cate yielded an SD of 5.5% of the reference interval value. In addition, 46 serum samples from healthy individuals were tested using the EFC-cAMP method (mean 94.4%, median 94.5%, and SD 6.8%). Results for the normal samples were 80% to 106% of the control (data not shown).

This EFC-cAMP assay is a nonradioactive surrogate for RIA-cAMP for studying antibody-mediated increases in cAMP concentrations in response to activation of the G-coupled TSH receptor.

Grant/Funding Support: This work was supported by the ARUP Institute for Clinical and Experimental Pathology®

Financial Disclosures: None declared.

Acknowledgments: We thank Leonard Kohn for providing the Chinese hamster ovary cell line expressing the TSH receptor (Leonard Kohn, The Interthyr Research Foundation), Linda Seiler for excellent technical expertise, Hayden Jeffreys for samples from Graves patients (Kronus), and Lois Hybl and Tom Martins for critical reading of the manuscript.

References


Tanya Sandrock1*
Alan Terry1
Jeff C. Martin1
Evrim Erdogan2
Wayne A. Meikle2,3

1 ARUP Institute for Clinical and Experimental Pathology
Salt Lake City, UT

* Address correspondence to this author at:
ARUP Institute for Clinical and Experimental Pathology
500 Chipeta Way
Salt Lake City, UT 84108
Fax 801-584-5207
E-mail tanya.sandrock@aruplab.com

DOI: 10.1373/clinchem.2007.100396

In Vitro Formation of Homocysteine in Whole Blood in the Presence of Anticoagulants

To the Editor:

We read with great interest the letter by Hübner et al. (1) on the stability of plasma sulfur-containing metabolites in the presence of anticoagulants (EDTA and citrate) and a homocysteine stabilizer. These authors observed time-dependent increases in plasma concentrations of homocysteine (Hcy)1 and similar increases in plasma S-adenosylhomocysteine (SAH) in EDTA- and citrate-containing whole blood after 24 h at room temperature. In discussing the mechanisms of these increases, they postulated that plasma Hcy is generated from SAH in erythrocytes by the SAH-hydrolase–catalyzed reaction, and Hcy and SAH leak into plasma from erythrocytes (1). They further noted that the inhibition of SAH hydrolase by citrate results in a smaller Hcy increase compared with EDTA, which they claimed does not inhibit this enzyme.

We recently reported the iron-dependent in vitro chemical formation of Hcy from methionine

\[ y = 1.0596x - 18.27 \]
\[ R = 0.96 \]

Fig. 1. Correlation between extracellular cAMP measured by RIA and intracellular cAMP measured by EFC-cAMP (n = 49) [SEE = 34.75; F = 513.15, P < 0.0001]. Assays were performed in triplicate; 130% of control is denoted by dashed lines. Normal is ≤109%; indeterminate is 110%–129%; a positive result is ≥130%.

1 Nonstandard abbreviations: Hcy, homocysteine; SAH, S-adenosylhomocysteine; Met, methionine.
(Met), SAH, and cystathionine, where EDTA or citrate (both 5 mmol/L) acted as a chelator (2). Based on these findings, we propose that a portion of the Hcy increases reported by Hübner et al. (1) results from the iron-dependent conversion of Met and SAH to Hcy. Furthermore, we showed that iron chelated by EDTA catalyzes the conversion of Met to Hcy more efficiently than iron chelated by citrate, and this should also be true for SAH (2). Although the inhibition of SAH hydrolase would prevent increases in plasma Hcy, this factor alone may not completely account for the differences in plasma Hcy increases of approximately 12 and 3 μmol/L in EDTA- and citrate-treated blood, respectively. One would expect that plasma SAH would be higher in the citrate-treated samples than EDTA-treated samples, if SAH hydrolase is inhibited by citrate. This is not true, because plasma SAH concentrations are 70–90 μmol/L at 24 h in samples containing EDTA or citrate. Plasma Hcy increases in whole blood treated with EDTA and 3-deazaadenosine (inhibitor of SAH hydrolase) after incubation at room temperature and 37 °C (3). Ubbink et al. (4) also reported that EDTA-treated blood produced a marked increase in plasma Hcy compared with Na-fluoride–treated blood. Na-fluoride may inhibit SAH production or SAH hydrolase; however, Na-fluoride is not an iron chelator and should not mobilize protein-bound iron. The amount of K3 EDTA in evacuated tubes is about 4 mmol/L (1.8 g/L of blood), which is similar to the EDTA concentration (5 mmol/L) that we used in our in vitro study (2). Therefore, iron in both plasma and erythrocyte would be exposed to chelation, because EDTA concentration is higher than that of protein that binds iron. Both SAH and the larger pool of Met could react with iron chelated by EDTA, resulting in a larger-than-expected increase in plasma Hcy. Serum samples contain no iron chelator and a small amount of iron and should not show an increase in Hcy.

In addition to the mechanistic points presented here, Hübner et al. (1) reported that the 24-h in vitro increases in plasma Hcy concentrations in the presence of EDTA were only 0.1–9.5 μmol/L in patients with renal disease whose baseline plasma Hcy values were far higher than those of healthy subjects. In contrast, the increases in plasma Hcy in healthy subjects were much higher, range 8.7–23.6 μmol/L. These findings might be explained by the fact that blood iron concentrations in patients with renal disease are much lower than those in healthy subjects, since patients with chronic renal disease often have iron deficiency (5). The amount of iron that would be chelated by EDTA may be lower in these patients, resulting in a reduced production of Hcy from Met and SAH. It may be of interest to examine the correlation between 24-h Hcy increase and blood iron concentrations in these patients and healthy individuals.

Hübner et al. (1) noted that the proprietary material in Primavette™ affected the fluorescence polarization immunoassay of Hcy. Although we have not evaluated Primavette, it may be found to interfere with other laboratory analyses. We recommend that whole blood samples should be collected with citrate rather than EDTA as an anticoagulant and centrifuged as soon as possible. If this is not possible, whole blood samples should be kept refrigerated until plasma separation. Further studies are needed to elucidate the mechanism of Hcy increase.

Grant/Funding Support: None declared.

Financial Disclosures: None declared.

References


Tsunenobu Tamura* Joseph E. Baggott

Department of Nutrition Sciences
University of Alabama at Birmingham
Birmingham, AL

* Address correspondence to this author at:
Department of Nutrition Sciences
University of Alabama at Birmingham
Birmingham, AL 35294-3360
Fax 205-996-5775
E-mail tamurat@uab.edu

DOI: 10.1373/clinchem.2007.101642

Estimation of Glomerular Filtration Rate by Use of Beta-Trace Protein

To the Editor:

Because we recently introduced beta-trace protein (BTP) for estimating glomerular filtration rate (GFR) in patients after kidney transplantation (1), we read with interest the report by White and coworkers (2). These authors proposed a BTP-based equation to calcu-late GFR in renal transplant recipients. Because a separate second

Clinical Chemistry 54:8 (2008) 1403