Results: were measured. Hemoglobin, indicators of iron and vitamin A status, and EPO placebo at baseline and at 5 mo. At baseline, 5 mo, and 10 mo, improved significantly compared with the control group (P < 0.02). Vitamin A treatment increased mean hemoglobin by 7 g/L (P < 0.001) and reduced the prevalence of anemia from 54% to 38% (P < 0.01). Vitamin A treatment increased mean corpuscular volume (P < 0.001) and decreased serum transferrin receptor (P < 0.001), indicating improved iron-deficient erythropoiesis. Vitamin A decreased serum ferritin (P < 0.02), suggesting mobilization of hepatic iron stores. Calculated from the ratio of transferrin receptor to serum ferritin, overall body iron stores remained unchanged. In the vitamin A group at 10 mo, we observed an increase in EPO (P < 0.05) and a decrease in the slope of the regression line of log10(EPO) on hemoglobin (P < 0.01).

Conclusion: In children deficient in vitamin A and iron, vitamin A supplementation mobilizes iron from existing stores to support increased erythropoiesis, an effect likely mediated by increases in circulating EPO.

KEY WORDS Vitamin A, iron, deficiency, supplementation, hemoglobin, anemia, erythropoietin, children, Morocco

INTRODUCTION

Vitamin A deficiency and iron deficiency anemia affect >30% of the global population (1, 2). The most vulnerable groups are women of reproductive age, infants, and children (2, 3). Vitamin A deficiency may cause anemia by impairing iron metabolism (4), but the mechanism of this effect is unclear. In vitamin A–deficient populations, improving vitamin A status reduces the prevalence of anemia in most (5–13) but not all (14, 15) studies.

Erythropoietin (EPO) is a 30 400-Dalton glycoprotein, produced mainly by renal peritubular cells. It acts on the late stages of erythropoiesis, primarily on colony-forming unit erythroid cells, and stimulates maturation through the normoblast into reticulocytes and mature erythrocytes (16). The enhancer region of the EPO gene contains a response element that is regulated by retinoic acid (17). In vitro and in animal models, vitamin A treatment stimulates production of EPO (17), but it is unclear whether vitamin A supplementation increases EPO concentrations in humans.

Two studies in malnourished populations have examined the effect of vitamin A supplementation on circulating EPO (15, 18). Compared with iron and folate supplementation, vitamin A, iron, and folate supplementation did not affect EPO concentrations in Malawian pregnant women (15). In Tanzanian children, a single dose of vitamin A decreased serum ferritin (SF) and EPO concentrations measured after 72 h (18). However, those studies did not have true controls and were done in regions endemic for malaria, which influences EPO concentrations (19, 20). Therefore, the present study was designed as a placebo-controlled trial of vitamin A supplementation in malaria-free school-age children with poor vitamin A and iron status. Its aim was to measure the effect of vitamin A repletion on hemoglobin, iron status, and EPO concentrations.

1 From the Human Nutrition Laboratory, Swiss Federal Institute of Technology, Zürich, Switzerland (MBZ, RB, FR, CZ, and RFH), and The Ministry of Health, Rabat, Morocco (AD and NC).
2 Supported by the Thrasher Research Fund (Salt Lake City, UT), the Foundation for Micronutrients in Medicine (Rapperswil, Switzerland), and the Swiss Federal Institute of Technology (Zürich, Switzerland). Vitamin A supplements were provided by Task Force Sight and Life (Basel, Switzerland).
3 Reprints not available. Address correspondence to MB Zimmermann, Human Nutrition Laboratory, Swiss Federal Institute of Technology Zürich, LFV E19, Schmelzbergstrasse 7, CH-8092 Zürich, Switzerland. E-mail: michael.zimmermann@ilw.agr.ethz.ch.
Received March 3, 2006.
Accepted for publication May 11, 2006.

Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron1–3

Michael B Zimmermann, Ralf Biebinger, Fabian Rohner, Abdeljawad Dib, Christophe Zeder, Richard F Hurrell, and Nourredine Chaouki

ABSTRACT

Background: Vitamin A deficiency impairs iron metabolism; vitamin A supplementation of vitamin A–deficient populations may reduce anemia. The mechanism of these effects is unclear. In vitro and in animal models, vitamin A treatment increases the production of erythropoietin (EPO), a stimulant of erythropoiesis.

Objective: We measured the effect of vitamin A supplementation on hemoglobin, iron status, and circulating EPO concentrations in children with poor iron and vitamin A status.

Design: In a double-blind, randomized trial, Moroccan schoolchildren (n = 81) were given either vitamin A (200 000 IU) or placebo at baseline and at 5 mo. At baseline, 5 mo, and 10 mo, hemoglobin, indicators of iron and vitamin A status, and EPO were measured.

Results: At baseline, 54% of children were anemic; 77% had low vitamin A status. In the vitamin A group at 10 mo, serum retinol improved significantly compared with the control group (P < 0.02). Vitamin A treatment increased mean hemoglobin by 7 g/L (P < 0.02) and reduced the prevalence of anemia from 54% to 38% (P < 0.01). Vitamin A treatment increased mean corpuscular volume (P < 0.001) and decreased serum transferrin receptor (P < 0.001), indicating improved iron-deficient erythropoiesis.

Conclusion: In children deficient in vitamin A and iron, vitamin A supplementation mobilizes iron from existing stores to support increased erythropoiesis, an effect likely mediated by increases in circulating EPO.


KEY WORDS Vitamin A, iron, deficiency, supplementation, hemoglobin, anemia, erythropoietin, children, Morocco
SUBJECTS AND METHODS

The study was done in rural villages in the Rif Mountains of northern Morocco. The villages are ∼600 m above sea level and have a temperate climate. Most available food is produced locally on small farms, and the dietary staples are whole-wheat bread, pulses, and olive oil (21). Per capita iron intakes in school-age children in this region are 9–14 mg/d, and iron bioavailability from the local diet is estimated to be ∼2% when adjusted for low body iron stores (21). When a conversion factor was used of 12 µg β-carotene to 1 µg retinol for a mixed fruit and vegetable diet (22, 23), vitamin A intakes (mean ± SD) are 206 ± 67 and 288 ± 71 µg retinol activity equivalents/d in children 6–8 y and 9–13 y, respectively. This is 48–52% of the recommended dietary allowance for vitamin A in these age groups (22). This region is malaria free and has a clean water supply, and diarrheal disease and hookworm are rare. There is a low rate of infection and inflammation; in children, the year-round prevalence of elevated C-reactive protein (CRP) is <5% (24).

The subjects were 5–13-y-old children from local primary schools. Informed written or oral consent was obtained from the parents and oral assent from the children. The Swiss Federal Institute of Technology Zürich and the Ministry of Health in Rabat gave ethical approval for the study. In a baseline screening, weight and height were measured in all consenting children (n = 81). Whole blood (5 mL) was collected by venipuncture to measure mean corpuscular volume (MCV) and concentrations of hemoglobin (Hb), serum retinol (SR), retinol binding protein (RBP), prealbumin, C-reactive protein (CRP), serum ferritin (SF), serum transferrin receptor (TIR), whole-blood zinc protoporphyrin (ZnPP), and plasma erythropoietin (EPO).

The children were randomly assigned into 2 groups. One group (n = 40) received an oral placebo capsule (sunflower oil) at 0 and 5 mo; the other group (n = 41) was given oral retinyl palmitate (200 000 IU) (RpScherer, Aprilia, Italy) at baseline and 5 mo (25). At 5 and 10 mo, all baseline measurements were repeated (Figure 1). After completion of the study, all children with low vitamin A status were treated with 200 000 IU vitamin A, and all children with iron deficiency anemia were treated with oral iron (60 mg iron as ferrous sulfate 4 d/wk for 12 wk).

Laboratory analyses

Hemoglobin and MCV were measured on the day of blood collection with the use of an AcT8 Counter (Beckman Coulter, Krefeld, Germany). Anemia was defined as hemoglobin <120 g/L in children aged ≥12 y and hemoglobin <115 g/L in children aged 5–11 y (26). ZnPP was measured on washed red blood cells within 48 h of blood collection with the use of a hematofluorometer (Aviv Biomedical, Lakewood, NJ). The usual reference cutoff for ZnPP on washed red blood cells is ≤40 µmol/mol heme. Serum samples were divided into aliquots and frozen at −20 °C until analyzed. SF and TIR were measured by using enzyme-linked immunosorbent assays (RAMCO, Houston, TX). Iron deficiency was defined as either SF <15 µg/L or TIR >7.6 mg/L and ZnPP ≥40 µmol/mol heme (27). Body iron was estimated by the method of Cook et al (28). SR was measured by HPLC (29). Vitamin A deficiency was defined as SR <0.70 µmol/L (30), and low vitamin A status was defined as SR <1.05 µmol/L (3). RBP was measured by an enzyme-linked immunosorbent assay (Immundiagnostik AG, Bensheim, Germany). Prealbumin and CRP were measured by using nephelometry (TURBOX; Orion Diagnostica, Espoo, Finland). There is no consensus on a cutoff value for RBP or the RBP-to-prealbumin ratio (31–34), so data were presented only as distributions. In subjects with CRP ≥10 mg/L, because of the confounding effects of inflammation, values for SR and SF were excluded from the analysis. Plasma EPO was measured by using an enzyme-linked immunosorbent assay (IBL ELISA; Immunobiological

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**FIGURE 1.** Flow diagram of the study. VA, vitamin A.
Labs, Hamburg, Germany); this assay has a reference range of 4–36 mIU/mL in adults and high-performance characteristics (35).

Statistical analysis

Data processing and statistics were done by using SPLUS (2000; Insightful Corporation, Seattle, WA), PRISM (version 3; GraphPad, San Diego, CA), and EXCEL (XP 2002; Microsoft, Seattle, WA). Group randomization was done by using a Bernoulli distribution \((P = 0.5)\) where random variables have the value \(0\) or \(1\). When data were not normally distributed, statistical analysis was done after log or square root transformation. A 2-factor repeated measures analysis of variance was done to compare effects of treatment \(\times\) time for hemoglobin, CRP, MCV, SF, TfR, ZnPP, body iron, SR, RBP, prealbumin, RBP: prealbumin, and EPO. If the interaction effect was significant \((P < 0.05)\), \(t\) tests between groups and paired \(t\) tests within groups over time were done and adjusted for multiple comparisons (Bonferroni correction). Logistic regression was done to compare effects of treatment \(\times\) time on the prevalence of anemia, vitamin A deficiency, and low vitamin A status. A mixed model controlling for repeated measures with subject as a random effect was used to compare the slopes between log10(EPO) and hemoglobin between groups and over time. Significance was set at \(P < 0.05\).

RESULTS

The mean \(\pm\) SD age of the children in the control and the vitamin A groups at baseline was 10.6 \(\pm\) 2.2 y and 10.2 \(\pm\) 1.7 y, respectively. The ratio of girls to boys in the control and the vitamin A groups was 19:21 and 19:22, respectively. All children completed the study. No significant differences were observed between the groups in age, sex ratio, or other baseline characteristics presented in Tables 1 and 2. The prevalence of an elevated CRP was 2–4% in both groups at all time points, and no significant difference was observed in mean CRP concentration or prevalence of an elevated CRP concentration between groups (data not shown). The means \(\pm\) SDs for weight in the control and vitamin A groups at baseline, at 5 mo, and at 10 mo were 10.2 \(\pm\) 3.0, 10.9 \(\pm\) 3.1, and 10.2 \(\pm\) 3.0, respectively. The means \(\pm\) SDs for height and vitamin A groups at baseline, at 5 mo, and at 10 mo were 0.1 \(\pm\) 0.2, 0.1 \(\pm\) 0.2, and 0.1 \(\pm\) 0.2, respectively. The overall prevalence of stunting in the study population was <5%. No significant differences were observed in weight or height between groups at baseline, at 5 mo, or at 10 mo.

The changes in vitamin A status and EPO concentrations during the study are shown in Table 1. At baseline, the prevalence of vitamin A deficiency, as defined by a low SR, was 17%, indicating moderate vitamin A deficiency in this group (30); 77% of children had low vitamin A status. No child exhibited clinical eye signs of vitamin A deficiency. At 10 mo, mean SR, RBP, and RBP:prealbumin concentrations increased significantly in the vitamin A group \((P < 0.02)\) and did not change significantly in the control group. At 10 mo, the prevalence of vitamin A deficiency and low vitamin A status significantly decreased in the vitamin A group compared with the control group \((P < 0.01)\).

A significant increase was observed in the geometric mean EPO concentration in the vitamin A group \((P < 0.05)\). Because renal EPO production is influenced in a feedback loop by hemoglobin, it is important to measure both the absolute change in circulating EPO and the change in the slope of the regression line between log10(EPO) and hemoglobin (36). The relation between log10(EPO) and hemoglobin in the vitamin A and placebo groups at baseline and 10 mo is shown in Figure 2. A and B. A decrease \((-0.0042)\) in the slope of the regression line of log10(EPO) was observed on hemoglobin in the vitamin A group between 0 and 10 mo. In the mixed model comparing the slopes of the regression lines between log10(EPO) and hemoglobin at baseline and 10 mo in the 2 groups, a significant time \(\times\) treatment interaction \((P < 0.01)\) was observed.

Changes in hemoglobin and iron status are shown in Table 2. Mean hemoglobin concentration at 10 mo was significantly greater in the vitamin A group than in the control group \((P < 0.02)\). Anemia was reduced by vitamin A \((P < 0.01)\); the prevalence of anemia in the control group at baseline, at 5 mo, and at 10 mo was 54%, 49%, and 59%, respectively; in the vitamin A group it was 54%, 43%, and 38%, respectively. Compared with the control group at 10 mo, vitamin A treatment increased mean MCV concentration \((P < 0.001)\), decreased geometric mean TfR concentration \((P < 0.001)\), and decreased geometric mean SF concentration \((P < 0.02)\). Calculated from the TfR:SF, no significant change in body iron stores was observed in either group over the course of the study.

DISCUSSION

In preschool and school-age children (5–9), as well as nonpregnant and pregnant women (10–13), improving vitamin A status generally increases hemoglobin concentrations and reduces anemia, although not all studies agree (14, 15). Several mechanisms may explain the effect of vitamin A status on anemia (4): 1) increased resistance to infection and, hence, the anemia of infection; 2) effects on iron absorption, metabolism, or both; and 3) direct modulation of erythropoiesis. A change in prevalence of the anemia of infection as a result of vitamin A repletion is unlikely to explain our findings. In the study region, malaria is absent, and the prevalence of intestinal parasites is low. Only 3–4% of the study children had an elevated CRP concentration, and no change in CRP concentrations was observed during the course of the study.

Vitamin A supplementation may influence iron absorption, but data from the 2 human isotope studies are conflicting. Vitamin A was reported to enhance nonheme iron absorption in Venezuelan adults (37), but this finding was not confirmed in a study in Europe (38). In the present study, vitamin A treatment improved iron-deficient erythropoiesis, as reflected by an increase in MCV, a sharp decrease in TfR concentrations, and a modest increase in hemoglobin concentrations. At the same time, SF concentrations fell, suggesting mobilization of hepatic iron (13, 18). Calculated from the TfR:SF, overall body iron remained unchanged. These findings argue against a vitamin A-mediated increase in iron absorption. Rather, they suggest vitamin A repletion causes redistribution of iron from stores to the marrow for erythropoiesis.

Our findings are consistent with previous studies in animals and humans. In animals with vitamin A deficiency, iron is retained in the liver and spleen, and it is less available for erythropoiesis (39–42). In vitamin A–deficient rats, iron uptake by the bone marrow is impaired (43), and erythrocyte incorporation
TABLE 1
Serum retinol (SR) and retinol binding protein (RBP) concentrations, RBP-to-prealbumin ratio, prevalences of vitamin A deficiency and low vitamin A status, and plasma erythropoetin (EPO) concentrations in children given 200,000 IU vitamin A or placebo control at baseline (0 mo) and 5 mo and followed for 10 mo.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Vitamin A</th>
<th>Control</th>
<th>Vitamin A</th>
<th>RBP:prealbumin</th>
<th>Control</th>
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<tr>
<td></td>
<td>µmol/L</td>
<td>mg/L</td>
<td>µmol/L</td>
<td>mg/L</td>
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<td>mIU/mL</td>
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<tr>
<td>0 mo</td>
<td>0.91 ± 0.23</td>
<td>21.7 ± 10.2</td>
<td>0.27 ± 0.15</td>
<td>28 (20)</td>
<td>30 (75)</td>
<td>14.8 (0.8–51.9)</td>
<td>14.9 (1.3–144.4)</td>
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<tr>
<td>5 mo</td>
<td>0.95 ± 0.25</td>
<td>26.2 ± 12.1</td>
<td>0.19 ± 0.19</td>
<td>1.07 (0.19–0.7)</td>
<td>0.19</td>
<td>32 (78)</td>
<td>18.3 (2.8–60.0)</td>
<td>19.2 (4.0–118.4)</td>
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<td>10 mo</td>
<td>0.97 ± 0.24</td>
<td>25.9 ± 11.4</td>
<td>0.33 ± 0.16</td>
<td>0.42 ± 0.15</td>
<td>12.7</td>
<td>27 (68)</td>
<td>21.5 (2.0–71.6)</td>
<td>21.8 (2.6–138.9)</td>
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1 Significant treatment × time interaction, P < 0.001 (ANOVA).
2 Significant time × treatment effect, P < 0.01 (logistic regression).
3 Geometric x; range in parentheses (all such values).
4 x ± SD (all such values).
5 Significantly different from baseline: a P < 0.01.
6 Significantly different from control: b P < 0.02.

TABLE 2
Hemoglobin, mean corpuscular volume (MCV), serum transferrin receptor (TIR), zinc protoporphyrin (ZnPP), serum ferritin (SF), and total body iron in children given 200,000 IU vitamin A or placebo at baseline (0 mo) and 5 mo and followed for 10 mo.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Vitamin A</th>
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<td>g/L</td>
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<tr>
<td>0 mo</td>
<td>112 ± 10</td>
<td>77 ± 6</td>
<td>7.3 (3.5–14.2)</td>
<td>74 (4.5–13.1)</td>
<td>40 (13–165)</td>
<td>19 (5–35)</td>
<td>1.90 ± 2.30</td>
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<td>5 mo</td>
<td>114 ± 9</td>
<td>78 ± 5</td>
<td>7.0 (3.3–14.1)</td>
<td>6.1 (3.8–12.1)</td>
<td>44 (14–124)</td>
<td>18 (6–39)</td>
<td>1.96 ± 2.05</td>
<td>2.12 ± 2.71</td>
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<td>10 mo</td>
<td>113 ± 9</td>
<td>84 ± 4</td>
<td>7.1 (4.3–14.4)</td>
<td>5.1 (3.1–9.2)</td>
<td>41 (15–109)</td>
<td>20 (8–58)</td>
<td>2.11 ± 2.01</td>
<td>1.90 ± 2.04</td>
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1 Significant treatment × time interaction, P < 0.001 (ANOVA).
2 Geometric x; range in parentheses (all such values).
3 Significantly different from baseline: a P < 0.05, b P < 0.01, c P < 0.001.
4,5,6,7 Significantly different from control: d P < 0.02, e P < 0.001.
A sequence homologous to DR-2, a steroid-responsive element. A status (17). The 30-enhancer region of the erythroid colonies (52). Synthesis of EPO is regulated by vitamin A and iron status. Vitamin A and iron status, vitamin A repletion modestly increased EPO concentrations and reduced the slope of the regression line between EPO and hemoglobin. Several mechanisms may explain the change in the slope of the regression line. It may represent physiologically appropriate EPO concentrations for children with hemoglobin at the lower range of the distribution after improvements in iron-deficient erythropoiesis. Alternatively, a direct vitamin A-mediated stimulus of erythropoiesis may have down-regulated the EPO response to lower hemoglobin concentrations. Enhanced erythropoiesis may lower circulating EPO concentrations in anemia (36, 58) because of internalization and degradation of the EPO-EPO receptor complex in maturing erythroid cells (59).

**FIGURE 2.** Regression lines of log10 erythropoietin (EPO) and hemoglobin in children with poor vitamin A and iron status given 200 000 IU vitamin A or placebo at 0 and 5 mo and followed for 10 mo. In the placebo group (A) and the vitamin A group (B), the solid lines (data points as circles) indicate the relation between log10(EPO) and hemoglobin baseline, and the broken lines (data points as triangles) indicate the relation at 10 mo. At baseline in the placebo group, the intercept was 4.020 and the slope was −0.02514 (P < 0.0001); in the vitamin A group, the intercept was 3.986 and the slope was −0.02436 (P < 0.0001). At 10 mo in the placebo group, the intercept was 4.081 and the slope was −0.02765 (P < 0.0001); in the vitamin A group, the intercept was 4.310 and the slope was −0.02013 (P < 0.0001). When the slopes of the regression lines between log10(EPO) and hemoglobin were compared at baseline and 10 mo, a significant time × treatment interaction was observed for the vitamin A group (P = 0.0008) (mixed model).

Two previous intervention trials in Africa, one in pregnant women and one in infants, investigated whether vitamin A supplementation would affect EPO concentrations. In Malawi, Sembda et al (15) randomly assigned pregnant women to receive daily for 38 wk either vitamin A (3 mg retinol equivalent), iron (30 mg), and folate (400 mg) or iron (30 mg) and folate (400 mg). At baseline, half of the subjects were anemic, and 25–35% were vitamin A deficient. At 38 wk, no significant differences were observed between the 2 groups in hemoglobin concentrations, vitamin A status, EPO concentrations, or the slope of the regression line between log EPO and hemoglobin. However, the control group received iron and folate, which may have modified vitamin A status (60) and improved erythropoiesis. Also, physiologic fluctuations in EPO concentration during pregnancy may have confounded the study (61). In an uncontrolled trial in severely anemic preschool children, Cusick et al (18) investigated the 72-h effects of a single high dose of vitamin A. Vitamin A increased the reticulocyte production index and decreased serum ferritin and EPO concentrations. The investigators suggested that the treatment mobilized the iron for erythropoiesis, which then lowered EPO concentrations. However, the lack of a control group and the short follow-up limit interpretation of the results. Moreover, both studies (15, 18) were done in areas where malaria is endemic, and malaria may decrease (19, 62) or increase (20, 63) EPO production.

Strengths of the current study were the absence of possible confounding of EPO concentrations by malaria, a clear and sustained improvement in vitamin A status, and a placebo control. Although our data suggest that increased circulating EPO may mediate improvements in hemoglobin during vitamin A repletion, vitamin A also influences other hormones and cytokines involved in erythropoiesis, including insulin-like growth factor 1 (17). Also, because vitamin A metabolites are ligands that regulate transcription of many hepatic genes (64), it is possible that vitamin A status could modulate synthesis or catabolism of proteins involved in hepatic iron storage and mobilization. Future
research should show the effect of vitamin A on other erythropoietic factors and more closely examine the mechanisms by which storage iron is mobilized by vitamin A treatment.

We thank the participating children and teachers, as well as the staff at the Brikha Center. Special thanks go to R. Rahmouni and A. Halhinni (Brikha, Morocco) and to L. Molinari, R. Wegmueller, S. Renggli, M-H. Balsat, and B. Hassler (Zurich, Switzerland).

Each of the authors contributed to the study design. MBZ, RB, AD, FR, CZ, and NC performed the fieldwork and the data collection. MBZ, FR, CZ, and RFH supervised the laboratory analysis and completed the data analysis. MBZ conducted the statistical analysis. The first draft of the manuscript was written by MBZ. All authors edited the manuscript. None of the authors had a financial or personal conflict of interest in regard to this study.

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