physical training. J Appl Physiol 1972;33: 602–6.

Mads Nybo*
Per Grinsted
Per E. Jørgensen

Department KKA
Clinical Biochemistry
Odense University Hospital
Odense, Denmark

*Address correspondence to this author at: Department KKA, Clinical Biochemistry, Odense University Hospital, DK-5000 Odense C, Denmark. Fax 45-6541-1911; e-mail mads.nybo@ouh.fyns-amt.dk.

DOI: 10.1373/clinchem.2005.051789

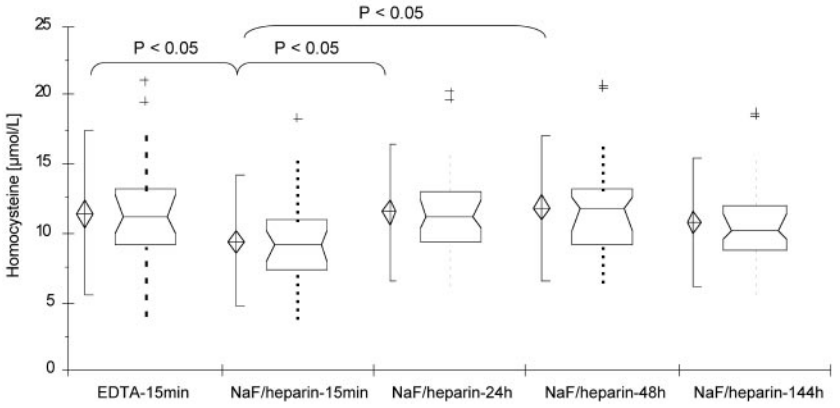

Fig. 1. Box-plots for NaF/heparin plasma homocysteine concentrations depending on the time interval between blood drawing and centrifugation.

The *left image* of each time group shows the mean (*center* of the *diamond*), the 95% confidence interval (*upper* and *lower tips* of the *diamond*), and the parametric percentile range (*vertical line*). The *right image* of each time group shows the box-plot with median (*horizontal line* within the *box*); 95% confidence interval of the median (*ends* of the *diagonal lines*); the interquartile range, corresponding to the 25th–75th percentiles (*lower* and *upper limits* of the *box*); nearest observations within 1.5 interquartile ranges (*dotted line*); and near outliers between 1.5 and 3.0 interquartile ranges away (+). One-way ANOVA computed significant differences between homocysteine concentrations for EDTA-15 min and NaF/heparin-15 min plasma, NaF/heparin-15 min and NaF/heparin-24h plasma, and NaF/heparin-15 min and NaF/heparin-48h samples ($P < 0.05$).

Effects of Prolonged Ambient Storage of Sodium Fluoride/Heparin Specimens on Plasma Homocysteine

To the Editor:

Increased plasma homocysteine is regarded as a risk factor for arterial and venous occlusive disease. Sample collection ideally requires use of cooled EDTA containers and immediate centrifugation of the blood *(1)*. These conditions may be hard to maintain in a clinic and are not possible in the many medical practices that lack a centrifuge. We tested whether analyzing uncentrifuged EDTA or NaF/heparin plasma bears a greater risk of giving false-positive homocysteine results under these conditions.

Blood was drawn from 50 healthy volunteers (40 female and 10 male) into 1 EDTA and 4 NaF/heparin Vacutainers (Becton Dickinson), which were stored at room temperature. The Vacutainers were randomly assigned to groups—EDTA-15min, NaF/heparin-15min, NaF/heparin-24h, NaF/heparin-48h, and NaF/heparin-144h—and centrifuged at 2700*g* for 7 min under cooling after the time stated in the group name. Homocysteine was analyzed by liquid chromatography–tandem mass spectrometry, as described by Arndt et al. *(2)*. Statistical analyses (box-plots and ANOVA) were done with the Analyze-it software (Analyze-it Software Ltd.), setting significance at $P < 0.05$.

We found (*a*) a significantly greater mean homocysteine concentration in EDTA-15min plasma (11.5 μmol/L) than in NaF/heparin-15 min plasma (9.5 μmol/L) and (*b*) a significant increase in homocysteine in NaF/heparin-15min (9.5 μmol/L) compared with NaF/heparin-24h (11.5 μmol/L, which is equal to the EDTA-15min value) but no further increase with greater time gaps between blood sampling and centrifugation for NaF/heparin blood (Fig. 1).

Our data show higher homocysteine results in EDTA-15min plasma than in NaF/heparin-15min samples. Plasma dilution by NaF-induced water loss from the erythrocytes has been reported *(1, 3)*. Hughes et al. *(3)* found lower mean homocysteine concentrations in NaF/heparin plasma stored at ambient temperature than in EDTA blood stored at 0 °C; however, after ~3 h, the difference in homocysteine concentrations was no longer significant *(3)*. We conclude that NaF/heparin blood stored for at least 3 h at ambient temperature shows homocysteine concentrations comparable to those from EDTA blood centrifuged immediately after blood sampling. Dilution effects by NaF should no longer be significant after this time.

Our most important finding, which had not been shown previously, was that a prolonged time interval of 48 h (and 144 h) between blood sampling and centrifugation, which is common under routine conditions, is not accompanied by a further homocysteine increase in NaF/heparin plasma (Fig. 1). In contrast, EDTA blood shows a steady increase in plasma homocysteine reaching 380% after 168 h *(4)*.

We conclude that in the absence of a centrifuge, NaF/heparin blood can be used for homocysteine analysis without the need for specific reference values. Therefore, we consider NaF/heparin blood to be a suitable material for homocysteine measurements in hospitals with commonly delayed sample transport to the in-house or reference laboratory or at medical practices without a centrifuge.

References

1. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004;50:3–32.
2. Arndt T, Guessregen B, Hohl A, Heicke B. Total plasma homocysteine measured by liquid chromatography–tandem mass spectrometry with

use of 96-well plates. Clin Chem 2004;50: 755–7.

3. Hughes MP, Carlson TH, McLaughlin MK, Bankson DD. Addition of sodium fluoride to whole blood does not stabilize plasma homocysteine but produces dilution effects on plasma constituents and hematocrit. Clin Chem 1998;44: 2204–6.
4. Clark S, Youngman LD, Sullivan J, Peto R, Collins R. Stabilization of homocysteine in unseparated blood over several days: a solution for epidemiological studies. Clin Chem 2003;49:518–20.

Ralf Scheidhauer*
Brunhilde Guessregen
Axel Hohl
Torsten Arndt

Bioscientia GmbH
Ingelheim, Germany

*Address correspondence to this author at: Bioscientia GmbH, Konrad-Adenauer-Strasse 17, D-55218 Ingelheim, Germany. Fax 49-6132-781-428; e-mail ralf.scheidhauer@bioscientia.de.

DOI: 10.1373/clinchem.2005.052852

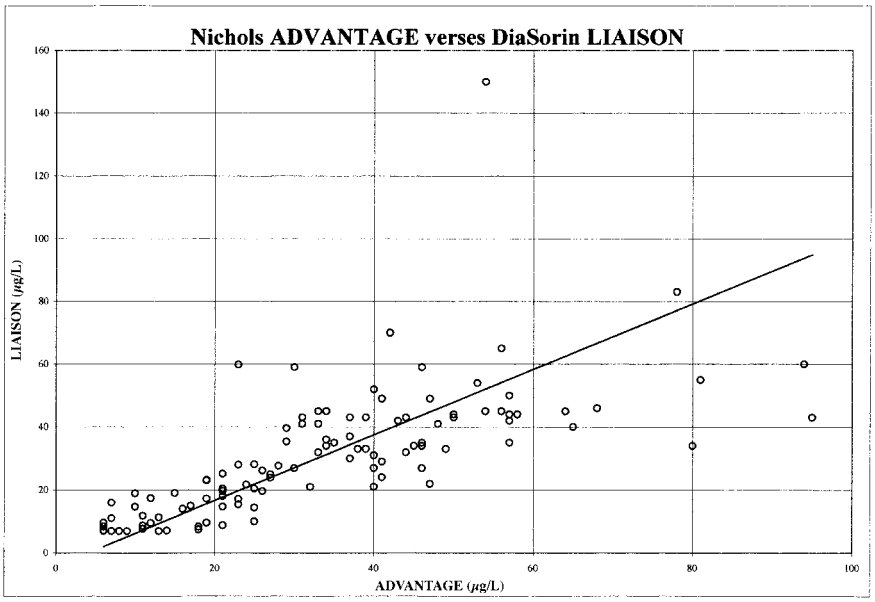

Fig. 1. Deming regression analysis of the Nichols ADVANTAGE vs the DiaSorin LIAISON 25-OH-vitamin D assay.

Equation for the regression line: $y = 1.04x - 4.2$ μg/L.

Measurement of 25-Hydroxyvitamin D by the Nichols ADVANTAGE, DiaSorin LIAISON, DiaSorin RIA, and Liquid Chromatography–Tandem Mass Spectrometry

To the Editor:

Vitamin D [ergocalciferol (D_2) and cholocalciferol (D_3)] is 25-hydroxylated by the liver and subsequently hydroxylated by the kidney to form 1,25-vitamin D, the active form of the vitamin. 25-Hydroxyvitamin D (25-OH-vitamin D) and 1,25-dihydroxyvitamin D have important effects on calcium absorption, bone calcium balance, and renal excretion of calcium and phosphorus *(1)*. Assessment of the monohydroxy form of vitamin D is important for identifying insufficient endogenous synthesis disorders that impair gastrointestinal absorption and to identify renal or hepatic abnormalities *(2)*.

Here we report on 25-OH-vitamin D as measured by the Nichols ADVANTAGE® 25-OH Vitamin D, DiaSorin LIAISON® 25-OH Vitamin D, DiaSorin 25-OH Vitamin D RIA, and liquid chromatography–tandem mass spectrometry (LC-MS/MS; Mayo Clinic). For measurement of vitamin D, the Nichols ADVANTAGE and DiaSorin LIAISON use a 2-site chemiluminescence assay; however, one important difference is that the Nichols ADVANTAGE relies on binding of vitamin D to a vitamin-D–binding protein purified from human sources and the DiaSorin LIAISON uses an antibody against 25-OH-vitamin D.

Problems have emerged with the Nichols ADVANTAGE 25-OH Vitamin D method. In patients supplemented with ergocalciferol (D_2) to improve their vitamin D concentrations, the Nichols ADVANTAGE failed to consistently show the expected increase in vitamin D *(3)*. Ergocalciferol (Drisdol; Sanofi Pharmaceutical) is the only vitamin D_2 pharmaceutical preparation available for physicians in the United States to use as a vitamin D supplement; it is extracted from plants or fungi and is processed in the body to 25-OH-vitamin D_2 and 1,25-dihydroxyvitamin D_2. The DiaSorin LIAISON was shown not to underestimate the D_2 concentration *(4)*.

To further analyze the various methods, we measured vitamin D from 110 deidentified samples (Institutional Review Board no. 7740), using the Nichols ADVANTAGE, DiaSorin LIAISON, and a manual DiaSorin RIA. Vitamin D measured by the Nichols ADVANTAGE and DiaSorin LIAISON gave a Deming *(5)* regression of $y = 1.04x - 4.20$ μg/L (Fig. 1). The DiaSorin RIA vs the Nichols ADVANTAGE gave a Deming regression of $y = 1.42x - 8.39$ μg/L, and the DiaSorin RIA vs the DiaSorin LIAISON gave $y = 1.37x - 9.64$ μg/L.

Eight samples with significant variation in vitamin D values between the Nichols ADVANTAGE and the DiaSorin LIAISON were measured by LC-MS/MS. Deming results were $y = 0.36x + 14.23$ μg/L for LC-MS/MS vs the Nichols ADVANTAGE; $y = 1.22x - 2.97$ μg/L for LC-MS/MS vs the DiaSorin LIAISON; and $y = 0.76x - 8.96$ μg/L for LC-MS/MS vs the DiaSorin RIA.

For 3 of the samples, the Nichols ADVANTAGE yielded values <40% of those obtained by LC-MS/MS. Although the LIAISON gave slightly higher values than LC-MS/MS, there was good agreement with LC-MS/MS for those 8 samples. For pa-