Comparison of methods to evaluate the quality of the Mediterranean diet in a large representative sample of young people in Southern Spain

M. Mariscal-Arcas¹, J. Velasco¹, C. Monteagudo¹, M. A. Caballero-Plasencia², M. L. Lorenzo-Tovar³, F. Olea-Serrano¹


Abstract

The objective of this study was to compare the usefulness of two methods to evaluate diet quality in young people in Southern Spain: a new Mediterranean Diet Pattern (MDP) and a modification of the Diet Quality Index-International (DQI-I) for the Mediterranean area. The study population was 3190 schoolchildren aged 8-15 yrs. The questionnaires used were first validated (Bland-Altman plot and Wilcoxon tests) in a randomized sample. The DQI gives a more detailed evaluation of food components, whereas the MDP gives global information on food groups but includes foods characteristically consumed in the Mediterranean region. Highly similar results were obtained using the MDP and the adapted DQI-I, which appear to be equally useful to evaluate diet quality in a Mediterranean population. The fact that we selected the same types of food for both indices may explain the similar overall evaluations. According to these results, both methods appear to be equally appropriate for evaluating diet quality in a Mediterranean population.

DOI:10.3305/nh.2010.25.6.4889

Key words: Diet quality. Diet Quality Index-International (DQI-I). Mediterranean Diet Pattern adherence (MDP). Young people’s diet. Southern Spain.

Correspondencia: Fatima Olea-Serrano
Department of Nutrition and Food Science
University of Granada
Campus de Cartuja, s/n.
18071 Granada, Spain
E-mail: folea@ugr.es
Recibido: 3-VII-2010.
Aceptado: 15-IX-2010.
Introduction

The traditional Southern Spanish diet corresponds to the typical Mediterranean dietary pattern (MDP)\(^1\)-\(^4\). The MDP is characterized by: a high intake of vegetables, pulses, fruits and nuts, and cereals (largely unrefined in the past); a high intake of olive oil but a low intake of saturated lipids; a moderately high intake of fish (depending on the proximity to the sea); a low-to-moderate intake of dairy products (mostly cheese or yoghurt); a low intake of meat and poultry; and a regular but moderate intake of wine, generally during meals\(^5\)-\(^8\). The MDP has been associated with better health and a longer life\(^9\) and has been promoted as a model for healthy eating\(^10\)-\(^11\). However, there is wide epidemiological evidence of a rapid change in dietary patterns in Mediterranean countries, with a higher consumption of animal products and saturated fats (SFA) to the detriment of vegetable foodstuffs\(^12\)-\(^14\). Departure from the MDP might be accompanied by loss of its protective effects on health, leading to a rise in diet-related diseases such as cardiovascular disease and cancer\(^15\).

In comparison with the prevalent dietary pattern at the beginning of the 20th century, there is a higher fat and saturated fat content and a lower consumption of fruit and vegetables\(^9\). Several indices have been developed to assess the diet quality of previously defined population groups. Since Kant et al.\(^17\) published the Dietary Diversity Score, based on the daily consumption of foods classified in five groups, there have been various proposals. Thus, Drewnosky et al.\(^18\) considered the consumption of up to 164 foods during a 15-day period. In the Healthy Eating Index (HEI)\(^19\), the first five components compare diet quality with the Food Guide Pyramid\(^20\) and the second five components examine dietary moderation. Kim et al.\(^21\) developed the Diet Quality Index-International (DQI-I), a score based on information from food frequency questionnaires and a quantitative assessment of reference nutrients. A correction to this index was recently applied to evaluate adherence to the Mediterranean Diet\(^22\)-\(^23\). New versions of methods used to evaluate the diet quality of a given population were recently published, using indices based on the Mediterranean diet\(^24\)-\(^25\) or the American pyramid model\(^26\)-\(^27\). However, no study has compared these approaches to establish whether one is more appropriate than the other\(^28\)-\(^31\). Diet quality indexes measure the overall diet quality based on food group consumption, the intake of nutrients related to chronic disease and the variety of the diet\(^32\)-\(^34\). The objective of this study was to compare the usefulness of two methods to evaluate diet quality (MDP adherence and DQI-I).

Subjects and Methods

A representative sample of young and adolescent schoolchildren in the city of Granada was recruited from schools in each of the eight administrative districts of the city, including both public and private schools among the 35 randomly selected centers.

This population-based cross-sectional nutritional survey included 3190 schoolchildren (1557 males and 1597 females) aged 8-15 yrs old from Granada province (Southern Spain). They were recruited between 2005 and 2006. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of University of Granada. Written informed consent was obtained from all subjects, parents or guardians.

Questionnaires

The questionnaire developed by Mariscal-Arcas\(^35\)-\(^36\) was used, comprising four sections: sociodemographic; food frequency questionnaire (FFQ); nutrition-related life habits, including both qualitative and quantitative variables; and three 24-h diet recalls (24-hR). Participants were identified by a six-digit number to preserve their anonymity. Studies were performed between February 2005 and May 2006.

Sociodemographic data gathered included sex, age, school year, and educational centre. The FFQ has been widely used and validated and was adapted to include items consumed by this population group\(^35\)-\(^36\)-\(^37\). It covered breakfast, mid-morning snack, lunch, afternoon snack, and evening meal. Data were gathered on the consumption or not of the item, the number of times it was consumed per day, week or month during the previous year, and the amount consumed each time in household measures (plates, glasses, spoons, etc.). The 24-hR was an open-format questionnaire that gathered information on the diet during the three days before the interview. Data were also gathered on the time of the intake, the amount of food consumed in household measures, its preparation, and the day of the week reported.

Questionnaires were administered at the school or in the young person’s home by a trained dietician between Tuesday and Friday. Days after the weekend or after a public holiday were avoided to ensure that all 24-hRs reported on a normal school day. The NO-VARTIS-DIETSOURCE v.1.2 program was used to convert foods into nutrients\(^38\).

Statistical analysis

SPSS-15 (SPSS Inc. Chicago, IL, USA) was used for the statistical analysis. P<0.05 was considered significant in all analyses.

Questionnaire validation

The questionnaires used were first validated in a randomized sample (n=241) that contained children...
(26.3%), male adolescents (37.5%), and female adolescents (36.6%) in the same proportions as in the whole series.

The Bland-Altman plot test and the Wilcoxon test for paired samples were used for the validation. Table I lists the macronutrient and energy values obtained.

The FFQ and 24h-R yielded highly similar mean energy and macronutrient values for the diet of this population, with no significant differences between them according to the Wilcoxon non-parametric test and with 95% of differences showing less than two standard deviations on the Bland and Altman plot.

Table I

<table>
<thead>
<tr>
<th>Energy/nutrient</th>
<th>FFQ Median</th>
<th>Interquartile amplitude</th>
<th>24h recall Median</th>
<th>Interquartile amplitude</th>
<th>Wilcoxon test*</th>
<th>p</th>
<th>Mean 24h recall - FFQ</th>
<th>Limits of Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (MJ)</td>
<td>8.20</td>
<td>1.99</td>
<td>8.06</td>
<td>2.14</td>
<td>-0.177</td>
<td>0.860</td>
<td>-0.013</td>
<td>-0.262 to -0.237</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>71.86</td>
<td>25.86</td>
<td>70.80</td>
<td>21.50</td>
<td>-0.204</td>
<td>0.838</td>
<td>0.638</td>
<td>-47.25 to 48.53</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>88.46</td>
<td>25.73</td>
<td>85.60</td>
<td>33.35</td>
<td>-1.192</td>
<td>0.233</td>
<td>-2.852</td>
<td>-59.71 to 54.00</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>215.51</td>
<td>74.55</td>
<td>224.40</td>
<td>76.05</td>
<td>-0.387</td>
<td>0.699</td>
<td>-0.414</td>
<td>-120.06 to 119.64</td>
</tr>
</tbody>
</table>

*Wilcoxon test for FFQ vs. 24-h recall.

Construction of adapted DQI-I (Range, 0-100)

The DQI-I was adapted to assess the Mediterranean diet, following the modification by Tur et al. and Mariscal-Arcas et al. of the method developed by Kim. Notably, whereas Kim recommended that fat intake should be ≤20% of total energy, Mariscal-Arcas et al. proposed a higher level (≤30%) for the consumption of fats in the Mediterranean region. The modified version of the index also includes Spanish recommended daily intakes (RDI) and changes the criterion for classifying empty-calorie foods. The adapted DQI-I focuses on four aspects of diet: variety, adequacy, moderation, and overall balance. The score for each category is the sum of the scores for each diet component that category. The total DQI-I score (range, 0-100 points) is the sum of the scores for the four categories.

Variety: Variety was evaluated both as overall variety and as variety of protein sources. The maximum overall variety score was achieved by intake of at least one serving per day from each of the five food groups (meat/poultry/fish/egg, dairy/pulses, cereals, fruit, and vegetables). The score for the variety of protein sources (meat, poultry, fish, dairy, pulses, and eggs) was based on intakes of more than half the serving size per day, using data gathered by the FFQ. Portions were based on portion-weight tables for each food group and household measures.

Adequacy: This category evaluates the adequacy of intake of dietary elements that are necessary to protect against under-nutrition and deficiency disorders. The adequacy of fruit, vegetable, cereal, and fiber intake is a function of the energy intake. Thus, for energy intakes of 7118 kJ (1700 kcal), 9211 kJ (2200 kcal), or 11304 kJ (2700 kcal), the maximum score is assigned to a diet containing 2, 3, or 4 portions of fruit and 3, 4, or 5 portions of vegetables, respectively. Likewise, the highest score for cereal and fiber categories was assigned to daily intakes of ≥6, ≥9, and ≥11 portions of cereal and ≥20, ≥25, and ≥30g of fiber for the three energy intake levels, respectively. Protein intake was considered adequate when the proportion of total energy from protein was >10%. Intakes defining the highest score for adequacy of iron, calcium, and vitamin C were derived from the RDIs for Spanish people, which vary according to age and gender.

Moderation: This category evaluates the intake of food and nutrients that are related to chronic diseases and may therefore need restriction. The importance of moderation in fat intake is emphasized in the DQI-I by applying more stringent cut-off values for total fat intake than found in other dietary indexes. In our modification, a score of 6 points was assigned when total fat was ≤30% of total energy/day, 3 points when 30-35% of total energy/day, and 0 points when >35% of total energy/day. The intake of saturated fats was evaluated as the percentage of energy from saturated fat, and levels of cholesterol and sodium intake were also recorded.

Overall balance: This category examines the overall balance of diet in terms of the proportions of energy sources and the fatty acid composition. Detailed cut-off values and corresponding scores were those proposed by Mariscal-Arcas et al.

Construction of Mediterranean dietary adherence (Range, 0-100)

We used a scale constructed by Trichopoulou et al. to evaluate the degree of adherence to the traditional Mediterranean diet, evaluating compliance with eight typical components of the Mediterranean diet: high consumption (median intake) of vegetables, fruit and nuts, pulses, cereals, fish, high MUFA:SFA ratio, and...
Diet quality of young people

The study sample comprised 3190 young people (49.4% males and 50.6% females). The sex distribution of the sample did not significantly differ from the current sex distribution in the population of Southern Spain\(^4\). The mean age was 10.89 yrs (SD: 1.84) (range: 8-15yrs). The sample comprised 36.7% male adolescents (10-15yrs), 36.9% female adolescents, and 26.9% children (<10yrs). Table II lists the mean nutrient and energy intakes and the corresponding RDI percentages with respect to the requirements of Spanish children and young people. The MDP adherence was calculated from data on the consumption (in g/day) of each food group gathered by the FFQ (table III).

The mean total modified DQI-I score was 58.37% of the possible score (100%). The highest score was for adequacy, followed by variety and moderation. The lowest score was for overall balance (table IV). Table V gives the percentage of the population above and below median intake of each food group as a function of the MDP adherence in tertiles (\(\leq 33.3\%\) poor compliance, MDP=33.01-65.51\% adequate compliance and MDP>65.52\% good compliance). Among the groups with poor compliance, we highlight the very low frequency of fish consumption in the children (<10yrs) and very high frequency in the female adolescents, the very high frequency of meat consumption in the male adolescents and the very high frequency of vegetable consumption in both male and female adolescents. The \(t\) test results showed significant differences in food intake values among the different compliance groups (MDP tertiles), except in the consumption of egg and cereals in male and female adolescents (p>0.05). The median MDP estimated for the whole population was 62.78. Table VI shows the distribution of the study population according to com-

**Table II**

<table>
<thead>
<tr>
<th>Percentage of nutrients with respect to Spanish RDI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children</strong> (8-10yrs)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>% RDI</td>
</tr>
<tr>
<td>Energy</td>
</tr>
<tr>
<td>Proteins</td>
</tr>
<tr>
<td>Ca</td>
</tr>
<tr>
<td>Fe</td>
</tr>
<tr>
<td>Zn</td>
</tr>
<tr>
<td>Se</td>
</tr>
<tr>
<td>Na</td>
</tr>
<tr>
<td>Iodine</td>
</tr>
<tr>
<td>Vit B1</td>
</tr>
<tr>
<td>Vit B2</td>
</tr>
<tr>
<td>Niacin</td>
</tr>
<tr>
<td>Vit C</td>
</tr>
<tr>
<td>Vit A</td>
</tr>
<tr>
<td>Vit D</td>
</tr>
<tr>
<td>Vit E</td>
</tr>
<tr>
<td>Folic acid</td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>Foods (g/day)</th>
<th><strong>Children</strong> (8-10yrs)</th>
<th><strong>Male adolescents</strong> (11-15yrs)</th>
<th><strong>Female adolescents</strong> (11-15yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (SD)</td>
<td>median</td>
<td>mean (SD)</td>
</tr>
<tr>
<td>Milk/dairy products (mL/day)</td>
<td>783.87 (289.95)</td>
<td>786.79</td>
<td>702.97 (267.53)</td>
</tr>
<tr>
<td>Eggs</td>
<td>22.42 (11.03)</td>
<td>22.00</td>
<td>22.58 (10.68)</td>
</tr>
<tr>
<td>Pulses</td>
<td>15.39 (8.61)</td>
<td>10.01</td>
<td>16.72 (8.15)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>204.59 (121.94)</td>
<td>203.57</td>
<td>194.60 (121.57)</td>
</tr>
<tr>
<td>Fruit/ nuts</td>
<td>176.58 (86.28)</td>
<td>162.86</td>
<td>163.45 (87.99)</td>
</tr>
<tr>
<td>Meat</td>
<td>78.48 (34.40)</td>
<td>76.79</td>
<td>79.44 (32.75)</td>
</tr>
<tr>
<td>Fish</td>
<td>54.16 (30.63)</td>
<td>42.86</td>
<td>52.04 (29.91)</td>
</tr>
<tr>
<td>Cereals</td>
<td>358.42 (110.69)</td>
<td>355.70</td>
<td>330.42 (103.46)</td>
</tr>
<tr>
<td>MUFA/SFA</td>
<td>1.25 (0.35)</td>
<td>1.21</td>
<td>1.25 (0.34)</td>
</tr>
<tr>
<td>Water (mL/day)</td>
<td>823.10 (213.20)</td>
<td>960.00</td>
<td>815.20 (214.20)</td>
</tr>
<tr>
<td>Soft drinks(mL/day)</td>
<td>108.50 (138.50)</td>
<td>57.20</td>
<td>122.20 (146.70)</td>
</tr>
</tbody>
</table>

MUFA/SFA: ratio of monounsaturated fatty acids to saturated fatty acids.

---

Note: Low consumption of meat and dairy products was adjusted intake of each of these food groups was standardized as a z value (value observed-mean/standard deviation\(^4\)). A moderate alcohol diet, also typical of the Mediterranean diet, was not considered for calculating the index in this group of children. Adherence to the MD was evaluated by means of the equation proposed by Sanchez-Villegas et al.\(^4\).
compliance with the Mediterranean diet. The two methods proposed to estimate diet quality were compared by applying the Altman-Bland plot and the Wilcoxon test for paired samples. Results obtained are compiled in table VI.

Discussion

The questionnaires used in this study were first validated in a randomized and representative sample of the whole study population, using the Bland-Altman plot and the Wilcoxon test for paired samples, finding a high level of agreement between the FFQ and 24-h recall for the intake of macronutrients and energy. As a result, we were able to evaluate the quality of diet using DQI and MDS and determine the more appropriate index for estimating the quality of the diet followed by a population.

The modified DQI-I index was used to evaluate the diet quality and food habits of a wide sample of 3190 schoolchildren in a Southern Spanish city23. Their mean score was 58.37% (SD: 7.74), slightly higher than the score obtained in similar studies in Spanish populations25,39. According to Kim et al.21, a score below 60% indicates a poor quality diet. However, this may not be the appropriate cut-off point in a Mediterranean setting, where the nutritional quality of fats should be considered differently23, because the Mediterranean diet has an elevated monounsaturated fatty acid (MUFA) content compared with diets (e.g., in

<table>
<thead>
<tr>
<th>Table IV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet Quality Index-International (DQI-I) scores and components</strong></td>
</tr>
<tr>
<td><strong>Component</strong></td>
</tr>
<tr>
<td>DQI-I total</td>
</tr>
<tr>
<td>VARIETY</td>
</tr>
<tr>
<td>Overall food group variety</td>
</tr>
<tr>
<td>Within-group variety for protein sources</td>
</tr>
<tr>
<td>ADEQUACY</td>
</tr>
<tr>
<td>Vegetable group</td>
</tr>
<tr>
<td>Fruit group</td>
</tr>
<tr>
<td>Cereal group</td>
</tr>
<tr>
<td>Fiber</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Iron</td>
</tr>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Vitamin C</td>
</tr>
<tr>
<td>MODERATION</td>
</tr>
<tr>
<td>Total fat</td>
</tr>
<tr>
<td>Saturated fat</td>
</tr>
<tr>
<td>Cholesterol</td>
</tr>
<tr>
<td>Sodium</td>
</tr>
<tr>
<td>Empty calorie foods</td>
</tr>
<tr>
<td>OVERALL BALANCE</td>
</tr>
<tr>
<td>Macronutrient ratio</td>
</tr>
<tr>
<td>Fatty acid ratio</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table V</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daily diet intake of Food Groups. % of population in each MDP</strong></td>
</tr>
<tr>
<td><strong>% Children</strong></td>
</tr>
<tr>
<td>≤33.00</td>
</tr>
<tr>
<td>Dairy products</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Eggs</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Pulses</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Vegetables</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Fruits and nuts</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Meat</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Fish</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Cereals</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>MUFA/SFA ratio</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Table VI
Comparison of DQI-I* and MDP**

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>MDP ADHERENCE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>62.98 (11.46)</td>
<td>63.25</td>
<td>62.20</td>
</tr>
<tr>
<td>Male adolescents</td>
<td>62.68 (11.17)</td>
<td>62.51</td>
<td>62.02</td>
</tr>
<tr>
<td>Female adolescents</td>
<td>62.73 (10.72)</td>
<td>62.74</td>
<td>62.11</td>
</tr>
<tr>
<td>Total population</td>
<td>62.74 (11.26)</td>
<td>62.78</td>
<td>62.35</td>
</tr>
<tr>
<td>DQI-I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children</td>
<td>58.91 (7.58)</td>
<td>59.00</td>
<td>58.30</td>
</tr>
<tr>
<td>Male adolescents</td>
<td>57.56 (7.69)</td>
<td>57.00</td>
<td>57.03</td>
</tr>
<tr>
<td>Female adolescents</td>
<td>58.88 (7.87)</td>
<td>59.00</td>
<td>58.35</td>
</tr>
<tr>
<td>Total population</td>
<td>58.37 (7.76)</td>
<td>58.00</td>
<td>58.10</td>
</tr>
</tbody>
</table>

Bland-Altman plot

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean DQI-I-MDP Adherence</th>
<th>Limits of agreement</th>
<th>Z Wilcoxon</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>-3.92</td>
<td>-4.92 to -2.92</td>
<td>-1.117</td>
<td>0.264</td>
</tr>
<tr>
<td>Male adolescents</td>
<td>-4.95</td>
<td>-5.82 to -4.08</td>
<td>-1.474</td>
<td>0.140</td>
</tr>
<tr>
<td>Female adolescents</td>
<td>-4.04</td>
<td>-4.88 to -3.20</td>
<td>-2.470</td>
<td>0.014</td>
</tr>
<tr>
<td>Total population</td>
<td>-4.34</td>
<td>-4.86 to -3.83</td>
<td>-0.893</td>
<td>0.372</td>
</tr>
</tbody>
</table>

*range of DQI-I and MDP adherence: 0 to 100

Figure 1.—Bland-Altman plot for questionnaire validation: a) energy intake, b) lipid intake, c) protein intake, d) carbohydrate intake.
USA, China) in which fats are largely of animal origin with a high SFA content.

The DQI-I components that showed the highest values were adequacy and variety, as observed in other Spanish studies. The adequacy of a diet is related to its compliance with current recommendations for a healthy diet. The present population had a high adequacy score for the intake of proteins, iron, calcium, and vitamin C, with a lower score for the intake of fruit, vegetables, cereals, and fiber, similar to results in other Spanish groups but different from findings in China and the USA, where the consumption of these foods is greater.

Adherence to the MDP was calculated according to the recommendations of Sanchez Villegas et al., but without considering alcohol intake, because a part of this young population never consumed wine or any other alcoholic drink, and taking account of fish consumption, which was not considered in the study by Sanchez Villegas et al., despite being a characteristic element of the MDP. Highly similar mean values were obtained by the two methods. Results of the Bland-Altman plot and Wilcoxon test for related samples confirmed that the diet quality evaluation by the two methods was similar in this study population.

The MDP directly estimates the intake of MUFA and SFA, whereas an adaptation of the original DQI is required to take account of the consumption of fats in a Mediterranean population. The DQI gives a more detailed evaluation of food components and specific values of Fe, vit. C and protein, etc., whereas the MDP adherence gives global information on food groups, although it includes foods characteristically consumed in the Mediterranean region. The fact that we selected the same type of foods for both indices may explain the similar overall evaluations obtained with the two methods. According to these results, both methods appear to be equally appropriate for evaluating diet quality in a Mediterranean population.

Acknowledgements

The authors’ responsibilities were as follows: M.M.-A. and F.O.-S. conceived, designed and devised the study and contributed to the analysis and writing of the manuscript. M.M.-A., J.V. and F.O.-S. analyzed the data. F.O.-S. and M.L.L.-T. supervised the study. J.V., C.M., M.A.C.-P. and F.O.-S. collected and supervised the samples. M.A.C.-P. and F.O.-S. obtained funding. All authors reviewed and approved the manuscript. None of the authors had any conflict of interest. The authors are grateful to Richard Davies for his revision of the English version. This study was supported by the Health Department of Granada City Council, Spain, the Junta de Andalucía, Spain (Research Group AGR-255) and the University of Granada, Spain (Postdoctoral Grant).

References