(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.

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**References**


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**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 calculate GFR in renal transplant recipients. Because a separate second
cohort was not available in the reported study, a validation of the BTP-based formula was not performed. However, we were able to validate the suggested equation in 85 kidney transplant recipient patients who had participated in our study mentioned above.

The following equation of White and coworkers was applied:

\[
\text{GFR (mL/min/1.73m}^2) = 167.8 \times \text{BTP}^{0.758} \times \text{creatinine}^{0.204} \times 0.871
\]

if the patient is female,

where BTP is given in milligrams per liter and creatinine is given in micromoles per liter. The computed results were compared to the reexpressed Modification of Diet in Renal Disease (MDRD) formula by determining correlation, bias, precision, and accuracy. As outlined in a previous report by White and coworkers, their creatinine is calibrated to the Cleveland Clinic laboratory (3). Thus, to provide comparability, we converted our isotope-dilution mass spectrometry–calibrated creatinine to the Cleveland Clinical Laboratory using an equation proposed by Levey et al. (4). GFR was determined by technetium-diethylentriamine pentaacetic acid clearance performed as a single-injection technique with a 2-point sampling approach at 1 and 3 h after intravenous injection, according to the method of Russell et al. (5). The mean GFR of the cohort was 38.6 (95% CI, 35.3–41.9) mL/min/1.73 m².

Correlation coefficients of the predicted GFRs by both formulae with the measured reference GFRs were high and did not differ significantly (BTP formula, \(r = 0.866\) vs MDRD equation, \(r = 0.863\)). Both equations overestimated measured GFR to a small but significant extent [mean GFR estimated by the BTP formula was 46.7 (95% CI, 43.0–50.4) mL/min/1.73 m² vs the mean GFR estimated by the MDRD equation, which was 45.3 (95% CI, 41.1–49.6) mL/min/1.73 m²; \(P < 0.001\) for both]. The bias of the MDRD equation was somewhat lower than that of the BTP formula (6.7 vs 8.1 mL/min/1.73 m², not significant). Precision (measured as SD of the mean difference between measured and estimated GFR) (6) tended to be better for the BTP formula (8.44 vs 10.03 mL/min/1.73 m²), but this difference in precision did not quite reach statistical significance \((P = 0.058)\). The rates of predicted GFRs within 10%, 30%, and 50% of the measured GFR were comparable (BTP formula: 24.7%, 63.5%, and 84.7%, respectively, vs MDRD equation: 24.7%, 71.8%, 87.1%; not significant by McNemar test). Bland and Altman plots of the measured vs predicted GFRs of both equations are given in Fig. 1.

In conclusion, this external validation provides evidence that the performance of the BTP formula suggested by White and coworkers (2) is comparable to that of the reexpressed MDRD equation. Nevertheless, some difficulties were associated with the validation process and may have affected our results: (a) the validation cohort was relatively small, (b) although the same BTP determination technique was used, no international standardization was available, (c) differences in methods used for creatinine determination were corrected mathematically but no samples were sent to the Cleveland Clinical Laboratory for analysis, and (d) the cohorts appeared to
have different degrees of graft function [mean (SD) GFR 59 (23) in the patient cohort of White vs 38.6 (15.1) in our patient cohort].

Finally, an additional economic aspect must also be taken into account: BTP is considerably more expensive than creatinine. Thus, the suggested BTP-based equation for GFR calculation will gain public recognition only when its performance is demonstrated to be clearly superior to the MDRD equation. Further studies are needed to elucidate this issue more clearly.

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Apolipoprotein A-II is a Negative Risk Indicator for Cardiovascular and Total Mortality: Findings from the Ludwigshafen Risk and Cardiovascular Health Study

To the Editor:

Apolipoprotein A-II (apoA-II)1 was recently shown by Birjmohun and coworkers to be inversely associated with future risk for coronary artery disease in 912 cases and 1635 controls (1). This finding is important, because prior data have suggested that apoA-II has poor antiatherogenic properties and may even be proatherogenic. We now confirm and extend the antiatherogenic association of apoA-II to cardiac and total mortality on the basis of data collected after 8 years of follow-up in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, which is investigating patients who underwent elective coronary angiography (2).

Complete clinical and biochemical data from 3261 participants in the LURIC study are available. For these study participants, 752 deaths were recorded, and causes of death were obtained from official death certificates. Death from cardiac causes was recorded in 431 cases. Patients with (n = 2535) and without (n = 726) coronary artery disease presented with mean (SD) apoA-II concentrations of 0.44 (0.10) and 0.41 (0.09) g/L (P < 0.001), respectively. To examine the effect of apoA-II on cardiac and total mortality, we used Cox proportional hazards regression models to calculate hazard ratios (HRs). The data of the LURIC trial showed that apoA-II concentrations were negatively associated with cardiac death (HR 0.95, 95% CI 0.94–0.96; P < 0.001) and total mortality (HR 0.96, 95% CI 0.95–0.96; P < 0.001). For further risk assessment, quartile ranges of apoA-II concentrations measured in study participants without coronary artery disease were defined as <0.37 g/L (reference), 0.37–0.43 g/L, 0.43–0.50 g/L, and >0.50 g/L. The unadjusted HR for cardiac death of the fourth vs the reference quartile was 0.38 (95% CI 0.27–0.52; P < 0.001), and the HR per SD increase of apoA-II was 0.60 (95% CI 0.54–0.67; P for trend <0.001).

However, in contrast to the findings of Birjmohun et al., we found that HRs of the fourth vs first quartile were 0.26 (95% CI 0.14–0.50; P < 0.001) for females (n = 990) and 0.45 (95% CI 0.31–0.64; P < 0.001) for males (n = 2271). For the total population, adjustment for age, sex, and body mass index; use of lipid-lowering drugs; presence of hypertension, diabetes, smoking, and/or alcohol consumption; and concentrations of triglycerides, LDL- and HDL cholesterol, and apoA-I resulted in HRs per SD increase of apoA-II of 0.66 (95% CI 0.57–0.78; P for trend <0.001) for cardiac death and 0.77 (95% CI 0.68–0.86; P for trend <0.001) for total mortality, respectively. If sensitive C-reactive protein was included, the HRs for the second and third quartile vs the 1st quartile remained significant only for cardiac death: 0.77 (95%

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Nonstandard abbreviations: apoA-II, apolipoprotein A-II; LURIC, Ludwigshafen Risk and Cardiovascular Health; HR, hazard ratio.