Dietary intakes of children with Crohn’s disease

Rachel Pons1, Kylie E. Whitten2, Helen Woodhead3, Steven T. Leach4, Daniel A. Lemberg5 and Andrew S. Day1,4,5,*,

1School of Health Sciences, University of Wollongong, Wollongong, Australia
2Department of Nutrition and Dietetics, Sydney Children’s Hospital, Randwick, Sydney, Australia
3Department of Endocrinology, Sydney Children’s Hospital, Randwick, Sydney, Australia
4School of Women’s and Children’s Health, University of New South Wales, Sydney, Australia
5Department of Gastroenterology, Sydney Children’s Hospital, Randwick, Sydney, Australia

(Received 27 August 2008 – Revised 17 March 2009 – Accepted 25 March 2009 – First published online 30 April 2009)

Approximately 25% of individuals with Crohn’s disease (CD), a life-long relapsing-remitting disease, are diagnosed during childhood and adolescence. Symptoms of CD, including abdominal pain, nausea and diarrhoea, can lead to reduced food intake, which may negatively have an impact on nutritional status during this critical period of growth and development. The aims of the present study were to assess the growth and adequacy of dietary intakes of children with CD at Sydney Children’s Hospital, Randwick, and compare with healthy controls. Sixty-three subjects aged 10–16 years were recruited, including: children with active CD (n 18), children with CD in remission (n 23) and healthy controls (n 22). Dietary intake was assessed using a FFQ and compared with current Australian recommended dietary intakes (RDI). Growth and dietary intakes were compared between groups. Subjects with active CD had lower weight and BMI Z scores than children in remission and controls. The energy intakes of children with active CD and those in remission were significantly lower than estimated energy requirements (P 0·001 and P 0·04 respectively). Children with active CD did not meet the RDI for Fe and their Ca intake was lower than the RDI (P 0·04). In conclusion, the dietary intake of children with active CD was impaired, with inadequate intakes of energy, Ca and Fe. Reduced energy intakes during active disease may contribute to poor weight gain and impaired growth. Quantifying nutrient intake and ascertaining requirements for nutritional supplementation are essential components of successful management in paediatric CD.

Children: Crohn’s disease: Inflammatory bowel disease: Diet: Nutrition

Crohn’s disease (CD) is a type of inflammatory bowel disease (IBD) involving chronic inflammation of any region of the gastrointestinal tract. It is a life-long disease without cure and cycles through periods of remission and relapse (active disease)(1). Its aetiology is suspected to be an uncontrolled immune response to endogenous or exogenous triggers as a result of genetic and/or other modifying factors (2). CD often presents during childhood and adolescence, with this age group accounting for 25% of newly diagnosed cases of IBD (2). Paediatric CD prevalence has increased around the world over the past few years, particularly during the period of late childhood and adolescence (3).

Inflammation of the gastrointestinal tract and the related symptoms of pain, nausea and diarrhoea, as seen in CD, often lead to a loss of appetite and reduced food intake (4). Bowel-wall thickening may lead to obstruction and contribute to pain as food is digested. As a result, children often experience early satiety and decrease their oral intake to avoid these symptoms, on both conscious and subconscious levels (5). Malnutrition is most common in the acute phases of CD (6); however, it has also been documented during periods of remission (6).

The nutritional inadequacies of reduced food intakes are intensified by the increased energy and nutrient requirements related to disease activity and the increased requirements during childhood and adolescence for healthy growth and development (5). Consequent to poor dietary intake in these children, micronutrient deficiencies are commonly seen. These include Ca, Fe, Zn, Mg (7), vitamin D (8), folate (9) and vitamin B12 (10) deficiencies. Reduced food intake in addition to malabsorption and increased intestinal losses of essential nutrients associated with inflammation may lead to chronic undernutrition in paediatric CD (5).

Reduced energy intake, increased weight loss and impaired linear growth in paediatric CD are associated with low lean body mass and chronic inflammation during disease activity (11). This can lead to a lower ideal body weight in children with CD (12) and affect the final height achieved.

Abbreviations: CD, Crohn’s disease; EER, estimated energy requirement; GF, growth factor; IBD, inflammatory bowel disease; NHMRC, National Health and Medical Research Council; PAL, physical activity level; PCDAI, paediatric Crohn’s disease activity index; RDI, recommended dietary intake; REE, resting energy expenditure.

* Corresponding author: Associate Professor Andrew S. Day, fax +61 2 9382 1787, email andrew.day@unsw.edu.au
Dietary intakes in paediatric Crohn’s disease

Especially when diagnosis is made during childhood and early adolescence
(11). Dietary intakes vary during the course of the disease depending on the severity and associated symptoms, where oral intake is reduced during active phases of CD, and increased during periods of remission (13).

Decreased bone mineral density and increased risk for developing osteopenia are also commonly identified in children and adolescents with CD (14,15). An inadequate dietary intake of specific nutrients is one of the proposed mechanisms responsible for increased osteoporotic risk in these children, in addition to malabsorption of Ca and vitamin D. Hormonal and genetic factors may also contribute as well as specific therapies (such as corticosteroids that may reduce Ca absorption) (16).

The aims of the present study were to assess the dietary intakes of children with CD at Sydney Children’s Hospital (Randwick, Sydney, Australia) in comparison with Australian recommended dietary intakes (RDI) and to determine if dietary intakes were affected by CD activity. Additionally, the present study aimed to compare growth and dietary intakes of children with CD with healthy controls.

Materials and methods

Subjects

Sixty-three subjects aged between 10 and 16 years were recruited. This included children with active CD (n 18), CD in remission (n 23) and healthy controls (n 22). Remission was classified as having a paediatric Crohn’s disease activity index (PCDAI) score of less than 15; not in remission (active disease) was defined as a PCDAI of 15 or higher (17).

The PCDAI is a validated instrument that assesses a combination of objective and subjective factors, including common laboratory tests, reported abdominal pain, growth parameters and history, to determine disease activity in paediatric CD (17). All forty-one children with CD attended the IBD Clinic at Sydney Children’s Hospital between November 2006 and December 2007 and were part of an ongoing prospective study to investigate the mechanisms of diminished bone strength in childhood IBD.

Healthy control subjects were recruited from out-patient clinics and the Ambulatory Care unit at Sydney Children’s Hospital during August and September 2007. Control subjects were excluded if they had any underlying gastrointestinal disease or a medical condition requiring a specialised or restricted diet.

Weight, height and age were recorded at the time of recruitment. Patients were weighed wearing light clothing and no shoes on the same calibrated digital scales to the nearest 0·1 kg. Height was measured to the nearest 0·1 cm, using the same fixed, calibrated stadiometer. BMI was calculated using available weight and height measurements. Age- and sex-specific z scores (Z scores) for weight, height and BMI were calculated using Epi Info (version 3.4.1, 2007; Centers for Disease Control and Prevention, Atlanta, GA, USA).

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving patients were approved by the South East Sydney Illawarra Area Health Service Research Ethics Committee. Written informed consent was obtained from all subjects or their parents.

Food-frequency questionnaire

Usual dietary intake was assessed for all subjects using a sixty-item FFQ based on data from the 1995 Australian National Nutrition Survey (NNS) (18). The food items were described in household portions (for example, slice of bread, tub of yoghurt), derived from median portions of common foods consumed by 12- to 15-year olds in the 1995 NNS (18). Portion sizes were able to be changed by subjects if they did not reflect their usual intake. Verbal instruction of FFQ completion and a written example were provided before self-administration of the questionnaire. Parents assisted their child to complete the questionnaire. The study investigator checked all questionnaires for completeness and clarified doubtful responses with both parent and child upon return of the questionnaire. The FFQ assessed the previous week’s intake by indicating how frequently each food item was consumed in times/d per week. Vitamin and mineral supplementation was not recorded.

The FFQ used was obtained, with permission, from the authors of a recent study assessing the dietary intakes of Ca, energy and fat in adolescents aged 12–16 years (19). Slight modifications were made to the original FFQ relevant to the purpose of the present study, including the addition of commercially available oral nutrition supplements that are commonly consumed by children attending the IBD clinic.

Data analysis

Differences in age between the groups were assessed by ANOVA and differences by sex assessed by the Fisher exact probability test. Data from the FFQ were manually entered into FoodWorks nutrient analysis software (V5.00.1369, Professional Edition, 2007; Xyris Software (Australia) Pty Ltd, Highgate Hill, Queensland, Australia). Demographic data and nutrient analysis data were then transferred and analysed using SPSS for Windows (version 15.0, 2007; SPSS Inc., Chicago, IL, USA). Significance between group means was set at the 0·05 level.

Within-group comparison. Estimated energy requirements (EER) were calculated by the method outlined in the Nutrient Reference Values for Australia and New Zealand (20). This method uses the Schofield equation (21), namely:

\[ EER = (0.074 \times wt) + 2.754 \times PAL \times GF \text{ in males;} \]

\[ EER = (0.056 \times wt) + 2.898 \times PAL \times GF \text{ in females;} \]

where PAL is physical activity level and GF is a growth factor. Individual subject weights (wt) in kg were entered into the equation. The PAL used for all subjects was ‘light’ (PAL = 1·6) and the standard GF used was 105 kJ/d for individuals aged between 9 and 18 years to include the extra energy required for normal growth (20). This GF was established by the National Health and Medical Research Council (NHMRC) based on research by Butte et al. (22) who estimated the energy content of tissue deposition, and Guo et al. (23) who established the 50th percentile for healthy weight gain at different ages.

Individual energy intakes taken from the FoodWorks analysis were compared as a percentage with EER. Similarly, dietary intakes of protein, Ca, Fe and Zn were compared as a
percentage with RDI\textsuperscript{20}. One-sample \( t \) tests were used to compare group mean percentage intake with EER/RDI using a test value of 100.

**Between-group comparison.** One-way ANOVA was used to compare intake of nutrients among the three groups, with \textit{post hoc} analysis using Tukey’s test to determine the significance of the mean difference between groups. Variables of interest included total energy intake, energy intake as a percentage of EER, protein, Ca, Fe and Zn intake as a percentage of RDI and the intake of protein, fat, carbohydrate (g), and their percentage of contribution to total energy intake (kJ). This method was also used to compare growth by testing weight, height and BMI \( Z \) scores for statistical difference between the three groups. Further analysis was also undertaken using linear regression, where active CD was coded as 0, inactive CD coded as 1 and controls coded as 2, to establish if there were any associations between groups.

**Results**

The three groups were of similar age and sex (\( P=0.4 \) for age and \( P=0.8 \) for sex). Ages and sex were 12.94 (SD 1.51) years in active CD (six males, twelve females), 13.65 (SD 1.95) years in CD in remission (fifteen males, eight females) and 13.59 (SD 2.11) years in healthy controls (twelve males, ten females). Mean \( Z \) scores for weight, height and BMI for active CD were all below the reference population mean, and were lower than children with CD in remission and controls (Fig. 1). Significant differences in weight \( Z \) scores were found between subjects with active CD and controls (\( P=0.04 \); Tukey’s test). In addition, there was a significant association in weight \( Z \) scores between the groups, with subjects with active CD having the lowest weight \( Z \) scores, followed by those with CD in remission and controls with the highest weight \( Z \) scores (\( \beta=0.300; P=0.017 \)). Height \( Z \) scores did not significantly differ between groups (\( P>0.05 \)) by ANOVA. However, there was a significant association between the groups for BMI \( Z \) scores by linear regression (\( \beta=0.235; P=0.045 \)).

The energy intakes (compared with EER) and intakes of protein, Ca, Fe and Zn (compared with RDI) of each group were compared (Fig. 2). Group averages show that no group met the EER; however, energy intake was lowest in the children with active CD. All children with CD had energy intakes significantly lower than EER (active CD, \( P=0.001 \); CD in remission, \( P=0.03 \); Tukey’s test); however, there was no association between the groups by linear regression. Total energy intakes of children with active CD were significantly lower than controls (\( P=0.047 \); Tukey’s test; Table 1). All groups on average consumed less than the RDI for Ca. However, the difference was only statistically significant for children with active CD (\( P=0.04 \)) and no association was found with linear regression. All subjects exceeded protein and Zn requirements. There was an association between the three groups for protein intake using linear regression (\( \beta=0.249; P=0.049 \)) but no association for Zn. Fe intake was adequate for CD subjects in remission and controls. Although not statistically significant by ANOVA or linear regression, subjects with active CD did not meet the RDI for Fe (86.3 (SD 49.5) % of RDI).

No difference was found in the percentage of energy intake from protein (\( P=0.83 \)), fat (\( P=0.22 \)) or carbohydrate (\( P=0.37 \)) between groups (Table 1). However, all macronutrient intakes (in g) were lower in active CD subjects, compared with children with CD in remission and controls. Carbohydrate intake was found to be significantly lower (\( P=0.03 \)) in children with active CD compared with controls. Intake of fat in children with active CD compared with controls approached statistical significance (\( P=0.06 \)), whereas protein intake was similar (\( P=0.09 \)) between the two groups.

---

*Fig. 1. Comparison of weight, height and BMI \( Z \) scores between groups: children with active Crohn’s disease (CD; ■); children with CD in remission (□); controls (●). Values are means, with standard errors represented by vertical bars. *Mean value was significantly different from that of the control group (\( P=0.04 \)). There was also a significant association between groups for weight \( Z \) scores (\( \beta=0.300; P=0.017 \)) and BMI \( Z \) scores (\( \beta=0.253; P=0.045 \)) by linear regression.*

*Fig. 2. Intakes of energy in comparison with estimated energy requirement (EER; - - -) and intakes of protein, Ca, Fe and Zn in comparison with recommended dietary intake (RDI; - - -) by group: children with active Crohn’s disease (CD; ■); children with CD in remission (□); controls (●). Values are means, with standard errors represented by vertical bars. Mean value was significantly different from that of the control group: *\( P=0.04 \), **\( P=0.03 \), ***\( P=0.001 \). There was a significant association between the groups for protein intake by linear regression analysis (\( \beta=0.249; P=0.049 \)) but not for any other nutrient.*
Macronutrient intakes were similar between children with CD in remission and controls.

No difference was observed between groups for intakes of energy, protein, Ca, Fe and Zn. However, when comparing the intakes of children with active CD (as a percentage of EER) with those with CD in remission and controls, there was a trend for the total energy and Fe intakes to be lower (Fig. 2).

Discussion

An important finding of the present study was that the dietary intake of children with active CD was inadequate. Children with active CD had significantly reduced energy intakes compared with EER, a likely consequence of reduced food consumption. The significantly lower weight and BMI Z score (Fig. 1) and inadequate intake of Ca (Fig. 2) in children with active CD are likely to be a result of reduced energy intake.

All energy intakes among the three groups were below EER, which suggests that the Schofield equation used may not accurately estimate EER. The Schofield equation (with weight variable only) has been shown to overestimate resting energy expenditure (REE) in healthy paediatric populations in comparison with other equations(24,25). However, the NHMRC EER equation(20), based on the Schofield equation, was applied to the present study for consistency of results. The PAL employed (1·6) may have contributed to the consistent overestimation of EER, and a PAL of 1·3 used by Filippi et al. (6) may have reduced the impact of this PAL. Additionally, the importance of a PAL is limited in the present study, since physical activity was not determined on an individual basis. Use of lean body mass as the primary variable in a REE prediction equation, as opposed to weight in the Schofield equation, may increase the accuracy of REE estimation in these children.

The lower energy intake observed in the active CD group compared with controls is consistent with the established literature(26,27). Thomas et al. (9) found that children with active CD had an energy intake deficit of 1764 kJ compared with controls; however, energy intake was normal during remission. Hendricks et al. (5) reported decreased energy intake in paediatric CD, which is suggested to be the key factor responsible for weight loss in CD(28). Children with CD have different energy requirements from healthy children, such as an increased REE during remission, probably due to a lower fat-free mass(29). Thus, an individual assessment of energy balance may be required in children with CD.

A key finding of the present study shows that children with active CD appeared to be growth impaired, with lower weight Z scores compared with those in remission or controls (Fig. 1). Further, there was an association with weight Z scores that in children with active disease have the lowest scores followed by children with CD in remission and then controls with the highest weight Z scores. A similar association was also found with BMI Z scores (Fig. 1). However, no significant difference was found with the height Z scores. This is consistent with the research of Burnham et al. (30), who found children with a higher PCDAI (i.e. active CD) had a reduced weight and lean tissue mass. The literature generally illustrates impaired growth of children with CD, particularly during periods of disease activity(11,14).

Interestingly, the results of the present study indicate that protein intake was adequate in children with CD, although children with active CD had a lower protein intake compared with inactive CD and controls as shown by the significant linear regression result. Nevertheless, subjects with active CD had impaired growth, which concurs with results by Hendricks et al. (7), where growth stunting was seen despite adequate protein intake in paediatric CD. Dietary protein is not an at-risk nutrient in Westernised countries; however, due to the increased demand during CD activity for normal growth and development as well as enhanced requirements due to the losses associated with chronic inflammation, protein consumption may still be inadequate(31). It is thought that increased intestinal and transcapillary losses are responsible for decreased protein stores and reduced serum albumin in up to 80% of CD patients(5).

There was a tendency toward lower fat intakes in children with active CD compared with those with CD in remission or controls. Dietary fat intake is significantly linked with intestinal mucosal events, suggesting a relationship between fat and CD activity(13). Dietary fat consumption has been found to be significantly lower in patients with CD(28) particularly during periods of disease activity in combination with impaired linear growth in childhood(7). Intakes of fat were similar between children with CD in remission and controls (Table 1), which is consistent with a study by Filippi et al. (6) who reported no difference in macronutrient intake between patients with CD in remission and controls. Other studies have reported depleted fat intakes in children with CD(32).

Table 1. Total energy intakes and comparison of macronutrient intakes and their contribution to total energy between groups (Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Active CD</th>
<th>CD in remission</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy from protein (%) total energy intake</td>
<td>16·83 3·07</td>
<td>16·48 2·56</td>
<td>16·32 2·42</td>
</tr>
<tr>
<td>Energy from fat (%) total energy intake</td>
<td>32·50 2·96</td>
<td>34·39 4·06</td>
<td>32·68 4·34</td>
</tr>
<tr>
<td>Energy from CHO (%) total energy intake</td>
<td>50·56 4·42</td>
<td>49·13 4·37</td>
<td>51·05 5·23</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>6556* 2959</td>
<td>8614 3384</td>
<td>9170 3658</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>64·78 29·33</td>
<td>84·13 37·50</td>
<td>88·73 36·64</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>59·22 29·82</td>
<td>82·35 37·74</td>
<td>83·73 35·55</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>196·94* 85·36</td>
<td>248·48 89·81</td>
<td>282·50 111·87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Active CD</th>
<th>CD in remission</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy from protein (%) total energy intake</td>
<td>16·83 3·07</td>
<td>16·48 2·56</td>
<td>16·32 2·42</td>
</tr>
<tr>
<td>Energy from fat (%) total energy intake</td>
<td>32·50 2·96</td>
<td>34·39 4·06</td>
<td>32·68 4·34</td>
</tr>
<tr>
<td>Energy from CHO (%) total energy intake</td>
<td>50·56 4·42</td>
<td>49·13 4·37</td>
<td>51·05 5·23</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>6556* 2959</td>
<td>8614 3384</td>
<td>9170 3658</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>64·78 29·33</td>
<td>84·13 37·50</td>
<td>88·73 36·64</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>59·22 29·82</td>
<td>82·35 37·74</td>
<td>83·73 35·55</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>196·94* 85·36</td>
<td>248·48 89·81</td>
<td>282·50 111·87</td>
</tr>
</tbody>
</table>

CD, Crohn’s disease; CHO, carbohydrate.

* Mean value was significantly different from that of the control group (P<0·05; post hoc Tukey’s test).
and adults with CD\cite{10,28,33}, particularly n-3 fatty acids, which may have beneficial anti-inflammatory properties\cite{34}.

Carbohydrate was the only macronutrient found to be consumed in a lower quantity by subjects with active CD than controls (Table 1). Rigaud et al.\cite{25} found a significantly decreased intake of carbohydrate in patients with CD, whereas Hendricks et al.\cite{7} found no significant difference in carbohydrate intake between children with CD and controls. In contrast, children with CD have been shown to consume significantly more carbohydrate than controls\cite{35}.

The varied results within the literature may be explained by the range of geographical locations studied, and may suggest that carbohydrate intake is not specifically linked with disease activity. The percentage of macronutrient contribution to total energy intake was similar between all groups, suggesting that the lower intake of macronutrients is a direct consequence of decreased food and energy intakes during active disease.

On average, no group met the RDI for Ca, which may be due to the increased recommendation for Ca requirements in the new nutrient reference values\cite{20}. The only group with a Ca intake significantly lower than the RDI was the children with active CD. This finding agrees with research by Hendricks et al.\cite{7} and Guerreiro et al.\cite{35} where children with CD consumed significantly less Ca than healthy controls. Poor dietary intakes of Ca may negatively impact on bone strength, increasing the osteoporotic risk in children with CD\cite{36}.

Fe is the most common micronutrient deficiency documented in paediatric CD, which partly stems from inadequate intake; however, it is more likely due to poor absorption, particularly in children with active small-bowel disease\cite{5}. Children with active CD were the only group who did not meet the RDI for Fe (86 % RDI), although this was not a statistically significant finding. The present study focused on dietary Fe intake and did not include the intake of Fe (or other vitamin and mineral) supplements. Inclusion of supplemental intakes would be useful in future studies, particularly when comparing intakes with markers of Fe status. Zn intake is also commonly reported to be low among the IBD population\cite{6,7}; however, this was not observed in the present study. High intakes of protein-containing foods, which are also major sources of dietary Zn, could be a reason for the adequate intakes of Zn observed in the present study.

RDI are designed to be used as a goal for individual intake, and have not been recommended to assess nutrient intake among groups\cite{20}. However, since the present study aimed to assess the dietary intake of children with CD who are unwell and more critically require sufficient nutrient intake, RDI were used as a means of comparing nutrient intake with recommendations. RDI were chosen since estimated average requirements (EAR) are much lower for nutrients that are already 'at risk' in this population, and consequently may inadequately reflect their true requirements. Future studies of this nature may benefit from comparing both RDI and EAR.

Although children with CD met RDI for protein and Zn, actual absorption of these nutrients may be limited, especially during disease activity and inflammation, since dietary intake is just one factor that affects the nutritional status of children with CD. Other confounding factors that affect nutritional status include malabsorption, intestinal losses, corticosteroid use and surgical resection\cite{2,27}.

The sample size of the present study limits the conclusions that can be taken from these results. A further limitation of the present study was the use of the NHMRC method of estimating energy requirements\cite{20} along with the inclusion of a standard PAL. This may have caused some inconsistencies with data from the previous literature. Additionally, limited nutrients were analysed, since the FFQ was not designed to accurately reflect dietary intakes of nutrients other than those reported here. The use of weighed food records would be beneficial in future studies to obtain a more accurate assessment of actual dietary intake.

In conclusion, dietary intakes of children with active CD were adversely affected. Reduced intake is a potential factor having an impact upon weight gain and linear growth. Low energy intake due to reduced food consumption is the primary reason for inadequate intakes of Ca and Fe in children with active CD. The results of the present study provide useful information of the potential impact of diet upon overall nutrient intake and growth in children with CD. Quantifying nutrient intake in paediatric CD can help to determine the extent to which nutrient supplementation and dietary intervention is required in the management of CD (and particularly active CD). An area of future research may be to observe the frequency of food group intakes (such as meat, dairy products, vegetables and fruit) to assess whether poor nutrient intakes correlate with specific food avoidance.

Acknowledgements

The present study was funded in part by the Sydney Children’s Hospital Foundation.

All of the authors were involved in the design, conceptualisation and conduct of the study. R. P. and K. E. W. had primary roles in the enrolment of patients, data collection, modification and administration of FFQ and analysis of dietary intakes. D. A. L. and A. S. D. were involved in patient recruitment. A. S. D. and S. T. L. assisted in the statistical analysis of data arising. D. A. L. and H. W. participated in the review and interpretation of data arising. R. P. prepared the first version of the manuscript and all of the authors participated in the drafting and subsequent reviews of the manuscript. All authors gave approval for the final version of the manuscript.

The authors have no conflict of interest to declare.

References


