and/or autoantibody-induced decreased clearance of the sialic acid-deficient transferrin isomers (CDT) via the asialoglycoprotein receptor.

The underlying mechanism for the increased CDT in our patient might be different from that in viral liver cirrhosis. To explore the etiology of this finding, measurements of neuraminidase and sialyltransferase activity (9) and of the sialyltransferase mRNA concentration (10) may be useful. We consider autoimmune hepatitis as a new cause for pathologic CDT results despite typical alcohol intake.

References


To the Editor:

Increased plasma total homocysteine (tHcy) is a recognized risk factor for cardiovascular diseases (1–3). Recommendations for accurate measurement of tHcy concentrations include collecting fasting blood samples (4, 5) with the patient in a sitting position (6), placing the (EDTA) tubes directly on ice, and immediately centrifuging the samples (4). There are no recommendations on whether to use a refrigerated or a nonrefrigerated centrifuge. We compared the tHcy concentrations in samples centrifuged at 4°C and at ambient temperature.

We studied 40 females (age range, 43–68 years) who participated in a side protocol of the SU.VI.MAX study (7). Two fasting venous blood samples were collected in EDTA Vacutainer Tubes (Becton Dickinson), which were not put on ice, but were centrifuged immediately for 15 min at 2000g, one at 4°C and the other at ambient temperature (−20°C). After centrifugation the plasma was immediately separated from the blood cells, and the plasma samples were stored at −20°C until the tHcy measurement.

The tHcy concentration, including the protein-bound and non-protein-bound fractions of homocysteine, was measured by HPLC with fluorescence detection (8). The within- and between-run CVs for this method were 3.4% and 4.8%, respectively, at a tHcy concentration of 8.4 μmol/L (5).

Stability studies indicate that the increase in tHcy in whole blood kept at room temperature (20–25°C) may be 0.009–0.023 μmol/L per min (9–13). This means that if there is an effect of centrifugation at 20°C, the expected increase in tHcy concentration in the samples centrifuged at room temperature would be 0.14–
0.35 μmol/L because we centrifuged for 15 min. With 40 women the study would be able to detect an increase of 0.20 μmol/L with a statistical power of 80% and a one-sided α of 0.10, with an assumed SD of the difference in tHcy concentrations of the matched pairs of 0.5 μmol/L.

A priori, a difference in the tHcy concentration <0.2 μmol/L was not regarded as relevant regarding misclassification of an individual with respect to risk of ischemic heart disease or stroke. The relevance of the difference was calculated with the data from a recent metaanalysis (2). From these data we can deduce that an increase in the tHcy concentration of 0.2 μmol/L is associated with an increase in risk of only 1% for ischemic heart disease and for stroke. A difference in the tHcy concentration <0.2 μmol/L would lead to a negligible increase in the risk of cardiovascular diseases; it was therefore not considered relevant.

All statistical analyses were done with the statistical package Analyze-it for Microsoft Excel (97 SR-2). Our results were based on data of 39 women (plasma was absent in one tube). The mean (SD) tHcy concentrations were 9.18 (3.7) μmol/L for the samples centrifuged at 4 °C and 9.25 (4.1) μmol/L for the samples centrifuged at 20 °C. The tHcy concentration of the samples centrifuged at 20 °C was thus on average 0.07 μmol/L higher. However, this somewhat higher value was not statistically significantly different from zero (95% confidence interval, −0.201 to 0.340), indicating no difference in tHcy concentration according to the centrifugation temperature. These results are supported by the Bland–Altman plot in Fig. 1, which shows the difference between the tHcy concentrations in samples centrifuged at 4 and 20 °C for each woman plotted against the mean tHcy concentration at the two different temperatures.

We conclude that use of nonrefrigerated centrifuges does not lead to misclassification of individuals according to their tHcy concentration if whole blood is centrifuged immediately after drawing. If immediate centrifugation is not feasible, whole blood samples should be placed on ice immediately after drawing.

This work was supported financially by a grant from the Ministry of Research NUTRIALIS (réseau RARE, no. 02 P 0565).

References

Angelika de Bree1*, Véronique Ducros2 Louise I. Mennen1,3 Isabelle Quéré4 Serge Herberg1,2 Pilar Galan1

1 UMR INSERM unit 557 INRA unit 1125 Institut Scientifique et Technique de la Nutrition et de l’Alimentation ISTNA/CNAM Paris, 75003 France
2 Département de Biologie Intégrée CHU Grenoble, 38043 France
3 Unité de Surveillance et d’Épidémiologie Nutritionnelle InVS-CNAM Paris, 75003 France
4 Département de Médecine Interne CHU Saint Eloi Montpellier, 34059 France

*Address correspondence to this author: ISTNA/CNAM, 5 rue du Vertbois, 75003 Paris, France. Fax 33-1-53018070; e-mail s_debree@vcnam.cnam.fr.