Serum Concentrations of Transferrin Receptor in Hereditary Hemochromatosis

To the Editor:

There is considerable literature on the relationship between serum concentrations of transferrin receptor (TfR), erythropoiesis, and iron status with regards to iron deficiency (1–3). Much less is known, however, about TfR concentrations in iron overload disorders such as hereditary hemochromatosis (HH) (4), an autosomal recessive disorder in humans of Northern European origin with an estimated population frequency of 3 to 5 per 1000 (5).

To investigate a potential relationship between TfR concentrations and HH, we measured TfR in sera from 7 subjects with HH (4 females, 3 males) and from 19 age- and sex-matched controls (11 females, 8 males), using the Quantikine ELISA (R&D Systems, Minneapolis, MN). Sampling procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983, and with the ethical standards of the Foundation for Blood Research Institutional Review Board. Laboratory diagnosis of HH was made by the presence of above-normal values for transferrin saturation (TS; cutoffs: TS >68 in males and >55 in females) on two consecutive occasions (sampling interval 5.8 ± 5.3 weeks). The median ferritin concentration in the seven HH subjects was 513 μg/L (range 115–2610 μg/L); ferritin was not determined in the controls. Clinical confirmation of HH was made by liver biopsy in five of the seven cases and by the demonstration of increased mobilizable iron on repeated phlebotomy in the two remaining cases. For these latter two cases, total body iron stores were estimated to be 3 and 5 g/L according to the method of Walters et al. (6). For one case—a 40-year-old man whose iron findings were 11,361 μg/g dry weight of liver (normal range 530–900 μg/g)—we also monitored serum concentrations of iron, transferrin, TS, TfR, and ferritin in serial samples over a period of 12 months of therapeutic phlebotomy (Table 1).

Mean TS concentrations among the HH subjects were nearly triple that of the controls (83% ± 11% vs 28% ± 8%; P <0.0001 by independent t-test). Concentrations of TfR were 29% lower among the HH subjects than in the controls (1.71 ± 0.6 mg/L vs 2.42 ± 0.8 mg/L; P = 0.03 by independent t-test, data log-transformed for analysis). Given the significant overlap in TfR values between cases and controls, however, it is unlikely that measurement of TfR alone would be useful as a screening test for HH. As shown in Table 1, during serial phlebotomy in the one case studied in detail, the number of phlebotomies performed was negatively associated with the measured concentrations of iron, TS, and ferritin, and positively associated with serum concentrations of transferrin and TfR and with TfR/ferritin.

The present data are consistent with a recent study by Thorstensen and Romolo (7) of 819 healthy men (ages 18–22 years), in which mean TfR concentrations were significantly lower (~20%) in 19 men with biochemical evidence of iron overload (iron >25 nmol/L, TS >60%) than in individuals without evidence of iron overload (TS <60%). In contrast, Huebers et al. (8) found no significant difference in the reported mean and SD for plasma TfR from 7 idiopathic hemochromatosis patients compared with that for 56 normal subjects. In that study, however, plasma samples for the estimation of TfR were obtained several years after phlebotomy had been initiated; thus, the increase in IR concentrations after phlebotomy (Table 1) probably confounded any association between TfR concentration and iron overload. Baynes et al. (9) also found no significant difference in mean iron receptor values between controls and subjects with evidence of nontransfusional iron overload identified on the basis of increased ferritin (>400 μg/L), either with increased TS (>55%, the HH group, n = 14) or without (secondary iron overload group, n = 60). In two of these three reports (7, 9), the diagnosis of homozygosity relied exclusively on laboratory tests, and in one report the diagnosis was based on laboratory results from a single serum specimen (7). Given that many factors are known to influence iron concentrations (e.g., normal diurnal variation, alcohol intake, iron supplements, heterozygote states), and that repeat assays are recommended for laboratory diagnosis (5), the cases reported were likely to have included a heterogeneous group of subjects, and contradictory results would not be unexpected.

Therapeutic monitoring of iron removal from the body is typically based on the measurement of hemoglobin (or hematocrit) and the volume of blood removed. Ferritin is frequently measured during the final stages of the phlebotomy protocol, to detect the onset of mild iron-deficiency anemia (indicated by ferritin <20 μg/L). However, interpretation of ferritin concentrations can be confounded by the presence of inflammation because ferritin is an acute-phase protein. Measurement of serum TfR concentrations, on the other hand, is unaffected by acute or chronic illness and may, in concert with ferritin, actually serve as a more reliable index of iron-deficiency anemia (10). Our present finding of a direct and significant relationship between the number of phlebotomies and the concentrations of transferrin and TfR is consistent with the data of Skikne et al. (1), who demonstrated in normal volunteers undergoing serial phlebotomy that TfR values increase after the appearance of iron-deficient erythropoiesis. In the only other published study of the effect of phlebotomy of TfR, Thorstensen et al. (11) monitored two HH patients undergoing therapeutic phlebotomy and suggested that TfR may be a better indicator than ferritin of when to end phlebotomy. In further agreement with Thorstensen et al., the data from our case indicate a sharp increase in the TfR/ferritin ratio as iron stores approach depletion (~25 phlebotomies), suggesting that the ratio of TfR/ ferritin may be a more sensitive marker for the induction of iron-de-
icient erythropoiesis than ferritin alone.

In conclusion, serum concentrations of TIR are ~30% lower in hemochromatotic individuals than in normal subjects. The present findings represent, to the best of our knowledge, the first study based on clinically confirmed, untreated cases. The application of the TIR assay as an aid in the management of HH patients, especially in phlebotomy schedules, requires further investigation.

References

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High-Resolution 1H-NMR Spectroscopy of Blood Plasma for Metabolic Studies

To the Editor:

In the recent report by Wevers et al. (1) on high resolution 1H-NMR spectroscopy of blood plasma for metabolic studies, instead of using the untreated sample (plasma) they perform a lengthy extraction procedure, finally dissolving the extract in D2O. Since 1989, we and others have published numerous papers demonstrating the advantages of using NMR to study metabolites in unextracted body fluids, especially plasma and urine, because of the speed and simplicity of analysis (2–5). To remove this advantage is to obviate one of the main justifications for using the technique. Foxall et al. have demonstrated clearly (e.g., 6) that at 600 MHz one can obtain high-quality plasma spectra without the need for prior extraction.

More importantly, we disagree in particular with Wevers et al. over their suggestion that 1H-NMR spectroscopy of plasma should be used for the diagnosis of inborn errors of metabolism in preference to urine. For the majority of these disorders, the plasma concentration of abnormal metabolites is very low on the NMR scale of sensitivity; much greater concentrations and variety of abnormal metabolites of diagnostic significance are usually observed in urine samples. We demonstrated clearly in 1985 in this journal (5) that 1H-NMR spectroscopy could be used to diagnose certain inherited organic acidurias within a very short analysis time (5–10 min) by analyzing untreated urine. Subsequently, we have published urine spectra for several additional disorders, e.g., 3-hydroxy-3-methylglutaryl aciduria (7) and urea-cycle disorders (8). Some of these spectra have been confirmed by others (9, 10). Nearly all of these spectra appear to be characteristic for the relevant disorder and, given the rapidity of the test, use of urine for NMR analysis is clearly a method of choice. In our experience, the statement of Wevers et al., that the many resonances in the urine spectra make interpretation more difficult, is quite incorrect.

Indeed, there are other disadvantages in the use of plasma (extracts) for such diagnostic work. As Wevers et al. themselves point out, the complex glucose spectrum obscures the 3.2–3.95 ppm region. Because glucose is normally absent from urine, such serious interference rarely occurs.

Several metabolic disorders show important resonances in this region (7, 8). Wevers et al. also point out a problem with the visibility of methylmalonic acid; because of deuterium exchange with the methine proton in this molecule, only a singlet from the methyl proton is left. In contrast, more than a dozen urine spectra from patients with methylmalonic aciduria have been published (e.g., 5, 11), clearly showing both the doublet and quartet resonances that act as a characteristic "signature" for this molecule. NMR analysis of (unextracted) plasma from one of our patients showed only a very small methylmalonate peak (1.2 ppm) and large signals from free propionate (12). Methylmalonate is rapidly cleared by the kidney, whereas propionate is reabsorbed in the renal tubules.

The single example of a metabolic disorder shown by Wevers et al., despite the title and context of their paper, is that of pyroglutamic aciduria (5-oxopropionuria). This disorder could have been much more easily and quickly diagnosed by analysis of urine. Their paper would have been much more convincing with valid examples of other more common and important metabolic disorders.

Wevers et al. raise an important point about the need for a standard pH for body fluid analysis. We, in discussion with others active in this field, have concluded that it is difficult to achieve an ideal pH: in solving some problems of metabolic interference, e.g., at pH 7–6 betaine and trimethylamine-N-oxide signals overlap, new ones are created. For diagnostic screening we have suggested (13) that optimal analyses may be obtained by running the sample at its natural pH initially, recording the results, and then repeating the analysis both at an acidic pH (2.5) and an alkaline pH (e.g., 8.5). However, if the spectrum from the untreated sample reveals either no abnormal signals or only easily interpretable ones, then the pH adjustment can be ignored and no time has been wasted. Use of extreme pH values alone can present disadvantages; e.g., low pH causes hydrolysis of acyl-carnitines and glutamine and promotes the (artificial) formation of cyclic anhydrides of argininoenecuacine, the last two of which are important in the context of urea-cycle defects (8). Measurement of metabolite chemical shifts in extracellular fluids such as plasma and cerebrospinal fluid at physiological pH is also more relevant to vivo...