Transferrin Synthesis Is Increased in Nephrotic Patients Insufficiently to Replace Urinary Losses

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Abstract. The urinary loss of transferrin is sufficient to reduce plasma transferrin concentrations in the nephrotic syndrome. Hypotransferrinemia may lead to iron loss and microcytic anemia. The mechanism responsible for the hypotransferrinemia in the nephrotic syndrome is, however, unknown. In the present study, synthesis rate of transferrin was measured in vivo in nephrotic patients (n = 7) compared with control subjects (n = 6) using L-[1-13C]-valine. Plasma transferrin and iron concentration in the patients were significantly lower than in control subjects (transferrin, 1.39 ± 0.08 versus 2.57 ± 0.11 g/L, P < 0.0001; iron, 10.2 ± 0.8 versus 21.1 ± 4.5 μmol/L, P = 0.02). Furthermore, albuminuria correlated with transferrinuria (r² = 0.901, P = 0.001). The absolute synthesis rate of transferrin was increased in the patients (10.0 ± 1.1 versus 7.4 ± 0.7 mg/kg per d, P = 0.07), although this value failed to achieve significance. C-reactive protein, plasma iron, and proteinuria did not correlate with transferrin synthesis. In contrast, transferrin synthesis correlated with albumin synthesis (r² = 0.648, P = 0.03; n = 7). The present study indicates that increased transferrin synthesis occurs in nephrotic patients but is insufficient to compensate for urinary losses. Because, overall, no significant relationship was found between transferrin synthesis and either C-reactive protein or iron, it is unlikely that inflammation suppresses or that iron deficiency stimulates increased transferrin synthesis in these patients. The correlation between transferrin synthesis and albumin synthesis suggests that transferrin synthesis is a component of a general response in hepatic protein synthesis in the nephrotic syndrome. This suggests that a therapeutic approach to maximize plasma transferrin concentrations in nephrotic patients should be aimed primarily at reducing urinary protein excretion.

Proteinuria is one of the hallmarks of the nephrotic syndrome. Although albumin is the most commonly measured urinary protein, other proteins, including transferrin, are also lost into the urine. Serum transferrin levels are also decreased in nephrotic patients (1–3). Transferrin is a negative acute-phase protein and is the most important glycoprotein for transport of iron between sites of absorption, storage, and use in the human body. Transferrin is synthesized mainly in the liver (4,5), although small amounts of transferrin are also synthesized in testis, spleen, kidney (6), and brain (7). It is an 80-kD glycoprotein with 2 N-linked carbohydrate chains. The protein has a half-life of 8 to 12 d and has the capacity to bind two ferric atoms (8–11). During childhood and in healthy adults, plasma transferrin concentrations range between 2 and 4 g/L and are not strongly influenced by age and gender (12,13). Genetic hypotransferrinemia and atransferrinemia are extremely rare (14).

Hypotransferrinemia in the nephrotic syndrome may induce low plasma iron concentrations that eventually result in microcytic anemia (15,16), although the urinary loss of erythropoietin may also be significant as a cause of anemia in this patient population (17,18). The pathophysiologic mechanism responsible for the decreased plasma transferrin concentration in the nephrotic syndrome is poorly understood. Transferrin synthesis may be modulated as a component of the acute-phase cascade (19) or as a consequence of iron deficiency (6). Furthermore, malnutrition causes downregulation of hepatic transferrin gene expression, resulting in decreased synthesis (20,21). Inflammation, iron deficiency, and malnutrition are often found in the nephrotic syndrome (22). Jensen et al. (23), using 125I-labeling studies, measured transferrin kinetics in eight patients with the nephrotic syndrome. Both synthesis rate (extrapolated from this indirect measurement of transferrin turnover) and transferrin fractional catabolic rate were increased. This was confirmed by Kaysen et al. (22), who studied transferrin kinetics in rats with Heymann nephritis. However, these radioactive kinetic studies may not represent the physiologic situation, as

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the studied protein is isolated, labeled, and reinjected. Labeling of the protein may alter its conformation; thus, kinetic studies that use stable isotopes for the direct evaluation of endogenously synthesized protein have an advantage over studies that use exogenous tracers.

The present study was undertaken to determine directly whether transferrin synthesis was increased in the nephrotic syndrome using endogenous labeling with $^{13}$C-valine. We further aimed to determine potential mechanisms that lead to the hypotransferrinemia.

**Materials and Methods**

**Patients**

Seven patients (five males, two females) were recruited for this study from the renal division of the University Medical Center Utrecht. Seven nephrotic patients and six control subjects were studied. Six of seven patients, among them five men, had membranous glomerulonephritis. One female patient was known to have focal glomerulonephritis. The clinical data of the patients and control subjects are shown in Table 1. A dietician prescribed a diet containing 0.8 g protein/kg body wt per d and 100 mmol of sodium/d for at least 2 wk before starting the infusion protocol. Besides diuretics, none of them received medication, or medication was stopped at least 2 wk before the infusion study.

Control studies were done in six healthy subjects (four males, two females) who were on a similar diet as the nephrotic syndrome patients. One d before the infusion protocol the subjects collected a 24 h urine sample that was analyzed for urea, creatinine, protein, transferrin, iron and albumin. All patients and volunteers agreed to participate after signing an informed consent form, in accordance with the Helsinki Declaration of Human Rights. The Institutional Ethical Committee for studies in humans approved this study.

**Materials**

$^{1-13}$C-valine (isotope mole fraction > 0.99; Mass Trace, Woburn, MA) was dissolved in sterile 0.9% saline and sterilized through a 0.22-$\mu$m filter. Cyanogen bromide (CNBr)-Sepharose 4B was from Pharmacia (Uppsala, Sweden). Human antitransferrin antibody was from Dade Behring (Marburg, Germany). O-phenylene-diamine (OPDA) was from Sigma (St. Louis, MO). N-methyl-N-(tertiary-butyl(dimethyl)silyl)trifluoroacetamide (MTBSTFA) and pyridine were from Pierce (Rockford, IL). All other chemicals were obtained from Riedel de Haën (Seelze, Germany).

**Infusion Protocol**

The subjects came to the research unit in the morning after a 12-h fast. Two intravenous cannulas were placed for blood sampling and infusion of the labeled valine. No food was given during the tracer infusion, and the subjects were allowed only to drink water.

At baseline ($t = 0$), a priming dose of 15 $\mu$mol/kg $^{1-13}$C-valine was administered intravenously in 2 min, followed by a continuous infusion of 15 $\mu$mol/kg per h $^{1-13}$C-valine during 6 h. Blood samples were taken in heparin-containing and ethylenediamine-tetraacetate-containing tubes from the contralateral arm at $t = 0$, 15, 30, 60, 120, 180, 240, 270, 300, 330, and 360 min. Samples were kept on ice until plasma was separated by centrifugation (20 min, 2000 x g, 4°C).

**Preinfusion Measurements**

Preinfusion plasma and urinary concentrations of transferrin were measured using a routine nephelometric assay (Dade Behring). Urinary iron was measured using the Keberle method (24).

Plasma albumin, total protein, and iron were measured with standard laboratory methods on a Vitros 950 (Johnson & Johnson, Clinical Diagnostics, Rochester, NY). Soluble transferrin receptor was measured using an Immunometric assay (Orion Diagnostica, Espoo, Finland). C-reactive protein (CRP) was measured using an enzyme-linked immunosorbent assay (Detection limit 0.2 $\mu$g/ml; Hemagen Diagnostics, Waltham, MA). Urinary urea, urinary creatinine, proteinuria, and albuminuria were measured with standard laboratory methods on a Hitachi 911 (Boehringer, Mannheim, Germany). The colloid osmotic pressure (COP) was measured using a 4400 colloid osmometer (Wescor Inc., Logan, UT).

**Isolation and Purification of Transferrin from Plasma**

From each sampling point, transferrin was purified by affinity chromatography according to van Eijk et al. (25) with some slight modifications. One ml of human antitransferrin antibody was coupled

| Table 1. Clinical data of the nephrotic patients and the control subjects$^a$ |
|-----------------|-------|-------|--------|-------|
|                 | Renal Disease | Age (yr) | Gender | Weight (kg) | BMI (kg/m²) |
| Nephrotic patients (N = 7) | Membr | 49 | M | 82.1 | 26.5 |
| 1                | Membr | 52 | M | 86.2 | 25.2 |
| 2                | Membr | 31 | M | 73.6 | 21.7 |
| 3                | Membr | 56 | M | 92.0 | 31.8 |
| 4                | Membr | 41 | M | 85.2 | 25.2 |
| 5                | Membr | 55 | F | 96.7 | 33.9 |
| 6                | Focal glom | 63 | F | 70.8 | 25.1 |
| mean             | 50    | 5M/2F | 83.8 | 27.1 |
| ±SEM             | 4     | 3.5   | 1.6  |
| Control subjects (N = 6) | Membr | 33 | 4M/2F | 79.9 | 24.9 |
| mean             | 3     | 5.8   | 1.7  |

$^a$ BMI, body mass index; membr, membranous glomerulonephritis; focal glom, focal glomerulonephritis.
to 7 ml of CNBr-activated Sepharose 4B. Sepharose 4B activation was prepared according to the instructions manual. In short, 2 g of CNBr-activated Sepharose 4B was suspended in 15 ml of 1 mM ice-cold HCl. After centrifugation (10 min, 1750 × g, 4°C), which was repeated each wash step, the gel was washed four times with 1 mM HCl and subsequently washed twice with 0.1 M Borate buffer (pH 8.5) containing 0.5 M NaCl (coupling buffer). After the supernatant was removed, 1 ml of human antitransferrin antibody was diluted 15 times in coupling buffer and added to the activated gel before an overnight incubation. The remaining active groups were blocked with 0.2 M glycine (pH 8.0) for 1 h at room temperature, followed by a washout procedure. The uncoupled human antitransferrin was washed out three times alternately with a high-pH buffer (0.1 M Borate buffer [pH 8.5] containing 1.0 M NaCl) and a low-pH buffer (0.1 M acetate buffer [pH 4.0] containing 1.0 M NaCl). Finally, the high- and low-wash buffers were washed out twice with 0.1 M Borate buffer (pH 8.5) containing 0.5 M NaCl. The gel was stored for a maximum of 4 wk at 4°C.

Ethylendiaminetetraacetate plasma containing approximately 400 μg of transferrin was diluted twice with 0.2 M Na₂HPO₄ containing 1.0 M NaCl. The diluted plasma sample was applied to a column containing 2 ml of gel suspension. Unbound substances were washed away with 30 ml of 0.1 M phosphate-citrate buffer (pH 8.5) containing 0.5 M NaCl. The sample was eluted in 7 ml of phosphate-citrate buffer (pH 2.8) containing 0.5 M NaCl and dried under N₂ at 45°C, followed by hydrolysis in 6 M HCl at 110°C for 24 h (26). The released amino acids were purified by cation exchange chromatography (AG-50W-X8; Bio-Rad Laboratories, Hercules, CA) and dried under N₂ at 50°C. Derivatization was done according to the method of Husek (27).

Isolation and Purification of Albumin

Albumin was isolated according to the method of Korner and Debroy (28). In short, 0.5 ml of heparin plasma was deproteinized with 0.5 ml 20% trichloric acid. After centrifugation (10 min, 1750 × g, room temperature), the pellet was dissolved in 3 ml of ethanol, after which an aliquot of the supernatant was used to monitor protein purity and identification using a 4 to 15% gradient sodium dodecyl sulfate-phastgels. To remove free amino acids, a second aliquot of the ethanolic protein solution, containing 500 μg of protein, was evaporated under N₂ at 45°C. The dried residue was redissolved in 1 ml of 0.3 M NaOH at 37°C for 30 min and cooled to room temperature. After a 5-min ice-incubation step, albumin was reprecipitated with 1 ml of 2 M HClO₄ at 4°C and, subsequently, twice with 1 ml of 0.2 M HClO₄ at 4°C before hydrolysis, cation exchange, and derivatization as described above.

Conversion of Valine into α-Keto-Isovaleric Acid

A standard curve (between 0 and 20% enrichment), prepared by enzymatic conversion of L-[1-13C]-valine into α-keto-isovaleric acid (α-KIV), was used. To each standard (100 μg), 500 μl of Tris-buffer (1.0 M [pH 7.7]) and 50 μl of α-amino acid oxidase (6 mg/ml in Tris buffer, freshly prepared) were added. The standards were incubated for 90 min at 37°C. After incubation, 450 μl of H₂O was added and the standards were derivatized with 1 ml of OPDA (20 mg/ml in 4 M HCl, freshly prepared) for 1 h at 90°C, cooled to room temperature in the dark, and extracted twice with 2 ml of ethylacetate. The ethylacetate phase was dehydrated with Na₂SO₄, transferred to new tubes, and dried under N₂ at 40°C. Next, 100 μl of CHCl₃ was added and dried under N₂ at 40°C. Finally, the samples were derivatized with 50 μl of MTBSTFA:pyridine (3:1) for 15 min at room temperature.

Isolation of α-KIV from Plasma

α-KIV was isolated from plasma according to the method of Rocchicciolo et al. (29) with some slight modifications. In short, 4 ml of absolute ethanol was added to 500 μl of plasma. After centrifugation (20 min, 1750 × g, 4°C), the supernatant was removed and dried under N₂ at 50°C. One ml of H₂O was added, and the samples were derivatized with 1 ml of OPDA for 1 h at 90°C, cooled to room temperature, extracted twice with ethylacetate, and dried. Next, 100 μl of CHCl₃ was added and the samples were dried again followed by derivatization with MTBSTFA:pyridine (3:1) as described above.

Measurement of Stable Isotope Enrichment

The O-t-butyldimethylsilyl-quinoxalinol derivatives of KIV were prepared, based on the procedure of Rocchicciolo et al. (29). Positive chemical ionization was used to measure the ions at m/z 245/246 for plasma [1-13C]-KIV enrichment using electron impact. KIV enrichment was measured by gas chromatography mass spectrometry (GC-MS) on a Hewlett-Packard HP 5890 type II gas chromatograph (Palo Alto, CA) interfaced to an HP 5989B mass spectrometer. The gas chromatograph was equipped with a coating CP Sil 19CB capillary column (Chrompack, Bergen op Zoom, The Netherlands). The injection (2 μl) was performed in the split mode (1:20). The flow rate of the carrier gas (helium) was 1 ml/min. The injector temperature was 240°C, and the oven temperature was programmed starting at 210°C for 1 min, then increased from 210°C to 280°C at 25°C/min and held at 280°C for 3 min. The KIV eluted at 2.6 min. Samples were measured in the selected ion monitoring (SIM) mode. The source and the quadrupole temperature were 250°C and 150°C, respectively.

Amino acids isolated from hydrolysates of transferrin and albumin (m/z 44/45) were analyzed as their N(O,S)-methoxy carbonyl methyl ester derivative by gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS) on a Varian 3400 gas chromatograph, equipped with a CuO combustion oven and interfaced to a DELTA S isotope ratio mass spectrometer (Finnigan-MAT, Bremen, Germany) as described by Reijnoud et al. (30). The GC/MS measurements were performed in duplicate, whereas the GC/C/IRMS measurements were performed only once.

Model and Calculations

Transferrin and albumin fractional synthesis rate (FSR; expressed as %/d) were calculated using a linear regression model. The following standard equation was used:

\[ \text{FSR} (%/day) = \frac{\text{PEt}_6 - \text{PEt}_0}{\text{Epl}} \times \frac{2400}{t_e - t_3} \]  

(1)

\( \text{PEt}_6 - \text{PEt}_0 \) is the increase in enrichment of transferrin-bound [1-13C]-valine over the time period 3 to 6 h \((t_e - t_3)\) of the infusion, and \(\text{Epl}\) is the steady-state plasma tracer/tracee ratio (% of KIV). In this calculation, the plateau enrichment of KIV in plasma is assumed to represent the enrichment of the intrahepatic valine pool from which transferrin is synthesized.

To determine kinetics of several proteins, a mono-exponential can also be used, as described below:

\[ A(t) = A_p[1 - e^{-k(t-d)}] \]  

(2)

\(A(t)\) is the tracer/tracee ratio (%) of valine at time \(t\) (in hours), \(A_p\) is the steady-state plasma tracer/tracee ratio (%) of KIV, \(d\) is the delay time, and \(k\) represents the FSR of the protein. Calculations were done using GraphPad Prism 2.0 software (San Diego, CA).
Comparison of both equations used for calculating protein kinetics, showed an unreliable equation for transferrin kinetics \( (y = 0.67x + 4.17) \) in contrast to albumin kinetics \( (y = 1.03x + 17.3) \). The lack of a mono-exponential model for studying transferrin kinetics is probably due to the enormous delay (±3 h) or to a too low enrichment of L-[1-\(^{13}\)C]-valine in transferrin in the first 3 h of the study protocol. Therefore, we used equation 1 to determine kinetics of both proteins.

The absolute synthesis rate (ASR), which is the amount of protein synthesized per day, was calculated as the product of the FSR and the plasma pool (plasma volume × plasma concentration). Plasma volume was determined using Evans blue as described previously (31). To adjust for different body weights, these quantities are expressed per kilogram of body weight.

**Statistical Analyses**

Data are expressed as mean ± SEM. The significance of differences was assessed by \( t \) test. If normality test or equal variance test failed, a Mann-Whitney rank sum test was performed. Correlations were performed by linear regression analysis.

**Results**

**Baseline Data**

The plasma values of total protein, COP, albumin, transferrin, iron, soluble transferrin receptor, CRP, and plasma volume in nephrotic patients and control subjects are shown in Table 2. Total protein, COP, albumin, transferrin, and iron are significantly different between both groups. Total protein, albumin, and COP are significantly decreased in nephrotic patients compared with control subjects (total protein, 45.5 ± 1.5 versus 68.2 ± 1.9 g/L, \( P < 0.0001 \); COP, 11.2 ± 0.4 versus 25.2 ± 0.4 mmHg, \( P < 0.0001 \); albumin, 22.3 ± 0.7 versus 38.2 ± 0.7 g/L, \( P < 0.0001 \)). Plasma transferrin and plasma iron values are also significantly decreased in nephrotic patients compared with control subjects (transferrin, 1.39 ± 0.08 versus 2.57 ± 0.11 g/L, \( P < 0.0001 \); iron, 10.2 ± 0.8 versus 21.1 ± 4.5 \( \mu \)mol/L, \( P = 0.02 \)). No significant difference is found in the soluble transferrin receptor, plasma CRP values, and plasma volumes in both groups (soluble transferrin receptor, 1.93 ± 0.28 versus 1.74 ± 0.14 mg/L; CRP, 3.6 ± 1.4 versus 3.5 ± 1.5 \( \mu \)g/ml; plasma volumes, 42 ± 2 versus 39 ± 2 ml/kg).

Proteinuria, albuminuria, transferrinuria, and urinary iron loss in nephrotic patients are shown in Table 3. Mean proteinuria in the nephrotic patients is 12.0 ± 2.3 g/d (range, 6.1 to 23.2 g/d). The loss of albumin and transferrin is significantly different between nephrotic patients and control subjects (albumin, 110.7 ± 16.4 mg/kg per d, \( P < 0.001 \); transferrin, 9.7 ± 1.6 mg/kg per d, \( P < 0.001 \)). Urinary iron concentration is significantly higher in the patient group (4 ± 1 versus 1 ± 0 \( \mu \)mol/L, \( P < 0.001 \)). A strong correlation is observed between the loss of albumin and transferrin in urine both expressed in g/d (\( r^2 = 0.901, P = 0.001 \); Figure 1) and both expressed in g/L (\( r^2 = 0.809, P = 0.006 \)). Furthermore, strong correlations are found between proteinuria and transferrinuria (\( r^2 = 0.887, P = 0.002 \)) and between transferrinuria and urinary iron (\( r^2 = 0.761, P = 0.01 \)) in nephrotic patients. Creatinine clearance (86 ± 7 versus 125 ± 8 ml/min per m\(^2\), \( P < 0.001 \)) is significantly decreased in the nephrotic patients. No significant difference is found for urinary urea in both groups (289 ± 37 versus 364 ± 40 mmol/d; Table 3).

**Isolation of Transferrin from Plasma**

Figure 2 shows the elution pattern of the isolated transferrin. The purity of eluted transferrin peak is checked by a 7.5% homogeneous sodium dodecyl sulfate phastgel (Pharmacia, Uppsala, Sweden) (Figure 3). Different fractions in the elution peak (lanes 3 to 6) show one band at approximately 80 kD and

**Table 2. Biochemical parameters in plasma from nephrotic patients and control subjects**

<table>
<thead>
<tr>
<th></th>
<th>Total Protein (g/L)</th>
<th>COP (mmHg)</th>
<th>Albumin (g/L)</th>
<th>Transferrin (g/L)</th>
<th>Iron (( \mu )mol/L)</th>
<th>Soluble Transferrin Receptor (mg/L)</th>
<th>CRP (( \mu )g/ml)</th>
<th>Plasma Volume (ml/kg)</th>
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<td>22.3( ^b )</td>
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\( ^a \) COP, colloid osmotic pressure; CRP, C-reactive protein; NS, not significant.

\( ^b \) \( P < 0.0001 \).

\( ^c \) \( P < 0.05 \).
run at the same size as commercially available transferrin (lane 7). In addition, no contamination with albumin is found in lanes 3 to 6. The transferrin fractions (21–24) were pooled, hydrolyzed, and derivatized as described in the Materials and Methods section.

Table 3. Biochemical parameters in urine from patients and control subjects

<table>
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<tr>
<th></th>
<th>Protein (g/d)</th>
<th>Albumin (mg/kg per d)</th>
<th>Transferrin (mg/kg per d)</th>
<th>Iron (μmol/L)</th>
<th>Creatinine Clearance (ml/min per m²)</th>
<th>Urea (mmol/d)</th>
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<td>110.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> P < 0.001.

Figure 1. Relationship between transferrinuria (g/day) and albuminuria (g/day) in nephrotic patients (n = 7). The 95% confidence limits of the whole group are shown on either side of the regression line (y = 9.92x + 1.20, r² = 0.901).

Figure 2. Elution pattern of purified transferrin using cyanogen bromide-activated Sepharose 4B. •, 1 ml fraction.

**Synthesis Rate of Transferrin in Nephrotic Patients and in Control Subjects**

Transferrin kinetics in nephrotic patients and control subjects are shown in Table 4. The ASR of transferrin in the patient group tends to be higher compared with control subjects (10.0 ± 1.1 versus 7.4 ± 0.7 mg/kg per d; Figure 4), although
statistical significance is not reached ($P = 0.07$). FSR of transferrin is, however, significantly higher in the patients compared with control subjects (17.3 ± 1.4 versus 7.4 ± 0.6%/d, $P < 0.0001$). The wide variation in both groups, however, is striking. Two patients (5 and 7) have lower ASR values (7.1 and 6.2 mg/kg per d, respectively) compared with the mean value in the control group. Both patients also have the lowest FSR (12.7 and 14.6%/d), although these FSR values are twice as high as those in control subjects. The same two patients also have the lowest creatinine clearance (67 and 62 ml/min per m²). Furthermore, patient 7 has the highest CRP value and patient 5 has the third highest CRP value. With the exclusion of these two patients from the group ($n = 5$), the difference in ASR between the nephrotic patients and control subjects was highly significant ($P = 0.007$).

**Synthesis Rate of Albumin in Nephrotic Patients and in Control Subjects**

Albumin kinetics in nephrotic patients and control subjects are shown in Table 4. The ASR of albumin in the patient group is significantly higher compared with control subjects (240.3 ± 26.3 versus 105.3 ± 12.2 mg/kg per d, $P = 0.0012$). Furthermore, FSR of albumin is also significantly higher in the patients compared with control subjects (25.7 ± 1.8 versus 7.2 ± 1.0%/d, $P < 0.0001$).

**Correlations**

Considering the data in control subjects and patients together, several parameters are tested. Transferrin synthesis rates do not correlate with plasma transferrin ($r^2 = 0.183$, $n = 13$), plasma albumin ($r^2 = 0.241$, $n = 13$), or COP ($r^2 = 0.243$, $n = 13$). Within the patient group ($n = 7$), the transferrin synthesis rate did not significantly correlate with plasma iron ($r^2 = 0.116$), plasma CRP ($r^2 = 0.136$), transferrinuria ($r^2 = 0.047$), urinary iron ($r^2 = 0.001$), and proteinuria ($r^2 = 0.074$). Exclusion of patients 5 and 7 also reveals no significant correlation between ASR of transferrin and CRP ($r^2 = 0.128$, $n = 5$). Furthermore, in the patient group ($n = 7$), no correlation was found between albumin synthesis rate and CRP ($r^2 = 0.005$). There was a positive correlation between the synthesis rate for transferrin and albumin in the patient group ($r^2 = 0.648$, $P = 0.03$, $n = 7$) and in the whole group ($r^2 = 0.631$, $P = 0.0012$, $n = 13$).

**Discussion**

Urinary albumin excretion, as well as urinary transferrin excretion, is often observed in the nephrotic syndrome (1). In the present study, a strong correlation was found between albuminuria and transferrinuria ($r^2 = 0.901$, $P = 0.001$), which is in agreement with the study by Howard et al. (32). This indicates that an increase in proteinuria directly reflects transferrinuria. The urinary loss of protein is sufficient to reduce plasma transferrin levels and may lead to iron loss and microcytic anemia.

In the present study, using stable isotopes, we showed that transferrin synthesis in nephrotic patients tended to be increased. Transferrin synthesis was increased 1.3-fold compared with control subjects, although statistical significance was not reached (10.0 ± 1.1 versus 7.4 ± 0.7 mg/kg per d, $P = 0.07$). The failure to reach statistical significance may be due to the wide variation in transferrin synthesis rate in both groups (Figure 4) and the low number of participants in both groups. In the present study, patients and control subjects were not matched for age. Although there is no strong effect of age on plasma transferrin concentration (12,13), no data are available.

**Figure 4.** Box plots showing the absolute synthesis rate (ASR, mg/kg per d) of transferrin in both patients ($n = 7$) and control subjects ($n = 6$). Shown are the mean (dotted line), median (solid line), and range (vertical bars).
on its effect of age on transferrin synthesis directly. The wide variation in transferrin synthesis rate within our control subjects (range, 5.0 to 9.0 mg/kg per d) was also reported by others (22,23,33–35). Two patients (5 and 7) had lower ASR values of transferrin (7.1 and 6.2 mg/kg per d) compared with the mean value in the control group and also had two of the lowest three albumin synthesis rates (169.7 and 187.6 mg/kg per d). The same two patients also had two of the highest three CRP values. Furthermore, both patients also had the lowest creatinine index (an index for muscle mass; 0.79 and 0.88, respectively). The reduction in muscle mass indicates that both patients are not well nourished. Because inflammation and malnutrition affect transferrin synthesis (20,36), this might suggest that the failure of these two patients to increase transferrin synthesis to a greater extent might have been a reflection of an underlying inflammatory event or that malnutrition prevents this response. Exclusion of both patients from the group (n = 5) revealed a significant difference in transferrin synthesis rate between the nephrotic patients and control subjects (P = 0.007). The increase in transferrin synthesis rate in five of seven nephrotic patients is not sufficient to compensate the low plasma transferrin concentrations as shown in this study (1.48 ± 0.08 versus 2.57 ± 0.11 g/L, P < 0.0001). In general, this seems to be what is observed with regard to other liver-derived proteins that are lost into the urine in the nephrotic syndrome. Although albumin synthesis rate is increased, it clearly does not increase plasma albumin levels to normal (31).

For therapeutic reasons, the mechanisms of hypotransferrinemia and the insufficient compensation by synthesis are of relevance. Transferrin is regulated by several control mechanisms. Iron depletion, proteinuria, inflammation, and malnutrition reduce plasma transferrin concentrations. The stimulus for increased transferrin synthesis in the nephrotic syndrome and its regulation are not known.

Our data suggest that iron depletion is not a main stimulus for transferrin synthesis in the nephrotic patients, as no significant correlation was found between plasma iron concentration and transferrin synthesis rate in nephrotic patients (r² = 0.116, n = 7). This is in accordance with the animal studies of Kaysen et al. (22), who described that both transferrin mRNA and transferrin gene transcription were increased in Heymann nephritis and Nagase analbuminemic rats (NAR) and were unaffected by the administration of iron. This suggests that in the nephrotic syndrome, a process other than iron depletion stimulates transferrin synthesis.

There was also no correlation between proteinuria and transferrin synthesis (r² = 0.074, n = 7) or transferrinuria and transferrin synthesis (r² = 0.047, n = 7). This suggests that loss of transferrin in urine is not directly responsible for increased transferrin synthesis in our nephrotic patients, which is in agreement with studies in Heymann nephritis and in NAR (22).

Alternatively, inflammation could play a role in transferrin metabolism, because transferrin synthesis is suppressed as a component of the acute-phase cascade (36). Because no significant relationship has been found between transferrin synthesis and CRP (r² = 0.136) or between albumin synthesis and CRP (r² = 0.005), it is unlikely that inflammation suppresses increased transferrin synthesis for the group as a whole, but it is possible that inflammation and/or malnutrition did significantly suppress the otherwise inadequate synthesis response in the two patients in whom there was overlap with the control group.

Finally, malnutrition could affect transferrin synthesis in nephrotic patients. We recently showed that both nephrotic patients and control subjects exhibited an identical relationship between valine oxidation and urea appearance (37), suggesting that this relationship is unaffected by external loss of protein. This implies that nephrotic patients are able to invoke a normal dietary adaptive response to a low-protein diet, e.g., reducing

### Table 4. Kinetics of transferrin and albumina

<table>
<thead>
<tr>
<th></th>
<th>ASR Transferrin (mg/kg per d)</th>
<th>FSR Transferrin (%/d)</th>
<th>ASR Albumin (mg/kg per d)</th>
<th>FSR Albumin (%/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrotic patients (N = 7)</td>
<td></td>
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</tr>
<tr>
<td>1</td>
<td>12.0</td>
<td>18.7</td>
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<tr>
<td>2</td>
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<td>6</td>
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<td>15.8</td>
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<td>14.6</td>
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<td>mean</td>
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<td>240.3c</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>mean</td>
<td>7.4</td>
<td>7.4</td>
<td>105.3</td>
<td>7.2</td>
</tr>
<tr>
<td>± SEM</td>
<td>0.7</td>
<td>0.6</td>
<td>12.2</td>
<td>1.0</td>
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</tbody>
</table>

a ASR, absolute synthesis rate; FSR, fractional synthesis rate.

b P < 0.0001.
c P < 0.001.
valine oxidation, and patients therefore are not at an increased risk for malnutrition (38). Moreover, malnutrition is associated with a low transferrin synthesis (20), whereas transferrin synthesis in the present study is increased for the nephrotic group as a whole.

We further tested the hypothesis that transferrin synthesis was related to albumin synthesis, because it was suggested that a group of hepatic proteins are upregulated coordinately in the nephrotic syndrome (1,39). Previously, we described a proportionate increase of albumin and fibrinogen synthesis (31), and we tested whether this is also true for albumin and transferrin. A correlation was found between the synthesis rates of both proteins ($r^2 = 0.648, P = 0.03, n = 7$), suggesting that regulation of synthesis of both proteins is interrelated. Other groups (22,23) also observed a strong correlation for transferrin synthesis and albumin synthesis.

In hereditary analbuminemic rats (NAR), transferrin mass was increased entirely as a consequence of increased synthesis (22), suggesting that a low COP could be a stimulus for increased synthesis. In our group, no significant correlation was found between transferrin synthesis rate and COP ($r^2 = 0.243, n = 13$), in contrast to albumin synthesis rate and COP (31).

The therapeutic approach to maximizing plasma transferrin concentrations in nephrotic patients should be aimed primarily at reducing urinary protein excretion rather than at treatment with anti-inflammatory agents, iron supplementation, or a high-protein diet. In addition, urinary loss of iron should not be ignored. In the present study, urinary iron correlated with urinary transferrin levels ($r^2 = 0.761, P = 0.01$), which is in accordance with the study by Howard et al. (32). At a pH of approximately 6, iron dissociates from transferrin, and depending on the state in which iron exists, it catalyzes the Haber-Weiss reaction with the formation of hydroxyl radicals (40,41). These radicals are toxic to virtually all biologic membranes and could play a role in the pathogenesis of the tubulointerstitial disease present in the nephrotic syndrome. Thus replacement of iron lost into the urine may not be risk free and should not be undertaken without clear evidence of iron deficiency.

In conclusion, transferrin synthesis is increased in the nephrotic syndrome, although less so than albumin, and this response does not compensate the low plasma transferrin concentrations. Transferrin synthesis correlates with albumin synthesis, indicating that several proteins synthesized in the liver are upregulated simultaneously in the nephrotic syndrome.

Acknowledgments
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References


