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Transcapillary colloid osmotic gradient, plasma volume and interstitial fluid volume in long-term Type 1 (insulin-dependent) diabetes

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Summary. Plasma and subcutaneous colloid osmotic pressure, transcapillary escape rate of albumin, plasma volume and extracellular fluid volume were measured in 10 long-term Type 1 (insulin-dependent) diabetic patients without clinical nephropathy. Interstitial colloid osmotic pressure was reduced compared with normal subjects (12.9 ± 3.0 versus 15.8 ± 2.7 mmHg, $p < 0.05$) and the transcapillary colloid osmotic gradient increased (17.0 ± 2.4 versus 12.8 ± 2.7 mmHg, $p < 0.01$). Plasma volume was in the normal range and interstitial fluid volume increased by approximately 21% compared with normal subjects ($p < 0.01$). Transcapillary escape rate of albumin was significantly increased compared with normal subjects (8.9 ± 1.9 versus 5.1 ± 1.6%/h, $p < 0.01$). A negative correlation was found between the transcapillary colloid osmotic gradient and interstitial fluid volume ($r = 0.6$, $0.01 < p < 0.05$). These results suggest that the increased small vessel permeability in long-term diabetes leads to wash-out of interstitial proteins and the resulting increased transcapillary colloid osmotic gradient tends to preserve the plasma volume and to limit the tendency to increased interstitial fluid volume.

Key words: Type 1 diabetes, interstitial colloid osmotic pressure, plasma colloid osmotic pressure, interstitial fluid volume, plasma volume, transcapillary escape rate of albumin.

Increased microvascular permeability to small [1-3] and large molecules [4, 5] is found in patients with long-term diabetes. This increased small vessel permeability can be demonstrated in various tissues and organs [3, 6, 7]. The findings of Alpert et al. [8] of increased capillary diffusion capacity and no significant change in capillary filtration coefficient measured in leg muscles in long-term diabetes suggest that small vessels are abnormally permeable to small molecules and that the increased diffusion capacity is not due to increased surface area of the microvasculature. Poulsen and Nielsen, however, found significantly increased capillary filtration coefficient measured in the forearm [9].

The distribution of fluid between the blood and the extravascular space is usually assumed to be determined according to Starling's hypothesis concerning fluid transfer. The disturbances in capillary permeability to proteins, as well as to small molecules in diabetic patients, could lead to changes in the transcapillary colloid osmotic gradient and to the distribution of the extracellular fluid. The aim of the present study was to evaluate the relationships between the transcapillary colloid osmotic gradient, plasma and interstitial fluid volume and the transcapillary escape rate of albumin in patients with long-term Type 1 diabetes.

Subjects and methods

Subjects

Ten Type 1 diabetic patients (seven females, three males, mean age 37 years) were studied. Age, duration of diabetes and clinical details are given in Table 1. Their mean height was 169 ± 8.4 cm and their mean body weight was 64.5 ± 7.9 kg. All patients had Albustix-negative urine. None were on anti-hypertensive drugs or diuretics and all were normotensive (blood pressure $< 145/90$ mmHg). Patient 1 was unable to collect 24 h urine reliably. Her urine was Albustix-negative and she had normal serum creatinine (85 μmol/l). All patients were treated by twice daily injections of insulin (Monotard, Rapitard, Actrapid, Novo Industries, Copenhagen, Denmark; Insulatard, Velosulin, Nordisk, Gentofte, Denmark) (Table 1) and none had ketosis at the time of investigation. Five had retinopathy; in three this was proliferative. They were all ambulant in full-time employment. None had symptoms or signs of heart or liver disease and clinical examination revealed no oedema.

Plasma and extracellular fluid volumes and transcapillary escape rate of albumin were measured in the morning after an overnight fast, in the supine position and before injection of insulin. The patients were recumbent for at least 30 min before the tracer injections were given. After breakfast and their usual insulin dose, measurements of plasma and interstitial colloid osmotic pressure were performed with the patients in the supine position.

Normal subjects

Colloid osmotic pressure in plasma and interstitial fluid was measured in 16 normal subjects (4 females, 12 males). Their age ranged

Table 1. Clinical characteristics and laboratory measurements of the diabetic patients studied

Diabetic patients	Age (years)	Sex	Height (cm)	Weight (kg)	Duration of diabetes (years)	Type of insulin, dosage (i.u. morning/afternoon)		HbA_1 (%)	Blood glucose (mmol/l)	Serum albumin (g/l)	Urinary protein excretion (g/24 h)	Creatinine clearance (ml/min per 1.73 m^2)
1	30	F	158	53	22	Insulatard Velosulin	20/4 8/4	13.1	8.2	34	-	-
2	35	F	160	62	16	Insulatard Velosulin	16/16 12/0	11.5	12.5	37	0.18	130
3	37	F	177	70	23	Monotard	16/12	12.2	9.9	43	0.13	138
4	25	F	176	64	11	Insulatard Velosulin	16/14 12/2	12.8	9.0	41	0.11	159
5	56	M	170	67	18	Insulatard Velosulin	16/12 2/0	12.3	8.8	41	0.17	69
6	21	M	180	72	14	Monotard Actrapid	28/16 12/8	9.8	9.6	42	0.11	114
7	35	M	174	70	30	Insulatard Velosulin	28/16 8/8	8.6	5.6	41	0.25	89
8	61	F	164	58	45	Monotard Actrapid	12/8 4/4	8.5	8.0	36	0.06	64
9	35	F	174	74	28	Rapitard	24/16	8.3	11.4	38	0.20	114
10	35	F	158	54	29	Monotard	24/12	11.9	5.8	37	0.09	70

Blood glucose was measured in non-fasting conditions at the time of the investigations

from 33 to 72 years (mean 51 years). All were normotensive (blood pressure $<145/90$ mmHg) and had normal serum albumin levels (mean 41.7 ± 3.4 g/l, range 35–48 g/l). Measurements were made in the recumbent position and at the same times of the day as in the diabetic patients. Plasma and extracellular fluid volume and transcapillary escape rate of albumin were measured in another group of 19 normal subject (11 females, eight males), aged 22–54 years (mean 36 years). Their mean height was 172 ± 9.2 cm and mean body weight 65.9 ± 11.1 kg. Measurements were made after an overnight fast in the morning with the subjects recumbent for at least 30 min before the start of the experiments. Urine analysis was normal in all the normal subjects in both groups and also they had normal serum creatinine levels and no symptoms or signs of heart or liver disease.

Informed consent was obtained from all subjects. The protocol was approved by the Ethical Committee, Norwegian Council for Science and the Humanities.

Methods

Colloid osmotic pressure. A colloid osmometer (Department of Physiology, University of Bergen) made for 5 µl samples was used [10]. The coefficient of variation within assays was 4.3% ($n=12$) and between assays was 4.9% ($n=10$). Plasma samples were obtained by venepuncture using short-term stasis.

Interstitial colloid osmotic pressure. Interstitial fluid was collected by the wick technique [11–13]. Under aseptic conditions four double nylon wicks (containing 600 filaments, diameter 1 mm) soaked in saline (0.154 mol/l) were sewn into subcutaneous tissue on the side of the thorax in a length of 5–8 cm. The skin was anaesthetized by intradermal injection of 0.1–0.2 ml of lidocaine (20 mg/ml) at the site of the entrance and exit of the wicks. Straight needles (75 mm long) without cutting edges were used for implantation (Acufirm, Dreieich, Hessen FRG). After 1 h implantation, the wicks were removed and transferred immediately to a 10 ml plastic tube containing 8 ml of mineral oil (Paraffin dickflüssig, 7160, Merck, Darmstadt, FRG). Only unstained or slightly pink wicks were accepted, red wicks being excluded. Wick fluid was isolated by centrifugation (1000 g for 15 min) under mineral oil [12] and colloid osmotic pressure measured by the colloid osmometer. All the interstitial fluid pressure values in this study represent the mean of at least two (two to four) separate measurements of wick fluid from two to four wicks.

Plasma volume. Plasma volume was determined as the initial ^{125}I-albumin distribution space. ^{125}I-labelled human serum albumin (4 µCi Amersham International, Amersham Bucks, UK) was injected intravenously and blood samples were taken after 15 and 30 min. The distribution space was calculated by dividing the injected amount of tracer into the calculated zero-time concentration. Zero-time concentration was calculated from the plasma disappearance curve using the sum of the least square method [14]. Values are given as ml/cm body height.

Extracellular fluid volume. Extracellular fluid volume was measured by administration of intravenous radiosulphate as by Walser et al. [15] but modified by McGrath et al. [16]. Approximately 25 µCi in a volume of 5 ml of $Na_2{}^{35}SO_4$ (Amersham International) was injected intravenously. Blood samples were drawn from the contralateral arm at 30 and 60 min after injection, and plasma was obtained by centrifugation at 2000 rpm for 10 min. Proteins were precipitated by adding trichloracetic acid (30%, 1 ml). The administered dose of radiosulphate was calculated from standards prepared for counting the same way as the blood samples to reduce the problem of quenching in the plasma samples compared to the standards. The radiosulphate space (S, lit) was calculated from the following fomula:

$$S = \frac{C_i \, 0.91}{T_o}$$

where C_i equals the total number of cpm injected, 0.91 is a fraction to correct for the Gibbs-Donnan effect [17] and T_o is the calculated zero time concentration. The zero time concentration of radiosulphate was calculated by means of sample counts using the sum of the least square method [14]. The values are given as percentage body weight.

Interstitial fluid volume. Interstitial fluid volume equals extracellular fluid volume minus plasma volume and the values are given as percentage body weight.

Transcapillary escape rate of albumin. The transcapillary escape rate of albumin (TER_{alb} %/h) was determined from the initial disappearance rate of ^{125}I-albumin [4, 5, 18, 19]. The disappearance of ^{125}I-albumin was assumed to be a monoexponential function during the first 30 min after injection. TER_{alb} was calculated as the disappearance rate constant by the least square method [14].

HbA_1, was measured using agar gel electrophoresis (Corning). The normal range is 5.0–7.5%.

Statistical methods

Wilcoxon's test for two independent samples was used. Correlations were calculated by least square linear regression analysis. Results are given as mean ± SD.

Results

Plasma and interstitial colloid osmotic pressure

Plasma colloid osmotic pressure was in the normal range and similar to the values in normal subjects (Table 2, Fig. 1). Mean interstitial colloid osmotic pressure was reduced by approximately 3 mmHg compared to normal subjects ($p<0.05$). The resulting transcapillary colloid osmotic gradient in the patients was increased by approximately 4 mmHg ($p<0.01$).

Plasma volume, extracellular fluid volume and interstitial fluid volume

Plasma volume in the patients was similar to the values in normal subjects (Table 2, Fig. 2). In the patients extracellular fluid volume was 20.1 ± 2.6 compared with 17.4 ± 2.2% body weight in the normal subjects ($p<0.01$). Mean interstitial fluid volume was in the patients was increased by approximately 21% compared with normal subjects (Table 2, Fig. 2). As a result, the distribution of fluid between the intravascular and the interstitial space was significantly different in the patients compared with normal subjects ($p<0.01$).

Transcapillary escape rate of albumin

The values for TER_{alb} were significantly increased in the patients compared to normal subjects ($p<0.01$, Table 2, Fig. 2), but with some overlapping values.

In the patients a negative correlation was found between transcapillary colloid osmotic gradient and interstitial fluid volume ($r=-0.6$, $0.01<p<0.05$). No correlation was found between interstitial colloid osmotic pressure and interstitial fluid volume.

Discussion

Methods for measurement of interstitial colloid osmotic pressure have recently been reviewed by Aukland and Nicolaysen [20]. The wick technique has been extensively investigated in both experimental animals and man by Fadnes and Aukland [21] and Noddeland [13] and the method gives representative and reproducible results. In experimental animals, reduced subcutaneous interstitial colloid osmotic pressure is paralleled by a reduction in colloid osmotic pressure in skeletal muscle interstitial fluid and lymphatic protein concentration [20]. Standard methods were used to measure plasma volume, TER_{alb} and extracellular fluid volume. Parving [19], using ^{125}I-albumin to determine plasma volume and TER_{alb}, found the coefficient of variation to be 1.5% (plasma volume) and 8.5% (TER_{alb}). Bauer et al. [22] determined plasma volume and extracellular fluid volume using ^{125}I-albumin and radiosulphate and found less than 4% mean variation for all body fluid measurements.

Table 2. Colloid osmotic pressures, body fluid volumes and transcapillary escape rate of albumin in diabetic patients and normal subjects

	Diabetic patients ($n=10$)	Normal subjects
Plasma colloid osmotic pressure (mmHg)	29.9 ±2.2	28.6 ±3.4 ($n=16$)
Interstitial colloid osmotic pressure (mmHg)	12.9 ±3.0[a]	15.8 ±2.7 ($n=16$)
Transcapillary colloid osmotic gradient (mmHg)	17.0 ±2.4[b]	12.8 ±2.7 ($n=16$)
Plasma volume (ml/cm)	17.0 ±2.2	17.3 ±1.5 ($n=19$)
Interstitial fluid colume (% body weight)	15.7 ±2.4[b]	13.0 ±2.0 ($n=19$)
Ratio of plasma volume to interstitial fluid volume	0.29 ± 0.05[b]	0.36 ± 0.06 ($n=19$)
Transcapillary escape rate of albumin (%/h)	8.9 ±1.9[b]	5.1 ±1.6 ($n=19$)

Results expressed as mean ± SD
[a] $p<0.05$; [b] $p<0.01$ compared with normal subjects

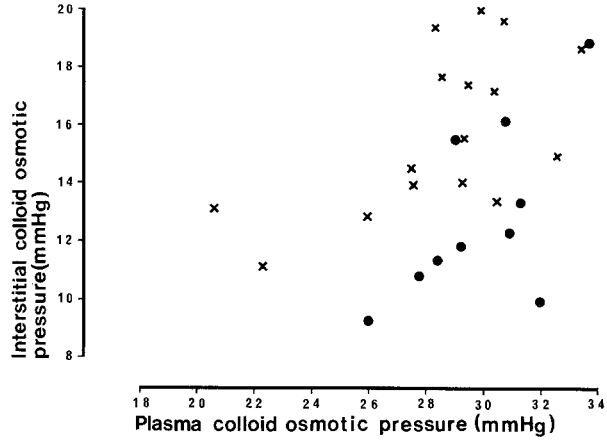

Fig. 1 Plasma colloid osmotic pressure and interstitial fluid colloid osmotic pressure in 10 diabetic patients (●) and 16 normal subjects (×)

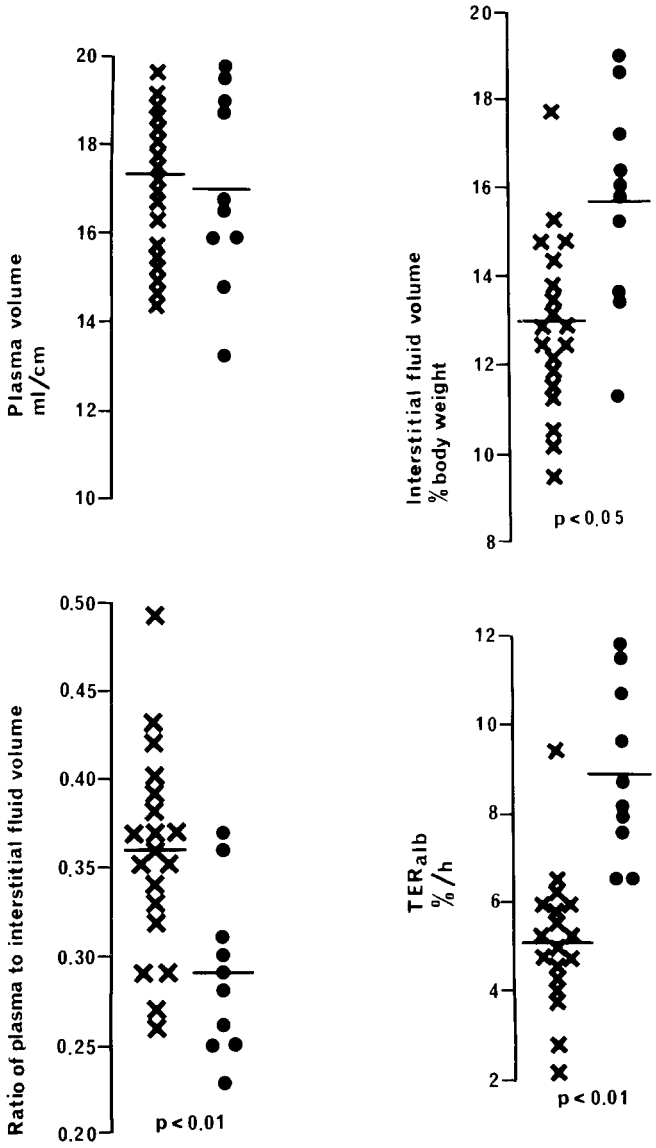

Fig. 2. Plasma volume, interstitial fluid volume, the ratio of plasma volume to interstitial fluid volume and transcapillary escape rate of albumin (TER_{alb}) in 10 diabetic patients (●) and in 19 normal subjects (×)

Measurements in the diabetic patients are only compared directly to the measurements in each of the two seperate groups of normal subjects. The control group for plasma volume, TER_{alb} and extracellular fluid volume was well matched to the patients regarding age, sex and body size. The control group for colloid osmotic pressures was older than the patients (mean age 51 and 37 years, respectively). Noddeland [13], however, in a younger group of healthy volunteers (mean age 22 years, range 19–29 years), using the same method, found values for plasma and interstitial colloid osmotic pressure of the same magnitude as in our older control group ($26.9 \pm 4.1/28.6 \pm 3.4$ mmHg and $15.8 \pm 2.3/15.8 \pm 2.7$ mmHg, respectively). In our normal subjects there were no differences between females and males in plasma colloid osmotic pressure (29.7 ± 2.9 and 28.6 ± 3.5 mmHg, respectively) or in interstitial colloid osmotic pressure (16.1 ± 2.3 and 15.7 ± 3.0 mmHg, respectively).

Reduced interstitial colloid osmotic pressure has been reported in patients with nephrotic syndrome [23] and in heart failure [24] and has been described as an oedema-preventing mechanism [25]. In 1973, Poulsen [26] reported reduced values for subcutaneous interstitial fluid albumin concentration in long-term diabetes. Our findings of reduced interstitial colloid osmotic pressure in diabetic patients are in accordance with his results. None of our patients had nephropathy or heart failure.

The unexpected finding of increased interstitial fluid volume cannot be explained by nephropathy, heart failure or liver disease. It may fit with the clinical impression that long-term diabetic patients have a tendency to oedema which is not due to kidney, heart or liver disease. As there was no negative correlation between interstitial colloid osmotic pressure and interstitial fluid volume, the reduced pressure was probable not caused by simple dilution of interstitial proteins.

The values for TER_{alb} in the diabetic and normal subjects confirm the findings of Parving et al. [4, 5]. The increased microvascular permeability to proteins would in itself tend to increase the interstitial protein mass and colloid osmotic pressure. The mechanism for reduction of interstitial colloid osmotic pressure is assumed to be increased net capillary filtration resulting in lymphatic wash-out of interstitial proteins [27]. The findings in our patients of reduced colloid osmotic pressure and increased TER_{alb} indicate that the net capillary filtration of fluid is proportionally more increased than the filtration of proteins. The increased net capillary filtration of fluid can be due either to increased capillary filtration coefficient as described by Poulsen and Nielsen [9] or to increased hydrostatic capillary pressure. Even if our long-term diabetic patients did not have clinical signs or symptoms of cardiac failure, a subclinical depression of myocardial function, leading to increased hydrostatic capillary pressure, should not be ruled out [24, 28].

Increased transcapillary colloid osmotic gradient as found in our patients can, in a state of increased capillary permeability and filtration, be seen as a plasma volume-preserving factor and also as an oedema-preventing mechanism. The negative correlation between transcapillary colloid osmotic gradient and interstitial fluid volume may indicate that the increased gradient is operating as an oedema-preventing factor.

The increased capillary permeability in long-term diabetic patients leads to a new steady state. Our results indicate that this new balance is characterised by a tendency to increased interstitial fluid volume limited by an increase in the transcapillary colloid osmotic gradient. The activation in long-term diabetic patients of the oedema-preventing mechanism with reduced interstitial colloid osmotic pressure can perhaps explain the

clinical impression that diabetic patients with fluid retention due to nephrotic syndrome or heart failure has an enhanced tendency to peripheral oedema.

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