Albumin and Fibrinogen Synthesis and Insulin Effect in Type 2 Diabetic Patients With Normoalbuminuria

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OBJECTIVE — Insulin stimulates albumin synthesis but inhibits that of fibrinogen in both type 1 diabetic and healthy subjects. In type 2 diabetes, fibrinogen production is increased both in the postabsorptive state and in response to hyperinsulinemia. No data exist on the rate of albumin synthesis and its response to insulin in type 2 diabetes.

RESEARCH DESIGN AND METHODS — We measured fractional synthesis rates (FSRs) and absolute synthesis rates (ASRs) of both albumin and fibrinogen in postabsorptive normoalbuminuric type 2 diabetic patients at their spontaneous glucose levels (study A), as well as albumin FSR and ASR before and after a hyperinsulinemic-euglycemic euaminoacidemic clamp (study B), using leucine isotope methods.

RESULTS — In postabsorptive type 2 diabetes (study A), albumin FSR (11.2 ± 0.9%/day) and albumin ASR (15.4 ± 1.2 g/day) were not different from control values (albumin FSR: 9.4 ± 0.7%/day; albumin ASR: 13.8 ± 1.2 g/day, $P > 0.1$ for both). In contrast, in the type 2 diabetic subjects, both fibrinogen FSR (24.9 ± 2.1%/day) and ASR (2.4 ± 0.2 g/day) were greater ($P < 0.025$ and $P < 0.007$, respectively) compared with the control subjects (FSR: 18.6 ± 1.51%/day; ASR: 1.6 ± 0.2 g/day). Worse metabolic control in the type 2 diabetic patients was associated with hyperfibrinogenemia and increased leucine rate of appearance, whereas neither the (increased) fibrinogen ASR nor the (normal) albumin production was affected. In study B, after hyperinsulinemia (raised to ~860 nmol/l), albumin FSR and ASR increased by ~25% versus basal ($P < 0.04$) and to the same extent in both type 2 diabetic and control subjects.

CONCLUSIONS — In normoalbuminuric type 2 diabetic patients, postabsorptive albumin synthesis and its response to insulin were normal, whereas fibrinogen synthesis was increased, irrespective of metabolic control. Furthermore, in normoalbuminuric type 2 diabetic patients, a normal insulin sensitivity with respect to albumin production but a selective hepatic dysregulation of fibrinogen metabolism were present.

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Albumin and fibrinogen are two liver-synthesized proteins differently regulated (1,2). Both insulin and diabetes affect their kinetics. In insulin-deprived type 1 diabetic patients, fibrinogen synthesis was increased and that of albumin decreased (3), with these changes being reversed by insulin (3). In type 2 diabetes, fibrinogen concentration (4) and synthesis (5) were increased, whereas insulin infusion paradoxically increased further fibrinogen synthesis in type 2 diabetic but not in control subjects (6). However, the rate of albumin synthesis, either in the fasting state or in response to hyperinsulinemia, has never been determined in type 2 diabetes. In addition, the possible effects of metabolic control on fibrinogen and albumin kinetics in type 2 diabetes are not known.

Therefore, this study was designed to measure synthesis rates of albumin and fibrinogen in normoalbuminuric type 2 diabetic patients and in matched control subjects, using leucine isotope methods, both in the postabsorptive state at the patients' spontaneous glucose levels after the overnight fast and after a hyperinsulinemic-euglycemic euaminoacidemic clamp. Because albuminuria per se can be associated with alterations of albumin and fibrinogen synthesis rates (7), in this study, we intentionally selected diabetic patients without nephropathy.

RESEARCH DESIGN AND METHODS

— Thirteen male type 2 diabetic subjects (disease duration of >2.5 years) were recruited from patients attending the Diabetes Center at the University Hospital of Padova, Italy. Fourteen male control subjects were also enrolled. The subjects' clinical characteristics are reported on Table 1. All the type 2 diabetic and the control subjects had a normal urinary albumin excretion rate (i.e., <20 μg/min), based on two 24-h determinations: normal albumin and creatinine plasma concentrations and normal oncotic pressure. All subjects had been adapted for at least 2 months to a carefully monitored standard weight-maintaining diet containing ~50% of calories as carbohydrates, ~20% as protein (>0.8 g/kg body wt, unrestricted), and ~30% as lipids. The patients' BMI was slightly (by ~10%), although significantly, greater than that of the control subjects (Table 1). The hypoglycemic therapy in the patients consisted of diet only in two subjects, insulin in one subject, and diet plus oral hypoglycemic agents (glyburide, glycyclamide, or tolbutamide) in the remaining

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Abbreviations: ASR, absolute synthesis rate; BCAA, branched-chain amino acid; FSR, fractional synthesis rate; HOMA, homeostatic model assessment.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical and biochemical characteristics of type 2 diabetic patients and control subjects*

	Type 2 diabetic patients	Control subjects
n	13	14
Age (years)	49 ± 2	43 ± 4
BMI (kg/m^2)	27.9 ± 1.5*	24.8 ± 0.6
Duration of disease (years)	10 ± 2	—
A1C (%)	9.1 ± 0.4†	4.6 ± 0.4
Fasting glucose (mmol/l)	9.7 ± 0.6†	4.8 ± 0.2
Insulin (nmol/l)	101 ± 8	75 ± 8
Glucagon (pg/ml)	141 ± 14*	109 ± 8
HOMA	6.02 ± 0.47†	2.26 ± 0.26
Erythrocyte sedimentation rate (mm)	10 ± 3	7 ± 2
Urinary albumin excretion rate (mg/24 h)	13.2 ± 2.6	10.2 ± 0.6
C-reactive protein (ng/ml)	3.29 ± 0.05	3.17 ± 0.02
White blood cells (per μl)	5,876 ± 396	5,020 ± 143
α_2-Macroglobulin (%)	8.8 ± 0.3	5.6 ± 0.1
Creatinine (μmol/l)	78 ± 3	86 ± 3
Albumin (g/l)	43 ± 2	43 ± 1
Fibrinogen (mg/dl)	325 ± 27*	255 ± 11
Total amino acids (mmol/l)	3.12 ± 0.11	2.84 ± 0.09
BCAAs (mol/l)	0.52 ± 0.05	0.44 ± 0.02
Total cholesterol (mmol/l)	5.1 ± 0.3	5.0 ± 0.3
HDL cholesterol (mmol/l)	1.1 ± 0.1	1.2 ± 0.1
Triglycerides (mmol/l)	1.7 ± 0.2	1.2 ± 0.2
KIC SA (in DPM/nmol) or TTR	3.80 ± 0.37	3.98 ± 0.60
Leucine SA (in DPM/nmol) or TTR	4.95 ± 0.61	4.97 ± 0.82
Albumin slope (dSA/dt, in DPM · μmol^{-1} · min^{-1}, or dTTR/dt [in min] × 1,000)	0.27 ± 0.03	0.27 ± 0.07
Albumin FSR (percent of circulating pool/day)	11.22 ± 0.91	9.42 ± 0.70
Albumin pool (g)	139.5 ± 7.7	148.5 ± 8.1
Albumin ASR (g/day)	15.4 ± 1.2	13.8 ± 1.2
Fibrinogen slope (dSA/dt, in DPM · μmol^{-1} · min^{-1}, or dTTR/dt [in min] × 1,000)	0.52 ± 0.04	0.53 ± 0.09
Fibrinogen FSR (percent of circulating pool/day)	24.9 ± 2.1*	18.6 ± 1.5
Fibrinogen pool (g)	11.1 ± 0.8	8.6 ± 0.4
Fibrinogen ASR (g/day)	2.4 ± 0.2†	1.6 ± 0.2

Data are means ± SE. The following were applicable in study A only (n = 12): plasma leucine and α-ketoisocaproate (KIC) pooled specific activities (SAs) and trace-to-tracee ratios (TTRs) at steady state, slopes of the increase of fibrinogen- and albumin-bound leucine SA (dSA/dt) or TTR (dTTR/dt) versus time, albumin and fibrinogen circulating pools, albumin and fibrinogen fractional synthesis rates, and absolute synthesis rates (see RESEARCH DESIGN AND METHODS and RESULTS for further details). *$P < 0.05$, †$P < 0.01$ vs. control subjects.

ten subjects. Three diabetic and two control subjects were on antihypertensive therapy, with combinations of ACE inhibitors, diuretics, antiadrenergic agents, and/or calcium antagonists. All drugs were suspended the night before the study day. The patients' metabolic control (HbA_{1c} [A1C]) on average was poor (Table 1), despite a wide range of A1C values. To assess the role of metabolic control on the parameters measured, the type 2 diabetic subjects were further divided in two groups, with either a worse (n = 7) or a better (n = 6) metabolic control, arbitrarily defined on the basis of an A1C level either above or below 8.2%. No patient had any clinical sign of either edema or pleural and abdominal liquid effusion. Background retinopathy and noncritical peripheral vascular stenoses were found in one and two diabetic subjects, respectively. No subject had any clinical or biochemical evidence of ongoing inflammatory disease, as shown by normal leukocyte counts, erythrocyte sedimentation rate, C-reactive protein (conventional assay), and/or α_2-macroglobulin concentrations. No subject was either a current or a recent smoker. The protocol was approved by the ethics committee of the Medical Faculty and by the Radiation Safety Officer of the University of Padova, and it complied with the Helsinki Declaration. Aims and potential risks of the study were explained in detail, and an informed consent was signed by each subject.

Experimental design

All subjects were admitted to the Clinical Study Unit at 0730 on the morning of the study after the overnight fast. Two studies were performed in two different partially overlapping groups of type 2 diabetic and control subjects.

Study A: Albumin and fibrinogen kinetics in the basal state. All the type 2 diabetic subjects and 12 control subjects were studied first in the postabsorptive state. The physical characteristics of the two groups were not vastly different, including BMI (type 2 diabetic patients: 27.9 ± 1.5 kg/m^2; control subjects: 25.3 ± 0.8 kg/m^2; NS). In 9 type 2 diabetic and 10 control subjects, a primed ($4–5 \times 10^5$ DPM · kg^{-1})-continuous ($8–10 \times 10^4$ DPM · kg^{-1} · min^{-1}) infusion of L-[4,5-^{3}H]-leucine (^{3}H-leu) (Amersham, Buckinghamshire, U.K.) (sterile and apyrogen) was started at 0730 by means of a calibrated pump and continued for 180–300 min. In the remaining four type 2 diabetic subjects and in two control subjects, a primed (4.8 μmol/kg)-continuous infusion (0.8 μmol · kg^{-1} · h^{-1}) of the stable isotope L-[5,5,5-^{2}H]-leucine (D_3-leu) (Mass Trace, Woburn, MA) (sterile and apyrogen) was used in the place of ^{3}H-leu. The use of two leucine isotopes was due to changes in regulation at our institution forbidding the use of radioactive isotopes after the first studies performed with the ^{3}H-leu tracer. The data from the subjects infused either with the ^{3}H-leu or the D_3-leu tracers within each group were pooled, since the results in both isotope subgroups were similar (data not shown). Two of the originally recruited control subjects were excluded from these analyses because of their younger age and a significantly lower BMI, which introduced differences between the two groups. Venous-arterialized blood samples were drawn every 30–60 min for 180–300 min.

Study B: Effects of hyperinsulinemia on albumin kinetics. Four type 2 diabetic subjects and four control subjects infused with the ^{3}H-leu tracer and four type 2 diabetic subjects and two control subjects infused with the stable isotope D_3-leu tracer of study A, plus two addi-

tional control subjects infused with the stable isotope D_3-leu tracer, were studied in both the basal state for 180 min and thereafter for a further 180 min after a euglycemic-hyperinsulinemic euaminoacidemic clamp. The physical characteristics in the two groups were not different, including BMI (type 2 diabetic subjects: 28.4 ± 1.7 kg/m^2; control subjects: 24.5 ± 0.8 kg/m^2; NS). The technical details of this study, as well as the fibrinogen kinetic data of this group, had been previously reported (6).

Biochemical determinations

The intravascular space was determined with dye injection (7,8). Plasma insulin, glucagon, amino acid (5,7), and fibrinogen concentration (9) were measured as referenced. Plasma glucose, albumin, triglyceride, total and HDL cholesterol, and creatinine concentrations were determined by automatic methods using a COBAS Mira Auto Analyzer (Roche Italia, Milan, Italy) and specific reagents (Dade-Behring, Marburg, Germany). Plasma protein profiles were determined with standard electrophoresis. Circulating blood cells and the erythrocyte sedimentation rate were determined by photometrical capillary stopped-flow kinetic analysis (Test1TH; Alifax, Padova, Italy). C-reactive protein was measured by nephelometry on a BN2 analyzer (Dade-Behring) (a non–high-sensitive assay). Urinary albumin was determined by radioimmunoassay (Immunotech; Beckman Coulter, Prague, Czech Republic) (10).

Albumin and fibrinogen were isolated from plasma and hydrolyzed and analyzed for the leucine specific activities as previously described (3,5–7,11,12). Plasma leucine and α-ketoisocaproate concentrations and specific activity (11), and plasma D_3-leu and α-D_3-ketoisocaproic acid enrichments (12), were measured as referenced. The D_3-leu enrichment in the albumin hydrolysate was determined after extraction with dimethoxypropane, esterification with acetyl chloride:propanol, acylation with acetone:trimethylacetic anhydride, and final resuspension with ethyl acetate. A Delta Plus GC-Combustion-Isotope Ratio Mass Spectrometer (GC-C-IRMS) (Thermoquest, Bremen, Germany) equipped with a hydrogen cap and an Ultradue GC column (length: 50 m, 0.25 μm i.d.; J&W Agilent Technology Italia SPA, Cernusco sul Naviglio, Milan, Italy) was used. Enrichments were expressed as tracer-to-tracee ratios (13).

Calculations and statistical analysis

Albumin and fibrinogen fractional synthesis rates (FSRs) (expressed as percent of the circulating pool per day) were calculated using standard precursor-product relationships (3,5–7,11,14). Plasma α-^{3}H-ketoisocaproic acid specific activity or α-D_3-ketoisocaproic acid tracer-to-tracee ratio was used as the precursor pool, when the ^{3}H-leu or the D_3-leu tracer was infused, respectively (3,5–7,11,14). The intravascular absolute synthesis rates (ASRs) of the two proteins (expressed in grams per day) were calculated by multiplying the FSR value times the corresponding intravascular pools (in grams), on turn obtained by multiplying the plasma protein concentration times plasma volume (in liters) measured with dye injection (8) (see above). Total ASR may be underestimated to the extent that the protein pool is extravascular, which occurs mainly (by 60%) for albumin (1). The leucine rate of appearance (total and endogenous, during the clamp) was calculated using the reciprocal pool model (15). The insulin sensitivity index in study A was calculated according to the homeostatic model assessment (HOMA) (16). In study B, insulin sensitivity was calculated from the rate of exogenous glucose infusion required to maintain euglycemia (*M* value).

All data were expressed as means ± SE. The statistical analysis was performed using the two-tailed Student's *t* test for unpaired data when two sets of data were compared. Two-way ANOVA for repeated measurements was used to analyze comprehensively the data of both the basal and the clamp periods in study B. The regression analysis to calculate the slopes of albumin- and fibrinogen-bound labeled leucine was performed using the Statistica Software (version 4; StatSoft, Tulsa, OK). A *P* value of <0.05 was considered statistically significant.

RESULTS

Albumin and fibrinogen kinetics in the postabsorptive state (study A)

The type 2 diabetic patients as a group had greater fasting glucose, A1C, glucagon, and fibrinogen concentrations and a greater HOMA index than the control subjects, whereas other substrate and hormone concentrations were normal (Table 1). In type 2 diabetic patients, the fibrinogen circulating pool was ~11% greater, albeit not significantly ($P > 0.1$), when compared with control subjects, whereas the albumin circulating pool was normal (Table 1). The steady state was attained in the precursor pool(s) (data not shown).

In the postabsorptive type 2 diabetic patients (study A), albumin FSR and ASR were slightly although insignificantly greater when compared with the control subjects, ($P > 0.1$) (Table 1). Normalization for body weight made these (insignificant) differences even smaller (FSR, type 2 diabetic patients: 0.13 ± 0.01; control subjects: 0.12 ± 0.01% · day^{-1} · kg^{-1}; $P = 0.53$; ASR, type 2 diabetic patients: 0.19 ± 0.01; control subjects: 0.18 ± 0.02 g · day^{-1} · kg^{-1}; $P = 0.84$). In contrast, both fibrinogen FSR and ASR were increased in the type 2 diabetic subjects (Table 1). Normalization for body weight of FSR (type 2 diabetic patients: 0.30 ± 0.03; control subjects: 0.24 ± 0.02% · day^{-1} · kg^{-1}; $P = 0.059$) and ASR (type 2 diabetic patients: 0.029 ± 0.003; control subjects: 0.021 ± 0.002 g · day^{-1} · kg^{-1}; $P < 0.02$) did not substantially alter the results.

There were no significant differences between the hypertensive and the normotensive type 2 diabetic subjects with regard to albumin FSR (11.1 ± 1.3 vs. 12.1 ± 1.9%/day), albumin ASR (15.6 ± 1.8 vs. 15.9 ± 1.9 g/day), fibrinogen FSR (21.8 ± 1.4 vs. 24.4 ± 4.4%/day), and fibrinogen ASR (2.2 ± 0.2 vs. 2.8 ± 0.5 g/day). Similar findings were obtained in the control subjects (data not shown).

Whole-body leucine turnover was similar between the type 2 diabetic patients (2.63 ± 0.17 μmol · kg^{-1} · min^{-1}) and the control subjects (2.37 ± 0.23 μmol · kg^{-1} · min^{-1}).

A poorer metabolic control in type 2 diabetes was associated with greater fibrinogen concentration, fibrinogen pool, and leucine R_a, with a (surprisingly) lower fibrinogen FSR but with comparable fibrinogen ASR, albumin FSR, and albumin ASR (Table 2).

In study B, after the euglycemic-hyperinsulinemic euaminoacidemic clamp (Table 3), albumin FSR and ASR increased by ~25% versus basal in both groups ($P < 0.037$ and $P = 0.022$, respectively) (NS between groups by ANOVA) (Fig. 1). Similar results were observed also when the data were normalized for body weight (data not shown). The data of fibrinogen kinetics, endogenous leucine R_a (suppressed to the same extent in both groups), and the insulin-mediated glucose disposal (*M*) (decreased by

Table 2—*Study A: Effect of metabolic control (A1C either greater or lower than 8.2%) on metabolic parameters in the type 2 diabetic patients*

	A1C >8.2%	A1C <8.2%
n	7	6
Age (years)	46 ± 4	52 ± 1
BMI (kg/m^2)	27.5 ± 2.2	28.1 ± 0.8
Duration of disease (years)	10.6 ± 2.6	7.6 ± 1.6
A1C (%)	10.3 ± 0.5*	7.8 ± 0.2
Fasting glucose (mmol/l)	10.1 ± 0.6	8.3 ± 0.7
Insulin (nmol/l)	105 ± 11	85 ± 11
Glucagon (pg/ml)	124 ± 12	161 ± 16
Fibrinogen (mg/dl)	361 ± 35†	268 ± 20
Albumin FSR (%/day)	11.8 ± 1.7	10.5 ± 0.7
Albumin pool (g)	135 ± 6	135 ± 11
Albumin ASR (g/day)	15.7 ± 2.0	14.1 ± 1.3
Fibrinogen FSR (%/day)	20.5 ± 1.5†	30.2 ± 3.1
Fibrinogen pool (g)	11.2 ± 0.7†	8.0 ± 0.9
Fibrinogen ASR (g/day)	2.3 ± 0.2	2.4 ± 0.4
Leucine R_a (μmol · kg^{-1} · min^{-1})	2.90 ± 0.14‡	2.31 ± 0.25

Data are means ± SE. *$P < 0.001$; †$P < 0.05$; and ‡$P < 0.038$ vs. the type 2 diabetic patients with A1C <8.2%.

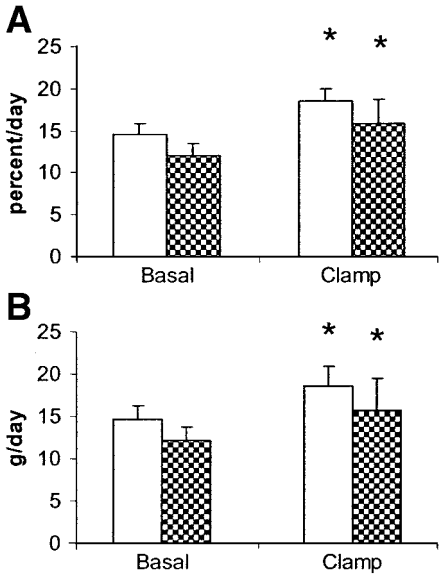

Figure 1—*Albumin FSR (A) and ASR (B) in the control subjects (□) and type 2 diabetes subjects (▨) in the basal postabsorptive state and after the euglycemic-hyperinsulinemic clamp.* *P < *0.05 vs. basal by ANOVA.*

~60% in the type 2 diabetic group) had been published earlier (6).

Correlations

Glucagon concentration was positively correlated with fibrinogen FSR ($r = 0.62$, $P < 0.01$) but not with albumin FSR. Fibrinogen concentration was positively correlated with both plasma glucose ($r = 0.55$, $P < 0.01$) and with the HOMA index ($r = 0.48$, $P < 0.03$). No correlation was found between fibrinogen and albumin FSR and ASR or between either blood glucose concentration or the HOMA value, albumin FSR, and fibrinogen FSR. No correlation was also found between total amino acid or the branched-chain amino acid (BCAA) concentrations and albumin FSR or between glucagon and the BCAA. However, a direct correlation was found between the BCAA concentrations and fibrinogen FSR ($r = 0.6$, $P < 0.01$).

CONCLUSIONS — This study shows that in normoalbuminuric type 2 diabetic subjects studied at their spontaneous hyperglycemic conditions after the overnight fast, albumin synthesis and concentration are normal, whereas fibrinogen concentration and synthesis are increased. With hyperinsulinemia, albumin synthesis was normally stimulated in the type 2 diabetic subjects, in contrast with the "paradoxical" insulin effect to increase fibrinogen synthesis in type 2 diabetic patients (by ~50%) but not in nondiabetic subjects, as previously reported (6). Thus, a defect in the regulation by insulin of fibrinogen but not of albumin synthesis is detectable in type 2 diabetes. These results differ from previous findings in insulin-withdrawn hyperglycemic type 1 diabetic subjects, in whom albumin synthesis was decreased but that of fibrinogen was increased (3), suggesting a different regulation of plasma protein kinetics in type 1 versus type 2 diabetes. The normal insulin and total amino acid concentrations (2,3,17) in the postabsorptive type 2 diabetic patients likely explain their normal postabsorptive albumin kinetics, in agreement also with their normal response to insulin.

Table 3—*Study B: Plasma glucose, insulin, total and BCAA concentrations, leucine and α-ketoisocaproate pooled specific activities and tracer-to-tracee ratio, at steady-state, and slopes of the increase of albumin-bound leucine specific activity (dSA/dt) or TTR (dTTR/dt) versus time in the basal and clamp periods*

	Type 2 diabetes ($n = 8$)		Control subjects ($n = 8$)	
	Basal	Clamp	Basal	Clamp
Plasma glucose (mmol/l)	9.5 ± 0.6*	4.9 ± 0.2	4.7 ± 0.2	4.5 ± 0.1
Plasma insulin (nmol/l)	100 ± 7*	832 ± 65	65 ± 14	876 ± 57
Total amino acids (mmol/l)	2,635 ± 91	2,936 ± 67	2,794 ± 109	2,842 ± 191
BCAA (mmol/l)	473 ± 36	576 ± 27	477 ± 24	547 ± 69
Leucine (in DPM/nmol) or TTR	6.51 ± 0.99	5.70 ± 0.83	7.57 ± 1.04	6.70 ± 0.98
α-Ketoisocaproate (in DPM/nmol) or TTR	4.98 ± 0.59	5.00 ± 0.63	6.29 ± 0.85	6.11 ± 0.88
Albumin (dSA/dt, in DPM/μmol/min, or dTTR/dt [in min] × 1,000)	0.29 ± 0.03	0.35 ± 0.06	0.43 ± 0.08	0.49 ± 0.07

Data are means ± SE. *$P < 0.025$ vs. control subjects. The fibrinogen data of this protocol had been previously reported (10). SA, specific activity; TTR, tracer-to-tracee ratio.

Insulin stimulates albumin gene expression and albumin secretion in isolated hepatocytes (18). Fibrinogen, a more complex molecule composed of three pairs of identical subunits (α, β, and γ) (19), is a positive acute-phase protein. Its synthesis is stimulated by stress hormones (20,21), positive feedback mechanisms (22), and inflammation (23), whereas albumin concentration and/or synthesis may be either normal or decreased under chronic inflammatory or stress conditions (1,2,22). Fibrinogen synthesis was less sensitive than that of albumin in response to dietary restriction or abundance in vivo or to serum, hormones, or other macromolecules supplements in vitro (17,24,25), being thus considered a "constitutive" liver function (25). Insulin in turn stimulated fibrinogen synthesis on vitro after prolonged exposure to concentrations 10 times higher than the (very low) doses to which albumin synthesis responded rapidly (25). Thus, insulin concentrations (and probably also glucose profiles) may need to be maintained at the lowest attainable level in type 2 diabetes to prevent increased fibrinogen synthesis and concentrations (6).

No inflammatory or stress condition was detectable in our subjects, and they were not current smokers (26). Therefore, these conditions should not be involved in the fibrinogen overproduction observed in type 2 diabetes, which could therefore be associated with hyperglucagonemia (21), insulin resistance, or other unknown factors. However, it cannot be excluded that in the type 2 diabetic patients there was a small change in the set point of the acute-phase responses, following a subtle undetected subinflammatory condition. Also, a mild increase of cortisol or interleukin-6 (not measured in this study) might be involved in the fibrinogen overproduction.

The positive relationship between the BCAA concentrations and fibrinogen synthesis suggests a role for these amino acids in the regulation of fibrinogen production, in broad agreement with the parallel changes in fibrinogen synthesis and BCAA concentrations observed in type 1 diabetic patients when either insulin deficient or insulin infused (3). This issue needs to be specifically investigated.

A possible effect on fibrinogen kinetics of the slightly greater BMI of our diabetic patients (Table 1) (27) seems unlikely, on the basis of data normalization over body weight (see RESULTS).

The acute metabolic control and/or insulin resistance may condition hyperfibrinogenemia (see "Correlations" in RESULTS), therefore exhibiting a procoagulant role, as discussed elsewhere (6). Whether better long-term metabolic control, achieved through improvement of insulin resistance and/or insulin secretion, would decrease both fibrinogen concentration and kinetics in type 2 diabetes remains to be established. With this respect, the type 2 diabetic subjects with the poorer metabolic control had greater fibrinogen concentrations, fibrinogen pool, and leucine R_a than the group with better control (Table 3). Surprisingly, however, FSR was lower in the group with the worse control, whereas fibrinogen ASR was similar between the two groups, although through slightly different mechanisms (Table 3). It is possible that a "constitutively" high fibrinogen FSR in type 2 diabetes is reduced to near-normal values when the metabolic control is worse, for a downregulation of hepatic fibrinogen synthesis in the presence of an expanded fibrinogen pool. However, this hypothesis is in contrast with the above-mentioned positive feedback between fibrinogen levels and hepatic production in animal models (23). Further studies are needed to clarify this complicated issue. Because this is a cross-sectional observation and not a follow-up study, it cannot be established whether the improvement of metabolic control in badly controlled type 2 diabetic subjects can decrease fibrinogen production to normal and through which mechanism(s). Nevertheless, this observation may help in understanding the interaction between metabolic control and the regulation of fibrinogen kinetics in type 2 diabetes. Worse metabolic control was also associated with a greater leucine R_a in the type 2 diabetic subjects (Table 3). This observation is in agreement with recent reports showing that moderate hyperglycemia in type 2 diabetes is associated with greater nitrogen flux, body protein synthesis, and degradation (28).

We intentionally selected type 2 diabetic patients with normal urinary albumin excretion rate, plasma albumin concentration, and oncotic pressure to exclude these potentially confounding factors that can affect albumin synthesis (7). Nevertheless, it would be highly interesting to also study albumin kinetics in albuminuric (either micro- or macroalbuminuric) type 2 diabetic patients to verify whether an upregulation of albumin synthesis occurs also in type 2 diabetes with albuminuria.

Although treatment with hypotensive drugs in three diabetic and two nondiabetic subjects might theoretically have affected the fibrinogen results (29), the nearly balanced number of hypertensive subjects in the two groups would blunt any possible bias.

In conclusion, this study demonstrates that albumin synthesis is normal, whereas that of fibrinogen is increased in type 2 diabetes with normoalbuminuria, both under fasting conditions and in response to hyperinsulinemia, thus showing a different regulation in the synthesis of these hepatic secretory proteins. The factors responsible for these specific effects, as well as the synthesis of other specific proteins in type 2 diabetes need to be investigated more extensively. The impact of an improvement in the metabolic control, as well as the presence of albuminuria, on albumin synthesis in type 2 diabetes will be specifically addressed in further studies.

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