

Magnesium Research

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Mg citrate found more bioavailable than other Mg preparations in a randomised, double-blind study

Volume 16, numéro 3, September 2003

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Résumé **Texte intégral** Références Illustrations

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Illustrations

Afficher les illustrations

INTRODUCTION

Magnesium intakes have decreased over recent years in many industrialised countries [1] and as a consequence, large segments of these populations may have chronic latent Mg deficiency [2]. Chronic suboptimal dietary Mg intakes have been implicated in the development of several disease states such as cardiovascular diseases, osteoporosis and asthma [3]; yet administration of oral Mg preparations has not always proved to be successful in their management. However, the efficacy of any nutrient depends on the bioavailability of its form. Lack of sound data on bioavailability of Mg preparations has hampered the understanding of the pharmacokinetics of the mineral and may explain the conflicting clinical findings reported for Mg intervention studies, despite suggestive epidemiological observations [4].

Mg absorption and bioavailability depend on a large number of variables, such as the type of Mg salt/compound used and the presence of other nutrients or substances that may enhance or interfere with its uptake or homeostasis. In Mg-replete, healthy individuals, renal Mg elimination (measured as 24-hour urinary Mg) after an oral Mg load, provides a clinically relevant measure of Mg salt bioavailability [5, 6]. This is because urinary Mg excretion is a major determinant of the body's magnesium balance. Serum Mg, albeit easily determined, may reflect body Mg status to a limited extent, although intracellular concentrations (e.g. of erythrocytes) are believed to serve as better biochemical indices.

Several human studies have attempted to investigate the bioavailability of different Mg preparations, however, it is not established yet which Mg preparation is superior. This is partly due to methodological differences used in the various reported studies, as the responses of different biochemical indices to similar supplementation often appear conflicting. In addition, many studies have not addressed the question of chronic daily administration [7], which is of most relevance for dietary supplementation. Other studies have failed to control for gender during data analysis [7, 8]. Consequently, robust evidence on the superiority of one Mg preparation over another does not exist. Nevertheless, preliminary data from human studies indicate that Mg oxide is less bioavailable than the organic forms [7, 8]. Despite this, the oxide continues to be used in Mg supplementation studies [9, 10] and in supplements available to the public. No doubt this is due to its low cost and mass, which make it favourable for one-a-day tablet formulations, favoured by the supplement industry.

This study was designed to compare the relative absorbability and bioequivalence of three forms of Mg (oxide, citrate and amino acid chelate) under 'acute' (24 h) and 'chronic' (2 months) administration of a daily dosage. Mg preparations containing 300 mg of elemental Mg were studied in healthy human subjects with daily Mg intakes from food equal to the Reference Nutrient Intake (RNI)¹¹ ± 20%.

METHODS

VOLUNTEERS

Volunteers were recruited through a poster advertising campaign at the University of Reading and were selected after an interview and analysis of a three-day diet diary. Subjects were only recruited if their dietary Mg intake was equal to the Reference Nutrient Intake (RNI) [11] ± 20%. The RNI is 270 mg for women and 300 mg for men. Those reporting a history of bone disease, diabetes, hypertension, chronic fatigue syndrome, premenstrual symptoms, peptic ulcer, intestinal resection, inflammatory disease of the GI tract or chronic diarrhoea, as well as alcoholics, caffeine abusers, smokers and pregnant or lactating women were excluded. Those using medications, which might interfere with Mg metabolism, those taking antibiotics, the contraceptive pill, mineral supplements or high doses of vitamin B₆, were also excluded from the study. Normal renal and hepatic function of each volunteer was confirmed by analysis of blood samples taken at the commencement of the study. The study was approved by The University of Reading Ethics and Research Committee. The participants were given oral and written information about the aims and procedures of the study, and written consent was obtained from them. Participation also required prior agreement from the volunteer's General Medical Practitioner.

DESIGN

The design of the study was a randomised, double-blind, parallel intervention. Each participant was issued a unique number after successful screening. To ensure the double-blind nature of the study, the project leader (AFW), who had no direct contact with the volunteers nor with the analysis of the data, undertook the randomisation: the other researchers involved in the study were not privy to the coding.

To effect randomisation, the volunteer numbers were written on individual pieces of paper, which were folded so that the number was not visible, being on the inside of the fold. These papers were mixed well and divided between two containers — one for men and one for women — from which volunteer numbers could be drawn blindly. Once drawn, each volunteer number was assigned to a treatment group by rotating allocation between treatments, but limiting the numbers in the two placebo groups to a maximum of 9 subjects in each. Blinding of the treatment codes was maintained throughout the study and its analysis.

The duration of the study was 60 days, and volunteers were required to provide a 24 h urine collection, 5 'spot' saliva samples and a fasting blood sample at baseline (day 1), 24 h after supplementation (day 3) and at the end of 60 days (day 61). Volunteers were advised to avoid Mg-rich foods (a list was provided) for 12 h before and on sampling days, to limit their caffeine and alcohol intakes during the whole study and to completely avoid these drinks 12 h before and on sampling days.

INTERVENTION

The five treatments were: 1) Mg amino acid chelate (300 mg elemental Mg/day), 2) Mg citrate (300 mg elemental Mg/day), 3) Mg oxide (300 mg elemental Mg/day) or 4) placebo (cellulose) or 5) placebo (sorbitol). These supplements were supplied to the volunteers in identical sealed plastic pots with instructions to take two tablets in the morning at 0800 h. The only identification written on the pots was the volunteer code. The supplements were manufactured and supplied by Lamberts Healthcare Ltd. (Century Place, Lamberts Road, Tunbridge Wells, Kent, TN2 3BE, United Kingdom).

Two placebos were used in this study to fulfil a further aim of this study, which was to show that, in healthy individuals, estimates of Mg status were no different following daily sorbitol placebo supplementation than following cellulose placebo supplementation. In a previous RCT on Mg intervention [12] we showed, for women suffering from premenstrual symptoms, that 60 days of daily supplementation with low-dose sorbitol (as placebo), resulted in reduction of premenstrual symptoms and a highly significant reduction in 24 h urinary Mg excretion, compared with baseline measurements. Hence, the data for the placebo groups derived from this present study confirmed a lack of effect on Mg status of a daily supplement of sorbitol in healthy individuals and have already been reported [12].

ANALYSES

Urine

Volunteers were issued with a kit containing two 1L plastic bottles, a plastic funnel and a timer, and detailed instructions to effect 24 h urine collections on day 1 (baseline), day 2 (acute supplementation) and day 60 (chronic supplementation). Ascorbic acid was used as a preservative (1g of ascorbic acid per 1L container). The volume of the urine collections was measured and aliquots were frozen at – 20°C pending analysis. The thawed samples were analysed for Mg concentration by MONARCH centrifugal analyser (Instrumentation Laboratories UK, Ltd - Kelvin Close, Birchwood Science Park, Warrington) which was equipped with an appropriate Magnesium kit (Instrumentation Laboratories UK, Ltd).

Blood

After each sampling day (and following a 14-h overnight fast), venous blood samples were collected at 0800 h or thereabouts at baseline (day 1), day 3 and at the end of the supplementation period (day 61) into heparinised tubes (Vacutainer® – Lithium heparin) (Becton Dickinson Vacutainer Systems Europe – B.P. 37-38241 Meylan, Cedex-France) and in a serum separator tube (Vacutainer® – SST™, Gel and Clot Activator) (Becton Dickinson Vacutainer Systems Europe). The blood samples were centrifuged at 3,000 rpm for 10 min and the plasma was stored at – 20°C until the time of the analysis. Plasma samples were analysed for magnesium concentration. After separating and removing the plasma, the erythrocytes were washed and lysed as described by Fischer *et al.* [13]. The supernatant haemolysate was stored at – 20°C pending analysis. The Mg content of the erythrocytes was determined by atomic absorption spectrophotometry (AAS) (Model PYE UNICAM SP9-800) with the emission mode at 285.2 nm using air-acetylene flame.

The SST™ tubes were processed at the Pathology Laboratory of the Royal Berkshire Hospital (Reading, Berkshire, UK), where total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-GT (GGT) were analysed from serum. These hepatic enzymes were monitored in order to ensure that subjects had normal hepatic function, that there were no toxic effects associated with the 2-month Mg supplementation and to detect whether there were any changes, which might be associated with excess alcohol consumption during the trial.

Saliva samples

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N°8 - Septembre 2018

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Spot saliva samples were collected on days 0, 2 and 60. Prior to saliva collection the subjects were requested to place on their tongue 2 drops of bitter-tasting Artemisia absinthum (wormwood) tincture 1:5 [made according to British Herbal Pharmacopoeia, 1983 standards [14]], in order to facilitate the collection of 5 ml saliva samples [15]. Saliva samples were requested at 2, 4, 8, 12 and 24 h following ingestion of the supplements. These samples were pooled and 1 ml aliquots stored at – 20C and later thawed and analysed for Mg by AAS.

DIETARY INTAKE

In addition to the 3-day diet diary completed at screening, two more three-day diet diaries were administered to the volunteers at the beginning and at the end of the study, to estimate any changes in the dietary habits during the study. The nutrient composition of the diets was analysed using computer software COMPEAT version 4.0 (Lifeline Nutritional Services Ltd, London, UK).

STATISTICS

A sample size of ten people in each of 5 treatment groups was calculated as required to detect a difference of 40 mg/d urinary Mg excretion per day between groups with a power of 80% at the 5% significance level, using data from a study of healthy women supplemented with magnesium [15]. All analyses of outcome data were carried out using analysis of covariance (ANCOVA). Adjustments were made for baseline values, irrespective of their statistical significance. The assumptions underlying the ANCOVA were examined using residual plots, and any potential influential observations were identified using Cook's D score. If an outlier was discovered, it was removed from the dataset, and the analyses were repeated to check the robustness of the results obtained. (An outlier was defined as a response datum whose value, when plotted against baseline, lay substantially outside the area occupied by the majority of comparable values for the full-set study population.) A comparison of each treatment with placebo was made and simultaneous 95% confidence intervals were calculated using Dunnett's adjustment [16].

The p-value from ANCOVA tested for any differences between the five treatments. In order to ascertain whether there was a difference at the 5% level of probability, between a given treatment and placebo, the 95% confidence interval was examined. If this did not contain zero, then the difference was statistically significant at the 5% level. All analyses were carried out using PROC GLM in SAS (Statistical Analysis Software) version 6.12.

RESULTS

VOLUNTEERS

Fifty one healthy volunteers were initially recruited for this study. One volunteer dropped out within 24 h of the commencement of the study and 5 others before the end of the study. Forty-six completed the study protocol. All withdrawals were for personal reasons associated with the inconveniences of the protocol routine – no withdrawals were due to adverse effects of treatment. Table 1 presents the personal characteristics of the volunteers in each treatment group at baseline.

Table 1. Mean (± sem) characteristics and baseline Mg intakes of the five treatment groups

Intervention	Age	Gender	BMI	Dietary Mg intake (mg/d)
Mg AAC	26.6 ± 1.2	4M/8F	22.2 ± 1.2	268.6 ± 34.3
Mg Citrate	24.6 ± 1.4	4M/6F	23.1 ± 1.1	255.3 ± 18.3
Mg Oxide	25.1 ± 0.7	4M/8F	22.2 ± 0.7	279.6 ± 29.8
Placebo (cellulose)	25.8 ± 1.2	3M/6F	21.5 ± 1.1	242.1 ± 16.6
Placebo (sorbitol)	27.4 ± 1.4	3M/5F	23.0 ± 1.1	291.8 ± 19.8

AAC, amino-acid chelate. Dietary Mg intake at baseline is given in table 1. There were no significant differences in the intakes of the 5 groups. Comparisons were made between baseline and end of study nutrient intake as recorded in 3-day diet diaries, focusing on Mg, Ca and vitamin B₆ intakes, in particular. No changes of significance in dietary intakes were found. At baseline, women had lower mean dietary Mg intakes (257.8 mg/day SD ± 76.7) compared to men (285.1 mg/day SD ± 82.2) (p = 0.061). Both these mean values are below the respective RNI's [11] of 270 mg/d Mg for women and 300 for men.

URINE

Most volunteers had Mg urinary excretion rates in the normal range of 72.9-109.4 mg/24h [17] at baseline. Although a lack of baseline differences between treatments groups with respect to urinary Mg output indicated that treatment group characteristics were adequately balanced at the study outset, gender appeared to be an important source of variation in the total study population (n = 51; p = 0.022). Mean urinary Mg output for women at baseline was 86.3 mg/24 h (± 27.9, SD), whilst men had higher mean urinary losses: 115.7 mg/24 h (± 43.5, SD).

ANCOVA revealed a lack of significant treatment differences in 24 h urinary Mg output following acute (n = 50) or chronic Mg (n = 46) supplementation. However, the residual plots showed that there were two outlying values in the chronic supplementation urinary Mg output dataset. Removal of these outliers and scrutiny of confidence intervals of treatment means showed that supplementation with the organic forms of Mg (the amino acid chelate and the citrate form) led to a significant increase (p = 0.033) in urinary Mg excretion compared to MgO and placebo treatment (figure 1) after 60 days.

PLASMA MG

A lack of baseline differences between treatments groups with respect to mean plasma Mg concentration meant that treatment group characteristics were adequately balanced at the study outset. However, gender contributed a substantial source of variation in the total study population, which just missed significance at the 5% level (n = 51; p = 0.057). Mean plasma Mg concentrations for women at baseline were 0.65 mmol/l (± 0.06, SD), whilst men had higher mean plasma levels: 0.69 mmol/l (± 0.05, SD).

The mean plasma concentrations of Mg are shown at baseline and following acute and chronic supplementation in table 2, according to treatment group. Scrutiny of ANOVA and treatment mean confidence intervals showed that Mg citrate significantly (n = 50; p = 0.026) increased plasma Mg concentrations after one day of supplementation (acute). However, there was no statistically significant difference between the treatments after 60 days of supplementation when all observations (n = 46) were included in the analyses. However, examination of the residual plots showed that one individual had outlying data, with an unusually large influence on the model. On repeating ANOVA, with this outlier removed, a statistically significant difference (n = 45; p = 0.006) was detected. An examination of 95% confidence intervals showed that this difference was due to the group treated with Mg citrate, who showed greater mean plasma Mg concentration at the end of the study than the other groups.

Table 2. Mean (± sem) plasma Mg concentrations (mmol/L) of the 5 treatment groups at various stages in the study

Intervention	Baseline	Acute supplementation	Chronic supplementation
Mg AAC	0.65 ± 0.02	0.70 ± 0.01	0.65 ± 0.01
Mg Citrate	0.68 ± 0.03	0.73 ± 0.02*	0.72 ± 0.04**
Mg Oxide	0.67 ± 0.02	0.68 ± 0.02	0.69 ± 0.01
Placebo (cellulose)	0.68 ± 0.01	0.65 ± 0.02	0.64 ± 0.02
Placebo (sorbitol)	0.69 ± 0.02	0.66 ± 0.02	0.62 ± 0.02

AAC, amino-acid chelate; *p = 0.026, **p = 0.006.

ERYTHROCYTE MG

Lack of baseline differences between treatments groups with respect to erythrocyte Mg concentrations indicated that treatment group characteristics were adequately balanced in the study. At baseline, women had lower, albeit not statistically different, mean erythrocyte Mg concentrations (1.87 mmol/L (± 0.53, SD)), compared to men (1.94 mmol/L (± 0.38, SD)).

No statistically significant differences in red blood cell Mg concentration among the treatments investigated were found by ANCOVA, either following acute supplementation (n = 50; p = 0.781) or chronic supplementation (n = 46; p = 0.448). However, the large variation in the data (shown by the relatively large SD in proportion to the mean values) may have contributed to lack of difference found.

SALIVA MG

There were no baseline differences between treatment groups with respect to salivary Mg concentrations, nor were there any statistically significant gender differences in baseline salivary Mg concentration.

The saliva concentrations of Mg are given in table 3 according to treatment group. ANCOVA showed no significant difference between groups following acute supplementation. However a significant difference (n = 46; p = 0.027) in salivary Mg concentration was observed following chronic supplementation. An examination of 95% confidence intervals showed that this difference was due to the greater mean concentration of salivary Mg in the group treated with Mg citrate compared with other treatments.

Table 3. Mean (± sem) salivary Mg concentrations (mmol/L) of the 5 treatment groups at various stages of the study

Intervention	Baseline	Acute	Chronic
Mg AAC	0.21 ± 0.04	0.26 ± 0.04	0.25 ± 0.04
Mg Citrate	0.26 ± 0.02	0.32 ± 0.03	0.33 ± 0.04*
Mg Oxide	0.26 ± 0.03	0.24 ± 0.02	0.23 ± 0.03
Placebo (cellulose)	0.26 ± 0.05	0.26 ± 0.04	0.27 ± 0.04
Placebo (sorbitol)	0.24 ± 0.03	0.27 ± 0.03	0.22 ± 0.04

AAC, amino-acid chelate; p = 0.027.

LIVER FUNCTION

Participants showed no abnormal liver parameters at baseline and these parameters were not affected after administration of any of the 5 supplements for 60 days. In particular, no significant differences were observed in any of the liver enzymes tested (i.e. bilirubin, alkaline phosphatase, ALT, AST and gamma-GT) either following acute or chronic supplementation.

DISCUSSION

In this study, daily Mg urinary output data shows that the organic preparations of Mg exhibited higher absorbability after 60 days of supplementation than Mg oxide (figure 1). This finding confirms data from previous *in vitro* and shorter duration human intervention studies [7]. It also adds to the body of information provided by a comparable study which showed equivalent and superior bioavailability of Mg chloride, Mg lactate and Mg aspartate compared with Mg oxide, also using 24 h urinary Mg output as outcome [18]. It has been speculated that low molecular weight organic acids, such as citric acid and amino acids, promote absorption of minerals by increasing their solubility. The lack of an acute response in urinary Mg excretion (after one day of supplementation) following administration of Mg citrate may have been due to a relative slow elimination from the body.

The large variability in the bioavailability of Mg between subjects in this study is in accordance with other studies [19]. In part this has been attributed to intra-subject variability of baseline Mg excretion rate, inter-subject variability in the capacity-limited absorptive process and Mg balance, as well as differences in stool transit time. A difference in transit time could partly explain the low bioavailability of Mg oxide compared to the other forms. Mg oxide is known to exert an osmotic effect, increasing stool volume and intestinal motility [20] and therefore potentially decreasing the full extent of Mg absorption. However, no differences in frequency of defecation were reported between the groups in a post-study questionnaire. A further source of variability might have been the lack of complete control for dietary Mg on sampling. Provision to all subjects of standard low-Mg meals prior to and during the days of sample collection, would have helped to avoid any bias introduced by diet which led to outlying data in this study. Even though financial and practical considerations meant that we were unable to follow this more exacting protocol, our volunteers were advised on which magnesium-rich foods to restrict prior to sample collection days.

Although contended in the literature, plasma Mg concentration proved in this study to be useful in assessing the relative bioavailability of different Mg preparations. Serum Mg concentration is subject to homeostatic control, albeit relatively weak compared with calcium, and this would tend to reduce variability of values, at the expense of Mg re-distribution in other body tissues (e.g. bone). However, since the effectiveness of oral Mg supplementation is determined by its rate of uptake from the intestine into the blood and then by its transfer into the tissues, plasma concentrations may serve as a valuable means of evaluating the immediate (acute) response to supplementation. According to Schimatschek, Rempis [21], serum Mg levels less than 0.76 mmol/L may provide evidence of a Mg deficiency. As shown in table 2, most subjects had plasma Mg below this, suggesting suboptimal Mg intakes among the volunteers. This supposition is supported by the mean dietary Mg intakes derived for analysis of records in the baseline 3-day diet diary. Mean values for both men and women were below the RNI. The relatively low Mg intake of our subjects reflects data obtained from nation-wide surveys in the UK [22, 23] and raises concerns for the health implications of possible marginal Mg deficiency in a large segment of the population.

Although, in this study, the organic forms of Mg were both superior to Mg oxide in bioavailability, according to 24-h urinary Mg output, only Mg citrate led to a greater increase in plasma Mg concentration (table 2), both acutely and chronically, compared with the other treatments. These findings are relevant to clinical situations, in which a rapid repletion of Mg deficiency might be needed, as well as those where effective long-term treatment is required (e.g. for patients with myocardial infarction or acute asthma). Our findings agree with a previous study on 8 men [24] which reported similar increases in plasma Mg concentrations after a single oral administration of trimagnesium dicitrate in normal therapeutic doses to volunteers.

The small increases in erythrocyte Mg concentration reported previously following Mg supplementation [25], were not observed in our study. One explanation might be the high variability in our data did not allow subtle changes to become evident. For future studies, means of reducing variability through improved laboratory procedures need to be sought.

Salivary sample analyses are not commonly used in Mg absorption studies. Nevertheless, Borella and co-workers [25] reported that changes in salivary Mg concentration appear to be fast and sensitive to Mg intake, but this may depend on the Mg status of the individual as well as the bioavailability of the Mg preparation. De Souza [15] observed that women suffering from premenstrual symptoms had significantly lower salivary Mg concentrations than control women. Moreover, she reported that Mg supplementation (200 mg/d Mg as MgO) failed to increase saliva Mg concentration in women suffering from premenstrual symptoms over a 2-month intervention period. However, this lack of response may have been due to the choice of MgO for supplementation.

In our hands, salivary Mg concentration proved to be a useful measurement for the evaluation of the relative bioavailability of Mg preparations. Published values for salivary Mg concentration of samples of mixed saliva, with no reference to age, sex and nutritional status have been reported to be in the range 0.19-0.51 mmol/L [26]. The values obtained in this present study lie within this range, although at the lower end (0.21-0.26 mmol/L). Following 60 days of daily supplementation, our data showed that Mg citrate resulted in a higher salivary Mg concentration than the other treatments (table 3). Although, based on these data, it could be suggested that Mg amino acid chelate is not as assimilable (available intracellularly) as Mg citrate, these data are preliminary and need to be confirmed in an adequately-powered study comparing the two Mg preparations. This is particularly so, as our findings are contradictory to the predictions of Polish researchers [27] who estimated, through molecular modelling, that Mg ions would penetrate better through biological membranes in the form of chelates.

In this study, it was found at baseline that male volunteers had a greater mean plasma Mg concentration than age-matched female volunteers. In addition, the mean 24 h urinary Mg output was significantly greater in men than women. Although reference limits for Mg blood levels do not currently differentiate between genders, our results suggest that in otherwise healthy women, Mg status is lower compared to age-matched men. This gender difference needs further exploration, in particular, to ascertain whether this effect was a consequence of: (a) lower dietary Mg intakes in women; (b) gender differences in Mg homeostasis; or a combination of (a) and (b). It may be that diet will prove to be the most important factor, if the results of a recent Japanese study are generally applicable. This study found no gender-specific differences in Mg handling [28].

Even though the UK RNI for Mg for women is set lower than for men [11], no evidence supports a view that women have a lower biological requirement for Mg than age-matched men. If a gender difference in Mg status, as found in this study, exists in the general population, this would have profound implications for women's health, particular for the many diverse disease conditions known to be associated with poor Mg status.

CONCLUSION

This study demonstrated that the organic forms of Mg (citrate and amino-acid chelate) are more absorbable than MgO or placebo, as assessed by the 24-h urinary excretion after 60 days of daily supplementation. However, Mg citrate was found to be the most bioavailable preparation compared with the other treatments studied, on account of it resulting in the greatest serum Mg concentration following both acute and chronic daily supplementation compared with the other treatments studied, including placebo.

Further support for the conclusion that Mg citrate had the best bioavailability of the Mg preparations studied came from the greater salivary Mg concentration after 60 days of this treatment compared with all other treatments studied. However, a further study, directly comparing bioavailability of Mg citrate and Mg amino-acid chelate is now required to confirm these preliminary findings of differences between them. Outcome data on MgO supplementation was no different from placebo – confirming its poor bioavailability. The lower Mg status of women in this study compared with men warrants further investigation.

ACKNOWLEDGEMENT

We are grateful to the volunteers who participated in this study and to the International Foundation for Promotion of Nutrition Research and Nutrition Education, Switzerland who provided a "top-up" fund and to Lamberts Healthcare Ltd for the manufacture and donation of the supplements. We would also like to thank Dr Miriam de Souza for her valuable comments on the design of the study, Mrs Marilyn Collins for her assistance in statistical analysis and Mr Paul Robinson for his assistance in the analyses of liver enzymes.

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