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Variation in Polyol Levels in Cerebrospinal Fluid and Serum in Diabetic Patients

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Summary. Cerebrospinal fluid (CSF) or CSF and plasma levels of sorbitol, 1,5-anhydroglucitol and myoinositol of diabetic and non-diabetic patients with normal kidney function and of diabetic and non-diabetic patients with impaired kidney function were measured by gas-liquid chromatography. The CSF sorbitol level correlated with the plasma glucose level ($p \leqq 0.05$) in diabetic patients with normal kidney function, having received insulin for less than 12 months. The correlation between CSF sorbitol and plasma glucose levels in patients not dependent on insulin was not significant. Sorbitol was not detected in the plasma. The highest sorbitol levels in CSF were seen in insulin-dependent diabetic patients with impaired kidney function. No rise was seen in non-diabetic uremia. 1,5-anhydroglucitol, normally present in plasma, was absent from CSF and plasma in diabetic patients receiving insulin. In non-diabetic uremic patients 1,5-anhydroglucitol levels in CSF and plasma were lower than in healthy subjects, but there was no correlation with plasma glucose levels. The myoinositol level was higher in CSF than in the plasma of both non-diabetic and diabetic patients with normal kidney function. Both plasma and CSF levels were significantly ($p < 0.001$) elevated in diabetic as well as in non-diabetic uremic patients, the plasma myoinositol increasing relatively more than the CSF levels. The elevation of plasma myoinositol correlated with the elevation of plasma creatinine and thus also with the impairment of kidney function. Plasma and CSF myoinositol levels were not influenced by the plasma glucose level.

Key words: 1,5-anhydroglucitol, myoinositol, sorbitol, diabetes, uremia.

The presence of the polyol pathway has been demonstrated in several tissues including the spinal cord and kidneys [1]. Sorbitol accumulation in the tissues has been regarded to be primarily controlled by the blood glucose level and the activity of aldose reductase (1.1.1.21.) [1, 2]. The activity of aldose reductase is increased in the Schwann cells in the nerves of diabetic rats [1, 3]. The accumulation of sorbitol in monkey kidney epithelial cell cultures by means of the polyol pathway has been demonstrated [4]. 1,5-anhydroglucitol is normally present in CSF and in plasma [8] but seems to be absent from CSF in diabetic patients receiving insulin and low in advanced stages of uremia. Myoinositol induces neural dysfunction in test animals [5] and diabetic patients are intolerant to orally administered myoinositol [6]. The plasma myoinositol level is elevated in uremia [7].

As an attempt to illuminate further the relationship between changes in the polyol levels in diabetic and uremic patients we measured sorbitol, 1,5-anhydroglucitol and myoinositol levels in CSF and plasma of diabetic and non-diabetic patients with normal kidney function. A correlation was sought between polyol levels and glucose levels in plasma and CSF. An increase in the concentration of CSF polyols was compared with the type of treatment and duration of diabetes. The results were also compared with those from diabetic and non-diabetic patients with impaired kidney function.

Material and Methods

Patients

61 diabetic patients with normal kidney function were studied. The mean age of the patients was 61.7 ± 15.5 years, their height 166.0 ± 9.5 cm and mean body weight 104% of the body weight normally considered ideal. 59 non-diabetic patients served as the control group. Their mean age was 61.2 ± 15.0 years, mean height 164.0 ± 7.6 cm and mean body weight 103% of the ideal body weight. 11 diabetic and 11 non-diabetic patients had signs of recent transient ischaemic attacks and 11 diabetic and 11 non-diabetic patients had a manifest stroke syndrome. 22 of the diabetic patients with normal kidney function received insulin, 23 patients received oral hypoglycemic drugs (14 of them were treated with sulfonylureas and 9 with phenformin) 16 patients were on a low carbohydrate diet only. These groups were compared with 6 diabetic patients (all on insulin) with impaired kidney function and with 18 non-diabetic uremic patients.

All diabetic patients were kept on a low carbohydrate and sorbitol-free diet for at least two days prior to lumbar puncture. The non-diabetic patients were kept on a sorbitol-free diet.

Cerebrospinal fluid (CSF) polyols, plasma and CSF glucose were determined from all patients. Also the

plasma polyol levels were determined from 25 of the diabetic and 27 of the non-diabetic patients.

Determination of Polyols

After at least one week of hospitalisation 2 ml of cerebrospinal fluid was taken by lumbar puncture. 4 ml of heparinized whole blood was taken within the same hour and the plasma was immediately separated by centrifugation. The samples were kept deep-frozen until prepared for gas-liquid chromatography (GLC). CSF polyols were measured by GLC according to a method described recently [9].

A modified method was used to determine the plasma polyols: protein were removed from 0.5 ml of plasma with 1.0 ml 10% TCA. 1.0 ml of the clear supernatant was taken after centrifugation and TCA was extracted with diethylether. From the neutral suspension glucose was removed with hexokinase (Boehringer, Mannheim). The removal of anions and cations was completed by using a mixed resin bed (Amberlite resin IR 120 CH® and Deacidite FF-IP SRA, Permutit®).

The remaining solution was dried in hot air and the polyols converted to acetylated forms with acetic anhydride [9]. Ribitol was used as an internal standard. A standard mixture of the following polyols: erythritol, ribitol, arabinitol, 1,5-anhydroglucitol, xylitol, mannitol, sorbitol and myoinositol was recorded at the beginning and the end of every GLC recording and the retention times were used as reference values. The detection limit for the polyols was 5 μmol/l.

Table 1. *Plasma glucose, CSF glucose and CSF sorbitol in diabetic patients and controls with normal kidney function*

Patients and treatment	Plasma glucose mmol/l[b]	CSF glucose mmol/l	CSF sorbitol μmol/l
Insulin (22)[a]	13.0 ± 4.0	5.8 ± 2.3	64.5 ± 70.5 $p < 0.001$[c]
Oral hypoglycemic drugs (23)	6.8 ± 2.9	4.7 ± 2.0	37.0 ± 17.0 $p < 0.001$
Carbohydrate restriction (16)	4.9 ± 1.6	3.6 ± 1.2	35.0 ± 11.0 $p < 0.002$
Non-diabetic patients (59)	4.1 ± 1.0	3.5 ± 2.0	22.0 ± 7.0

[a] Figures in parenthesis indicate the number of patients
[b] Mean and SEM
[c] p values indicate the significance of difference between the test group and non-diabetic controls

Determination of Plasma and CSF Glucose

The capillary plasma and cerebrospinal fluid glucose levels were measured with a Beckman glucose analyzer using glucose oxidase (Boehringer, Mannheim). Plasma creatinine concentrations were determined in an autoanalyzer (Technicon) according to the method suggested in the manual (Method AA II-11). The significance of the results was tested with the Mann-Whitney U-test for non-parametric statistics and by calculating the correlation coefficient r. The significane of r was tested with the following formula:

$$t_{n-2} = \frac{r \cdot \sqrt{n-2}}{\sqrt{1-r^2}}$$

were t_{n-2} is distributed according to the student distribution with n-2 degrees of freedom when $\varrho = 0$

Results

CSF Polyol Levels in Diabetic Patients with Normal Kidney Function

1. CSF Sorbitol. The mean level of CSF sorbitol was 40.5 ± 30.0 μmol/l in the diabetic group compared with 22.0 ± 7.0 μmol/l in the non-diabetic control group. The difference is significant ($p < 0.001$) (Table 1).

The insulin-receiving diabetic patients had higher sorbitol levels (mean 64.5 ± 70.5 μmol/l) and plasma glucose levels (mean 13.0 ± 4.0 mmol/l) than did patients receiving oral hypolgycemic drugs (mean 37.0 ± 17.0 μmol/l for sorbitol and 6.8 ± 2.9 mmol/l for plasma glucose). Patients on carbohydrate restriction only had the lowest mean levels of CSF sorbitol (35.0 ± 11.0 μmol/l) and plasma glucose (4.9 ± 1.6 mmol/l) (Table 1).

CSF sorbitol correlated positively with the plasma glucose level in patients who had received insulin for less than 12 months (r = 0.65). There was, however, no correlation between plasma glucose and CSF sorbitol levels in patients who had been insulin-dependent for more than 10 years. The CSF sorbitol level was low in all cases (Fig. 1).

The patients were grouped according to duration and type of diabetes and the state of kidney function (Fig. 2). High CSF sorbitol levels were seen in insulin-dependent diabetic patients with long duration of the disease and impaired kidney function (Fig. 2, Group A). Another group with high sorbitol levels in CSF consisted of old patients who had suffered from maturity onset type of diabetes for many years and were not dependent on insulin, but had recently been

put on insulin treatment because of severe hyperglycemia during an acute illness (pyelonephritis, sepsis or cerebral ischemic attack) (Fig. 2, Group B). None of these patients were given intravenous infusions when lumbar puncture was performed. A third group with high CSF sorbitol levels consisted of 5 patients whose diabetes had been diagnosed recently (less than 6 months duration) and who had been insulin-dependent from the beginning (Fig. 2, Group C). Fig. 2 also presents data obtained from other insulin-dependent diabetics with normal kidney function (Fig. 2, Group D) and 18 non-diabetic uremic patients (Fig. 2, Group E).

The dashed area in Fig. 2 indicates the normal range for CSF sorbitol in non-diabetic control patients. The sorbitol level in CSF was significantly elevated in all diabetic groups compared with non-diabetic and uremic patients.

In order to find out whether artificially sustained hyperglycemia may result in a change in the sorbitol level in CSF, samples were taken from four non-diabetic patients (fasting blood sugar normal, oral glucose tolerance test normal) who, as part of their treatment, received 1000–1500 ml 5% glucose per day intravenously for 2–3 days. A sample was taken for CSF sorbitol and plasma glucose determination at the end of the period. An almost two-fold increase of CSF sorbitol was noted in these non-diabetic patients, suggesting that hyperglycemia per se, sustained for this short period, resulted in an increased sorbitol production (Table 2).

2. CSF 1,5-Anhydroglucitol. 1,5-anhydroglucitol was absent from CSF in all diabetic patients receiving insulin (Table 3, Fig. 2, Group A and B). Those diabetic patients receiving oral hypoglycemic drugs or who were under carbohydrate restriction had normal anhydroglucitol levels in the CSF samples. The anhydroglucitol level in CSF showed no correlation with plasma glucose level or duration of diabetes.

3. CSF Myoinositol. The myoinositol levels in CSF were similar in diabetic and non-diabetic patients (Table 3). Insulin-dependent diabetic patients had the lowest mean level of CSF myoinositol, but the difference between this level and the mean level of the non-diabetic patients was not significant. CSF myoinositol did not correlate with plasma glucose level or duration of the disease.

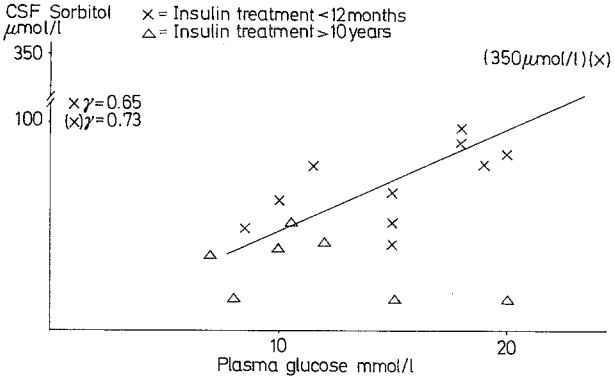

Fig. 1. The correlation between CSF sorbitol and plasma glucose levels in diabetic patients receiving insulin and having been treated with insulin for either less than 12 months or more than 10 years. One very high sorbitol concentration (x) was included. The correlation coefficients given refer to the data with or without inclusion of this one high value

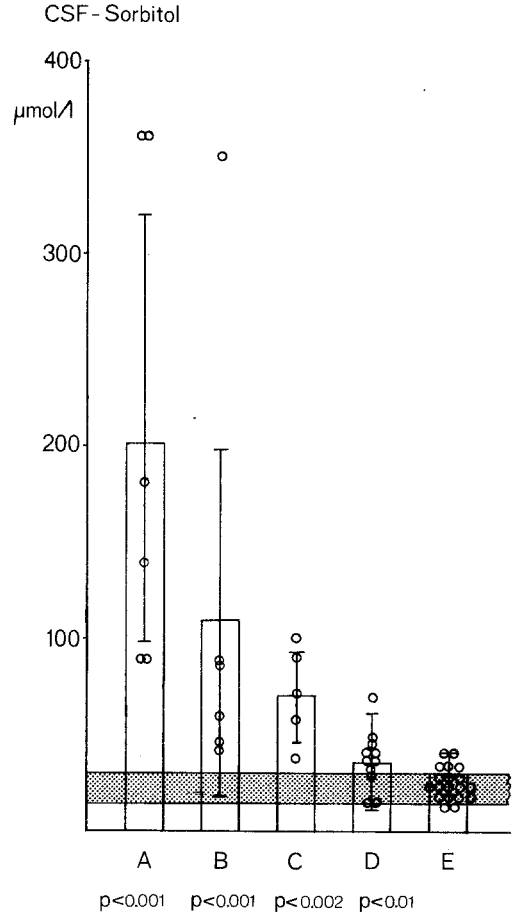

Fig. 2. The CSF sorbitol level in diabetic patients receiving insulin and in uremic non-diabetic patients. A = diabetic patients with impaired kidney function. B = diabetic patients not suffering from insulin-dependent diabetes, but shifted to insulin treatment because of severe hyperglycemia during an acute illness. C = diabetic patients suffering from insulin-dependent diabetes and treated with insulin for less than 12 months. D = other diabetic patients receiving insulin. E = non-diabetic uremic patients. The dashed area indicates mean ± SEM for non-diabetic controls: the p values indicate significance of difference between test groups and the control group

CSF Polyol Levels in Diabetic and Non-Diabetic Patients with Impaired Kidney Function

The patients with impaired kidney function, both diabetic and non-diabetic, had significantly elevated mean levels of myoinositol in CSF ($p < 0.001$) (Table 4). The CSF sorbitol level was significantly elevated in the diabetic patients. 1,5-anhydroglucitol was absent from the CSF of the diabetic group and very low in the non-diabetic uremic patients.

Comparison between Polyol Levels in Plasma and CSF

Both plasma and CSF samples were obtained from 22 diabetic and 23 non-diabetic patients with normal kidney function, and from 2 diabetic and 5 non-diabetic patients with elevated plasma creatinine, respectively.

1. Patients with Normal Kidney Function. Sorbitol was undetectable (below detection limits for GLC) in the plasma of these patients. 1,5-anhydroglucitol was present in the plasma of all non-diabetic patients and in the plasma of diabetic patients not receiving insulin. In contrast, it was absent from the plasma of diabetic patients receiving insulin (Table 5). The plasma myoinositol was significantly lower in the plasma ($p < 0.001$) than in the CSF of patients with normal kidney function.

2. Patients with Impaired Kidney Function. The plasma myoinositol level was elevated in all patients with impaired kidney function (Table 6). The CSF myoinositol level was also elevated but less so than the plasma level. In 3 out of 7 patients the myoinositol level was higher in the plasma than in the CSF. Sorbitol was undetectable in the plasma, and 1,5-anhydroglucitol low or undetectable.

The plasma myoinositol level was correlated with the plasma creatinine level in the patients with impaired kidney function (Fig. 3).

Discussion

Sorbitol

Our present study supports previous reports [6, 9, 10, 12] that diabetes profoundly changes the polyol metabolism in man.

Hyperglycemia is followed by an increase in CSF sorbitol in diabetic and obviously also in non-diabetic patients. CSF sorbitol was correlated significantly with plasma glucose in diabetic patients chronically receiving insulin and having recently been put on insu-

Table 2. *CSF sorbitol and 1,5-anhydroglucitol levels in four non-diabetic patients who received 1000–1500 ml 5% glucose intravenously daily for 2–3 days before lumbar puncture*

Patients[a]	Plasma glucose mmol/l	CSF sorbitol μmol/l	CSF 1,5-anhydroglucitol μmol/l
1.	8.3	47.0	68.5
2.	10.7	39.0	69.0
3.	7.0	39.0	105.0
4.	13.7	30.0	75.0
Normal values	4.1 ± 1.0	22.0 ± 7.0	87.0 ± 30.0

[a] Oral GTT and fasting blood glucose levels normal, plasma creatinine level normal.

Table 3. *Cerebrospinal fluid levels of 1,5-anhydroglucitol and myoinositol in diabetic and non-diabetic patients with normal kidney function (for footnotes see Table 1)*

Patients and treatment	CSF 1,5-anhydroglucitol μmol/l[b]	CSF myoinositol μmol/l[b]
Insulin (22)[a]	below 5	113.0 ± 52.0
Oral hypoglycemic drugs (23)	64.0 ± 70.0	152.0 ± 51.0
Carbohydrate restriction (16)	90.0 ± 70.0	149.0 ± 26.0
Non-diabetic patients (59)	87.0 ± 30.0	138.0 ± 50.0

Table 4. *CSF sorbitol, 1,5-anhydroglucitol and myoinositol levels in diabetic patients with impaired kidney function and uremic non-diabetic patients*

Patients	CSF sorbitol μmol/l	CSF 1,5-anhydroglucitol μmol/l	CSF myoinositol μmol/l
Diabetic patients[a] (6) plasma creatinine 250 ± 40 μmol/l	201.0 ± 128.0 $p < 0.001$	below 5.0 $p < 0.001$	216.0 ± 78.0 $p < 0.001$
Uremic patients not on dialysis (8) plasma creatinine 905 ± 200 μmol/l	30.0 ± 7.0	20.0 ± 17.0 $p < 0.001$	196.0 ± 56.0 $p < 0.002$
Uremic patients on dialysis (10) plasma creatinine 820 ± 300 μmol/l	27.5 ± 6.0	below 10.0 $p < 0.001$	273.0 ± 58.0 $p < 0.001$
Normal values (59)	22.0 ± 7.0	87.0 ± 30.0	138.0 ± 50.0

[a] p values indicate the significance of difference between the test group and non-diabetic non-uremic controls. The number of patients in each group is given in parentheses.

Table 5. *Plasma levels of sorbitol, 1,5-anhydroglucitol and myo-inositol in diabetic and non-diabetic patients with normal kidney function*

Patients and treatment	Plasma sorbitol µmol/l	Plasma 1.5-anhydro-glucitol µmol/l	Plasma myoinositol µmol/l
Insulin (12)	below 5.0	below 5.0 $p < 0.001$	31.0 ± 10.0 $p < 0.001$[a]
Oral hypolgycemic drugs or carbohydrate restriction (10)	below 5.0	96.0 ± 50.0	25.0 ± 5.0 $p < 0.001$
Non-diabetic patients (23)	below 5.0	99.5 ± 30.5	28.0 ± 12.0 $p < 0.001$
Normal CSF values	22.0 ± 7.0	87.0 ± 30.0	138.0 ± 50.0

[a] p value indicates significance of difference between plasma level and CSF level of myoinositol.

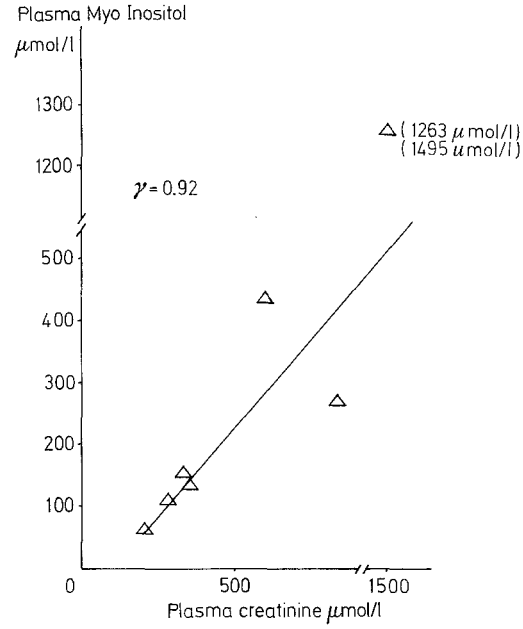

Fig. 3. The correlation between plasma creatinine level and plasma myoinositol level in 7 patients with impaired kidney function

Table 6. *CSF and plasma levels of myoinositol in two diabetic and five non-diabetic patients with impaired kidney function*

		Plasma myoinositol µmol/l	CSF myoinositol µmol/l	Plasma creatinine µmol/l
Diabetic	I.P.	66.0	144.0	200.0
Diabetic	E.S.	110.0	168.0	280.0
Non-diabetic	G.L.	135.0	187.0	351.0
Non-diabetic	S.I.	150.0[a]	140.0	341.0
Non-diabetic	H.H.	268.0	297.0	830.0
Non-diabetic	P.P.	435.0[a]	253.0	600.0
Non-diabetic	E.J.	1263.0[a]	1030.0	1495.0
Normal values		28.0 ± 12.0	138.0 ± 50.0	

[a] Plasma myoinositol level > CSF myoinositol level.

lin. We found no correlation, however, between plasma glucose levels and CSF sorbitol levels in insulin-dependent patients who had received insulin for more than 10 years; in the latter group of patients CSF sorbitol did not rise above the normal range. These findings are in agreement with experimental studies concerning the activity of aldose reductase [1] which demonstrate that aldose reductase activity is high at the beginning of experimental diabetes.

Diabetic patients with impaired kidney function had significantly elevated CSF sorbitol levels. The mechanism of this elevation is unclear. Schofield *et al.* [4] have recently demonstrated the role of the kidneys in sorbitol metabolism by showing that monkey kidney epithelial cells accumulate sorbitol by means of the sorbitol pathway. In uremic patients many substrates are retained in the body because of impaired urine excretion or impaired degradation in the kidneys, but since the plasma levels were not elevated and since uremia per se did not cause sorbitol elevation in CSF, this explanation is improbable. Thus the sorbitol elevation in diabetic patients with nephropathy must be caused by the diabetic state.

Osmolar Effects of Sorbitol. Although we noted maximum CSF sorbitol concentrations of 360 µmol/l, i.e. more than 10 times the normal level, the concentrations were still low in relation to possible osmolar effects. However, the elevation of CSF sorbitol must be a product of cellular metabolism and since penetration of sorbitol through cell membranes is slow, the elevation of CSF sorbitol may be a reflection of very high cellular levels.

1,5-Anhydroglucitol

This anhydride form of sorbitol has recently been demonstrated in human [8]. The present study verifies the previous findings that this polyol is absent from the plasma and CSF in insulin-receiving diabetic patients and low in advanced stages of uremia. The metabolism and role of this polyol is unknown. We found no correlation with the severity of the complications of either the diabetic or uremic state.

Myoinositol

Myoinositol was elevated both in the plasma and CSF of patients with impaired kidney function. The plasma level was correlated with plasma-creatinine level, Urine excretion of myoinositol is increased in uremia and diabetes [12]. In normal man only 0.8% of orally administered myoinositol is excreted in the urine; the remaining 99.2% is degraded [10]. Our studies support the theory that the kidneys [12] degrade myoinositol normally and that this degradation is impaired in uremic patients. Thus the elevation of plasma myoinositol in uremia can partly be explained by an impaired degradation in the kidneys.

We have shown that there are similarities as well as differences in the polyol metabolism in diabetic and uremic patients. In both groups the 1,5-anhydroglucitol level decreases in the plasma and CSF, but in diabetic patients this decrease is seen only in those patients receiving insulin. The myoinositol level is elevated in relation to the impairment of kidney function in uremia as well as in diabetes. The sorbitol level is significantly elevated in the CSF of diabetic patients, but no elevation was noted in the uremic patients. The elevation of sorbitol correlated significantly with the plasma glucose level in the early stages of insulin-dependent diabetes and in non-diabetic subjects receiving glucose infusions, indicating that the sorbitol elevation was caused by an increase in the glucose level. However, no such relationship was observed in cases of diabetes of long duration. The reasons for this is unknown, but it may be related to the changes in aldose reductase levels which have been observed in studies of diabetes in experimental animals [1]. The myoinositol excretion was increased in diabetes; the plasma and CSF levels of myoinositol were however, normal. Whether the increased excretion was due to increased synthesis or decreased degradation in the kidneys is not known.

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