able in borderline cases. The routine use of other biomarker assays, such as HFABP in conjunction with tGST activity, could provide complementary information on the potential allograft "viability". This is illustrated by the fact that there was better segregation of donor categories (controlled vs uncontrolled) with HFABP than with tGST activity. This finding therefore warrants continued evaluation.

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**Total Iron-binding Capacity Calculated from Serum Transferrin Concentration or Serum Iron Concentration and Unsaturated Iron-binding Capacity,** *Hachiro Yamanishi,[1,2*] Shigeru Iyama,[1] Yoshihisa Yamaguchi,[1] Yuzuru Kanakura,[1] and Yoshinori Iwatani[2,3]* ([1] Laboratory for Clinical Investigation, Osaka University Hospital, 2-15, Yamadaoka, Suita, Osaka 565-0871, Japan; [2] Division of Laboratory Science, Course of Health Science, Graduate School of Medicine, and [3] Department of Clinical Laboratory Science, School of Allied Health Sciences Faculty of Medicine, Osaka University, 1-7 Yamada-oka, Suita, Osaka 565-0871, Japan; * author for correspondence: fax 81-6-6879-6635, e-mail yamaha@hp-lab.med.osaka-u.ac.jp)

Total iron-binding capacity (TIBC) indicates the maximum amount of iron needed to saturate plasma or serum transferrin (TRF), which is the primary iron-transport protein *(1)*. Theoretically, 1 mol of TRF [average molecular mass, 79 570 Da *(2)*] can bind 2 mol of iron (55.8 Da) at two high-affinity binding sites for ferric iron *(3)*. Therefore, TIBC correlates well with TRF concentration, and the theoretical ratio of TIBC (in $\mu$mol/L) to TRF (in g/L) is 25.1: TIBC ($\mu$mol/L) = 25.1 $\times$ TRF (g/L) *(4, 5)*.

Measurements of TIBC, serum iron, and the percentage of iron saturation of TRF are useful for the clinical diagnosis of iron-deficiency anemia and chronic inflammatory disorders *(6, 7)* and as screening tests for other clinical conditions *(8)*. TIBC is routinely determined *(9–12)* by saturation of TRF with an excess predetermined amount of iron, removal of the unbound iron, and measurement of the iron that is dissociated from TRF. For removal of the unbound iron, magnesium carbonate *(9)*, ion-exchange resin *(10)*, alumina columns *(11)*, or magnetic particles *(12)* are used. Most direct TIBC measurement methods require manual procedures that involve centrifugation or pretreatment of serum samples. As an alternative to direct measurement methods, TIBC values are also calculated from the sum of serum iron and unsaturated iron-binding capacity (UIBC), both of which are determined by colorimetric methods (calculation method).

We developed a direct and fully automated TIBC (DTIBC) assay for use with an automated multipurpose analyzer *(13, 14)*. A fully automated TIBC measurement method is also commercially available *(15)*. In our previous study *(14)*, TIBC values obtained by DTIBC assay correlated strongly with serum TRF concentrations ($r$ = 0.984; n = 59), and the slope of the regression line was consistent with the theoretical TIBC/TRF ratio. We also observed good correlation between our DTIBC values and values calculated from the sum of serum iron and UIBC, but the calculated TIBC values were lower than the DTIBC-determined values and TIBC values obtained by an automated assay. Similar results have been reported in a comparison of a calculation method and a direct automated TIBC assay *(16)*. In the present study, we reevaluated the relationships between TRF concentrations and TIBC values obtained by the DTIBC assay and by calculation methods in a large sample (n = 188) and used

multiple regression analysis to investigate the difference between TIBC values converted from TRF concentrations and calculated TIBC values.

In addition to TIBC, TRF, serum iron, and UIBC, we measured serum ferritin and ceruloplasmin concentrations in the sera of 188 patients. Ferritin plays important roles in iron metabolism, storage, and detoxification *(17)*. Ceruloplasmin has ferroxidase activity as well as ferritin H subunits, and patients with a congenital absence of ceruloplasmin develop severe iron overload *(17, 18)*. The DTIBC assay was performed with the Hitachi Model 7070 automated analyzer. Both serum iron and UIBC were determined by direct colorimetric methods (Wako Pure Chemical Industries and Roche Diagnostics). The serum iron/UIBC assays from Wako use bathophenanthroline as chromogen, and those from Roche use ferrozine as chromogen. Calibration was with physiologic saline (zero calibrator) and an iron solution (36 μmol/L $Fe^{3+}$ in 0.9 g/L $H_2SO_4$). TIBC was then calculated as the sum of serum iron plus UIBC ($CTIBC_{batho}$ and $CTIBC_{ferro}$). Serum TRF and ceruloplasmin concentrations were determined by nephelometric assay with the Behring Nephelometer II analyzer (Dade Behring Marburg GmbH). Serum ferritin was measured with the ADVIA Centaur Ferritin Assay Reagent and ADVIA Centaur Analyzer (Bayer Diagnostics).

Shown in Fig. 1 are the correlations between serum TRF concentrations and TIBC determined by DTIBC assay and by the calculation methods (panels A–C) and between the DTIBC assay values and the calculated TIBC values (panels D and E). We found highly significant correlations ($P < 0.001$) between the DTIBC assay values, the calculated TIBC values, and the serum TRF concentrations. The DTIBC and calculated TIBC values also correlated well, but the TIBC calculation method showed a negative difference (slope = 0.87 and 0.88). The mean (SD) TIBC values were as follows: DTIBC, 63.8 (14.7) μmol/L; $CTIBC_{batho}$, 56.8 (13.2) μmol/L; and $CTIBC_{ferro}$, 56.4 (13.3) μmol/L. The calculated TIBC values were significantly lower than the DTIBC values ($P < 0.001$) regardless of which chromogen was used in the colorimetric assays. Correlation between the two calculation methods was: $CTIBC_{batho} = 0.994(CTIBC_{ferro}) + 0.80$ μmol/L ($r = 0.999$; $S_{y|x} = 0.70$ μmol/L). We observed no difference between the $CTIBC_{batho}$ and the $CTIBC_{ferro}$ values.

We also calculated the difference between the TIBC values converted from TRF concentrations and the calculated TIBC values [25.1 × TRF (g/L) − CTIBC (μmol/L)] and determined the relationships between the difference and serum concentrations of iron, UIBC, ferritin, and ceruloplasmin by multiple regression analysis. Sex was included as a covariate in the multiple regression models,

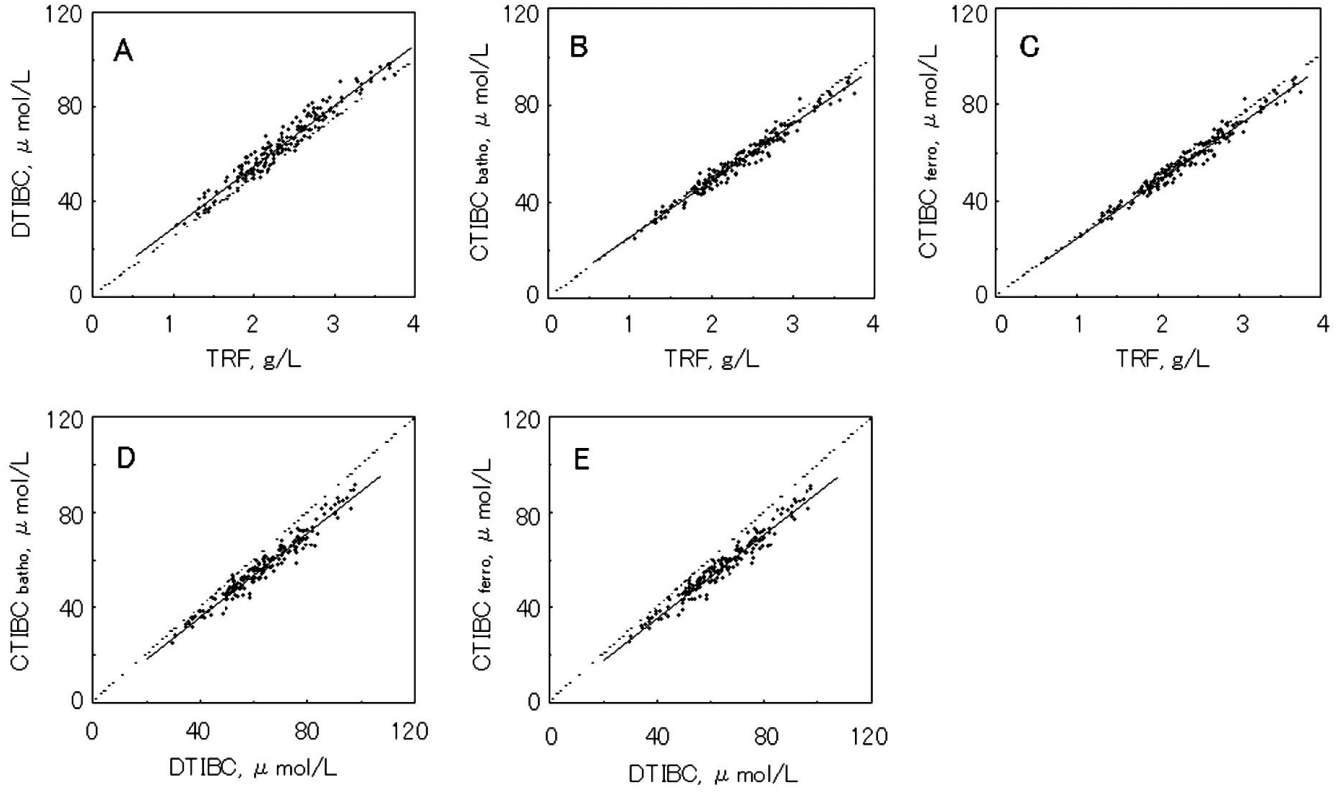


Fig. 1. Correlation between TRF concentrations and TIBC values and between DTIBC assay values and the calculated TIBC values.

(*A*), TRF (*x*) vs DTIBC (*y*): $y = 25.7x + 3.3$ μmol/L ($r = 0.973$; $S_{y|x} = 3.4$ μmol/L; n = 188). (*B*), TRF (*x*) vs $CTIBC_{batho}$ (*y*): $y = 23.3x + 2.0$ μmol/L ($r = 0.982$; $S_{y|x} = 2.4$ μmol/L; n = 188). (*C*), TRF (*x*) vs $CTIBC_{ferro}$ (*y*): $y = 23.4x + 1.2$ μmol/L ($r = 0.983$; $S_{y|x} = 2.4$ μmol/L; n = 188). (*D*), DTIBC (*x*) vs $CTIBC_{batho}$ (*y*): $y = 0.87x + 0.90$ μmol/L ($r = 0.976$; $S_{y|x} = 2.9$ μmol/L; n = 188). (*E*), DTIBC (*x*) vs $CTIBC_{ferro}$ (*y*): $y = 0.88x + 0.16$ μmol/L ($r = 0.977$; $S_{y|x} = 2.8$ μmol/L; n = 188). The *solid lines* are the regression lines. The *dashed lines* represent $y = 25.1x$ in *panels A–C* and $y = x$ in *panels D* and *E*.

and all continuous variables were transformed to gaussian distribution. The mean (SD) difference was 2.0 (2.6) μmol/L for $CTIBC_{batho}$ and 2.4 (2.6) μmol/L for $CTIBC_{ferro}$. As shown in Table 1, only UIBC had an independent influence on the difference in both models. UIBC was statistically associated with the difference in linear regression analysis. Serum iron was statistically associated with the $CTIBC_{batho}$ difference in linear regression analysis ($P = 0.015$); this was not, however, a significant variable in the multiple regression model that included ferritin. When UIBC was calculated as the difference in TIBC determined by DTIBC assay and the amount of serum iron determined by the colorimetric methods ($CUIBC_{batho}$ and $CUIBC_{ferro}$), the regression equation for the measured UIBC ($y$) and the $CUIBC_{batho}$ ($x$) was: $y = 0.90x - 2.4$ μmol/L ($r = 0.98$). The regression equation for $CUIBC_{ferro}$ ($x$) was: $y = 0.88x + 0.17$ μmol/L ($r = 0.97$). The UIBC values determined by the colorimetric methods were lower than both of the CUIBC values.

The slope of the regression line in which the DTIBC values are plotted against the TRF values is consistent with the theoretical TIBC/TRF ratio. However, the slopes of the regression lines for the calculated TIBC values plotted against the TRF values were ~7% lower than the theoretical ratio (Fig. 1, B and C). The results of multiple regression analysis suggest that the UIBC is independently associated with an increase in the difference between the converted TIBC and the calculated TIBC. Moreover, the slopes of the regression lines for the calculated UIBC values plotted against the measured UIBC were similar to the slopes of the regression lines in which the DTIBC values were plotted against the calculated TIBC. These results indicate that the differences in the DTIBC and the calculated TIBC can be attributed to the low UIBC value determined by the colorimetric assay. The UIBC concentrations were determined as the differences between a known amount of iron added for saturation of TRF and the amount of iron that was left after binding to unsaturated sites on TRF in the colorimetric UIBC methods. However, the binding of iron to TRF is not instantaneous, it takes many minutes to reach completion. As the number of unsaturated sites increases, more time is required for the reaction to come to completion. The low UIBC result obtained by the automated UIBC method was probably attributable to insufficient time allowed for the saturation of TRF.

Gambino et al. *(5)* reported that a two-point calibration method with a protein-based calibrator should be used for colorimetric UIBC methods and that this calibration method provides a more accurate calculated TIBC value. Although the availability of the proposed calibration method for the colorimetric UIBC methods was not evaluated in this study, the results of our multiple regression analysis provide support for the conclusions of the study by Gambino et al. *(5)*. We anticipated that ferritin and ceruloplasmin, which are iron-related proteins, would be associated with the difference between methods, but the results of our multiple regression analysis showed that these factors did not account for a significant variance in the difference. However, ferritin may be a confounding factor in the difference between the converted TIBC and the calculated TIBC. For calculation of an accurate TIBC value from the sum of serum iron and UIBC, it is likely that a multipoint calibration method with human protein-based calibrators for colorimetric UIBC assays will be useful. However, the total amount of iron that is really bound to TRF iron-binding sites should probably be measured as TIBC. When the slope of the regression line for calculated TIBC values plotted against TRF concentrations is significantly lower than the theoretical TIBC/TRF ratio, UIBC should be computed as the differences between TIBC measured by a direct TIBC method and serum iron. With the calculation method, when either the

**Table 1. Multiple regression analysis of the difference between the TIBC converted from TRF and the calculated TIBC.**

| Dependent variable | Independent variable | $\beta$[a] | SE | *t* value | *df*[b] | *P* | Slope[c] (*P*) |
|---|---|---|---|---|---|---|---|
| $CTIBC_{batho}$ difference[d] | Sex | −0.617 | 0.457 | 1.349 | 181 | 0.179 | |
| | Serum iron[f] | −0.100 | 0.220 | 0.452 | 181 | 0.652 | −0.445 (0.015) |
| | UIBC[f] | 0.042 | 0.020 | 2.102 | 181 | 0.037 | 0.040 (0.001) |
| | Ferritin | 0.173 | 0.193 | 0.899 | 181 | 0.370 | −0.221 (0.061) |
| | Ceruloplasmin | 0.113 | 0.126 | 0.898 | 181 | 0.371 | 0.183 (0.135) |
| $CTIBC_{ferro}$ difference[e] | Sex | −0.559 | 0.451 | 1.239 | 181 | 0.217 | |
| | Serum iron[g] | 0.196 | 0.218 | 0.895 | 181 | 0.372 | −0.180 (0.318) |
| | UIBC[a] | 0.052 | 0.020 | 2.566 | 181 | 0.011 | 0.035 (0.005) |
| | Ferritin | 0.206 | 0.190 | 1.086 | 181 | 0.279 | −0.178 (0.122) |
| | Ceruloplasmin | 0.047 | 0.124 | 0.376 | 181 | 0.707 | 0.100 (0.401) |

[a] Partial regression coefficient.
[b] Degrees of freedom.
[c] Regression coefficient of linear regression analysis.
[d] Difference between the converted TIBC from TRF and the $CTIBC_{batho}$.
[e] Difference between the converted TIBC from TRF and the $CTIBC_{ferro}$.
[f] Values measured by the colorimetric method from Wako.
[g] Values measured by the colorimetric method from Roche.

serum iron concentration or UIBC value is below detectable limits, TIBC cannot be determined.

In conclusion, TIBC values determined by DTIBC assay were strongly correlated with TRF concentrations. The slope of the regression line for the DTIBC values plotted against the TRF is consistent with the theoretical TIBC/TRF ratio. TIBC values calculated from the sum of serum iron and UIBC were significantly lower than those obtained by the DTIBC assay. This can be attributed to underestimation of UIBC values by the colorimetric assay. UIBC is an independent factor that influences the difference between the converted TIBC values and the calculated TIBC values. We suggest that TIBC be measured by direct TIBC methods and that UIBC be calculated as the difference between TIBC and serum iron.

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**Short-Term Variations in Enterolactone in Serum, 24-Hour Urine, and Spot Urine and Relationship with Enterolactone Concentrations,** *Katariina Stumpf* and Herman Adlercreutz* (Folkhälsan Research Center, Institute for Preventive Medicine, Nutrition and Cancer, and Division of Clinical Chemistry, Biomedicum, PB 63, University of Helsinki, FIN-00014 Helsinki, Finland; * author for correspondence: fax 358-9-191-25452, e-mail katariina.stumpf@helsinki.fi)

Enterolactone, a mammalian lignan, is produced by colonic microflora from precursors present in food plants. Because intake of vegetables, fruits, berries, or whole grains is related to the enterolactone concentration in blood *(1, 2)* and excretion in urine *(3, 4)*, enterolactone may function as a biomarker of fiber-rich foods. Important characteristics for a biomarker include convenient, low-risk collection of samples; specific, reliable laboratory measurement; and a high ratio of between-person to total variability [intraclass correlation (ICC)] *(5)*. In epidemiologic studies, analytes with a low ICC often show weak associations with any disease *(6)*.

The present study describes the short-term variation in enterolactone in serum, 24-h urine, and spot-urine enterolactone:creatinine ratio and the relationship between enterolactone concentrations in serum, 24-h urine, and spot urine.

The study protocol was approved by the Ethics Committee on Epidemiology and Public Health in the hospital district of Helsinki and Uusimaa. Twenty volunteers (13 females and 7 males) were recruited among university students. Exclusion criteria included age <18 years, antibacterial treatment during the preceding 3 months, and any major illness or regular medication, except contraceptive pills. The average age of the participants was 22.2 years [95% confidence interval (CI), 21.4–22.9 years], and the average body mass index was 22.3 (20.9–23.6) kg/m$^2$. Of the female participants, seven took oral contraceptives. The female participant who regularly took an antidepressant reported this only at the end of the study. One participant dropped out because of antibacterial treatment for a urinary tract infection. One spot-urine sample was missing for one female participant, who was thus excluded from that analysis.

The samples were collected on 5 successive days (Monday to Friday) for within-week variation and on the following 3 Mondays for within-month variation. Participants began collecting their urine 24 h before serum and spot-urine samples were collected. No specific timing was demanded for spot-urine collection. The venous samples were drawn after a 4-h fast, starting at 1600 h. The blood samples were collected by venipuncture and centrifuged before separation of the serum. The volumes of the 24-h urine samples were measured before storing. Some participants reported the approximate amount of urine not included in the 24-h collection, and this reported volume was added to the measured urine volume. All samples were stored frozen at −20 °C.

Enterolactone concentrations were analyzed by time-