P. Tessari · E. Kiwanuka · R. Barazzoni ·
M. Vettore · M. Zanetti

Diabetic nephropathy is associated with increased albumin and fibrinogen production in patients with type 2 diabetes

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Abstract *Aims/hypothesis:* Hyperfibrinogenaemia and albuminuria are cardiovascular risk factors, often coexisting in diabetic and non-diabetic people. Albuminuria in turn is associated with a compensatory albumin overproduction in non-diabetic patients. It is not known whether the presence of albuminuria in patients with type 2 diabetes mellitus is associated with greater albumin and fibrinogen production rates than in normoalbuminuric patients. *Subjects, materials, and methods:* Using leucine isotope methods, we measured fractional and absolute synthesis rates (FSR, ASR) of albumin and fibrinogen in post-absorptive type 2 diabetic patients with either normal ($n=11$) or increased ($n=10$) urinary albumin excretion. *Results:* In albuminuric patients, albumin FSR ($16.2 \pm 1.5\%/day$) and ASR (20.5 ± 1.9 g/day) were greater ($p < 0.02$ and $p < 0.05$, respectively) than in normoalbuminuric patients (FSR = $11.5 \pm 1.1\%/day$; ASR = 15.7 ± 1.2 g/day). Fibrinogen FSR was similar between patients with normal and increased albumin excretion, but concentration, the circulating pool and ASR of fibrinogen were 40 to 50% greater ($p < 0.035$) in patients with albuminuria. Albuminuria was positively correlated with albumin ASR, with fibrinogen concentration, the fibrinogen pool and ASR, whereas albumin synthesis was inversely correlated with calculated oncotic pressure. *Conclusions/interpretation:* Synthesis of albumin and fibrinogen is upregulated in type 2 diabetic patients with increased urinary albumin excretion. Albuminuria is associated with enhanced fibrinogen and albumin synthesis.

Keywords Absolute synthesis · Albuminuria · Fractional synthesis · Hepatic protein synthesis · α -Ketoisocaproate · Leucine · Precursor pool

Abbreviations Alb⁺: patients with increased AER · Alb⁻: patients with normal AER · ASR: absolute synthesis rate · FSR: fractional synthesis rate · HOMA: homeostatic model assessment · KIC: α -ketoisocaproic acid · SA: specific activity

Introduction

Hyperfibrinogenaemia and albuminuria are established cardiovascular risk factors in diabetic and in non-diabetic populations [1–3]. Hyperfibrinogenaemia is common in type 2 diabetes, and is often associated with albuminuria [4–9]. The mechanism(s) of such an association in diabetes are not known. Fibrinogen and albumin are two liver-synthesised proteins, with different functions and responses to both acute and chronic stimuli [10–17]. In non-diabetic nephrotic syndromes, production of albumin and fibrinogen is increased [18, 19], suggesting coordinate changes in hepatic protein production in response to albuminuria. In type 2 diabetes patients with normoalbuminuria, fibrinogen production is increased [20], whereas that of albumin is normal [21]. At present it is not known whether hepatic albumin production in albuminuric type 2 diabetes patients is also increased, and whether fibrinogen production is further increased in these patients. Knowledge of these potential relationships is important to understand both the mechanistic associations between albuminuria and hyperfibrinogenaemia, and whether the liver in people with diabetes is capable of counteracting the urinary albumin loss with increased production, to maintain the plasma albumin concentration at normal levels.

Therefore, this study was designed to measure fibrinogen and albumin synthesis using leucine isotope methods in patients with type 2 diabetes who had normal or increased urinary albumin excretion; we also investigated the relationships between albuminuria and plasma protein kinetics.

P. Tessari (✉) · E. Kiwanuka · R. Barazzoni ·
M. Vettore · M. Zanetti
Department of Clinical and Experimental Medicine,
Metabolism Division, University of Padua,
Padua, Italy
e-mail: paolo.tessari@unipd.it
Tel.: +39-049-8211748
Fax: +39-049-8754179
e-mail: ekiwanuka@uiss.tv.it
e-mail: barazzon@units.it
e-mail: vettore.monica@unipd.it
e-mail: zanetti@units.it

Subjects, materials and methods

Subjects

We recruited 21 male subjects with type 2 diabetes (disease duration >2.5 years) from patients attending the Diabetes Centre at the University Hospital of Padua, Italy. Eleven patients had a normal urinary AER (<30 mg/day; Alb⁻), whereas the remaining ten had an increased AER (>30 mg/day; Alb⁺), based on two 24-h urine collections. The albuminuric patients were slightly older and had a slightly greater BMI, than the non-albuminuric patients, although the differences were not significant (Table 1). Diabetes duration was, however, longer in the patients with albuminuria. All subjects had been adapted for at least 2 months to a standard weight-maintaining diet containing ~50% of calories as carbohydrates, ~20% as proteins and ~30% as lipids. Their dietary intake was carefully monitored by an expert dietician at the Diabetic Centre. Daily protein intake was unrestricted and was at least >0.8 g/kg body weight in both patients' groups. The hypoglycaemic therapy comprised diet only in one Alb⁻ subject, diet plus oral hypoglycaemic agents (glyburide or gliclazide) in nine Alb⁻ and in two Alb⁺ subjects, oral hypoglycaemic agents plus insulin in five Alb⁺ subjects, and split insulin doses in the remaining subjects. The patients' metabolic control was poor, as shown by the elevated HbA_{1c} concentration (Table 1), and it was not different in the two groups. Plasma creatinine concentration was normal in the Alb⁻ patients, whereas it was moderately increased in the Alb⁺ patients (Table 1). Four Alb⁻ and all the Alb⁺ patients were on anti-hypertensive therapy, with combinations of angiotensin-converting-enzyme inhibitors, diuretics, anti-adrenergic agents and/or calcium antagonists. All drugs were suspended the night before the study day. No patient had clinical signs of either oedema or pleural or abdominal liquid effusion. Background retinopathy and non-critical peripheral vascular stenoses were found in one and two, respectively, of the Alb⁻, and in six and five, respectively, of the Alb⁺ patients. No Alb⁻ subject had any clinical or biochemical evidence of ongoing inflammatory disease, as shown by normal leucocyte counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and α_2 -macroglobulin concentration. In the Alb⁺ patients, the ESR was elevated in seven subjects and CRP was mildly elevated (although less than two-fold the upper normal range) in two subjects. No subject was a current smoker or had been smoking for at least 6 months prior to the study. The protocol was approved by the Ethics Committee of the Medical Faculty at the University of Padua and it complied with the Helsinki Declaration and the recommendations of the local radiation safety officer. The aims and the potential risks of the study were explained in detail to the patients and an informed consent was signed by each subject.

Table 1 Clinical and biochemical characteristics of the type 2 diabetic patients with a normal (Alb⁻) or an increased (Alb⁺) urinary albumin excretion

	Alb ⁻ type 2 diabetes	Alb ⁺ type 2 diabetes
Age (years)	49±3	55±2
BMI (kg/m ²)	28.0±1.3	31.5±1.4
Duration of disease (years)	10±2	19±3 ^b
HbA _{1c} (%)	9.4±0.5	10±1
Fasting glucose (mmol/l)	9.7±0.6	12.3±1.2 ^a
Insulin (nmol/l)	101±8	111±8
Glucagon (pg/ml)	141±14	160±24
C-peptide (ng/ml)	1.82±0.17	3.44±0.6 ^a
HOMA	6.02±0.51	8.60±1.15 ^a
Erythrocyte sedimentation rate (mm)	9.9±2.8	56.4±13.4 ^b
Urinary albumin excretion rate (mg/24 h)	14.1±2.7	3,140±1,250 ^b
C-reactive protein (ng/ml)	3.17±0.02	4.88±0.75
Creatinine (μmol/l)	78±3	155±25 ^a
Albumin (g/l)	43±1	38±2 ^a
Albumin pool (g)	139±6	129±8
Fibrinogen (g/l)	3.25±0.27	4.86±0.41 ^a
Fibrinogen pool (g)	10.5±0.8	16.1±1.2 ^b
Total amino acids (mol/l)	3.11±0.11	3.07±0.18
Branched-chain amino acids (mol/l)	0.51±0.04	0.46±0.05
Total cholesterol (mmol/l)	5.37±0.28	5.38±0.28
HDL cholesterol (mmol/l)	1.11±0.08	0.96±0.08
Triglycerides (mmol/l)	1.59±0.25	1.34±0.17

^a*p*<0.05; ^b*p*<0.02 or less, Alb⁺ vs Alb⁻ type 2 diabetes

Experimental design

All subjects were admitted to the Clinical Study Unit on the morning of the study after an overnight fast. A primed (4×10^5 to 5×10^5 dpm/kg), continuous (8×10^4 to 10×10^4 dpm kg⁻¹ min⁻¹) infusion of L-[4,5-³H]leucine ([³H]Leu) (Amersham, Buckinghamshire, UK) was started at 07.30 h using a calibrated pump and was continued for 180 to 300 min. Venous arterialised blood samples were drawn every 30 to 60 min for the total duration of the study, for measurement of plasma substrates, hormone and isotope concentrations and specific activities (SA), as well as for albumin and fibrinogen isolation.

Biochemical determinations, calculations and statistical analysis

Blood samples (10–12 ml) were collected into tubes containing EDTA (6% w/v), rapidly centrifuged, and the plasma was stored at -20°C until assay. Between 60 and 90 min after the start of isotope infusion, a bolus injection of a dye (Infracyanine, SERB, Paris, France) was administered for the determination of plasma volume [22]. Plasma fibrinogen concentration was measured using

a nephelometer (Behring Nephelometer Analyser, Dade-Behring, Marburg, Germany) [23]. Plasma glucose, albumin, triglyceride, total and HDL cholesterol, creatinine, CRP (not the high-sensitivity assay) and urinary albumin concentrations were all determined by standard laboratory methods. Albumin and fibrinogen were isolated from plasma and hydrolysed using standard and validated methods as previously described [19, 20, 24]. Plasma leucine and α -ketoisocaproate (KIC) concentrations and SA, as well as leucine SA in the albumin and fibrinogen hydrolysate, were determined by HPLC (Waters Spa Italia, Milano, Italy) [24]. Insulin, glucagon and C-peptide concentrations were determined by radioimmunoassay as described elsewhere [20, 21]. Plasma amino acid concentrations were measured with a Beckman Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA, USA).

The albumin and fibrinogen fractional synthesis rates (FSR), expressed as a percentage of the circulating pool of each per day, were calculated from the increment of the SA values of leucine incorporated into either protein with respect to time, and divided by average plasma KIC SA, between 120 min and the end of the infusion (Table 2), using a standard linear relationship [13, 18, 20, 24, 25]. Within this interval, the chosen plasma precursor pool (i.e. KIC) SA, as well as plasma leucine SA, had achieved steady-state conditions (data not shown). Plasma α -[3 H] KIC SA was used as the precursor pool. The absolute synthesis rates (ASR) of the two proteins (expressed in g/day) were calculated by multiplying the FSR value by the corresponding intravascular pools (in g), obtained by multiplying each protein's concentration in plasma by the plasma volume (in litres) [22] (see above). The insulin-sensitivity index was calculated according to the homeostatic model (HOMA) [26].

All data were expressed as means $\pm$ standard error (SE). The two-tailed Student's *t*-test for unpaired data was employed for data analysis and comparisons. The regression analysis to calculate the slopes of albumin- and fibrinogen-bound labelled leucine was performed using Statistica Software (Version 4; StatSoft, Tulsa, OK, USA). A *p* value <0.05 was considered statistically significant.

Table 2 Plasma leucine and α -ketoisocaproate (KIC) specific activities at steady-state; slopes of the increase of albumin-bound and fibrinogen-bound leucine SA vs time (dSA/dt)

	Alb ⁻ type 2 diabetes	Alb ⁺ type 2 diabetes
Leucine SA (dpm/nmol)	4.20 $\pm$ 0.45	4.39 $\pm$ 0.31
KIC SA (dpm/nmol)	3.38 $\pm$ 0.28	3.80 $\pm$ 0.27
Albumin slope (dpm μ mol ⁻¹ min ⁻¹)	0.27 $\pm$ 0.03	0.40 $\pm$ 0.03 ^a
Fibrinogen slope (dpm μ mol ⁻¹ min ⁻¹)	0.53 $\pm$ 0.05	0.53 $\pm$ 0.04

^a*p* <0.01 or less, Alb⁺ vs Alb⁻ type 2 diabetes

Results

The patients with albuminuria had greater concentrations of plasma glucose, fibrinogen, C-peptide, creatinine and a greater HOMA index and fibrinogen pool than the patients without albuminuria (Table 1). Albumin concentration was lower in the albuminuric group (Table 1). Total and branched-chain amino acid concentrations were similar in the two groups (Table 1).

In Table 2, plasma SA of leucine and KIC, as well as the slopes of the increase of albumin-bound and fibrinogen-bound leucine SA vs time, are reported. The albumin slope was greater in the Alb⁺ than in the Alb⁻ patients.

In the patients infused for 300 min, i.e. four Alb⁻ and all the Alb⁺ (both micro- and macro-) patients, we calculated the slopes of the increase of protein-bound SA for both fibrinogen and albumin, in the time intervals between 120 and 180 min, as well as between 120 and 300 min. In the Alb⁻ patients the fibrinogen slope was $0.50 \pm 0.07 \times 10^{-3}$ (mean $\pm$ SE) between 120 and 180 min, and $0.50 \pm 0.05 \times 10^{-3}$ between 120 and 300 min whereas the albumin slope was $0.32 \pm 0.07 \times 10^{-3}$ between 120 and 180 min, and $0.33 \pm 0.07 \times 10^{-3}$ between 180 and 300 min. Similarly, in the Alb⁺ (both micro- and macro-) patients the fibrinogen slope was $0.53 \pm 0.05 \times 10^{-3}$ between 120 and 180 min, and $0.53 \pm 0.04 \times 10^{-3}$ between 120 and 300 min, whereas that of albumin was $0.40 \pm 0.04 \times 10^{-3}$ between 120 and 180 min, and $0.40 \pm 0.03 \times 10^{-3}$ between 180 and 300 min. Therefore, no differences were observed between the 120–180 and the 120–300 min values. Conversely, the plasma KIC SA were stable throughout the study period (data not shown). Therefore, no bias had been introduced in the protein FSR calculated either within the 120–180-min interval or within the 120–300-min interval.

In the Alb⁺ patients, both albumin FSR ($16.2 \pm 1.5\%$ /day) and ASR (20.5 ± 1.9 g/day) were significantly greater (*p* <0.02 and *p* <0.05 , respectively) than in Alb⁻ patients ($11.5 \pm 1.1\%$ /day and 15.7 ± 1.2 g/day) (Fig. 1a,b). When 24-h albumin excretion was added to the daily albumin ASR, to estimate total 24-h albumin turnover and to account for the urinary loss of albumin, the differences between the two groups were even more marked (Alb⁺ patients: 24.2 ± 1.8 g/day; Alb⁻ patients: 15.7 ± 1.2 g/day, *p* <0.001).

Fibrinogen FSR did not differ between the Alb⁺ ($20.4 \pm 1.2\%$ /day) and the Alb⁻ patients ($22.9 \pm 1.8\%$ /day) (Fig. 1c). However, in the Alb⁺ patients the fibrinogen ASR (3.3 ± 0.3 g/day) was greater (*p* $=0.03$) than that in the Alb⁻ patients (2.4 ± 0.2 g/day) (Fig. 1d).

When the two groups of diabetic subjects were compared with a healthy control group (age 43 ± 4 years, BMI 24.9 ± 0.6 kg/m²; data largely published in [21]), albumin FSR in the Alb⁺ patients was greater than in either the Alb⁻ patients or the healthy controls, even when FSR was normalised for body weight because of the lower BMI of the latter group (Table 3). The same results were found for albumin ASR (with data of albuminuria included). Conversely, fibrinogen FSR was not different among the three groups, whereas fibrinogen ASR was greater in the

Fig. 1 Albumin fractional synthesis rate (FSR) (a) and absolute synthesis rate (ASR) (b) in the type 2 diabetic patients without (white bars) and with (hatched bars) albuminuria. $p=0.019$ (a); $p=0.048$ (b). c, d Fibrinogen FSR and ASR, respectively, in the type 2 diabetes patients without (white bars) and with (hatched bars) albuminuria. $p=0.035$ (d)

Alb⁺ patients than in both the Alb⁻ patients and the healthy controls, also after normalisation for body weight (Table 3).

When the diabetic patients were divided into two groups by age, either younger or older than 52 years, no difference in any biochemical or protein kinetics parameters was detected, except for a greater prevalence of albuminuria in the older group (Table 4). In addition, when the albuminuric patients were divided according the presence of either microalbuminuria (30–300 mg/day) or macroalbuminuria (>300 mg/day), albumin ASR was greater in the microalbuminuric than in the macroalbuminuric group (25.1 ± 3.2 vs 17.5 ± 1.7 g/day, $p<0.05$), whereas fibrinogen ASR tended to be greater in the macroalbuminuric group, although the difference was not significant (3.6 ± 0.5 vs 2.8 ± 0.3 g/day, $p=0.28$). In contrast, no differences in albumin FSR (16.4 ± 1.9 vs 16.1 ± 2.4%/day) and fibrinogen FSR (19.7 ± 1.9 vs 20.8 ± 1.7%/day) were observed between the two groups.

Correlations

A direct relationship between albuminuria and albumin ASR was found ($r=0.59$, $p<0.005$). Direct relationships between albuminuria and fibrinogen concentration ($r=0.65$, $p<0.002$), fibrinogen pool ($r=0.66$, $p<0.002$) and fibrinogen ASR ($r=0.53$, $p<0.01$) were found. Fibrinogen FSR was also positively correlated with HbA_{1c} ($r=0.42$, $p<0.05$). Inverse correlations were found between oncotic pressure and either albumin FSR ($r=-0.43$, $p<0.05$) or ASR ($r=-0.44$, $p<0.05$). No correlations were found between oncotic pressure and either fibrinogen FSR or ASR.

Table 3 Albumin and fibrinogen FSR (expressed as %/day) and ASR (in g/day) in the type 2 diabetic patients with a normal (Alb⁻) or an increased (Alb⁺) urinary albumin excretion, compared to a healthy control group

	Alb ⁻ type 2 diabetes (n=11)	Alb ⁺ type 2 diabetes (n=10)	Healthy controls (n=15)
Albumin FSR	11.5±1.1	16.2±1.5 ^{a,b}	9.1±0.8
Albumin ASR	15.5±1.2	20.5±1.9 ^{a,b}	13.9±1.3
Albumin ASR ^d	15.5±1.2	24.2±1.8 ^{a,b}	13.9±1.3
Albumin FSR/kg	0.14±0.01	0.18±0.02 ^{a,b}	0.12±0.01
Albumin ASR/kg	0.19±0.01	0.23±0.02	0.18±0.02
Albumin ASR/kg ^d	0.18±0.01	0.27±0.02 ^{a,b}	0.18±0.02
Fibrinogen FSR	22.9±1.8	20.4±1.2	20.2±2.1
Fibrinogen ASR	2.38±0.24	3.30±0.31 ^{a,b}	1.78±0.19
Fibrinogen FSR/kg	0.28±0.02	0.22±0.01	0.27±0.03
Fibrinogen ASR/kg	0.028±0.003	0.038±0.005 ^{b,c}	0.024±0.003

The control data have been reported in part elsewhere [21]. Data are expressed also after normalisation to body weight (per kg)

^aStatistically significant difference vs the Alb⁻ type 2 diabetic patients ($p<0.05$ or less), whereas ^bdenotes a statistically significant difference vs the healthy control subjects ($p<0.025$ or less), by one-way ANOVA and the post hoc Bonferroni test. ^c $p<0.1$ vs the Alb⁻ type 2 diabetic patients

^dRefers to ASR with the addition of albuminuria

Table 4 Clinical, biochemical and protein kinetics characteristics of the type 2 diabetic patients, when these were divided into two groups (younger than/older than 52 years), irrespective of the rate of increased urinary albumin excretion

	Type 2 diabetes <52 years old	Type 2 diabetes >52 years old
Number of subjects	11	10
Age (years)	46±2	58±2 ^a
BMI (kg/m ²)	29.1±1.2	30.0±1.6
Duration of disease (years)	13±3	16±3
HbA _{1c} (%)	10.2±0.7	9.5±0.4
Fasting glucose (mmol/l)	10.0±0.9	12.0±1.0
Insulin (nmol/l)	109±9	102±6
Glucagon (pg/ml)	124±11	167±22
C-peptide (ng/ml)	1.98±0.26	3.26±0.61
HOMA	6.68±0.72	7.77±1.08
Erythrocyte sedimentation rate (mm)	27.1±9.5	39.2±14.7
Urinary AER (mg/24 h)	690±638	2,396±1,213
Number of subjects with either normal or increased AER	7/4	3/7
Creatinine (μmol/l)	89±11	142±26
Albumin (g/l)	42±1	39±2
Albumin pool (g)	127±14	129±9
Fibrinogen (g/l)	4.00±0.44	4.03±0.41
Fibrinogen pool (g)	13.1±1.4	13.2±1.2
Albumin FSR (%/day)	14.1±1.3	13.5±1.7
Albumin ASR (g/day)	17.4±2.6	16.3±1.3
Albumin ASR + albuminuria (g/day)	18.2±2.8	18.7±1.6
Fibrinogen FSR (%/day)	23.2±1.7	19.9±1.2
Fibrinogen ASR (g/day)	3.0±0.4	2.6±0.2

^a*p*<0.006, <52- vs > 52-year-old type 2 diabetic subjects

Discussion

Albuminuria is a marker of renal damage and a hallmark of progression to renal insufficiency [27–29]. It increases cardiovascular risk in type 2 diabetes mellitus [30, 31], probably because it reflects widespread increased vascular permeability causing organ damage [28, 31, 32]. Albuminuria and hyperfibrinogenaemia, another cardiovascular risk factor, are frequently associated [7, 8] in diabetes. Such an association is important in that fibrinogen, an acute-phase protein, is a powerful and independent cardiovascular risk factor [1–3]. However, the mechanism(s) of the association between hyperfibrinogenaemia and albuminuria, as well as the response of hepatic albumin synthesis to albuminuria in type 2 diabetes, are not known.

In non-diabetic nephrotic syndromes [18, 19] albuminuria is associated with an upregulation of albumin synthesis, probably mediated by the decreased oncotic pressure at the hepatic level [33, 34], which counteracts the increased urinary albumin loss. In these conditions, fibrinogen synthesis is also increased [18, 19], suggesting an upregulation of hepatic secretory proteins. Thus, a link between albuminuria and the altered fibrinogen metabolism

can be suspected also in type 2 diabetes, possibly at the site of liver production. However, whether these mechanisms are operating in type 2 diabetes as well is not known.

In this study we show that both albumin and fibrinogen productions are greater in type 2 diabetic patients with albuminuria than in patients with a normal urinary excretion rate. Positive correlations between the degree of albuminuria and both albumin and fibrinogen synthesis have been found. These observations suggest that upregulation of hepatic protein synthesis, probably in response to the increased urinary albumin loss, operates in type 2 diabetes with nephropathy. Albuminuria was also directly correlated with fibrinogen concentrations and the circulating pool of fibrinogen. Conversely, an inverse relationship between albumin production and oncotic pressure was demonstrated. Taken together, these data indicate that albuminuria in type 2 diabetes may represent a trigger for increased albumin production, as well as for a further increase of fibrinogen concentrations and production, and that the decreased oncotic pressure, secondary to albuminuria, may mediate the increased hepatic albumin synthesis. However, because these conclusions are based on statistical associations rather than on a direct pathophysiological demonstration, the intrinsic mechanism(s) of these associations remains elusive.

Among the albuminuric patients, albumin FSR was similar in the micro- and macroalbuminuric subgroups, indicating no further increase of hepatic albumin synthesis with macroalbuminuria. However, from our data it is not possible to conclude whether these patients can increase their albumin synthesis rates further. In contrast, albumin ASR was lower in the macro- than in the microalbuminuric subjects, probably because the albumin pool (i.e. a factor in the calculation of ASR from FSR) was reduced in these patients because of the urinary loss of albumin.

That fibrinogen synthesis is increased in type 2 diabetes, even in the absence of micro- or macroalbuminuria, has been previously demonstrated [20, 35]. The present study adds to this information, showing that a further increase not only of fibrinogen concentration but also of its synthesis occurs when type 2 diabetes patients are also albuminuric (Table 1) [7, 8]. The slightly higher rate of fibrinogen absolute synthesis rate in the macro- vs the microalbuminuric patients may indicate some sort of consumptive coagulopathy or an increased rate of disappearance of fibrinogen in the former. On the whole, the additional CV risk associated to albuminuria may be, at least partly, the result of the increased fibrinogen concentration and production of these patients. In contrast to fibrinogen, because albumin production is normal in normoalbuminuric type 2 diabetes subjects [21], an increased hepatic albumin production in type 2 diabetes occurs when albuminuria also occurs.

We also compared the protein kinetics data of both the Alb[−] and the Alb⁺ diabetic patients with those of a healthy control group, largely published before in a companion study (ref. [21] and Table 3). In the albuminuric, type 2 diabetic patients, both albumin FSR and ASR, as well as fibrinogen FSR, were greater than in either the normo-

albuminuric patients or the healthy controls, further confirming the association between albuminuria and the increased rates of these plasma proteins.

The mechanism(s) possibly associated with the increased fibrinogen production in type 2 diabetes have been previously discussed in detail [20, 21, 35], and may include insulin resistance, hyperglucagonaemia, increased fibrinogen degradation products acting as stimulators of fibrinogen production in the liver, and possibly, also a subclinical inflammatory state otherwise not detectable by standard assays. In our albuminuric type 2 diabetes patients, the increased ESR, which is a common finding in albuminuria [36], may indicate the occurrence of a mild inflammatory state, despite the normality of other biochemical (leucocyte counts, urinalysis, α_2 -globulins) and clinical indices of inflammation, with the exception of a mild, albeit insignificant, increase of CRP. Therefore, in addition to the previously mentioned factors, a subclinical inflammatory condition, with the associated expected changes in inflammatory cytokines (that were not measured in this study), may represent a stimulus towards increased fibrinogen production. On the other hand, that inflammation cannot be the only cause of the observed metabolic increase in hepatic protein synthesis is supported by the fact that albumin is a negative acute-phase protein [10, 37], therefore its synthesis should be depressed, and not increased, by inflammation, which is in contrast with our present findings. Since diabetes duration was greater in the albuminuric patients than in the non-albuminuric patients, disease duration could represent an additional variable in the observed findings.

Albumin synthesis is physiologically stimulated by insulin and amino acids [10, 13–15, 38]. Although insulin concentration was similar in both groups, the increased C-peptide concentration, an index of increased endogenous insulin secretion, observed in the albuminuric subjects could constitute a factor contributing to their increased albumin synthesis. Conversely, because total and branched-chain amino acid plasma concentrations were similar in the two groups, they should not have contributed to the albumin overproduction in the albuminuric subjects. The degrees of metabolic control (HbA_{1c}) and insulin resistance (HOMA index) were not associated with the increased albumin production (data not shown). However, a direct relationship between HbA_{1c} level and fibrinogen FSR was found, suggesting that metabolic control may be linked to fibrinogen production.

Although there were small, albeit insignificant, differences in age between the two groups, rearrangement of the data on the basis of an age either below or above 52 years resulted in no differences between the two groups in either albumin or fibrinogen kinetics, supporting the conclusion that age per se did not have any confounding role on albuminuria-associated increased albumin and fibrinogen production in type 2 diabetes.

The albuminuric diabetic patients had a moderate increase of creatinine concentration (Table 1). Although renal failure may theoretically affect plasma protein synthesis, albumin fractional synthesis rate was found to be normal in end-stage

renal disease patients undergoing haemodialysis [39], contrary to the present finding of an increased albumin FSR in the diabetic group with nephropathy. Thus, it is unlikely that the relatively modest (i.e. two-fold) increase in creatinine concentration in the albuminuric type 2 diabetes group had any significant effect.

The fact that all the albuminuric patients were receiving hypotensive drugs, as opposed to a lower number of subjects being treated in the normoalbuminuric group, might have affected the results because fibrinogen concentration can be reduced by angiotensin-converting-enzyme inhibitors [40]. However, because the fibrinogen concentration was greater in the albuminuric patients despite their drug therapy, their 'spontaneous' fibrinogen levels might have been even greater than those observed here. The results of this study should therefore be considered conservative. As a matter of fact, our study provides a picture of plasma protein synthesis in normoalbuminuric diabetic subjects in their usual clinical and therapeutic setting.

In conclusion, this study demonstrates that albumin and fibrinogen synthesis are increased in albuminuric type 2 diabetes subjects compared with type 2 diabetes patients with normoalbuminuria, showing an upregulation of hepatic secretory proteins in this clinical condition. Such an upregulation seems to be responsible for the (relative) hyperfibrinogenaemia observed in the albuminuric diabetic patients. Albuminuria, through as yet unknown mechanisms, could thus represent a key factor. The increased albumin production in turn, may be inversely associated with oncotic pressure. This study casts new light on the pathophysiological mechanisms of the association between albuminuria and hyperfibrinogenaemia in type 2 diabetes, as well as on possible therapeutic interventions.

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