Intravenous Lipid Emulsion Only Minimally Influences Bupivacaine and Mepivacaine Distribution in Plasma and Does Not Enhance Recovery from Intoxication in Pigs

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BACKGROUND: The reported successful use of IV lipid emulsions in local anesthetic intoxications is thought to be due to lipid sequestration of local anesthetics. However, controlled efficacy studies were lacking and other mechanisms of action have also been suggested. We investigated the effect of lipid infusion on plasma concentrations and cardiovascular effects of 2 local anesthetics differing in lipophilicity, bupivacaine, and mepivacaine.

METHODS: Bupivacaine (n = 20) or mepivacaine (n = 20) was infused into a central vein of anesthetized (isoflurane 1%, FIO2 0.21) pigs until mean arterial blood pressure decreased to 50% from baseline. Isoflurane was discontinued and FIO2 was increased to 1.0. Ten pigs in each local anesthetic group were treated with 20% lipid emulsion (ClinOleic®), and 10 pigs with Ringer's solution: 1.5 mL/kg in 1 minute followed by an infusion of 0.25 mL·kg⁻¹·min⁻¹ for 29.5 minutes. Five additional pigs were infused bupivacaine and Intralipid®. Total and nonlipid-bound local anesthetic concentrations were determined from repeated blood samples.

RESULTS: There were no overall differences in total or nonlipid-bound plasma local anesthetic concentrations between the lipid and Ringer's groups. However, plasma median total bupivacaine concentration was 21% and 23% higher at 20 and 30 minutes, respectively, in the lipid group (P = 0.016 without Holm–Bonferroni correction). There was also no overall difference between lipid and Ringer's groups in the rate of recovery of hemodynamic and electrocardiographic variables. Median mean arterial blood pressure in the lipid group with bupivacaine intoxication was 16 mm Hg and 15 mm Hg higher than in the corresponding Ringer's groups at 10 and 15 minutes, respectively (P = 0.016 and P = 0.021, respectively, without Holm–Bonferroni correction). Intralipid® also caused no difference between total plasma and nonlipid-bound concentrations of bupivacaine with no apparent enhancement of recovery.

CONCLUSIONS: Lipid emulsion neither had any measurable effect on the disposition of the studied local anesthetics in plasma, nor did it improve the rate of recovery from intoxication by either local anesthetic as measured by hemodynamic variables. (Anesth Analg 2012;114:901–6)

Triggered by several successful case reports, IV lipid emulsion (lipid rescue) has been adopted as a recommended treatment in systemic local anesthetic toxicity. However, the mechanism of action remains undetermined. One hypothesis is that IV lipid emulsions act as a "lipid sink," sequestering the lipophilic local anesthetic and decreasing the plasma concentration of nonlipid-bound bupivacaine.

The success of lipid emulsion therapy appears to be dependent on the lipophilicity of the toxin, because in isolated rat hearts, cardiac arrest caused only by bupivacaine, but not by the less lipophilic ropivacaine or mepivacaine, was reversed with lipid emulsion. Also, in almost every published case report of successful treatment of intoxication using lipid emulsion, the toxin has been markedly lipophilic. However, at least theoretically, molecular structure, e.g., molecular weight or the size of the alkyl substituents on the heterocyclic ring, could also be the underlying reason.

Direct support for the lipid-sink theory was provided in a letter containing a post facto analysis during lipid emulsion treatment of rats, bupivacaine concentration, measured at a single time point, was higher in the lipid phase than in the aqueous phase of plasma. In a swine model comparing lipid emulsion with saline, however, there was no difference in the free-bupivacaine concentration in plasma, indirectly suggesting that no sequestration occurred. Hicks et al. showed an increase in plasma bupivacaine concentrations in pigs receiving lipid emulsion, but no improvement in survival when compared with treatment with vasopressors.

Thus, the dependence of drug sequestration on lipophilicity and the overall efficacy of lipid emulsion in the treatment of local anesthetic toxicity warrants further research. We therefore measured the plasma concentrations and cardiovascular effects of bupivacaine and mepivacaine in anesthetized pigs during IV administration of either lipid emulsion or Ringer’s acetate solution to determine whether lipid emulsion has a significant antidotal effect on the toxicity of either drug.
Lipid Does Not Bind Bupivacaine or Mepivacaine in Pigs

![Diagram](https://via.placeholder.com/150)

**Figure 1.** Grouping. The 40 pigs in the main study were randomized into 4 groups of 10 each.

Our hypothesis was that bupivacaine, as the more lipophilic drug, would be sequestered to a higher extent than mepivacaine by lipid emulsion. Second, we expected that hemodynamic and electrocardiographic (ECG) variables would return to baseline values faster in the pigs treated with lipid emulsion than those treated with Ringer's acetate solution.

**METHODS**

**Animals and Settings**

The study was approved by the Finnish National Animal Experiment Board (ESHL-2008-04793/Ym-23) and conducted in accordance with the European Community guidelines for the care and use of experimental animals.

Forty Landrace pigs of either sex were fasted for at least 12 hours before the experiments, with free access to water, and were randomized into 4 equal groups of 10 to receive either bupivacaine (Bican®; Orion Pharma, Espoo, Finland) or mepivacaine (Scandican®; AstraZeneca GmbH, Wedel, Germany), followed by either 20% lipid emulsion (ClinOleic® [olive oil:soybean oil 80:20], 1.2% egg phosphatide, 2.25% glycerol; 0.03% sodium oleate; Baxter S.A., Lessines, Belgium) or Ringer's acetate solution (Ringer–Acetat Baxter Viaflo®; Baxter Medical, Sweden) (Fig. 1). The local anesthetic solutions were prepared by an anesthesiologist who was not involved in the study, and the researchers performing the experiment were blinded to the type of anesthetic administered.

**Experiment Protocol**

Anesthesia was induced with IV ketamine (400 mg, with additional 50 to 100 mg boluses as needed). The trachea was intubated, and volume-controlled ventilation was initiated with 2% isoflurane in 21% oxygen (ServoVentilator 900C, Siemens-Elema, Sweden). The respiratory rate was fixed at 20 minutes per minute, and minute ventilation adjusted to keep end-tidal PCO2 between 5.0% and 5.5%. Oxygenation was monitored via a pulse oximetry probe attached to the tail. Core temperature was maintained at 37.5°C to 39.0°C (measured via esophageal probe) using external radiant heating (OPN Ceiling Control Unit Type VII; Aragana, Sweden) and by infusing warmed Ringer's acetate solution.

Central venous pressure measurement and local anesthetic infusion were performed via a catheter in the jugular vein (Leader Cath 19G; Vygon, Ecouen, France). Continuous arterial blood pressure monitoring and periodic blood sampling were performed via the surgically prepared and cannulated femoral artery (Arterial Cannula with Flo-Switch 20G; Becton-Dickinson, Singapore). Five-lead ECG was recorded continuously for offline analysis of PQ interval, QRS width and QTc interval. The end-tidal isoflurane concentration was decreased to 1.0% (±0.1%) during the stabilization period (approximately 30 minutes). Hemodynamic and respiratory variables were measured using a multimodal patient monitor (Datex-Ohmeda Division; Instrumentarium Corp, Helsinki, Finland) and recorded during the experiment on a computer connected to the monitor using data collection software (iCentral® and S/5 Collect®; GE Health Care, Helsinki, Finland).

Baseline values were recorded at the end of the stabilization period (Table 1). The experiment began with an infusion of either 2 mg · kg⁻¹ · min⁻¹ bupivacaine HCl or 6 mg · kg⁻¹ · min⁻¹ mepivacaine HCl until mean arterial blood pressure (MAP) decreased to 50% of its baseline value (defined as the Toxic Point).

At the Toxic Point, the local anesthetic infusion and inhaled isoflurane were discontinued, and inhaled oxygen concentration increased to 100%. Simultaneously, we injected a 1.5 mL/kg IV bolus of either 20% lipid emulsion (lipid group) or Ringer's acetate solution (Ringer's group) in 1 minute via a peripheral vein. The bolus injection was followed by a continuous infusion of the same solution at a rate of 0.25 mL · kg⁻¹ · min⁻¹ for 29 minutes (Fig. 2). Arterial plasma concentrations of the local anesthetics were assessed at baseline, Toxic Point, and 5, 10, 20, and 30 minutes after the Toxic Point.

If spontaneous movement or breathing attempts occurred after the Toxic Point, 1% isoflurane was restarted. In case of cardiac arrest (defined as the ECG and arterial blood pressure monitoring showing >5 seconds asystole or ventricular fibrillation), we performed external manual chest compressions at a rate of 100 per minute. Additionally, IV injections of epinephrine (0.5 mg in case of asystole, electromechanical dissociation, or ventricular fibrillation) and electrical defibrillation (150 J in case of ventricular fibrillation) were administered when indicated. The cardiopulmonary resuscitation (CPR) was continued for at least 10 minutes, during which we took blood samples for local anesthetic concentration measurements as scheduled. After the experiment, the anesthetized pigs were euthanized by a rapid IV bolus of potassium concentrate.

**Intralipid® Experiment**

After analyzing the results of the main study, we performed an additional open study following the same protocol. Five pigs of similar characteristics as the main group were given bupivacaine followed by Intralipid® (Fresenius Kabi AB, Uppsala, Sweden) to determine whether there would be any difference in the sequestration of bupivacaine in comparison to that observed with ClinOleic®. Intralipid® is a mixture of soybean oil (20%), egg phosphatides (1.2%), and glycerol (2.25%).

**Local Anesthetic Concentration Measurement**

The plasma was separated from blood samples by centrifugation (10 minutes at 3000 rotations per minute [rpm]) and stored at −21°C. After thawing and careful mixing of plasma samples, the concentration of the local anesthetics...
Table 1. Baseline Data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bupivacaine + Lipid (n = 10)</th>
<th>Bupivacaine + Ringer's (n = 10)</th>
<th>Meptivacaine + Lipid (n = 10)</th>
<th>Meptivacaine + Ringer's (n = 10)</th>
<th>Bupivacaine + Intralipid (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>29 (27-29)</td>
<td>29 (27-30)</td>
<td>29 (28-30)</td>
<td>28 (28-30)</td>
<td>28 (25-29)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>78 (67-101)</td>
<td>82 (65-90)</td>
<td>77 (65-89)</td>
<td>72 (68-81)</td>
<td>93 (79-98)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>112 (101-124)</td>
<td>109 (82-125)</td>
<td>110 (88-116)</td>
<td>100 (85-109)</td>
<td>109 (96-123)</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>4 (3-4)</td>
<td>4 (3-5)</td>
<td>2 (2-2)</td>
<td>3 (2-4)</td>
<td>2 (2-5)</td>
</tr>
<tr>
<td>Oxygen saturation (%)</td>
<td>97 (97-98)</td>
<td>98 (97-99)</td>
<td>98 (95-98)</td>
<td>98 (97-96)</td>
<td>97 (97-99)</td>
</tr>
</tbody>
</table>

Values presented as median (25%-75% interquartile range).

Figure 2. Experiment protocol. The pigs were given a local anesthetic infusion until mean arterial blood pressure decreased to 50% of baseline, followed by treatment with either lipid emulsion or Ringer's acetate solution. C = time of baseline measurements.

was determined. Additionally, aliquots of plasma from pigs that received lipid emulsion were centrifuged for 10 minutes at 14,000 rpm (20,800 g) using an Eppendorf centrifuge at room temperature to separate the lipid from plasma. An aliquot from the lipid-poor aqueous fraction was then taken for the determination of the nonlipid-bound local anesthetic concentration.

The concentrations of local anesthetics were measured using a SCIEX Q Trap LC/MS/MS system (Sciex Division of MDX, Toronto, Ontario, Canada).12 The interday coefficients of variation for bupivacaine at 8, 15, and 30 mg/L were less than 3.6%, 3.8%, and 0.9% (n = 8, 8, and 4). The coefficients of variation for meptivacaine at 8, 20, and 75 mg/L were less than 2.3%, 2.5%, and 0.8% (n = 5, 7, and 5).

Statistical Analysis

Six outcome variables were analyzed: local anesthetic concentration, MAP, heart rate, PQ interval, QRS width, and QTc interval. Values are presented as the median (25%-75% interquartile range). Differences among groups are presented as the median difference (95% confidence interval of the median difference). Mann–Whitney’s U test was used for comparison of treatment groups at each time point, with significance set at α = 0.05. The Holm–Bonferroni method was used to correct α for the multiple comparisons.13 Survival was evaluated using the Fisher Exact test. SPSS 19.0.1 for OS X (IBM Corporation, Somers, NY) was used for all analyses except the calculation of confidence intervals for median differences, for which we used R for OS × 2.12.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Toxicity

The total dose of local anesthetic infused to decrease MAP by 50% (i.e., to the Toxic Point) was 10 mg/kg (9 to 11 mg/kg) in the bupivacaine groups and 45 mg/kg (38 to 50 mg/kg) in the meptivacaine groups.

Within 5 minutes after reaching the Toxic Point, 2 pigs given bupivacaine followed by Ringer’s acetate solution (bupivacaine doses 11 mg/kg and 13 mg/kg), and 1 given meptivacaine followed by lipid emulsion (meptivacaine dose 52 mg/kg) developed asystole and could not be resuscitated. There was no effect of lipid emulsion infusion on survival (bupivacaine P = 0.474, meptivacaine P = 1.000).

Plasma Concentrations

No overall differences in plasma bupivacaine or meptivacaine concentrations (total or nonlipid bound) were observed among groups during the treatment infusion (Fig. 3). Differences in median local anesthetic concentrations were only found in the bupivacaine pigs at 20 and 30 minutes using Mann–Whitney’s U test without Holm–Bonferroni correction. The median total bupivacaine concentration was 1.8 mg/L (0.5 to 2.9 mg/L, P = 0.016) and 1.9 mg/L (0.6 to 3.3 mg/L, P = 0.016) higher in the lipid group at 20 minutes and 30 minutes, respectively. The median concentration of nonlipid-bound bupivacaine was 1.2 mg/L (0.5 to 2.4 mg/L, P = 0.016) and 1.6 mg/L (0.7 to 2.9 mg/L, P = 0.016) higher in the lipid group at 20 minutes and 30 minutes, respectively.

Recovery of Hemodynamic and ECG Variables

At its lowest, MAP was 25 mm Hg (21 to 35 mm Hg) in the bupivacaine group 90 seconds (60 to 140 seconds) after the Toxic Point, and 29 mm Hg (25 to 33 mm Hg) in the meptivacaine group 40 seconds (40 to 70 seconds) after the Toxic Point.

Comparison of MAP (Fig. 4) and heart rate among treatment groups at each measured time point revealed no overall effect of lipid emulsion in comparison with Ringer’s acetate solution. Differences in median MAP were only
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![Graphs showing the concentration of lipid and bupivacaine/mepivacaine in plasma](image)

**Figure 3.** Local anesthetic concentration. The total plasma and nonl lipid-bound local anesthetic concentrations in plasma of the pigs intoxicated by bupivacaine (A) or mepivacaine (B) and treated with lipid emulsion (ClinOleic®) or Ringer’s acetate solution. Values displayed are medians with 25%-75% interquartile range. *Difference compared with the Ringer’s group concentration P < 0.05 (without Holm-Bonferroni correction; nonsignificant after the correction).

![Graphs showing mean arterial pressure](image)

**Figure 4.** Mean arterial blood pressure. Mean arterial blood pressure in the pigs intoxicated by bupivacaine (A) or mepivacaine (B). Values displayed are medians with 25%-75% interquartile range. Values from animals undergoing cardiopulmonary resuscitation are excluded from the relevant time points. *Median difference among groups P < 0.05 (without Holm-Bonferroni correction; nonsignificant after the correction).

**Table 2. Electrocardiographic Variables**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Toxic point</th>
<th>End of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PQ interval (ms)</td>
<td>Bupivacaine + Ringer’s</td>
<td>90 (69-104)</td>
<td>125 (120-153)</td>
</tr>
<tr>
<td></td>
<td>Bupivacaine + lipid</td>
<td>90 (75-105)</td>
<td>123 (113-143)</td>
</tr>
<tr>
<td></td>
<td>Mepivacaine + Ringer’s</td>
<td>92 (86-100)</td>
<td>117 (107-128)</td>
</tr>
<tr>
<td></td>
<td>Mepivacaine + lipid</td>
<td>93 (84-96)</td>
<td>126 (97-135)</td>
</tr>
<tr>
<td>QRS width (ms)</td>
<td>Bupivacaine + Ringer’s</td>
<td>50 (43-54)</td>
<td>81 (71-98)</td>
</tr>
<tr>
<td></td>
<td>Bupivacaine + lipid</td>
<td>54 (53-55)</td>
<td>78 (74-84)</td>
</tr>
<tr>
<td></td>
<td>Mepivacaine + Ringer’s</td>
<td>60 (44-54)</td>
<td>60 (59-69)</td>
</tr>
<tr>
<td></td>
<td>Mepivacaine + lipid</td>
<td>48 (44-51)</td>
<td>60 (56-81)</td>
</tr>
<tr>
<td>QTc interval (ms)</td>
<td>Bupivacaine + Ringer’s</td>
<td>343 (341-375)</td>
<td>342 (339-356)</td>
</tr>
<tr>
<td></td>
<td>Bupivacaine + lipid</td>
<td>357 (349-372)</td>
<td>302 (297-333)</td>
</tr>
<tr>
<td></td>
<td>Mepivacaine + Ringer’s</td>
<td>354 (340-381)</td>
<td>296 (278-307)</td>
</tr>
<tr>
<td></td>
<td>Mepivacaine + lipid</td>
<td>356 (342-370)</td>
<td>295 (286-337)</td>
</tr>
</tbody>
</table>

Values displayed as median (25%-75% interquartile range).

found in the bupivacaine pigs at 10 and 15 minutes using Mann-Whitney’s U test without Holm-Bonferroni correction. Median MAP in the pigs with bupivacaine intoxication was 16 mm Hg (0 to 39 mm Hg, P = 0.016) and 15 mm Hg (4 to 26 mm Hg, P = 0.021) higher in the lipid group at 10 minutes and 15 minutes, respectively.

The PQ interval and QRS width increased at the Toxic Point in all groups in comparison with baseline and returned to normal at the end of the experiment. No significant differences among treatment groups were observed (Table 2).

**Intralipid Experiment**

The total dose of bupivacaine needed to reach the Toxic Point was 10 mg/kg (9 to 11 mg/kg). One pig developed cardiac arrest right before the 5-minute sampling time, and CPR for 10 minutes was not successful.
There was no difference in median bupivacaine concentration in total plasma in comparison with the nonlipid-bound bupivacaine concentration (Fig. 5). MAP followed a course similar to the findings of the main study (Fig. 6).

**Side Effects**

In all pigs receiving either ClinOleic® or Intralipid®, there was a variable degree of reddish, partly mottled skin coloring at the end of the experiment. This was not accompanied by any changes in airway pressure or any decrease in arterial blood pressure.

**DISCUSSION**

In this model of local anesthetic intoxication, the IV lipid emulsion caused no marked changes in the total or nonlipid-bound plasma concentration of either local anesthetic. We used ClinOleic® for the main study because it is the 20% lipid emulsion used for parenteral nutrition in our hospital district. Despite some dissimilarities in lipid contents between ClinOleic® and Intralipid®, e.g., regarding the amount of soybean oil and oleic acid, it seems that the bupivacaine-entrapment effect of the 2 emulsions is similar. However, this was not directly assessed.

Considering an abundance of promising case reports and animal studies of the possible benefit of Intralipid®, our findings suggesting no marked sequestration of bupivacaine were unexpected.

Previously, the simultaneous infusion of ClinOleic® with amiodarone resulted in differences between total plasma and nonlipid-bound amiodarone concentrations up to 85%. The sequestration of amiodarone prevented its hypotensive effect, whereas in the present study the lipid emulsion did not enhance hemodynamic recovery. The lipid solubility of amiodarone is very high (octanol/water partition $\log P$ 8), resulting in a large volume of distribution (approximately 60 L/kg) in the body irrespective of its extensive (96%) binding to plasma proteins. Although bupivacaine is more lipophilic (octanol/water $\log P$ is 3.4) and more extensively (95%) bound to plasma proteins than mepivacaine (octanol/water $\log P$ is 2, protein binding 77%), both of them are approximately 100,000-fold less lipophilic than amiodarone. Both of them also have a small volume of distribution (approximately 1 L/kg) in comparison with that of amiodarone.

The effective sequestration of amiodarone and the lack of marked sequestration of bupivacaine and mepivacaine by lipid emulsion suggest that the lipophilicity of a drug needs to be very high in order for it to be markedly sequestered in vivo. However, we cannot exclude the possibility that the difference in the timing of the lipid infusion, simultaneous lipid infusion with amiodarone versus lipid infusion immediately after bupivacaine or mepivacaine in the present study may have contributed to the difference in both sequestration and hemodynamic response.

The rapidly infused lipid emulsion could provide its therapeutic effect through other mechanisms than sequestration, including reversal of bupivacaine-induced inhibition of lipid substrate oxidation in cardiac mitochondria as suggested by Weinberg et al. A strong direct myocardial effect of the lipid emulsion was not evident in our studies, because MAP, heart rate, and ECG variables returned to baseline at a similar rate in all treatment groups.

The reddish mottling of the pig skin during the lipid infusion, not accompanied by systemic hemodynamic responses or increase in airway pressure, could have been caused by the release of vasoactive prostaglandins. A lipid emulsion infusion can cause an increase in the synthesis and release of both vasodilatory and vasoconstrictive prostaglandins.
The direct applicability of our results to clinical practice is limited because of the fact that the experiments were performed in anesthetized pigs. The pig was chosen as a model for this study because its cardiovascular system, as well as physiology in general, is considered similar to those of humans, and instrumentation intended for humans can be adapted for use in pigs.\(^9\)\(^,\)\(^10\) There is little agreement on which species is most appropriate for the study of lipid resuscitation and it is possible that some species would not respond to the therapy in the same way as humans. Two previous studies on bupivacaine intoxication performed on pigs also found no beneficial effect of lipid emulsion when compared with vasopressors,\(^9\)\(^,\)\(^10\) whereas in studies using rats\(^9\) or dogs,\(^7\) lipid emulsion was superior to vasopressin and saline, respectively.

The typical clinical situation in the published case reports has been refractory cardiac arrest with continuing CPR. In the present study the end-point for the local anesthetic infusion was set at 50% baseline MAP, because pilot experiments indicated that no pigs survived if the infusion was allowed to continue until asystole. Even with this precaution, some pigs died during the resuscitative phase of the experiment. Our bupivacaine dose was the same as in an earlier toxicity study in pigs.\(^8\)\(^,\)\(^9\) The concentration and infusion rate of mepivacaine, at which 50% MAP would be reached after approximately the same time as with bupivacaine, were determined in pilot experiments.

Although the doses and infusion rates we used led to severe intoxication within less than 10 minutes, this obviously does not mimic the common reason for an intoxication in human patients, i.e., rapid intravascular injection. We cannot exclude the possibility that lipid emulsion would affect local anesthetic pharmacokinetics and toxicity differently after a rapid intravenous injection.

Although the study was randomized among all groups, and blinded regarding the local anesthetics, the researchers were not blinded to treatment fluids, which had different colors. To minimize possible bias, we standardized and controlled the technical procedures.

In conclusion, we found no support for the lipid-sink theory, or for the use of lipid emulsion as a treatment for local anesthetic intoxication in this model of local anesthetic intoxication. An IV infusion of the recommended toxicity-treatment dose of lipid emulsion influenced nonlipid-bound concentrations of bupivacaine only to a minimal extent, and mepivacaine even less. In addition, we saw no improvement in hemodynamic recovery from intoxication in the groups receiving lipid emulsion in comparison with those receiving Ringer’s acetate solution.\(^\#\)\(^\#\)

DISCLOSURES

Name: Per H. Rosenberg, MD, PhD.

**Contribution:** This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.

Name: Terese T. Horlocker, MD.

**Contribution:** This author helped design the study, conduct the study, analyze the data, and prepare the manuscript.

REFERENCES