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Effects of Hyperlipidemia on Plasma Sodium, Potassium, and Chloride Measurements by an Indirect Ion-Selective Electrode Measuring System

To the Editor:

We used indirect ion-selective electrode (ISE) methods (Roche/Hitachi Modular) to investigate the effect of hyperlipidemia (cholesterol plus triglycerides) on measurements of sodium, potassium, and chloride and the ability of published formulas to correct for the decrease in measured Na^+, K^+, and Cl^-. As recommended by Kroll *(1)*, we did not use enriched samples but instead used 21 patient samples (lithium-heparin plasma and serum) with triglyceride concentrations >15 mmol/L, total lipid concentrations (cholesterol plus triglycerides; Roche/Hitachi Modular) >20 mmol/L, a lipemic index (Modular) >3, and a total protein concentration within the reference interval (62–83 g/L). We analyzed samples before and after ultracentrifugation (Airfuge; Beckman Coulter; 15 min at 107 000*g*) by indirect ISE potentiometry (Modular) and direct ISE (Rapidlab 865; Bayer).

The ultracentrifuged indirect ISE result (y) and nonultracentrifuged direct ISE result (z) were subtracted from the nonultracentrifuged indirect ISE result (x; see Fig. 1 in the Data Supplement that appears with the online version of this letter at http://www.clinchem.org/content/vol52/issue1/).

The lipid concentration varied inversely, not only with Na^+ but also with Cl^- and (to a lesser degree) K^+ concentrations, as measured by the indirect ISE system (see Fig. 1 in the online Data Supplement). A 10 mmol/L increase in total lipid concentration decreased the Na^+ and Cl^- concentrations by ~1 mmol/L and K^+ by ~0.04 mmol/L.

We developed formulas to calculate corrected Na^+ (F3), Cl^- (F5), and K^+ (F7) results and compared the results with those from published formulae (listed below):

F1: Corrected Na^+ = Measured Na^+ + [measured serum lipids/4.63 g/L (~5.23 mmol/L)][2]
F2: Corrected Na^+ = Measured Na^+ + [{[0.21 × triglycerides (g/L)] − 0.6} × (Na^+/100)][3]
F3: Corrected Na^+ = Measured Na^+ + (total lipids/10)
F4: Corrected Cl^- = Measured Cl^- + [{[0.21 × triglycerides (g/L)] − 0.6} × (Cl^-/100)][3]
F5: Corrected Cl^- = Measured Cl^- + (total lipids/10)
F6: Corrected K^+ = Measured K^+ + [{[0.21 × triglycerides (g/L)] − 0.6} × (K^+/100)][3]
F7: Corrected K^+ = Measured K^+ + (total lipids × 0.004)

In a second set of 24 hyperlipidemic patient samples, analyzed prospectively, we observed effects of total lipids similar to those seen in the first set; we also demonstrated the utility of the formulas derived from the first set (Table 1).

The published formula by Ionescu-Tirgoviste and Cheta *(2)* leads to overestimation of the decrease in sodium. The Steffes–Freier formula *(3)* is based on triglyceride concentration alone; thus, there is potential for error in cases in which the hyperlipidemia is caused predominantly by hypercholesterolemia or mixed hypertriglyceridemia/hypercholesterolemia. In samples from patients with confirmed hypercholesterolemia, electrolytes should be measured with a direct ISE system.

The new formulas are simpler than published ones and allow operators to perform the calculations promptly. Thus, laboratories not equipped with computers, direct ISE systems, Airfuges, or lipid-clearing agents can estimate the true electrolyte concentrations. Care must be taken, however, if hyperlipidemia is complicated by hypoproteinemia or hyperproteinemia *(4)*.

In our organization, we have adopted a standardized approach to the processing of hyperlipidemic samples. We visually check turbid samples to determine whether it is possible to see through the sample. If not, the sample is analyzed for cholesterol, triglycerides, and lipemic index, and these results are reported irrespective of whether a lipid profile is requested. Triglycerides or cholesterol >20 mmol/L on first presentation are critical for proper patient management and are promptly phoned to the clinical unit. In cases in which the lipemic index is ≥3, the sample is optically turbid, and the total lipid concentration is >25

Table 1. Differences observed with the recommended and our correction formulas for decreases in the indirect ISE electrolytes with increasing total lipid concentration in the second subset of samples.

Analyte and correction formula[a]	Difference, mean (SD): Indirect ISE (ultracentrifuged) − formula-corrected value (mmol/L)	Difference, mean (SD): Direct ISE (mmol/L) − formula-corrected value (mmol/L)
Na^+		
F1	−4 (1.90)	−3 (2.4)
F2	−2 (2.3)	−1 (2.6)
F3	0 (1.9)	1 (3.2)
Cl^-		
F4	−1 (1.8)	2 (2.6)
F5	−1 (1.5)	2 (2.5)
K^+		
F6	−0.11 (0.14)	0.01 (0.16)
F7	−0.16 (0.08)	0.08 (0.22)

[a] F1, F2, F4, and F6 are published correction formulas. F3, F5, and F7 are correction formulas derived from the first subset in this study.

mmol/L, the processing varies depending on the laboratory. Smaller laboratories analyze the sample on a direct ISE system for Na^+, K^+, and Cl^-, and then forward the remaining sample to the nearest laboratory in the network with the capacity for ultracentrifugation and analysis of the remaining requested test profile. In the larger laboratories equipped with Airfuges, the sample is ultracentrifuged, and all results, including Na^+, K^+, and Cl^-, are reported from the ultracentrifuged sample. The results are reported with a comment indicating that the results were obtained after ultracentrifugation to remove lipids. No lipid-clearing agents are used.

In summary, hyperlipidemia caused errors in indirect ISE electrolyte measurements. All 3 electrolytes (Na^+, Cl^-, and K^+) determined by the indirect ISE system were affected, showing artifactual decreases as a result of hyperlipidemia. The Na^+ and Cl^- were decreased by ~1 mmol/L and K^+ by ~0.04 mmol/L for each 10-mmol/L increase in total lipid concentration. When direct ISE methods and ultracentrifuges are unavailable to handle severely lipemic samples, corrective formulas can be used.

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Larger Columns and Change of Lysis Buffer Increase the Yield of Cell-Free DNA Extracted from Amniotic Fluid

To the Editor:

Cell-free fetal nucleic acids (cffDNA) are present in maternal plasma and serum *(1)*, but amniotic fluid (AF) also provides an attractive source of cffDNA. The concentration of cffDNA is 100- to 200-fold higher in AF than in maternal plasma/serum *(2)*, but low yields of cffDNA compromise testing by techniques such as genomic microarrays, which require a minimum of 100 ng of DNA *(3)*.

For protocol optimization, we used 5 large-volume AF supernatant samples from patients who had undergone therapeutic amnioreduction for twin-twin transfusion syndrome. After optimization, we compared the DNA yield of the old and new protocols for freshly discarded AF supernatant samples from 29 euploid singleton pregnancies. Approval for this study was obtained from the Institutional Review Boards of Tufts-New England Medical Center and Women and Infant's Hospital. The median gestational age at amniocentesis was 16.9 weeks (25th–75th percentiles, 16.4–18.1 weeks).

We changed our original method *(3)* in the following 3 ways: we increased the vacuum extraction pressure to 800 mbar; we replaced the volume-overloaded mini spin columns with maxi spin columns (Qiagen) to allow for larger starting volumes; and we replaced the QiaAmp Lysis (AL) buffer with proprietary QiaAmp Viral Lysis (AVL) buffer (Qiagen), which has a high chaotropic salt concentration, on the basis of the similarities of AF and urine, a body fluid for which AVL buffer is recommended.

Quantitative PCR analysis of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) locus was performed in triplicate for each sample *(4)*, with the mean result of the 3 reactions used for further calculations. The results were expressed as genome equivalents (GE) per milliliter, using a conversion factor of 6.6 pg of DNA per cell *(5)*.

For large-volume AF samples, replacing AL with AVL buffer (using mini columns with high vacuum) led to a mean (SD) DNA yield of 1470 (456) GE/mL, and replacing mini with maxi columns (using AL buffer) led to a mean DNA yield of 1564 (623) GE/mL. Finally, substituting AL with AVL buffer and replacing mini with maxi columns led to a mean DNA yield of 1972 (786) GE/mL. DNA extraction with phenol, chloroform, and isoamyl alcohol *(6)* did not further improve the yield from 1 large-volume AF sample.

For euploid singleton pregnancies (n = 29), the median amount of *GAPDH* DNA extracted from 10 mL of AF with the new protocol was 1700 GE/mL (25th–75th percentiles, 1071–4938 GE/mL) compared with 246 (93–524) GE/mL obtained with the original protocol *(3)* ($P < 0.0001$, Wilcoxon signed-rank test). The proportion of samples that had a sufficient yield of extracted DNA for subsequent chromosome microarray analysis (i.e., ≥100 ng) also increased compared with the original protocol, from 39% (28 of 72) *(3)* to 86% (25 of 29; $P < 0.0001$, χ^2 test).

The new protocol allowed extraction of cffDNA from ≤10 samples in less than 3 h. The replacement of AL with AVL buffer eliminated the need for a heating bath during the lysis step, and fewer overall steps are involved in the protocol, which may reduce the potential for contamination. The cost of reagents and supplies for cffDNA extraction from a 10-mL AF supernatant sample is ~10-fold higher for the new