

# Liquid Heparin Anticoagulant Produces More Negative Bias in the Determination of Ionized Magnesium than Ionized Calcium

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The ionized calcium level in blood is known to be falsely decreased when self-prepared liquid heparin anticoagulant is used, due to dilution and binding effects. The effect of liquid heparin on the determination of ionized magnesium is not as well understood. We compared the effect of liquid sodium heparin on the determination of ionized calcium and magnesium in 44 clinical samples using two types of user-prepared heparin syringes which differed in the amount of residual heparin from the BD Preset<sup>TM</sup> reference syringe. With the type 1 syringe, the liquid heparin was expelled once or twice such that some heparin could be left in the dead space at the syringe hub, while the liquid sodium heparin was thoroughly expelled from the type 2 syringe. The ionized magnesium levels obtained with the type 1 syringe were significantly lower than the reference value (by 0.068 mmol/L) ( $p < 0.0001$ ), while the value obtained with the type 2 syringe differed less from the reference, by only 0.014 mmol/L ( $p < 0.0001$ ). The heparin binding effect resulted in more negative bias in ionized magnesium ( $-0.026 \pm 0.032$  mmol/L) than in ionized calcium ( $-0.009 \pm 0.042$  mmol/L,  $p < 0.0001$ ). In conclusion, we recommend using lyophilized, calcium-balanced, heparinized syringes for the determination of ionized magnesium and ionized calcium due to the increased negative bias in ionized magnesium determinations. When user-prepared syringes are used, the thorough evacuation of heparin solution should be strictly prescribed.

**Key Words:** Calcium, heparin, ion, magnesium

## INTRODUCTION

The ionized calcium level in blood is known to

be falsely decreased due to dilution and binding effects when self-prepared liquid heparin anticoagulants are used.<sup>1</sup> The CSLI (Clinical and Laboratory Standard Institute, formerly the National Committee for Clinical Laboratory Standards or NCCLS) recommends the use of lyophilized calcium-balanced heparin syringes<sup>2</sup> for collecting specimens for ionized calcium measurement. Although excess heparin has been suggested to affect ionized calcium levels,<sup>3</sup> such an effect on the measurement of ionized magnesium has not been documented. Some commercial calcium-balanced heparin syringes have been reported to affect ionized magnesium levels, although the clinical significance was believed to be minimal.<sup>4</sup> Liquid heparin may still be used for the determination of ionized calcium or magnesium by some clinical laboratories in developing countries due to budgetary constraints. In this study, we set out to systematically determine the liquid heparin effect on both ionized calcium and magnesium in clinical samples by head-to-head comparison.

## MATERIALS AND METHODS

Samples were obtained from the 44 patients to evaluate the effect of heparin on ionized magnesium levels. Informed consent was obtained from the patients and the study protocol was approved by the institutional review board.

We made user-prepared syringes using sodium heparin, 1000 U/mL (Choongwae Pharma Corporation, Seoul, Korea), which concentration was

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specially manufactured to reduce the heparin concentration for blood sampling and was lower than the common value prescribed for therapeutic heparinization. Heparin in the amount of 0.5 to 1.0 mL was drawn into a 2-mL syringe to coat the whole inner surface of the syringe, and was then expelled back into the original vial. Before and after coating the inner surface of the blood gas syringe with heparin, we tared the syringe on the electronic balance to calculate the amount of heparin solution remaining in the syringe after expulsion. The needle was then changed to one appropriate for collecting the sample. Two types of syringes which differed in their heparin coating protocols were compared to the reference syringe (lyophilized calcium balanced lithium heparin, BD Preset™ syringe, 30 I.U., BD, Franklin Lakes, NJ, USA) according to the behavioral pattern of the users as follows. With the type 1 syringe, heparin and air was ejected once or twice such that heparin may have been left in the dead space of syringe hub. In contrast, heparin and air were ejected methodically several times from the type 2 syringe until heparin was not detectable in either the syringe or the needle. Although bias may remain for the calcium balanced lithium heparin syringe compared to whole blood without anticoagulation,<sup>4</sup> we used it as the reference. Drawing 1.5 ml of whole arterial blood from each subject into each syringe, the samples were then recapped until analysis. Ionized calcium and magnesium were measured in all samples within 20 minutes after collection using Stat Profile M (Nova Biomedical, Waltham, MA, USA). The 'bias corrected by dilution effect' was calculated by subtracting the expected ion concentration from the actual value. The expected ion concentration was calculated as the ion concentration of the ref-

erence syringe multiplied by the following factor.

$$\text{Factor} = 1.5 / (1.5 + V)$$

where '1.5' is the volume (mL) of whole blood drawn from the patient and 'V' is the volume (mL) of the liquid heparin remaining in the syringe.

We analyzed the agreement of the three kinds of blood gas syringes for both ionized calcium and magnesium by Passing and Bablok analysis, linear regression analysis, and Pearson correlation using Analyse-it software (Leeds, England, UK). The bias of type 1 or 2 syringes was calculated against the calcium balanced lithium reference.

## RESULTS

Table 1 shows that traces of heparin were found in user-prepared syringes, with type 2 syringes containing much less heparin than type 1.

The type 1 syringe showed significant negative bias in both ionized calcium and magnesium levels (Table 2). However, the type 2 syringe, which contained less heparin, showed different results between the two tests; there was a statistically significant negative mean bias (-0.007 mmol/L) for ionized magnesium, but no significant mean bias (0.006 mmol/L) for ionized calcium (Table 2). The user-prepared heparinized syringes exhibited more than 10% negative bias above the level of 40 U/ml of heparin for ionized calcium (Fig. 1A), and above the level of 14 U/ml of heparin for ionized magnesium (Fig. 1B) with the user-prepared heparinized syringes. Table 3 shows that significant negative bias of the ionized magnesium compared to the ionized calcium could be observed if the dilution effect was excluded.

**Table 1.** The Mean Volume and Concentration of Heparin Remaining in a Blood Gas Syringe after Blood Drawing According to Syringe Type

Variable	Type 1* syringe	Type 2† syringe	p value
Residual volume of heparin‡ (µL)	75.5 ± 25.7	21.0 ± 6.7	< 0.0001
Final heparin concentration (U/mL)	47.7 ± 15.6	13.8 ± 4.3	< 0.0001

\*Type 1 syringe: heparin and air ejected once or twice such that the heparin could remain in the dead space of the syringe hub.

†Type 2 syringe: heparin and air ejected carefully several times until no heparin was observed in either the syringe or the needle.

‡Volume of heparin solution that remained in syringes (µL).

**Table 2.** The Bias of Ionized Calcium and Ionized Magnesium Values Measured in Type 1 or Type 2 User-Prepared Syringes Compared to a Reference Type 3 Syringe Containing Calcium Balanced Lithium Heparin

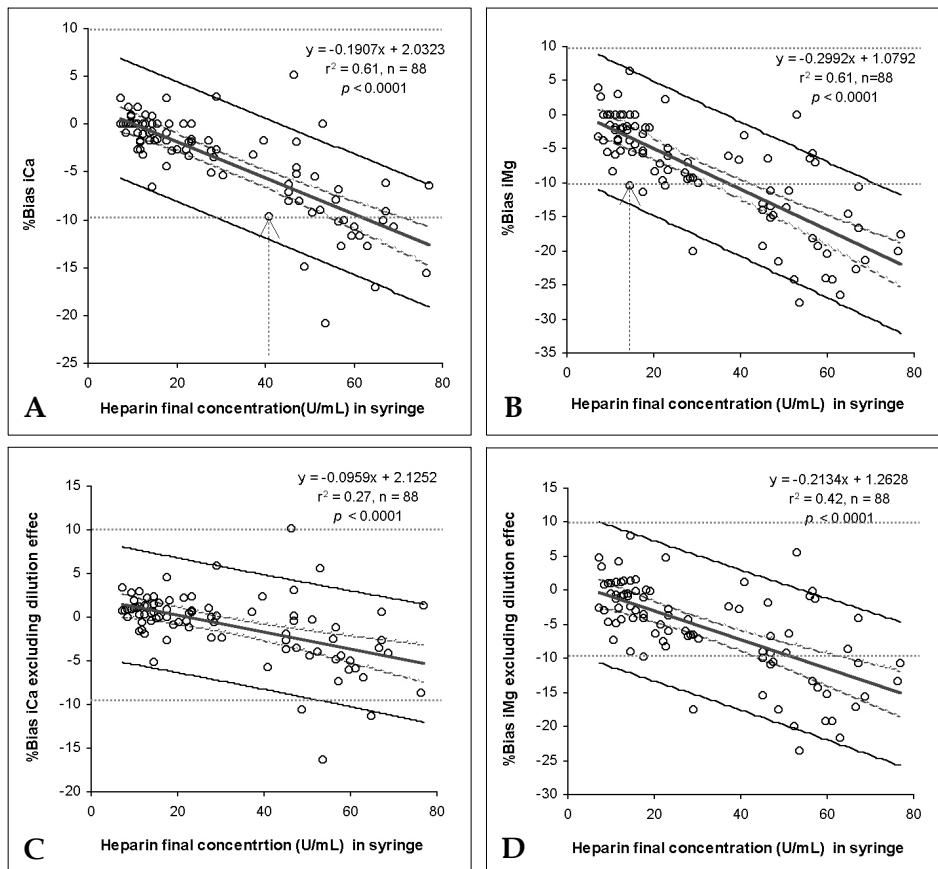
Test item	Bias	Mean Bias (mmol/L)	Range of Bias (mmol/L)	*p value
Ionized calcium	Type 1 syringe	0.079 (-0.098 to -0.060)	-0.25 to 0.05	< 0.0001
	Type 2 syringe	-0.010 (-0.016 to -0.004)	-0.08 to 0.03	< 0.005
	† Corrected bias of type 2 syringe	0.006 (0.000 to 0.012)	-0.06 to 0.05	‡ NS
Ionized magnesium	Type 1 syringe	-0.068 (-0.079 to -0.056)	-0.17 to 0.00	< 0.0001
	Type 2 syringe	-0.014 (-0.020 to -0.009)	-0.05 to 0.02	< 0.0001
	† Corrected bias of type 2 syringe	-0.007 (-0.012 to -0.002)	-0.04 to 0.02	< 0.01

Type 1 and Type 2 syringes were previously described in Table 1.

\*p values were derived from a paired t-test between the observed and reference syringe (the commercially available blood gas 'BD Preset™ syringe').

† Corrected bias for the dilution effect is described in the text, which represents only the heparin binding effect. The numbers in parentheses are the mean bias at the 95% confidence interval.

‡ NS, 'not significant' (p > 0.05)



**Fig. 1.** The percent bias of ionized calcium or ionized magnesium according to the final heparin concentration in a user-prepared syringe. A: percent bias of ionized calcium. B: percent bias of ionized magnesium. C: percent bias of ionized calcium excluding the dilution effect. D: percent bias of ionized magnesium excluding the dilution effect.

**Table 3.** Comparison of the Mean Bias of Ionized Calcium and Magnesium Measurements with Heparin Concentration

Effect	Mean bias (mmol/L)		p value
	Ionized calcium	Ionized magnesium	
Heparin binding plus dilution	- 0.044 ± 0.058	- 0.041 ± 0.04	NS
Heparin binding only	- 0.009 ± 0.042	- 0.026 ± 0.032	< 0.0001

## DISCUSSION

As the ionized fraction of magnesium is the biologically active part, its accurate determination is an important issue in critically ill patients.<sup>5,6</sup> The CLSI recommends that whole blood determinations of ionized calcium be performed on specimens transferred into commercially available, ready-to-use syringes or capillaries anticoagulated with one of the modified preparations of heparin designed to minimize its effects on the ionized calcium.<sup>3</sup> However, the performance of these syringes with ionized magnesium has not been documented. We found a clear reduction in measured ionized magnesium levels with increasing concentrations of heparin in syringes, confirming previous reports.<sup>2,7</sup> We postulate that ionized magnesium measurements are more greatly impacted by liquid heparin than ionized calcium values, as the absolute amount of ionized magnesium is much less than that of ionized calcium. We did not, however, confirm its mechanism. The maximum allowable heparin concentration in syringes was proposed to be 40 U/mL or 15 U/mL for ionized magnesium.<sup>7,8</sup> For ionized calcium, the CLSI recommends that the calcium balanced heparin concentration should be 70 U/mL or less. We found a greater than 10% negative bias above the clinical prescribed level of 40 U/mL of heparin for ionized calcium, and above the level of 14 U/mL of heparin for ionized magnesium in user-prepared heparinized syringes, due to both dilution and heparin binding effects. If the liquid heparin was deliberately and thoroughly expelled from the blood gas syringe, the mean bias in values did not exceed -0.08 mmol/L for ionized calcium and -0.05 mmol/L for ionized magnesium, putting the values within a clinically acceptable range.

Although Chantler and Cox showed that self-

prepared heparinized syringes for measuring ionized magnesium and calcium were sufficiently close to dry balanced heparinized syringes to justify their use under economically constraining conditions,<sup>9</sup> the protocol of thoroughly expelling the heparin solution should be mandated to minimize dilution and heparin binding effects.

In conclusion, we recommend using lyophilized calcium balanced heparinized syringes for the determination of ionized magnesium as well as ionized calcium, as we observed a greater negative bias of ionized magnesium values compared with ionized calcium in the presence of residual heparin. In the case of using user-prepared syringes, a protocol for thorough evacuation of heparin solution should be standardized and mandated.

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