every two months. Also, care must be taken when collecting fingerstick samples, because any hemolysis of the blood droplet—e.g., from excessive pressure—could affect the cholesterol value.

References

Intra-Individual Biological Variation in the N-Acetyl-β-glucosaminidase/Creatininum Ratio for First Morning Urine, J. Huguet, X. Fuentes-Arderiu, and C. Ferré (Servei de Bioquímica Clínica, Hospital de Bellvitge "Prínceps d'Esanya," Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain)

Biochemical quantities within an individual vary randomly about a homeostatic set-point (1). This fluctuation, described as "intra-individual biological variation," is useful for assessing any change between two consecutive values for a biochemical quantity in a patient (2).

We studied intra-individual biological variation for the ratio between the catalytic concentration of N-acetyl-β-glucosaminidase (EC 3.2.1.30) in first morning urine (Urine-N-acetyl-β-glucosaminidase, b) determined with the 3-cresolsulfophthalein method in a Hitachi 704 analyzer and the substance concentration of creatininum (the sum of creatinine and the creatininum ion actually measured) in first morning urine (Urine-Creatininum, c, Jaffé kinetic method, Hitachi 704 analyzer). b is an extremely sensitive index of renal parenchymal damage (3), a reliable quantity for monitoring the progression of kidney diseases (4), and an early indicator of immune rejection in patients with renal allografts (5). Because variations in urine flow may be compensated for by relating b to c, we calculated the ratio between the two.

We studied a group of 12 supposedly healthy individuals (seven women and five men, ages 12-76 y) for 12 weeks. Each week we measured b and c in each specimen and calculated the ratio between the two (Table 1). From this, we estimated the overall intra-individual variance of the ratio ($s^2_{2b}$). We also studied the relation between the between-days analytical variance and concentration for both biochemical quantities over a wide range of concentrations: b from 38.69 to 860.21 nkat/L, and c from 1.284 to 20.565 mmol/L. These results showed that both quantities were homooscedastic variables (Barlett's test), although for c this homoscedasticity was observed only after naperian logarithm transformation. Furthermore, we evaluated the between-days analytical variance of the ratio ($s^2_{2b}$) (see Table 1). The intra-individual biological variance ($s^2_{2b}$) of the ratio for each subject was calculated from this last value and from $s^2_{2b}$ as follows:

$$s^2_{2b} = s^2_{2b} - s^2_{2b}$$

From these, we obtained for each individual the value of the intra-individual biological variation of the ratio, expressed as a CV; the mean of these values was 35.7%. Moreover, we calculated for each individual the CV corresponding to the intra-individual biological variation of b; the mean of these values was 43%.

References


The cloning of the complete dystrophin cDNA has made it possible to identify molecular deletions in Duchenne (DMD) and Becker (BMD) muscular dystrophy patients (1). Utilizing eight cDNA probes, which span the entire message, deletions have been found in 67% of affected patients. Identification of a deletion in the proband permits direct carrier diagnosis of the female relatives by gene dosage (2). If the female exhibits single-copy intensity for the deleted band or bands on the autoradiograph, then she must be carrying the deletion on one of her X chromosomes and would therefore be a carrier. However, if the band or bands are of two-copy intensity, suggesting homozygosity for the fragment, this would indicate noncarrier status.

Variability in the amount of DNA applied onto the gel and differences in the retention of the high-molecular-mass fragments to the filter during Southern transfer can make it difficult to determine visually whether a band is of single- or double-copy intensity. To increase the accuracy of the gene dosage analysis, we have recently started quantifying the autoradiographic bands by scanning with a CS-9000 scanning densitometer (Shimadzu Corp., Kyoto, Japan).

Hybridization of a Hind III digest to cDNA 8 revealed that the DMD proband (A) was deleted for the 10-, 1.6-, and 1.2-kb fragments (Figure 1). To determine whether the relative intensities of these fragments were reduced in the mother (B), the fragments were analyzed densitometrically (Figure 2), and the 10/7-kb absorbance peak ratios for the

| Table 1. Summary of Data from the 12 Analyses on the 12 Healthy Individuals |
|-------------------------|-----------------|-----------------|
| **Quantity**            | $\bar{x}$       | $s_0$           | $s_{2b}$        |
| c, mmol/L               | 12.5            | 3.5             | 0.091           |
| b, nkat/L               | 52.6            | 15.8            | 139.6           |
| Ratio c/b, μkat/mol     | 4.4             | 1.06            | 0.074           |
| $\bar{x}$ = mean of the mean values for each individual; $s_0$ = standard deviation of the mean values for each individual; $s_{2b}$ = between-days analytical variance. |
Discordant RIA Results for Thyroxin-Binding Globulin (TBG): a New Indicator of Variant TBG Molecules with Decreased Affinity for Thyroxin, Jerald C. Nelson,1 Louis G. Linarelli,2 and M. R. Pandian1 (1 Dept. of Intern. Med., Loma Linda Univ. School of Med., Loma Linda, CA 92350; 2 Children's Hospital, San Diego, CA 92101; and 3 Nichols Institute, San Juan Capistrano, CA 92675)

Seven congenital molecular variants of human thyroxin-binding globulin (TBG) have been reported (I). Some have decreased affinity for T4 and cause a euthyroid hypothyroxinemia resembling TBG deficiency (I). The most recently described TBG variant is TBG-San Diego, a variant with reduced affinity for T4 (I). Two brothers and their maternal grandfather carry the hypothyroxinemic form of this X-linked abnormality, which is named after the city in which the affected family lives (I). When total T4 concentrations of 32, 41, and 45 mg/L were detected in these euthyroid males by T4 screening, TBG deficiency was suspected. However, TBG determinations with different RIA methods gave discordant results. TBG concentrations were 22, 26, and 17 mg/L as measured with a 125I-labeled TBG displacement RIA (Nichols Institute, San Juan Capistrano, CA 92675), but 5, 6, and 3 mg/L as measured with a 125I-T4-binding sandwich RIA (Clinical Assays, Baxter Travenol Diagnostics, Cambridge, MA 02139).

This discordance provides a new method, based on clinical laboratory procedures, for distinguishing euthyroid hypothyroxinemias due to TBG deficiency from euthyroid hypothyroxinemias due to molecular variants of TBG with reduced affinity for T4. Experience with this family indicates that the ratio of TBG-binding activity (determined by 125I-T4-binding RIA) to TBG immunoreactivity (determined by 125I-labeled TBG displacement RIA) can be used to indicate TBG affinity. Figure 1 shows the ratios for 100

mother and a nonaffected control (C) were calculated. The comparison of band ratios, rather than single bands, decreases the error caused by differences in the amount of DNA in each lane. The 10/7-kb absorbance ratio in the mother was 0.39, approximately half the absorbance ratio of the control (0.76). This ratio indicates that she has a single copy of the 10-kb fragment and is a DMD carrier, and therefore the proband was not the result of a sporadic mutation.

Use of the densitometer to quantify dosage helps to address concerns regarding the subjectivity of qualitative visual analysis of the autoradiographic bands. We find densitometric scanning to be a valuable tool in determination of DMD and BMD carrier status.

References