OVERT NEPHROTIC SYNDROME is associated with altered profiles of several plasma proteins. Reduced albumin levels and increased concentrations of both fibrinogen and apolipoprotein (apo)B-100-containing lipoproteins are frequently observed (25). These changes in plasma protein concentrations reflect a resetting of their kinetics. Indeed, increased hepatic albumin synthesis has been shown to occur in humans in the presence of massive albuminuria and/or associated hypoalbuminemia (4, 13, 22). This finding is further supported by the observation that albumin transcript levels are increased (28, 40). The potential stimulus responsible for the upregulation of hepatic protein synthesis in this syndrome has not been clearly identified; however, multiple factors have been proposed, such as a reduced plasma oncotic pressure (24, 47) and/or proteinuria itself (9). In addition, to a coordinated upregulatory response of hepatic synthesis, a decreased body catabolism is felt to occur for selected plasma proteins. Patients with marked proteinuria with or without impaired creatinine clearance exhibit reduced apoB-100 removal (14, 44, 45). A similar finding has been reported for fibrinogen (13), although an increased synthetic rate of this protein in nephrotic patients has also been described (41).

Whether these adaptive mechanisms occur also in milder forms of nephrotic syndrome with normal creatinine clearance is not known. Interest in such investigation arises from the fact that albuminuria, hyperfibrinogenemia, and hyperlipidemia are important risk factors of cardiovascular disease, and they are frequently associated (15). Furthermore, albumin, fibrinogen, and very low density lipoprotein (VLDL) apoB-100 are proteins of hepatic origin. Thus the presence of albuminuria, even at an early stage of disease evolution, may affect the kinetics of some hepatic proteins secreted in plasma and ultimately result in an alteration of their plasma concentrations. It is also important to study patients without signs of renal failure and/or metabolic acidosis to rule out other confounding factors involved in the modulation of protein turnover (3).

Therefore, the aim of this study was to assess simultaneously fractional (FSR) and absolute synthetic (ASR) rates of albumin, fibrinogen, and VLDL apoB-100 in a group of patients with normal creatinine clearance and moderate proteinuria (4 g/day on average) compared with matched healthy control subjects. Leucine isotope precursor-product models were employed to measure protein synthesis.
MATERIALS AND METHODS

Isotopes. L-[4,5-3H]leucine ([3H]Leu) and L-[1-14C]leucine ([14C]Leu) were purchased from Amersham (Amersham, Buckinghamshire, UK). L-[1-13C]leucine ([13C]Leu) was purchased from Tracer Technologies (Masstrace, Woburn, MA). All isotopes were dissolved in saline, filtered through a 0.2-µm filter (Millipore, France), and proven to be sterile and pyrogen-free before use.

Patients. Six adult patients with proteinuria without apparent edema (P; 5 males, 1 female) and with histologically proven glomerulonephritis, and ten age-, sex- and body mass index-matched control subjects (C; 9 males, 1 female) were studied in the postabsorptive state. The clinical and biochemical characteristics of the patients are reported in Table 1. Primary renal disease included membranous (n = 3) and mesangio-proliferative glomerulonephritis (n = 1), IgA disease (n = 1), and diffuse glomerulonephritis (n = 1). The diagnostic biopsies were performed ≥6 mo before the study. Daily proteinuria ranged between 3.5 and 7 g/day (average 4.4 ± 0.6 g/day). For ≥1 mo before the study, all subjects had been consuming a standard diet containing ≥0.45 g of protein/kg body wt −1·day −1 and 100 mmol sodium/day. In the 3 wk before the study, no steroids or hypolipidemic medications were given, and all the other drugs (anti-hypertensive and diuretic agents) were discontinued on the day before the study. No patient had any biochemical sign of either metabolic acidosis or altered bicarbonate concentration. The protocol was approved by the Ethics Committee of the medical faculty at the University of Padova, Italy, and it was performed according to the Helsinki Declaration. The aims and potential risks of the study were explained in detail, and informed written consent was obtained from each subject before the study.

Experimental design. All subjects were admitted to the Clinical Study Unit at 0700 on the morning of the study. A polyethylene catheter was inserted into a dorsal vein of the hand for blood withdrawal. This hand was kept in a heated box at 55°C to obtain arterialized venous blood. Then, a hemoglobin filter (Millipore, France), and proven to be sterile and pyrogen-free before use.

To determine baseline natural

| Table 1. Clinical and biochemical characteristics of proteinuric and control subjects |
|----------|----------|
| Proteinuric | Control |
| Age, yr  | 48 ± 6 | 44 ± 5 |
| Gender, M/F | 5/1 | 9/1 |
| Body mass index, kg/m² | 27.6 ± 1.4 | 24.0 ± 0.7 |
| Plasma creatinine, µmol/l | 97 ± 10 | 90 ± 8 |
| Creatinine clearance, ml/s | 1.8 ± 0.3 | 1.7 ± 0.6 |
| Azotemia, mmol/l | 6.5 ± 0.7 | 5.4 ± 0.7 |
| Plasma albumin, g/l | 31.4 ± 3.3± | 42.6 ± 1 |
| Proteinuria, g/day | 4.4 ± 0.6 | 3.7 ± 0.6 |
| Plasma fibrinogen, g/l | 5.0 ± 0.5 | 2.7 ± 1.4 |
| VLDL apoB-100, mg/dl | 14.1 ± 1.1 | 7.5 ± 0.6 |
| Plasma COP, mmHg | 17.9 ± 1.8 | 25.1 ± 1.1 |
| Triglycerides, mmol/l | 2.5 ± 0.5 | 1.3 ± 0.2 |
| Total cholesterol, mmol/l | 9.1 ± 1.5 | 5.2 ± 0.4 |
| HDL cholesterol, mmol/l | 1.4 ± 0.2 | 1.2 ± 0.2 |
| LDL cholesterol, mmol/l | 6.6 ± 1.2 | 3.6 ± 0.6 |

Values are means ± SE. VLDL, very low density lipoprotein; apoB-100, apolipoprotein B-100; COP, colloid oncotic pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *P < 0.05 vs. control.
liquid chromatography (HPLC) as referenced (34). MPE of $[^{13}C]Leu$ and $[^{13}C]KIC$ in plasma, as well as of $[^{13}C]Leu$ in the VLDL apoB-100 hydrolysate, were determined as t-butyldimethylsilyl derivatives (39) by GC-MS with the use of a Hewlett-Packard model 5988 MS, a capillary column, and electron impact ionization. Plasma insulin and glucagon were determined by radioimmunoassay, as previously described (42).

Isolation of the leucine tracer incorporated into fibrinogen and albumin. Fibrinogen and albumin were simultaneously isolated from plasma samples as previously described (21, 42). Briefly, fibrinogen was extracted by addition of 100 µl of 1 mol/l CaCl$_2$ and 10 IU of thrombin in 100 µl of deionized water to 2 ml of plasma to activate the formation of the fibrin clot. After 1 h of incubation at room temperature, the clot was removed from the residual sample, gently squeezed and washed on filter paper, and then hydrolyzed in 4 ml of 4 N HCl for 48 h at 110°C. The fibrinogen-derived free amino acids were further purified through cation exchange Ag50 $\times 8$ column (21, 42). Removal of amino acids from the column was accomplished by means of 1 × 6 ml of 25% (vol/vol) ammonium hydroxide. The [3H] or $[^{13}C]Leu$ SA derived from fibrinogen was then determined by HPLC as described by Horber et al. (21).

To isolate albumin from the defibrinated plasma, a supersaturated solution of ammonium sulfate was added to remove contaminating globulins. The supernatant was then transferred to clean tubes and acidified with 1 ml of 1 N HCl. After centrifugation at 4°C at 3,000 rpm, the pellet containing albumin was washed twice with 0.1 N HCl to remove the free amino acids in the supernatant. In preliminary studies, the purity of albumin was verified by SDS-PAGE with a 3.5–10% gradient. A single band, corresponding to albumin standard, was detected. The pellet was then hydrolyzed and analyzed by HPLC for leucine SA as described for fibrinogen.

Isolation of VLDL apoB-100 and $[^{13}C]Leu$ analysis. VLDL apoB-100 kinetics was determined using the $[^{13}C]Leu$ stable isotope tracer. Lipoproteins were separated from 4 ml of plasma by means of standard procedures of preparative ultracentrifugation (18) using a L5–65 ultracentrifuge (Beckman Instruments) and a Ti 50.3 rotor at densities of 1.006, 1.063, and 1.21 g/ml to obtain the VLDL, LDL, and HDL subfractions, respectively. Protein content in each sample was quantified by the Lowry assay (31). After delipidation, VLDL apoB-100 was separated from other proteins (mainly apoE) by use of a 3.5–10% gradient SDS-PAGE and staining with Coomassie blue. A single band corresponding to apoB-100 molecular size was recovered and compared with commercially available standard molecular markers. The bands were cut out of the gels, sliced, and acid-hydrolyzed for 24 h. VLDL apoB-100-derived free leucine was then further separated as described for albumin and fibrinogen and was processed for GC-MS analysis.

The concentration of apoB-100 in the VLDL fraction was determined by radioimmunnoassay (Pharmacia & Upjohn, Milan, Italy).

Calculations. Albumin and fibrinogen FSR values (expressed as percent of pool per day) were calculated from the slope of the increase of protein-bound leucine SA vs. time between 120 and 420 min. By that time, the chosen plasma precursor pool (i.e., radioactive KIC) SA, as well as leucine SA, had achieved steady-state conditions (data not shown). The standard equation (20, 42) was used

$$\text{FSR} \times 1,440 \times 100 = \frac{SA_{(t2)} - SA_{(t1)}}{t(2) - t(1)} \times \text{KIC SA}$$

where $SA_{(t1)}$ and $SA_{(t2)}$ represent the slope of protein-bound leucine SA (in dpm/nmol) over time points (t2) and (t1) (in min), respectively (Table 2); SA precursor is plasma KIC SA at steady state; the factor 1,440 is used to normalize data per one day (i.e., to 24 h, or 1,440 min); 100 is to convert FSR to percentages. Plasma KIC SA is classically used as an index of intrahepatic precursor pool for fibrinogen, albumin and apoB-100 synthesis (8, 20, 21, 37, 42).

The apoB-100 leucine MPE in VLDL was analyzed using a monoeponential model (37), described by the equation

$$E_{\text{MPE}} = \lim_{t \rightarrow \infty} \left[ 1 - e^{-k(t-d)} \right]$$

where $E_{\text{MPE}}$ represents the isotopic enrichment of $[^{13}C]Leu$ incorporated into VLDL apoB-100; EP plasma is $[^{13}C]KIC$ MPE at steady-state value; k is the fractional rate at which $[^{13}C]Leu$ appeared into the apoB-100 VLDL pool in the unit of time (minutes). By multiplying k times 1,440 (i.e., the minutes in a day), the number of times the circulating VLDL apoB pool turns over in 1 day is calculated, and d accounts for the delay in appearance of VLDL apoB-100 in plasma. VLDL apoB-100 FSR was also calculated using as precursor the $[^{13}C]Leu$ MPE in the VLDL apoB-100 at its maximum value (see results).

Absolute secretion/synthesis rate (ASR) of each protein (intravascular pools, expressed in mg/day) was calculated by multiplying the corresponding FSR or k value times the plasma pool (Table 2). The intravascular pools were calculated by multiplying plasma concentrations (in mg/l) of each protein times the plasma volume (in l) measured with the dye (see above) (17). Oncotic pressure was calculated using standard formulas (7).

Statistical analysis. All data were expressed as means ± SE. The data of the two groups were compared using the two-tailed Student’s t-test for unpaired data. Log transformation or nonparametric tests were employed when data distribution was not normal. A P value <0.05 was considered statistically significant.

RESULTS

Plasma albumin and fibrinogen concentrations and kinetics. Plasma albumin concentration in proteinuric subjects was reduced by 30% (P < 0.003), whereas fibrinogen was increased by 50% (P < 0.001; Table 1). Plasma volume was increased by ∼15% in the patients (3.9 ± 0.3 vs. 3.4 ± 0.2 l in control, P = nonsignificant (NS)). As a result, the intravascular albumin pool was slightly decreased in the patients (126 ± 20 vs. 144 ± 9 g, P = NS), and that of fibrinogen was markedly increased by twofold (19.5 ± 1.9 vs. 9.1 ± 0.7 g, P <
In the proteinuric group, albumin FSR was about threefold greater (22.3 ± 5.5 vs. 7.6 ± 0.6%/day, P < 0.01; Fig. 1), whereas fibrinogen FSR was not different (P: 21.7 ± 2.2%/day; C: 19.7 ± 1.8%/day, P = 0.2; Fig. 1). Albumin ASR was more than twofold greater (P < 0.001) in P (4.3 ± 0.6%/day) than in C (1.8 ± 0.2%/day; Fig. 2). Fibrinogen ASR was more than twofold greater (P = 0.028) in P than in C (6.6 ± 0.8 vs. 14.2 ± 3.6 pools/day; Fig. 1). As a result, there was no statistical difference in VLDL apoB-100 ASR between the two groups (P: 2.8 ± 0.4 g/day; C: 3.1 ± 0.6 g/day; P = NS; Fig. 2). Similarly, by use as precursor of the maximum value of [13C]Leu VLDL apoB-100 enrichment, FSR was 7.3 ± 0.05 pools/day in P and 12.9 ± 1.8 in C (P < 0.05), whereas ASR was 4.1 ± 0.5 and 3.5 ± 0.1 g/day, respectively (P = NS between the two groups).

**DISCUSSION**

The results of this study show that complex and apparently unrelated alterations of hepatic protein synthesis occur in patients with hypoalbuminemia, modest proteinuria, and normal creatinine clearance. We found that albumin FSR as well as ASR was markedly increased (>2- to 3-fold) in the patients. Albumin FSR was inversely related to calculated oncotic pressure, either including all subjects together (r = −0.74, P < 0.05) or in the proteinuric group only (r = −0.79, P = 0.05; Fig. 4). However, this association was not found in the control group alone. No other correlations were found among either the FSR or the ASR of the three proteins. Also, no correlation between albumin ASR and proteinuria was detected (data not shown).

**Plasma substrates and hormone concentrations.** Plasma total cholesterol and triglyceride concentrations were significantly higher in P than in C (Table 1). HDL cholesterol was normal, whereas LDL cholesterol levels were increased (P = 0.05) in the patients (Table 1). Insulin concentrations were similar in both groups (P = 14 ± 3 μU/ml, C = 12 ± 1 μU/ml, P = NS), whereas plasma glucagon was significantly increased in P (153 ± 27 ng/l vs. 104 ± 11 in C, P < 0.05).

**Fig. 1.** Fractional synthesis/secretion rate (FSR) of albumin, fibrinogen, and very low density lipoprotein apoB-100 (VLDL apoB-100; expressed as %/day) and very low density lipoprotein apoB-100 (VLDL apoB-100; expressed as g/day) in proteinuric and control subjects. *P < 0.01; **P < 0.001 vs. control.

**Fig. 2.** Absolute synthesis/secretion rate (ASR) of albumin, fibrinogen, and VLDL apoB-100 (expressed as g/day). *P < 0.03; **P < 0.001 vs. control.

**Fig. 3.** VLDL apoB-100 [13C]leucine ([13C]Leu) mole percent enrichment (MPE) in the proteinuric (dotted line) and control (solid line) subjects over the experimental period.
whereas fibrinogen FSR was normal. In contrast, VLDL apoB-100 FSR was decreased by 50% in the patients, whereas VLDL apoB-100 ASR was normal because its plasma pool was increased (2-fold). Finally, no correlations were found among the synthetic rates (either fractional or absolute) of any of the three proteins examined, as well as among the rates of synthesis and plasma concentrations. These data indicate that an independent and possibly multifactorial mechanism(s) is responsible for increased synthesis of albumin, hyperfibrinogenemia, and hyperlipidemia occurring in modest, almost subnephrotic, proteinuria.

To our knowledge, this is the first study in which the turnover of three different plasma proteins (albumin, fibrinogen, VLDL apoB-100) has been directly and simultaneously assessed in a group of patients with normal creatinine clearance and moderate proteinuria. We selected a nephrotic population characterized by a fairly homogenous low-to-moderate degree of proteinuria. At variance with other reports, special care was taken to match patients with control subjects, because some anthropometric factors (such as age) are known to affect per se estimates of plasma protein synthesis (16).

Interestingly, we found a marked increase (by ~3-fold) of fibrinogen ASR (i.e., the product of FSR and intravascular pool) in the patients, because both plasma concentration and pool were increased whereas FSR was normal. Recently, De Sain-van der Velden et al. (13) found that fibrinogen ASR was increased by only 60% in a group of nephrotic subjects with heavy proteinuria and impaired creatinine clearance, whereas its FSR was reduced by 30%. However, fibrinogen concentration was increased to an extent similar to that in our study (Table 1). One possible explanation for these different findings is that our population represents an early model of nephrotic syndrome and that concurrent changes in fibrinogen production and disposal occur as the disease progresses. In advanced stages of disease evolution, such as the one described by De Sain-van der Velden et al., a simultaneous decrease of both fibrinogen FSR and catabolism may occur, possibly associated with the massive proteinuria and/or the impaired renal function. The former change may be adaptive, i.e., possibly limiting a further increase in fibrinogen concentration. The mechanism(s) of such regulation is, however, hypothetical and needs to be substantiated by direct experimental evidence. Whatever the explanation, understanding the mechanism(s) responsible for increased fibrinogen concentration in nephrotic syndrome is important, because hyperfibrinogenemia is a very well known risk factor of vascular disease, which has an increased incidence in this syndrome (32, 35). With regard to the possible role of hormones, fibrinogen production is inhibited by insulin (10) but is stimulated by glucagon (42). In our study, insulin concentrations were not different in the two groups, whereas glucagon was significantly increased in the patients. This finding may account, at least in part, for the increased fibrinogen ASR in the nephrotic subjects.

The reason(s) for the increased plasma glucagon concentrations in these patients is unclear. Increased glucagon production is a classic feature of stress (29). The primary glomerular disease in our population was caused by an inflammatory process, which is commonly associated with elevated plasma levels of acute-phase reactants and may be considered a stress situation. Alternatively, impaired renal turnover of glucagon, which may occur in the presence of nephrotic syndrome, could be responsible for this phenomenon. A major role for the kidney in the regulation of glucagon turnover both in normal conditions and during renal failure has been previously demonstrated (5). However, these possibilities are speculative and need to be substantiated by direct experimental evidence.

At variance with fibrinogen kinetics, our results of a marked increase in albumin synthesis are in keeping with previous findings obtained in patients with advanced-stage nephrotic syndrome (4, 13, 22). Thus hepatic albumin overproduction is a feature of proteinuria regardless of its degree. Furthermore, the lack of relationship between daily albumin excretion and either its FSR or ASR in this as well as in previous reports (4, 13, 22) supports the concept that hepatic albumin synthesis may not be modulated by proteinuria. Rather, plasma oncotic pressure, probably at the hepatocyte level, may represent the most relevant reg-

Fig. 4. Inverse correlations between albumin FSR and calculated oncotic pressure in the proteinuric group (left) as well as in all subjects together (right).
ulatory factor (24, 47). This hypothesis is supported by the finding of a negative relationship between oncotic pressure and albumin FSR in the patient group. Interestingly, the lack of association between these two parameters in the control group suggests that this correlation may have physiological relevance only in the presence of reduced oncotic pressure. Obviously this hypothesis needs to be tested directly.

The lack of correlation between proteinuria and kinetic parameters of albumin underscores the complexity of the regulation of the synthesis of this plasma protein. It is known that albumin production is apparently modulated not only by protein loss but also by dietary protein intake (26, 27), physical activity (46), hormonal milieu (10), and plasma oncotic pressure (24). In addition, whole body protein turnover has been shown to adapt successfully to increased urinary protein losses to achieve nitrogen conservation (30, 33).

With regard to lipid and apolipoprotein metabolism, our subjects exhibited increased plasma cholesterol and triglyceride concentrations as expected (23, 25, 36). VLDL apoB-100 concentration was almost doubled in the patients, whereas its FSR was, surprisingly, nearly halved. As a result, VLDL apoB-100 ASR was normal, suggesting that a decreased catabolic rate is mainly responsible for its increased concentrations in these patients, in agreement with previous reports (12, 14). Therefore, these data strongly indicate that the altered metabolism of apoB-100 is not driven by increased albumin synthesis, proteinuria, or decreased plasma oncotic pressure and that it is also not related to fibrinogen kinetics. This finding is further supported by the lack of correlation among either FSR or ASR of albumin, fibrinogen, and VLDL apoB-100. Thus different mechanisms are likely to regulate hepatic protein synthesis as well as whole body plasma protein clearance in nephrotic patients with low-grade proteinuria. Our findings suggest that impaired VLDL apoB-100 catabolism may be an early event in nephrotic syndrome, without any compensatory change in its synthetic rate, as opposed to fibrinogen. At variance with our report, previous studies have shown that VLDL apoB-100 FSR is either increased, normal, or only modestly decreased (12, 14, 43, 45) in patients with marked proteinuria with or without impaired creatinine clearance. It is likely that clinical differences in the selected population may explain, at least in part, the discrepancies in VLDL apoB-100 kinetics among these studies. In particular, it is not known whether an impaired renal function may per se determine an increased plasma concentration of VLDL (2).

In this study, we used a one-compartment model to measure VLDL apoB-100 FSR; however, linear regression analysis and a multicompartmental model have also been used to this end by others (1, 37). Although the latter is commonly considered to better fit some features of VLDL apoB-100 metabolism, the three methods of analysis have been shown to yield comparable results when applied to the calculation of VLDL apoB-100 production rate (37).

In conclusion, our study indicates that patients with modest proteinuria and normal creatinine clearance display a markedly increased albumin synthesis and an atherogenic pattern of plasma fibrinogen and of VLDL apoB-100 concentrations due to complex rearrangements of their kinetics. The increased concentration of fibrinogen is likely due to increased synthesis, whereas that of VLDL apoB-100 is likely due to decreased catabolism. The hyperlipidemia associated with proteinuria is known to be involved in the rate of progression of renal failure (2). Therefore, early therapeutic interventions should be considered to normalize the pattern of hepatic as well as whole body plasma protein turnover, with the aim to affect positively the long-term clinical outcome of these patients.

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