Original article

Omega 3:6 ratio intake and incidence of glaucoma: The SUN cohort

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S U M M A R Y

Background & aims: Omega 3 and omega 6 polyunsaturated fatty acids have been related to many diseases, especially to cardiovascular diseases. We aimed to assess the association between the intake of omega 3, omega 6 fatty acids and their ratio and the incidence of glaucoma in this prospective cohort study.

Methods: We followed-up 17,128 participants initially free of glaucoma for a median time of 8.2 years. Validated data of diet were collected at baseline with a 136-item semi-quantitative food-frequency questionnaire and information of new diagnosis of glaucoma in biennial follow-up questionnaires. Multivariable Cox regression models were fit to assess the relationship between omega 3, omega 6 and their ratio and the incidence of glaucoma in the total sample and in participants aged ≥40 years at baseline.

Results: During a median follow-up time of 8.2 years, 156 new cases of glaucoma were identified. No significant association was observed for the omega 3 or the omega 6 intake and the risk of glaucoma. Participants in the highest quintile of omega 3:6 ratio intake had a significantly higher risk of glaucoma than participants in the lowest quintile {hazard ratio (HR): 1.91 [95%CI: 1.05–3.46], p for trend 0.03}. The association became stronger (HR for the comparison of the 5th versus the 1st quintile: 2.43 [95%CI: 1.17–5.03], p for trend 0.02) when we considered only those participants who were ≥40 years old.

Conclusions: Our data suggest an association between omega 3:6 ratio intake and incident glaucoma in our cohort, especially among older participants.

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1. Introduction

Glucoma is the second most common cause of blindness worldwide, and it is estimated that the number of people with glaucoma will increase from 60.5 million in 2010 to 79.6 million in 2020. Primary open-angle glaucoma (POAG), the most common type of glaucoma, is characterized by progressive optic nerve degeneration thus affecting the visual field leading to blindness. Although its pathogenesis is poorly understood, non-modifiable risk factors include older age, ethnicity and a family history of POAG. At the present time the only established modifiable risk factor for the development of POAG and progression of POAG is elevated intraocular pressure (IOP). Because IOP levels may be modulated by lifestyle factors, efforts to identify activities that may reduce IOP levels may be promising in developing primary prevention strategies for POAG.

Epidemiologic studies on lifestyle factors are few and controversial, and up to now no environmental factor has been significantly associated with POAG. Nevertheless, risk factors such as dietary fat are believed to potentially influence the IOP. In fact, the omega 6 derived eicosanoids include prostaglandin F2α, which has been demonstrated to lower IOP. Indeed, an analogue of prostaglandin F2α called latanoprost is a widespread antiglaucomatous drug that decreases IOP by 25–35% by increasing uveoscleral outflow.

Both omega 3 and omega 6 are polyunsaturated fatty acids (PUFAs), which are essential and therefore must be ingested in our diet. As both PUFAs compete for enzymes, the balance between
omega 3 and omega 6 may modulate the availability of endogenous omega 6 prostaglandins, and differences in physiologic concentrations of omega 6 may influence IOP.\textsuperscript{12} In that way, as the ratio between both PUFA can be modified in the diet, it seems important to investigate the relationship between the dietary omega 3:6 ratio and the risk of glaucoma.

The aim of this study was to evaluate the association between the intake of omega 3, omega 6 and their ratio and the risk of glaucoma in a cohort study of university graduates conducted in Spain.

2. Materials and methods

2.1. Study population

The SUN ("Seguimiento Universidad de Navarra" [University of Navarra Follow-up]) study is a multipurpose dynamic Spanish cohort of university graduates. The study methods were published in detail elsewhere.\textsuperscript{14} Briefly, information on exposure and outcome is gathered by mailed questionnaires collected biennially. Persons who do not reply to follow-up questionnaires are sent up to 5 additional mailings. The recruitment of participants started in December 1999 and it is permanently on-going. The overall follow-up rate is approximately 90%. Before March 2010, 20,572 participants had answered their baseline questionnaire (Fig. 1). After excluding participants with no follow-up, with energy intake out of the predefined limits (<800 kcal/d for men, <500 kcal/d for women or >4000 kcal/d for men, >3500 kcal/d for women\textsuperscript{15}), or with prevalent glaucoma, our effective sample size for the analyses was 17,128 participants.

The study was approved by the Human Research Ethical Committee at the University of Navarra. Voluntary completion of the first questionnaire was considered to imply informed consent.

2.2. Assessment of dietary exposure

The dietary exposure was ascertained through a 136-item semi-quantitative food-frequency questionnaire previously validated in Spain.\textsuperscript{16} Food consumption was calculated as the self-reported nutrient data bank using the latest available information included in food composition tables for Spain. The omega 3:6 ratio was categorized into quintiles.

2.3. Assessment of other covariates

The validated physical activity questionnaire included information about 17 activities.\textsuperscript{17} To quantify the volume of activity during leisure time, an activity metabolic equivalent (MET) index was computed by assigning a multiple of the resting metabolic rate (MET score) to each activity, and the time spent in each of the activities was multiplied by the MET score specific to each activity, and then summed over all activities obtaining a value of overall weekly MET-h.

Body mass index (BMI), defined as weight (kg) divided by the square of height (m\textsuperscript{2}), was computed using the self-reported information on weight and height from the baseline questionnaire. The validity of self-reported weight was assessed in a subsample of the cohort.\textsuperscript{18}

2.4. Assessment of the outcome

Glaucoma was assessed through a question included in the follow-up questionnaire. Participants responded to the question: “Have you ever been diagnosed of glaucoma by a health professional?” The question also specified the date of diagnosis. Even though this question has not been specifically validated in our cohort, validation studies on other outcomes have provided reassuring results on the validity of our data.\textsuperscript{19–21}

2.5. Statistical analysis

Cox regression models were fit to assess the relationship between the intake of omega 3, omega 6 or their ratio and the
incidence of glaucoma. We calculated nutrient densities of omega 3 and omega 6 fatty acids intake.13,15 Hazard ratios (HRs) and their 95% CI were calculated considering the lowest quintile of omega 3, omega 6 or omega 3:6 ratio intake as the reference category. Tests of linear trend across increasing quintiles were conducted by assigning the medians to each quintile and treating the resulting variable as a continuous variable. Participants contributed to the follow-up period until the date of return of their last questionnaire, death, or diagnosis of glaucoma, whichever came first.

In addition to stratifying by age, other potential confounders included as covariates in the multiple Cox models were age, sex, body mass index (kg/m^2), smoking, hypertension, diabetes mellitus, physical activity (tertiles), coffee consumption (4 categories), alcohol consumption (quintiles), adherence to the traditional Mediterranean diet12— which is characterized by a high consumption of fruits and nuts, vegetables, legumes, fish and cereals, a high intake of monounsaturated to saturated fatty acids, a moderate consumption of wine and a low consumption of meat and meat products and dairy products— and total energy intake.

As a sensitivity analysis, we restricted our analysis to participants who were at least 40 years of age at baseline because they are at a higher risk of glaucoma.13,23

All p values presented are two tailed; p < 0.05 was considered a priori as statistically significant.

3. Results

During follow-up (median 8.2 years), 156 incident cases of glaucoma were identified over a total of 132,606 person-years.

Baseline characteristics of the participants according to the quintiles of omega 3:6 ratio intake are presented in Table 1. The mean age of the participants was 39 years (S.D.: 12) and the mean omega 3:6 intake was 0.18 (S.D.: 0.13).

Participants in the highest quintile of omega 3:6 ratio intake were more likely to be women and older, less likely to be never smokers, and more prone to have a lower total energy intake, a lower total fat intake, a higher adherence to the traditional Mediterranean dietary pattern, hypertension or diabetes.

Participants with the highest intake of omega 3:6 ratio showed a significantly higher risk of glaucoma compared with those participants in the lowest quintile after controlling for potential confounders (HR: 1.91 [95% IC: 1.05–3.46], p for trend 0.029) (Table 2).

When we restricted our analyses to the 7332 participants who were at least 40 years of age at baseline, analyses were based on 126 incident cases among 54,274 person-years of follow-up. The association between the omega 3:6 ratio and the risk of glaucoma became stronger even after adjusting for potential confounders (HR for the comparison of the 5th quintile versus the 1st quintile: 2.43 [95% IC: 1.17–5.03], p for trend 0.021).

Neither omega 3 intake nor omega 6 intake were significantly associated to the risk of glaucoma (Table 3).

4. Discussion

In this large prospective study, we found that the omega 3:6 ratio was significantly associated with a higher risk of developing glaucoma.

To our knowledge there are few studies that have assessed the association between omega 3:6 ratio and incidence of glaucoma. Our results are consistent with those previously reported by Kang et al.,13 who studied the association between fat intake and primary open angle glaucoma (POAG) in two large cohorts and found that a high dietary omega 3:6 ratio appears to increase the risk of POAG, particularly high-tension POAG. In a prospective population-based cohort, the Rotterdam Study,12 a total of 3502 participants for whom dietary and ophthalmic data were available were followed up for 9.7 years. The omega 3:6 ratio was higher in participants with POAG (mean 0.19) compared to participants without POAG (0.12), but this difference did not reach statistical significance (p = 0.022).

Some studies that have assessed the association between omega 3 intake and the risk of glaucoma have reported an inverse association between omega 3 intake and glaucoma.25–29 Cellini et al.25 showed that a support therapy with a combination of DHA, vitamin E and vitamin B complex (TROFINERV) significantly improved all the perimetric indices and, in particular, the MD and CPSD, in glaucomatous patients. Connor et al.26 demonstrated that Rhesus

Table 1

<table>
<thead>
<tr>
<th>N (Omega 3:6 ratio (range))</th>
<th>Omega 3:6 ratio</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3426</td>
<td>0.00–0.107</td>
<td>56.3</td>
<td>57.5</td>
<td>59.3</td>
<td>60.8</td>
<td>62.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3426</td>
<td>0.107–0.136</td>
<td>56.3</td>
<td>57.5</td>
<td>59.3</td>
<td>60.8</td>
<td>62.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3425</td>
<td>0.136–0.169</td>
<td>56.3</td>
<td>57.5</td>
<td>59.3</td>
<td>60.8</td>
<td>62.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3436</td>
<td>0.169–0.236</td>
<td>56.3</td>
<td>57.5</td>
<td>59.3</td>
<td>60.8</td>
<td>62.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3425</td>
<td>0.236–4.58</td>
<td>56.3</td>
<td>57.5</td>
<td>59.3</td>
<td>60.8</td>
<td>62.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex (female%)</th>
<th>56.3</th>
<th>57.5</th>
<th>59.3</th>
<th>60.8</th>
<th>62.7</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36 (12)</td>
<td>37 (11)</td>
<td>38 (12)</td>
<td>40 (12)</td>
<td>42 (13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>23.3 (3.4)</td>
<td>23.4 (3.4)</td>
<td>23.6 (3.5)</td>
<td>23.6 (3.4)</td>
<td>23.9 (3.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity (METS-h/week)</td>
<td>19.1 (20.9)</td>
<td>20.4 (22.7)</td>
<td>20.6 (21.0)</td>
<td>22.9 (22.5)</td>
<td>23.6 (24.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total energy intake (Kcal/day)</td>
<td>2406 (650)</td>
<td>2418 (607)</td>
<td>2397 (592)</td>
<td>2370 (596)</td>
<td>2153 (613)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total fat intake (% total energy)</td>
<td>37.8 (6.7)</td>
<td>37.3 (6.1)</td>
<td>36.8 (6.1)</td>
<td>36.2 (6.4)</td>
<td>34.3 (7.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Saturated fat intake (% total energy)</td>
<td>13.4 (3.2)</td>
<td>12.8 (2.8)</td>
<td>12.5 (2.9)</td>
<td>12.2 (3.2)</td>
<td>11.3 (3.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monounsaturated fat intake (% total energy)</td>
<td>16.2 (3.8)</td>
<td>15.9 (3.6)</td>
<td>15.8 (3.5)</td>
<td>15.7 (3.7)</td>
<td>14.7 (3.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Omega 3 intake (% total energy)</td>
<td>0.8 (0.3)</td>
<td>1.0 (0.5)</td>
<td>1.0 (0.4)</td>
<td>1.1 (0.3)</td>
<td>1.2 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Omega 6 intake (% total energy)</td>
<td>10.6 (5.9)</td>
<td>8.1 (3.8)</td>
<td>6.5 (2.4)</td>
<td>5.4 (1.7)</td>
<td>3.7 (1.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Coffee (cups/day)</td>
<td>1.2 (1.3)</td>
<td>1.2 (1.2)</td>
<td>1.2 (1.2)</td>
<td>1.2 (1.3)</td>
<td>1.2 (1.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Alcohol intake (g/day)</td>
<td>6.6 (10.0)</td>
<td>6.7 (9.7)</td>
<td>6.7 (10.1)</td>
<td>7.1 (10.9)</td>
<td>6.9 (11.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adherence to the Mediterranean diet (0–9 score)</td>
<td>3.5 (1.7)</td>
<td>3.8 (1.7)</td>
<td>4.2 (1.7)</td>
<td>4.7 (1.7)</td>
<td>5.0 (1.7)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* The percentages do not add to 100% because this information was missing in 436 subjects.
monkeys developed reduced visual acuity at 8 and 12 weeks of age and a pathologic electroretinogram (ERG) after postnatal deprivation of dietary n-3 fatty acids. Accordingly, Ren et al.27 found decreased omega 3 PUFA levels in glaucoma patients compared with their healthy siblings. In a review article Huang et al.30 postulated that cod liver oil, as a combination of vitamin A and omega 3 fatty acids, should be beneficial for the treatment of glaucoma. As they explain, vitamin A is an antioxidant which prevents the oxidative damage that contributes to the etiology and progression of glaucoma, and on the other hand, omega 3 fatty acids increase ocular blood flow, and improve optic neuroprotective function. The Photograft study25 is a French nationwide case-control study that evaluated risk factors for POAG. In this study, 339 cases with high-tension POAG were compared to 339 controls with ocular hypertension (OHT). In the final multivariate model POAG was significantly associated with low consumption of fatty fish (OR = 2.14, 95% CI: 1.10–4.17, p = 0.02) and walnuts (OR = 2.02, 95% CI: 1.18–3.47, p = 0.01). These results suggested a protective effect of omega 3 fatty acids against POAG. Contrarily, we found no significant association between omega 3 intake and the risk of glaucoma and a direct association between omega 3:6 ratio intake and risk of glaucoma.

The mechanism by which a lower omega 3:6 ratio may contribute to reduce IOP and thereby to reduce the risk of glaucoma is not clear. Our findings are consistent with the hypothesis that diets based on a lower omega 3:6 ratio, thus, with a higher dietary omega 6 fat intake might lead to greater availability of omega 6 derived prostaglandins,13 such as prostaglandin F2α, which may reduce IOP to levels that are less harmful to the optic nerve and therefore may help to reduce the occurrence of POAG. In a murine model, mice fed with an omega 6 polysaturated fatty acid-rich diet had higher plasma 8-iso-prostaglandin F2α levels than mice fed with a monounsaturated fatty acids acid-rich diet.31 Contrarily, consuming an energy restricted diet rich in salmon, which is an important source of omega 3 fatty acids, decreased serum concentrations of prostaglandin F2α.32 As prostaglandins have been reported as therapeutic agents for glaucoma, these results suggest that variations in the physiologic concentrations of ocular prostaglandins in healthy populations may influence IOP and therefore may be related to their future risk of developing glaucoma.

The observed associations between adherence to the Mediterranean diet and diabetes or hypertension in the cross-sectional analyses (Table 1) are most likely to be explained by lifestyle changes recommended after the medical diagnosis of diabetes or hypertension (i.e. reverse causality). In fact, in prospective analyses conducted in the SUN Project, with the proper adjustment for confounding and with the use of longitudinal designs, a higher adherence to the Mediterranean diet was associated with a lower risk of diabetes16 and with reduced age-related changes in systolic and diastolic blood pressure.34 Thus, the observed results in Table 1 may be due to reverse causation.

The strengths of this study are the large sample size, long duration of follow up, its prospective design that could avoid inverse causation bias, and the control of a wide variety of potential confounders. In addition, dietary exposure was ascertained through a validated questionnaire.

However, there are some limitations in our study as the self-reported nature of exposure and outcome. Although previous validation studies showed acceptable correlations on the food-frequency questionnaire,16 some degree of misclassification is likely to exist. However, we expect it to be non-differential and, therefore, drive the association toward the null value. In addition, new cases of glaucoma have not been specifically validated in our cohort. This fact could bias our results. However, we expect this bias to be non-differential and not very large since several validation studies in our cohort have provided reassuring accuracy of our data.17–21,35 On the other hand, only university graduates are recruited in the cohort. This could induce a selection bias.

### Table 2

Hazard ratios (95% CI) of glaucoma according to quintiles of omega 3:6 ratio intake.

<table>
<thead>
<tr>
<th>Quintiles of omega 3:6 ratio intake</th>
<th>Cases/person-years</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and sex-adjusted</td>
<td>1 (Ref.)</td>
<td>1.15 (0.64–2.06)</td>
<td>1.12 (0.63–2.00)</td>
<td>1.06 (0.69–2.05)</td>
<td>1.06 (0.69–2.05)</td>
<td>1.06 (0.69–2.05)</td>
<td>0.30</td>
</tr>
<tr>
<td>Multiple-adjusted model</td>
<td>1 (Ref.)</td>
<td>1.13 (0.63–2.02)</td>
<td>1.08 (0.61–1.94)</td>
<td>1.06 (0.62–2.04)</td>
<td>1.06 (0.62–2.04)</td>
<td>1.06 (0.62–2.04)</td>
<td>0.046</td>
</tr>
<tr>
<td>Multiple-adjusted model</td>
<td>1 (Ref.)</td>
<td>1.12 (0.62–2.00)</td>
<td>1.09 (0.61–1.96)</td>
<td>1.03 (0.89–2.00)</td>
<td>1.03 (0.89–2.00)</td>
<td>1.03 (0.89–2.00)</td>
<td>0.047</td>
</tr>
</tbody>
</table>

### Table 3

Hazard ratios (95% CI) of glaucoma according to quintiles of omega 3 and omega 6 intake.

<table>
<thead>
<tr>
<th>Quintiles of omega 3 intake</th>
<th>Cases/person-years</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and sex-adjusted</td>
<td>1 (Ref.)</td>
<td>0.61 (0.35–1.08)</td>
<td>1.00 (0.61–1.63)</td>
<td>0.88 (0.54–1.43)</td>
<td>0.95 (0.60–1.51)</td>
<td>0.912</td>
<td></td>
</tr>
<tr>
<td>Multiple-adjusted model</td>
<td>1 (Ref.)</td>
<td>0.62 (0.35–1.09)</td>
<td>1.01 (0.62–1.64)</td>
<td>0.84 (0.51–1.37)</td>
<td>0.96 (0.60–1.52)</td>
<td>0.921</td>
<td></td>
</tr>
<tr>
<td>Multiple-adjusted model</td>
<td>1 (Ref.)</td>
<td>0.73 (0.40–1.30)</td>
<td>1.18 (0.71–1.97)</td>
<td>0.94 (0.56–1.58)</td>
<td>1.07 (0.64–1.79)</td>
<td>0.694</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4

Hazard ratios (95% CI) of glaucoma according to quintiles of omega 6 intake.

<table>
<thead>
<tr>
<th>Quintiles of omega 6 intake</th>
<th>Cases/person-years</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude model</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age and sex-adjusted</td>
<td>1 (Ref.)</td>
<td>1.01 (0.65–1.56)</td>
<td>0.67 (0.41–1.09)</td>
<td>0.71 (0.44–1.16)</td>
<td>0.66 (0.40–1.07)</td>
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</tr>
<tr>
<td>Multiple-adjusted model</td>
<td>1 (Ref.)</td>
<td>0.99 (0.63–1.54)</td>
<td>0.64 (0.41–1.09)</td>
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<td>0.67 (0.41–1.11)</td>
<td>0.076</td>
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</tr>
<tr>
<td>Multiple-adjusted model</td>
<td>1 (Ref.)</td>
<td>1.07 (0.67–1.69)</td>
<td>0.64 (0.37–1.08)</td>
<td>0.75 (0.45–1.26)</td>
<td>0.69 (0.40–1.17)</td>
<td>0.070</td>
<td></td>
</tr>
</tbody>
</table>

### Notes

a Adjusted for age, sex, body mass index (kg/m²), smoking, hypertension, diabetes mellitus.
b Adjusted for age, sex, body mass index (kg/m²), smoking, hypertension, diabetes mellitus, physical activity (turtles), coffee consumption (4 categories), alcohol consumption (quintiles), adherence to the Mediterranean diet and total energy intake.
Nevertheless, this decision is convenient and methodologically adequate in order to obtain a better control for confounding due to economical and socio-demographic factors. Also, we had no information on family history of glaucoma, which is an important risk factor for glaucoma. Another consideration is that most of the participants are white. Thus, our results may not be widely generalizable to other ethnicities, particularly those of African or Caribbean heritage who are at greater risk of POAG.

In conclusion, in this prospective cohort study we found that a diet with a high omega 3:6 ratio intake, thus low in omega 6, is associated with a higher occurrence of glaucoma. Recommending a diet with a high omega 3:6 ratio intake, thus low in omega 6, is associated with a higher occurrence of glaucoma. Another consideration is that most of the participants are white. Thus, our results may not be widely generalizable to other ethnicities, particularly those of African or Caribbean heritage who are at greater risk of POAG.

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Statement of authorship

Each participant sufficiently participated, intellectually and practically, in the work to take public responsibility for the content of the article. ET and MAMG designed the study; MAMG was in charge of collecting the data; MPA, ET and CS analyzed the data; MPA and ET wrote the manuscript; and AG and JMM provided significant advice. All authors read and approved the final manuscript.

Conflict of interest statement

No conflict of interest.

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