. Based on foodstuff questionnaires, it was concluded that all participants had comparable dietary habits with an average daily intake of quercetin of approximately 15 mg. None of the participants took any medication or vitamin or food supplements either prior to or during the study. Randomisation occurred by first dividing all participants into trios based on their age and gender and then by randomly giving placebo treatment to one individual out of each trio.

### *Participants*

Eighteen non-smoking patients with symptomatic sarcoidosis (age  $45 \pm 10$ ) were enrolled in this study. Sarcoidosis had been diagnosed based on both clinical features and bronchoalveolar lavage (BAL) fluid analysis results (data not shown) (15). Moreover, a biopsy confirmation of the disease had been performed in 11 out of the 18 sarcoidosis patients. The clinical symptoms of all patients included respiratory symptoms, i.e. dyspnea, coughing and chest pain. The characteristics of the study population are summarized in table 9.1.

### *Lung function measurement*

Lung function measurements included FEV<sub>1</sub> and DLCO. FEV<sub>1</sub> was measured with a pneumotachograph, DLCO by the single-breath method (Masterlab, Jaeger, Würzburg, Germany). Values were expressed as a percentage of those predicted based on age and gender (16).

### *Supplementation study*

Prior to the actual supplementation period, participants were subjected to a two-day wash-out period. During this period, they were not allowed to consume food rich in flavonoids in general or quercetin in particular. This food included non-organic onions, apples, red wine, tea, organic and freshly pressed fruit juices, berries (e.g. blueberries and elderberries), grapes, cherries, raisins, parsley, broccoli, cabbage, green beans and tomatoes (6). Participants also had to minimise the use of herbs and spices during this period.

The wash-out period was followed by a 24-hour supplementation period during which all participants had to take 4 capsules containing either 500 mg

quercetin or a placebo. The capsules were taken throughout the day, i.e. during lunch, during dinner, just before bedtime and the last during breakfast the following morning, three hours before the second blood withdrawal. Before and after this supplementation period, venous blood samples were drawn into EDTA-containing vacutainer tubes (Vacutainer, Becton-Dickinson, Belgium) and kept on ice prior to processing which occurred within 1 hour after blood collection. During supplementation, the same dietary restrictions as during the wash-out period were applied.

Table 9.1 Characteristics of the participants.

	Quercetin-receiving group	Placebo-receiving group
number (m/f)	12 (8/4)	6 (4/2)
age	31-69 (46 ± 3)	34-59 (44 ± 3)
length	158-186 (174 ± 3)	158-182 (175 ± 4)
weight	64-96 (81 ± 3)	69-96 (84 ± 4)
body mass index	23-32 (27 ± 1)	24-35 (28 ± 2)
time since diagnosis	1-10 (5 ± 1)	1-11 (4 ± 2)
biopsy taken	yes: 7 no: 5	yes: 4 no: 2
DLCO	55-109 (84 ± 5)	85-107 (91 ± 6)
FEV <sub>1</sub>	36-133 (96 ± 7)	51-101 (86 ± 7)
FVC	75-128 (104 ± 5)	65-112 (97 ± 7)
chest radiograph stage 0/I/II/III/IV (n)	3/3/3/3/1	2/2/1/1/0

Controls are matched by age and gender and do therefore not significantly differ from the patients regarding these parameters. Age is expressed by year, length by cm, weight by kg, and both DLCO (diffusing capacity of the lung for carbon monoxide) and FEV<sub>1</sub> (forced expiratory volume in 1 second) by % of the predicted value based on age and gender. Data are expressed as range (mean ± SEM).

#### *Preparation of the blood samples*

Blood was aliquoted into eppendorfs for both the ascorbic acid and the GSH/GSSG analysis: for the former 10% TCA was added to the whole blood, whereas 1.3% SSA in 10 mM HCl was used to preserve the samples for the latter. Another aliquot of blood was used for the incubations required for the blood-based cytokine production assay as described in that section below. The remaining blood was centrifuged (3000 rpm, 5' at 4°C) to obtain plasma. Deproteinization of an aliquot of this plasma, using 10% trichloro-acetic acid (TCA) 1:1, followed by centrifugation (13,000 rpm, 5' at 4°C), was carried for

the trolox equivalent antioxidant capacity measurement (TEAC). All samples were stored at -80°C prior to analysis.

#### *Determination of total plasma quercetin concentration*

Total quercetin (sum of quercetin aglycone and quercetin glucuronides/sulfates) concentrations in plasma were analysed by means of HPLC with coulometric array-detection after enzymatic hydrolysis as described previously (17). 3'-O-methoxy quercetin (isorhamnetin) and 4'-O-methoxyquercetin (tamarixetin) aglycone and glucuronides/sulfates were not detected in the plasma.

#### *Total antioxidant capacity (TEAC)*

The trolox equivalent antioxidant capacity (TEAC) value is a measure for the total antioxidant status, relating the free radical scavenging properties of a solution or a compound to that of the synthetic antioxidant trolox. The assay is performed as previously described (18). The relative contribution of uric acid, vitamin C and quercetin to the total TEAC value is calculated using the TEAC value described for each individual antioxidant, i.e. 1, 1 and 6.24 respectively (19).

#### *Ascorbic acid measurement*

Calibrators were prepared freshly, containing the same amount of TCA as the samples. Samples and calibrators were processed identically as described previously. (20).

#### *Uric acid measurement*

Uric acid was measured in the plasma of all samples as described previously. (21).

#### *GSH, GSSG and haemoglobin measurement*

Both GSH and GSSG calibrators were prepared freshly and contained the same concentrations of SSA as the samples. Samples and calibrators were treated identically and GSH and GSSG levels measured were related to the haemoglobin content as described previously (22).

#### *Malondialdehyde (MDA) measurement*

Malondialdehyde (MDA) was measured in the plasma of all samples as described previously (23).

#### *Blood-based cytokine production assay*

Within one hour after blood collection, the blood-based cytokine production assay was performed as described previously (24). Care was taken that handling of the blood prior to LPS-stimulation did not influence the cytokine release.

### *Enzyme linked immune sorbent assay (ELISA) measurement*

TNF $\alpha$ , IL-8 and IL-10 were quantified using PeliKine Compact human ELISA kits (CLB/Sanquin, the Netherlands) based on appropriate and validated sets of monoclonal antibodies. Assays were performed as described in the manufacturer's instructions.

### *Statistics*

The data of the quercetin and placebo group were compared using a Mann Whitney U test. The relation between the basal or LPS-induced cytokine production and the effect of the quercetin supplementation on these cytokine levels has been appraised using Spearman's rank correlation coefficient. A one-tailed probability value (P-value) equal to or less than 0.05 was considered to be statistically significant.

## Results

Baseline plasma quercetin levels were below the lower limit of detection of 0.054  $\mu\text{M}$  in all sarcoidosis patients prior to supplementation. After supplementation, the quercetin plasma concentration, reaching  $0.27 \pm 0.04 \mu\text{M}$ , could be quantified in all patients (Figure 9.1A).

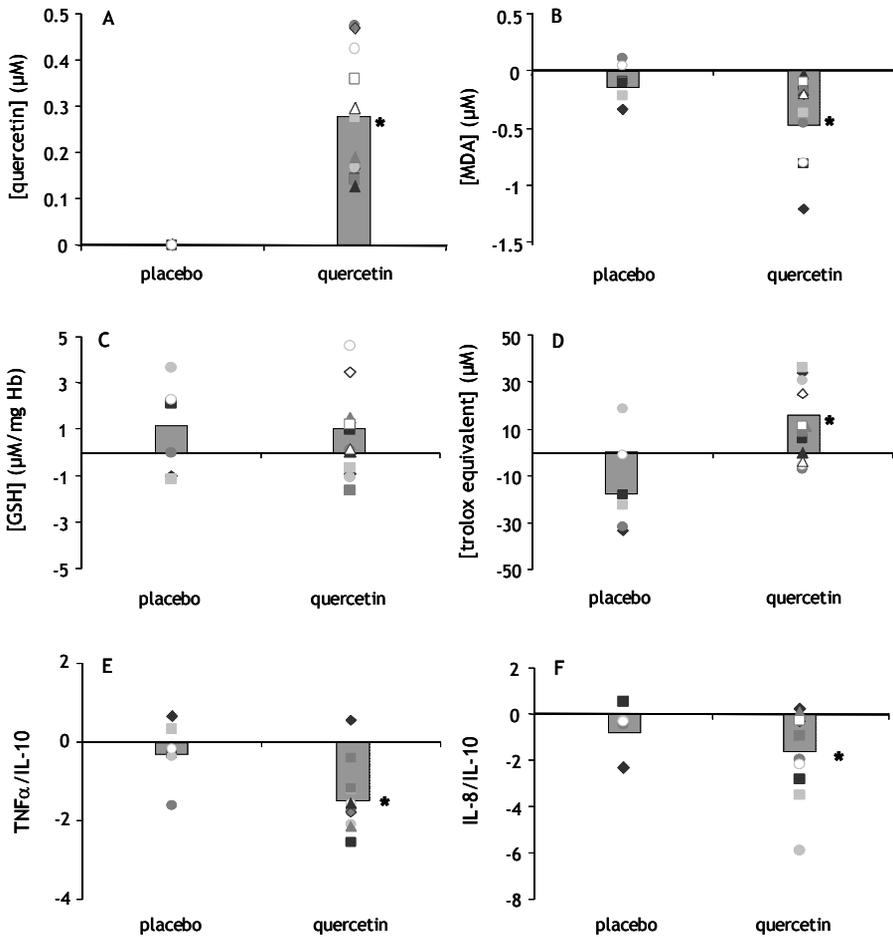
Malondialdehyde (MDA), a marker of oxidative damage to lipids, was significantly reduced after supplementation with quercetin (Figure 9.1B).

Endogenous blood glutathione (GSH) levels were unaffected by supplementation in all patients (Figure 9.1C). The total plasma antioxidant capacity, i.e. the total sum of all plasma antioxidants that is expressed as Trolox equivalent, was significantly enhanced after supplementation in the quercetin-receiving patients (Figure 9.1D).

The plasma levels of the endogenous antioxidants uric acid and vitamin C were not altered by supplementation (data not shown). For the quercetin-receiving group, the relative contribution of these endogenous antioxidants, as well as that of the exogenous quercetin to the total plasma antioxidant status is depicted in Figure 9.2. This reveals that the relative contribution of quercetin to the total antioxidant capacity was extremely small (0.3%) and that a major part of the antioxidant capacity was due to plasma antioxidants other than vitamin C and uric acid, such as low molecular protein thiols (Figure 9.2A). This residual antioxidant capacity was significantly increased (3%,  $P < 0.01$ ) after quercetin supplementation.

The ratios of pro- versus anti-inflammatory cytokines TNF $\alpha$ /IL-10 and IL-8/IL-10, used as inflammatory marker, were significantly decreased by supplementation (Figures 9.1E and 9.1F).

The ratios of TNF $\alpha$ /IL-10 and IL-8/IL-10 after *ex vivo* addition of LPS were also significantly reduced by quercetin supplementation (Figure 9.3).

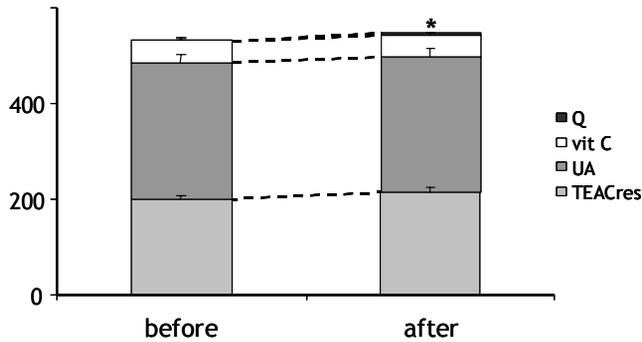


**Figure 9.1** The effect of quercetin supplementation on plasma quercetin concentration (panel A), plasma malondialdehyde (MDA) levels (panel B), blood glutathione (GSH) levels (panel C), the total plasma antioxidant capacity (TEAC) (panel D) and the levels of the ratios TNFα/IL-10 (panel E) and IL-8/IL-10 (panel F) in sarcoidosis patients. Data are individually expressed for all 12 sarcoidosis patients receiving quercetin and all 6 sarcoidosis patients receiving placebo; light grey bars represent the mean. \* P<0.05 compared to the “before” measurement prior to the quercetin supplementation

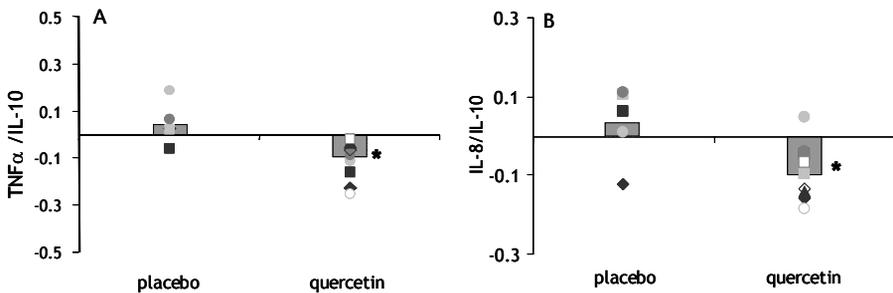
In the placebo-receiving group, no significant differences were found between the values before and after supplementation regarding any of the parameters measured.

No correlations were found between the increase of the total quercetin plasma concentration or of the increase in total plasma antioxidant capacity

with the effect of supplementation on the markers of oxidative stress and inflammation measured. The *ex vivo* effect of quercetin supplementation on LPS-induced TNF $\alpha$  or IL-8 level was dependent on the level of the LPS-induced pro-inflammatory cytokine prior to supplementation (Figure 9.4 A-B). This indicates that the higher the cytokine production evoked by LPS is, the more prominent the inhibiting effect of quercetin on this cytokine level. Quercetin supplementation did not affect the LPS-induced production of the anti-inflammatory cytokine IL 10 (Figure 9.4C).

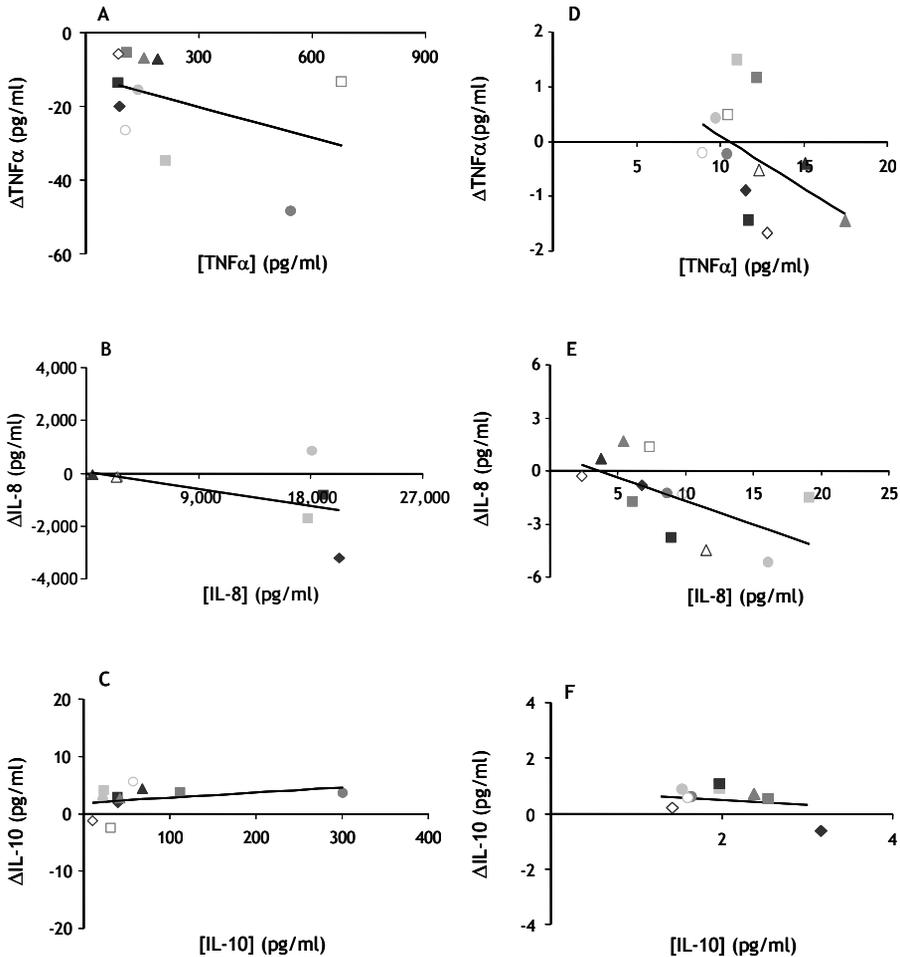


**Figure 9.2** The relative contribution of uric acid (UA), vitamin C (vit C) and quercetin (Q) to the total plasma antioxidant capacity in sarcoidosis patients before and after quercetin supplementation. Data are expressed as mean  $\pm$  S.E.M (n=12). TEACres= residual plasma antioxidant capacity.  
 \*= P<0.05 compared to the “before” measurement prior to the quercetin supplementation.



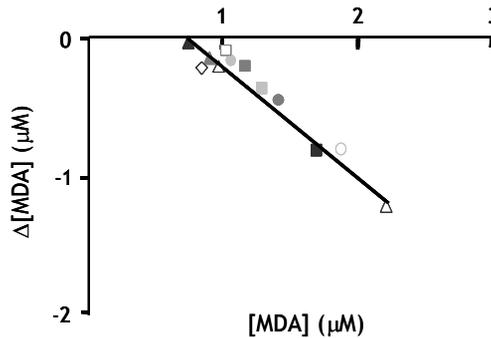
**Figure 9.3** The effect of quercetin supplementation on LPS-induced cytokine production. Data are individually expressed for 11 sarcoidosis patients receiving quercetin and 5 sarcoidosis patients receiving placebo; light gray bars represent the mean. Results are expressed in ratios of percentage, with 100% representing the cytokine-release under stimulation of LPS prior to the supplementation.  
 \*= P<0.05 compared to the “before” measurement prior to the quercetin supplementation.

A similar dependency as described in the previous paragraph for the *ex vivo* effect was found for the *in vivo* effect of quercetin supplementation; the higher the  $\text{TNF}\alpha$  and IL-8 levels before supplementation, the more pronounced the reduction of the plasma concentration of the cytokines by quercetin (Figure 9.4 D-E). Quercetin supplementation did not affect the plasma level of the anti-inflammatory cytokine IL 10 (Figure 9.4F).



**Figure 9.4** The relation between the LPS-induced (panel A-C), the basal amount (panel D-F) of cytokine and the effect of the quercetin supplementation on these cytokine levels in sarcoidosis patients. The cytokine level (x-axis) is plotted against the effect on this level caused by the quercetin supplementation (y-axis) for  $\text{TNF}\alpha$  (panel A and D), IL-8 (panel B and E) and IL-10 (panel C and F). Data are individually expressed for 11 (panel A-C) or all 12 (panel D-F) sarcoidosis patients receiving quercetin.

The efficacy of quercetin supplementation on the MDA levels is comparable to that on the pro-inflammatory cytokines; the higher the MDA level at baseline, the more pronounced the reduction of this marker of oxidative stress by quercetin supplementation (Figure 9.2B).



**Figure 9.5** The relation between the amount of malondialdehyde (MDA) present and the effect of the quercetin supplementation on these MDA levels in sarcoidosis patients. MDA levels (x-axis) are plotted against the effect on these level caused by the quercetin supplementation (y-axis). Data are individually expressed for all 12 sarcoidosis patients receiving quercetin.

## Discussion

Sarcoidosis is an inflammatory disorder in which oxidative stress appears to be involved (1-3), as indicated by several biomarkers including a reduced antioxidant capacity. To the best of our knowledge, this is the first study demonstrating a positive effect of a one day antioxidant supplementation on this reduced antioxidant capacity in sarcoidosis.

After *ex vivo* LPS-stimulation of blood,  $\text{TNF}\alpha/\text{IL-10}$  and  $\text{IL-8}/\text{IL-10}$  levels, two markers of inflammation, are significantly reduced by the *in vivo* quercetin supplementation. These *ex vivo* results are in agreement with our previous *in vitro* findings. Quercetin decreased LPS-induced  $\text{TNF}\alpha$ - and IL-8 production when added to the blood of sarcoidosis patients in the test tube (unpublished data). Interestingly, a one day quercetin supplementation also resulted in a significant decrease of the ratios of pro- versus anti-inflammatory cytokines  $\text{TNF}\alpha/\text{IL-10}$  and  $\text{IL-8}/\text{IL-10}$  as well as of the plasma levels of the lipid peroxidation product malondialdehyde (MDA), a marker of oxidative stress. These results confirm that quercetin is capable of

empowering the compromised antioxidant defence system of sarcoidosis patients as well as of mitigating the inflammation present in sarcoidosis.

A possible explanation for the anti-inflammatory effects of quercetin can be found in the interplay between oxidative stress and inflammation. ROS are directly involved in the occurrence of oxidative stress. Moreover, ROS are also capable of promoting inflammation by activating the transcription factors NF- $\kappa$ B and activator protein-1. These transcription factors induce pro-inflammatory cytokines such as TNF $\alpha$  (25,26). By scavenging ROS, quercetin might not only offer protection against oxidative stress, the flavonoid may simultaneously mitigate inflammation. It has been reported that quercetin is capable of inhibiting TNF $\alpha$  production as well as TNF $\alpha$  gene expression via modulation of NF- $\kappa$ B in human peripheral blood mononuclear cells (12).

During the scavenging of ROS, quercetin is converted into oxidation products. These products might react with critical sulfhydryl groups, thereby impairing vital cellular functions (27,28). The level of glutathione (GSH), the most abundant endogenous thiol, is not affected by quercetin supplementation. This indicates that the possible formation of the reactive oxidation products has no significant impact on the applied dosing regime. This outcome could have been anticipated based on the relatively low plasma quercetin concentration achieved by the current supplementation ( $0.27 \pm 0.04 \mu\text{M}$ ) compared to the high cellular concentrations of GSH (1 to 10 mM). However, in a chronic disorder such as sarcoidosis, supplementation has to proceed over a much longer time period. The safety, tolerability and efficacy of the long-term use of quercetin in sarcoidosis remains to be investigated.

Interestingly, the effects of quercetin supplementation appear to be more pronounced when the baseline levels of MDA, TNF $\alpha$  and IL-8 are increased. Only when MDA levels are high does supplementation reduce this marker of oxidative stress. Similarly, only when the TNF $\alpha$ /IL-10 ratio or the IL-8/IL-10 ratio is high does supplementation reduce these markers of inflammation. The extent of the effect of the applied quercetin supplementation appears to be predominantly governed by the individual level of oxidative stress and inflammation in the patient. This high correlation might conceal the anticipated dependency of the effects on the degree of the quercetin plasma level. No correlation with the quercetin level was observed in the present study. These results indicate that beneficial effects of antioxidant supplementation can only be expected in people with enhanced oxidative stress or inflammation. Although this seems trivial, it is often not realised and might explain the negative outcome of many clinical studies where antioxidants were supplied to healthy subjects (29). The

present study shows that the baseline value of oxidative damage and inflammation is a major discriminator in the beneficial effect of antioxidant supplementation.

In several diseases associated with enhanced oxidative stress, the use of antioxidants, i.e. N-acetyl cystein and lipoic acid, is proven to be clinically beneficial (30,31). In these antioxidant supplementation studies, besides having an antioxidant effect, the reduction of the occurring inflammation has also been implicated (31).

Recently, it has been demonstrated that Infliximab, a  $\text{TNF}\alpha$  antibody, improved lung function in stable pulmonary sarcoidosis (32). This finding is in line with studies regarding the use of Infliximab in the treatment of other chronic inflammatory diseases such as Bechterew (33,34), Chron's disease (35) and rheumatoid arthritis (36-38). The latter studies report an inverse correlation between the effects of this anti- $\text{TNF}\alpha$  therapy and the severity of the pathologies, i.e. the more severe the disease, the less effective this anti- $\text{TNF}\alpha$  antibody therapy is. This might be due to the limited capacity of the anti- $\text{TNF}\alpha$  therapy that was restricted by the use of a fixed dosing regime independent of the  $\text{TNF}\alpha$  level at baseline. In the present study, no limitation of the effect of quercetin was seen. In contrast to regular anti- $\text{TNF}\alpha$  therapy, it was found that the more outspoken the markers of oxidative stress and inflammation, the more effectively quercetin reduces these markers, including the  $\text{TNF}\alpha$  level.

In conclusion, the present study indicates that the supplementation of a high dose of the antioxidant quercetin over a 24-hour period is able to reduce markers of oxidative stress and inflammation in sarcoidosis, provided these markers are elevated.

## References

1. Drent M, van den Berg R, Haenen GR, van den Berg H, Wouters EF, Bast A. NF-kappaB activation in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 2001;18:50-56.
2. Kanoh S, Kobayashi H, Motoyoshi K. Exhaled ethane: an in vivo biomarker of lipid peroxidation in interstitial lung diseases. *Chest.* 2005;128:2387-2392.
3. Montuschi P, Ciabattoni G, Paredi P, Pantelidis P, du Bois RM, Kharitonov SA, Barnes PJ. 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases. *Am J Respir Crit Care Med.* 1998;158:1524-1527.
4. Lenz AG, Costabel U, Maier KL. Oxidized BAL fluid proteins in patients with interstitial lung diseases. *Eur Respir J.* 1996;9:307-312.
5. Heijnen CG, Haenen GR, Oostveen RM, Stalpers EM, Bast A. Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited. *Free Radic Res.* 2002;36:575-581.
6. Wilms LC, Hollman PC, Boots AW, Kleinjans JC. Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. *Mutat Res.* 2005;582:155-162.
7. Ziegenhagen MW, Schrum S, Zissel G, Zipfel PF, Schlaak M, Muller-Quernheim J. Increased expression of proinflammatory chemokines in bronchoalveolar lavage cells of patients with progressing idiopathic pulmonary fibrosis and sarcoidosis. *J Investig Med.* 1998;46:223-231.
8. Baughman RP, Iannuzzi M. Tumour necrosis factor in sarcoidosis and its potential for targeted therapy. *BioDrugs.* 2003;17:425-431.
9. Meloni F, Caporali R, Marone Bianco A, Paschetto E, Morosini M, Fietta AM, Patrizio V, Bobbio-Pallavicini F, Pozzi E, Montecucco C. BAL cytokine profile in different interstitial lung diseases: a focus on systemic sclerosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 2004;21:111-118.
10. Moller DR. Cells and cytokines involved in the pathogenesis of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* 1999;16:24-31.
11. Manjeet KR, Ghosh B. Quercetin inhibits LPS-induced nitric oxide and tumor necrosis factor-alpha production in murine macrophages. *Int J Immunopharmacol.* 1999;21:435-443.
12. Nair MP, Mahajan S, Reynolds JL, Aalinkeel R, Nair H, Schwartz SA, Kandaswami C. The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kappabeta system. *Clin Vaccine Immunol.* 2006;13:319-328.
13. Read MA. Flavonoids: naturally occurring anti-inflammatory agents. *Am J Pathol.* 1995;147:235-237.
14. de Boer VC, Dihal AA, van der Woude H, Arts IC, Wolffram S, Alink GM, Rietjens IM, Keijer J, Hollman PC. Tissue distribution of quercetin in rats and pigs. *J Nutr.* 2005;135:1718-1725.
15. Drent M, Nierop MAMF, van Gerritsen FA, Wouters EFM, Mulder PGH. Computer program using BALF analysis results as diagnostic tool in interstitial lung diseases. *Am. J. Respir. Crit. Care Med.* 1996;153:736-741.
16. Quanjer PH, Tammeling GJ, Cotes JE, Pedersom OF, Peslin R, Yernaut JC. Lung volumes and forced ventilatory flows. Report working party. Standardization of lung functions tests. European Commission for Steel and Coal. Official statement of the European Respiratory Society. *Eur Respir J.* 1993;16:5-40.
17. Arts ICW, Venema DP, and Hollman PCH. Quantitative determination of flavonoids in plant foods and biological fluids. In: Santos-Buelga, C. *Methods in polyphenols analysis.* Cambridge: The Royal Society of Chemistry; 2003.
18. Fischer MA, Gransier TJ, Beckers LM, Bekers O, Bast A, Haenen GR. Determination of the antioxidant capacity in blood. *Clin Chem Lab Med.* 2005;43:735-740.
19. Arts MJTJ, Dallinga JS, Voss HP, Haenen GRMM, Bast A. A new approach to assess the total antioxidant capacity using the TEAC assay. *Food Chem.* 2004;88:567-570.

20. Speek AJ, Schrijver J, Schreurs WHP. Fluoremetric determination of total vitamin C in whole blood by high performance liquid chromatography with pre-column derivatization. *J Chrom Biomed Appl.* 1984;305:53-60.
21. Benzie IF, Chung W, Tomlinson B. Simultaneous measurement of allantoin and urate in plasma: analytical evaluation and potential clinical application in oxidant:antioxidant balance studies. *Clin Chem.* 1999;45:901-904.
22. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem.* 1969;27:502-522.
23. Lepage G, Munoz G, Champagne J, Roy CC. Preparative steps necessary for the accurate measurement of malondialdehyde by high-performance liquid chromatography. *Anal Biochem.* 1991;197:277-283.
24. Swennen EL, Bast A, Dagnelie PC. Immunoregulatory effects of adenosine 5'-triphosphate on cytokine release from stimulated whole blood. *Eur J Immunol.* 2005;35:852-858.
25. Rahman I. Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. *Biochem Pharmacol.* 2002;64:935-942.
26. MacNee W. Oxidative stress and lung inflammation in airway diseases. *Eur J Pharmacol.* 2001;429:195-207.
27. Galati G, Moridani MY, Chan TS, O'Brien PJ. Peroxidative metabolism of apigenin and naringenin versus luteolin and quercetin: glutathione oxidation and conjugation. *Free Radic Biol Med.* 2001;30:370-382.
28. Boots AW, Kubben N, Haenen GR, Bast A. Oxidized quercetin reacts with thiols rather than with ascorbate: implication for quercetin supplementation. *Biochem Biophys Res Commun.* 2003;308:560-565.
29. Halliwell B. The antioxidant paradox. *Lancet.* 2000;355:1179-1180.
30. Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, MacNee W, Thomeer M, Wallaert B, Laurent F, Nicholson AG, Verbeken EK, Verschakelen J, Flower CD, Capron F, Petruzzelli S, De Vuyst P, van den Bosch JM, Rodriguez-Becerra E, Corvasce G, Lankhorst I, Sardina M, Montanari M. High-dose acetylcysteine in idiopathic pulmonary fibrosis. *N Engl J Med.* 2005;353:2229-2242.
31. Hofmann MA, Schiekofer S, Isermann B, Kanitz M, Henkels M, Joswig M, Treusch A, Morcos M, Weiss T, Borcea V, Abdel Khalek AK, Amiral J, Tritschler H, Ritz E, Wahl P, Ziegler R, Bierhaus A, Nawroth PP. Peripheral blood mononuclear cells isolated from patients with diabetic nephropathy show increased activation of the oxidative-stress sensitive transcription factor NF-kappaB. *Diabetologia.* 1999;42:222-232.
32. Baughman RP, Drent M, Kavuru M, Judson MA, Costabel U, du Bois R, Albera C, Brutsche M, Davis G, Donohue JF, Muller-Quernheim J, Schlenker-Herceg R, Flavin S, Lo KH, Oemar B, Barnathan ES. Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med.* 2006;174:795-802.
33. Baraliakos X, Listing J, Brandt J, Zink A, Alten R, Burmester G, Gromnica-Ihle E, Kellner H, Schneider M, Sorensen H, Zeidler H, Rudwaleit M, Sieper J, Braun J. Clinical response to discontinuation of anti-TNF therapy in patients with ankylosing spondylitis after 3 years of continuous treatment with infliximab. *Arthritis Res. Ther.* 2005;7:R439-444.
34. Sidiropoulos P, Kritikos HD, Siakka P, Mamoulaki M, Kouroumalis H, Voudouris K, Boumpas DT. Low dose of infliximab is inadequate in most patients with spondylarthropathies. *Clin. Exp. Rheumatol.* 2005;23:513-516.
35. Menachem Y, Avidan B, Lavy A, Lang A, Bardan E, Fidler H, Bar-Meir S, Chowers Y. Increasing the infliximab dose is beneficial in Chron's disease patients who responded to a lower dose and relapsed. *Digestion* 2005;72:124-128.
36. Edrees AF, Misra SN, Abdou NI. Anti-tumour necrosis factor (TNF) therapy in rheumatoid arthritis: correlation of TNF-alpha serum level with clinical response and benefit from changing dose or frequency of infliximab infusions. *Clin. Exp. Rheumatol.* 2005;23:469-474.
37. Berger A, Edelsberg J, Li TT, Maclean JR, Oster G. Dose intensification with infliximab in patients with rheumatoid arthritis. *Ann. Pharmacother.* 2005;39:2021-2025.

38. Collantes-Estevez E, Munoz-Villanueva MC, Zarco P, Torre-Alonso JC, Gratacos J, Gonzalez C, Sanmarti R, Canete JD. Effectiveness of reducing infliximab dose interval in non-responder patients with refractory spondyloarthropathies. An open extension of a multicentre study. *Rheumatology* 2005;44:1555-1558.