Effect of dietary protein manipulations in subclinical portal-systemic encephalopathy

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SUMMARY Eight stable cirrhotic patients with mild or subclinical portal-systemic encephalopathy (PSE) were studied after shunt surgery when they were off all antiencephalopathic therapy. Equal amounts of mixed proteins were alternated with animal or vegetable protein in a crossover protocol under metabolic conditions for five consecutive, one week periods. The different dietary periods were not associated with either a change in the neurological impairment score or the Trailmaking Tests, which showed a learning effect. The peak frequencies of the computer analysed EEG (CAEEG) were lower during the animal (6.58 ± 0.42 Hz) than the vegetable (7.10 ± 0.44 Hz) diet ($p < 0.01$). Neither arterial ammonia levels nor plasma amino acid ratios changed with the diets, whereas urinary 3-methyl-histidine excretion increased during the animal diet. During the vegetable diet the apparent nitrogen balance tended to be more positive than during either the mixed or animal diets associated with a decrease in the urinary nitrogen excretion. The peak frequency of the CAEEG is the most sensitive test to monitor methods of treatment in portal-systemic encephalopathy. A vegetable protein diet, rather than overall protein restriction, should be considered in the management of this disorder, particularly when the nutritional state is poor.

Treatment of mild portal-systemic encephalopathy focuses on prevention of symptoms by protein restriction and, if necessary, by adding lactulose or neomycin. Recently Greenberger¹ showed that not only the quantity but also the quality of the dietary protein may be important in the management of the chronic condition. Vegetable derived protein was claimed to be superior to animal protein. Only three patients were studied, however, and two received additional anti-encephalopathic treatment with neomycin, lactulose, or sorbitol. Furthermore, the protocol of the protein administration varied.

To investigate the role of dietary protein in portal-systemic encephalopathy we undertook a controlled trial with elimination of all variables apart from the source of protein in the diet. The

effect of animal vs vegetable protein on manifestations of the disorder was assessed in a crossover protocol with mixed protein using neurological assessment, conventional as well as computer analysed EEG recordings, performance on the Trailmaking Test, arterial ammonia, plasma amino acids, and nitrogen balance studies.

Methods

PATIENTS

Patients were accepted for this study only if they fulfilled *all* of the following five criteria: (1) shunt surgery at least one year before the study, for recurrent bleeding from oesophageal varices; (2) at least one documented attack of spontaneous portal-systemic encephalopathy after shunt surgery; (3) stable cirrhosis for a minimum of six months before the study. The clinical condition was recorded during the regular visits to the Shunt Clinic follow-up programme, when blood for biochemical liver function tests was also obtained; (4) patients could

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enter only if they did not require lactulose, neomycin, diuretics, or sedatives for the duration of the study; (5) consent from the patient and his/her family to participate in this investigation was needed, knowing the hardships of a five week metabolic study; it was approved by the University of Toronto Ethics Committee, April 1979.

In addition, we tried to study equal numbers of patients with alcoholic and post-necrotic cirrhosis. The alcoholic patients needed to be abstinent for at least two months before admission, as assessed by (1) the patient's history, (2) confirmatory history from his/her family, (3) observations during clinic visits including, in one patient, daily screening of the patient's urine for alcohol.²

The clinical data of the eight patients who entered the study are summarised in Table 1. Under close clinical observation, patients on lactulose had their therapy gradually withdrawn and replaced with psyllium mucilloid powder together with a 10 mg bisacodyl suppository to ensure a daily bowel action.

The biochemical liver function tests are summarised in Table 2. There were no significant changes in the biochemical tests during the study, although the bilirubin and globulin concentrations tended to decrease slightly.

PROTOCOL

Initially, after an interview with the dietician, patients were maintained on the amount of protein of their home diet. According to the individual patient's likes and dislikes, a meal plan was made to ensure that every item of all three diets was totally acceptable to the patient. The three different meal plans contained equal amounts of protein, and calories for the entire study period. The patient received each meal in weighed boats on individual trays and was supervised while eating in the Clinical

Research Unit. If the patient could not finish a meal completely, the residue was returned to the kitchen to be reweighed and the protein intake corrected. During the entire study each patient received one multivitamin tablet a day to avoid differences in the composition of microconstituents of the diets.

During the first, third, and fifth weeks the same control 'mixed' diet of 50:50 animal to vegetable protein was administered. During the second and fourth weeks all the protein was either vegetable only, or >90% was derived from animal sources. Fish and milk were considered to be animal protein. Four patients (two with alcoholic and two with non-alcoholic cirrhosis) received the vegetable diet in the second and the animal diet in the fourth week, while, for the other patients, the diet order was reversed. Patients no. 1 and 8 had an additional carbohydrate restriction and patients no. 1, 3, 4, 5, 7, and 8 an additional salt restriction.

The actual amino acid composition of the different diets was calculated for patients no. 5, 6, 7, and 8 from published tables.^{3,4} Only those amino acids in which the amounts were significantly different are shown (Table 3). It should be noted that the amounts of branched chain amino acids were not different.

The day after admission, a fasting arterial ammonia was measured using an enzymatic method.⁵ Each Friday, 1½ hours after breakfast, a non-fasting arterial ammonia was drawn, together with a venous sample for amino acid determination. Amino acids were measured on an automated Beckman amino acid analyser, Model 116/119. Methionine, valine, leucine, isoleucine, tyrosine, and phenylalanine were measured in all samples. In patients no. 5-8 a complete plasma amino acid spectrum was also obtained during the vegetable and animal diets.

Table 1 Description of patients

Patient no.	Sex	Age (yr)	Cause of cirrhosis	Shunt		PSE treatment on admission			Comments
				Type	Interval to study (yr)	Protein (g/24 h)	Other	(g/day)	
1	F	51	Postnecrotic	Portocaval	5	50	—	—	Maturity onset diabetes, hypothyroidism
2	F	19	Postnecrotic	Distal splenorenal	1-5	65	—	—	Pre-existing intellectual impairment
3	M	65	Alcoholic	Mesocaval	0-5	40	Lactulose neomycin	40 2	Portal-systemic myelopathy, multiple ventricular extrasystoles
4	F	57	Alcoholic	Mesocaval	2	45	Lactulose	20	—
5	M	61	Alcoholic	Distal splenorenal	2	80	Lactulose	80	—
6	F	55	Alcoholic	Portocaval	1	40	Lactulose	20	Propyl thiouracil study
7	F	63	Postnecrotic	Portocaval	2	40	Lactulose	100	Irritable bowel syndrome, psychoneurosis
8	F	63	Postnecrotic	Portocaval	4-5	40	Lactulose	40	Insulin dependent diabetes (65 U/day)

Table 2 Liver function tests*

	Normal range	Admission	Discharge	p-value paired t test
SGOT (IU/l)	8-30	40 (27-59)	38 (12-58)	0.67
SGPT (IU/l)	8-30	22 (16-27)	25 (8-41)	0.33
αGT (IU/l)	<45	46 (23-111)	36 (17-62)	0.17
Alkaline phosphatase (IU/l)	56-244	330 (227-580)	307 (216-405)	0.49
Bilirubin (total) (μmol/l)	<17	60 (12-224)	51 (7-185)	0.11
Total protein (g/l)	64-80	69 (60-82)	65 (55-70)	0.09
Albumen (g/l)	35-50	33 (25-37)	32 (26-40)	0.18
Prothrombin time (deviation from control) (s)	<2.5	2.6 (1.0-5.0)	2.7 (1.0-4.5)	0.75

* Mean values and, in parentheses, ranges of the biochemical liver function tests at the beginning and the end of the study period.

From each sample, the branched chain over aromatic amino acid ratio⁶ was calculated:

$$\frac{(\text{VAL}) + (\text{LEU}) + (\text{ILE})}{(\text{PHE}) + (\text{TYR})}$$

Urinary 3-methyl-histidine (3MH) excretion during the vegetable and animal protein diets was measured with an automated Beckman amino acid analyser, model 116/119.

The patients collected their urine and stool from 0800 h of day 5 to 0800 h of day 7, stool as one 48 hour sample and urine as two 24 hour samples. Homogenised stool samples and urine aliquots were stored at -18°C until analysis with modified Berthelot reaction,⁷ expressed in grams of nitrogen (N) per 24 hours. The urine creatinine excretion was also measured⁸ to check for completeness of the

collection. To calculate the apparent N-balance, protein was converted to nitrogen using the average conversion factor 6.25.⁹

Conventional EEGs were recorded every Thursday, 1½ hours after breakfast, and CAEEGs every Friday, 1½ hours after lunch. In addition, conventional and computer analysed EEGs were obtained from age- and sex-matched controls. These volunteers did not have a history of epilepsy, alcohol abuse, liver disease, nor did they take any medication at the time of study.

Clinical evaluation with a 30 item standard neurological screening protocol¹⁰ took place in combination with the conventional EEG, and after the Friday blood sampling the patient performed a Trailmaking Test part A and part B¹¹ while supervised by a psychometrist.

Table 3 Protein and amino acid intake*

	Mixed	Vegetable	Animal	Veg vs Ani p-value paired t test
Protein intake (g/24 h)	56.9±9.98	57.1±10.10	57.6±9.90	0.29
Acids (μmol/l)				
Aromatic†				
PHE (μmol/24 h)	11.60×10 ³ ±3.34×10 ³	17.79×10 ³ ±3.17×10 ³	13.75×10 ³ ±2.48×10 ³	0.03
(mg/24 h)	(1917±552)	(2939±525)	(2272±410)	
TYR	3.09×10 ³ ±0.79×10 ³	4.06×10 ³ ±0.58×10 ³	3.58×10 ³ ±0.54×10 ³	0.005
	(560±144)	(737±106)	(650±99)	
Basic				
ARG	12.02×10 ³ ±2.50×10 ³	24.61×10 ³ ±4.87×10 ³	14.38×10 ³ ±2.57×10 ³	0.05
	(2094±436)	(4288±849)	(2505±449)	
LYS	18.82×10 ³ ±4.79×10 ³	16.06×10 ³ ±2.98×10 ³	27.21×10 ³ ±4.24×10 ³	0.01
	(2752±701)	(2348±437)	(3978±621)	
Sulphur containing				
CYS	5.93×10 ³ ±1.86×10 ³	8.23×10 ³ ±1.09×10 ³	5.03×10 ³ ±0.75×10 ³	0.01
	(719±226)	(997±133)	(610±91)	
MET	6.52×10 ³ ±1.70×10 ³	6.19×10 ³ ±0.88×10 ³	9.85×10 ³ ±1.32×10 ³	0.03
	(973±254)	(924±132)	(1471±198)	

* Protein and amino acid intake during the mixed, vegetable, and animal protein diets calculated for patients 5-8. The intake of branched chain amino acids (ILE, LEU, VAL) and neutral amino acid (THR) did not change during the different diet periods.

† Considered as the base - that is, not salt form.

ELECTROENCEPHALOGRAPHY

For the conventional EEG, recordings were made with eyes closed, eyes open, during hyperventilation, and light stimulation. The EEG was scored for the dominant frequency and an overall score by the criteria of Parsons-Smith *et al*,¹² refined with the Mayo Clinic criteria.¹³

For the computer analysed EEG (CAEEG) silver-silver-chloride electrodes were applied with collodion in the temporal and occipital positions because in portal-systemic encephalopathy EEG abnormalities are most pronounced in those areas.¹⁴ The CAEEG consisted of four six minute recordings at 30 minute intervals with eyes open or closed alternatively. Smoothed power spectra were calculated as follows: the six minute recording was divided into 22 16 second ensembles, the first and last eight seconds of the six minutes having been discarded. For each ensemble, the mean was removed and the result windowed using a 10% extended cosine bell window.¹⁵ The frequency spectrum was computed by Fast Fourier Transform and the power spectrum calculated by the direct method. The smoothed power spectrum was obtained by ensemble averaging of the 22 power spectra and frequency using a 5 point unit weighed moving average digital filter. The averaged power spectrum had a band width of <0.25 Hz. By visual examination of the power spectral plot the peak frequency (arrow in Fig. 1) in the alpha and/or theta bands – that is, between 4 and 13 Hz – was determined as the mean of the right and left channels. The delta band (<4 Hz) was not included because patients with mild portal-systemic encephalopathy were not expected to have such low peak frequencies.

STATISTICAL ANALYSIS

Only mean scores and standard errors of the mean

(SEM) are given here. (Upon request, tables with the individual patient scores are available from the authors (LMB).) The diets were compared with the paired *t* test, two-tailed, and correlations analysed with the Pearson's correlation coefficient. A difference was accepted to be significant at the $p < 0.05$ level.

Results

On admission, three patients presented with grade 1 encephalopathy according to the modified¹⁶ Parsons-Smith criteria (Table 4). The others had no evidence for a clinical diagnosis of portal-systemic encephalopathy. The neurological examinations did not change with the dietary manipulations.

EEG

The conventional EEGs of the control subjects disclosed no abnormalities (Table 5). In contrast, seven patients had EEG abnormalities, with portal-systemic encephalopathy grades ranging from 1 to 3.5. These grades did not change for the different diets (Table 6), nor did the dominant frequencies for the various diet periods differ from each other (Table 6).

The peak frequency of the CAEEG correlated strongly with the dominant frequency of the conventional EEG ($r = 0.97$, $p < 0.0001$). All CAEEG recordings of the control subjects yielded peak frequencies in the normal alpha range – that is, between 8 and 12 Hz (Table 5). In contrast, seven patients had abnormally low peak frequencies (Tables 4 and 5).

During the animal protein diet, the peak frequencies were significantly lower than during the vegetable diet (Table 6). This effect did not depend on the order in which the diets had been administered.

Fig. 1 Examples of power spectra from six minute EEG recordings in a control subject (left) and a patient with grade 1 portal-systemic encephalopathy (right). The peak frequencies are indicated with arrows. Both top panels show recordings with eyes closed and both bottom panels with eyes open. With eye opening the alpha (like) activity disappears and low frequency power increases due to eye movement artefacts.

Table 4 Test scores on admission, according to criteria listed below each column

Patient no.	Neurological examination	PSE-grade EEG	CAEEG peak frequency (Hz)	Trailmaking Part A (s)	Fasting ammonia ($\mu\text{mol/l}$)
1	±	+	+	+	±
2	±	±	0	±	±
3	+	++	++	+++	+
4	0	++	++	+	±
5	0	0	+	++	+
6	0	+	+	+	±
7	+	+	+	+++	+
8	+	+++	++	+++	+
	<5 = 0 5-10 = ± 10-20 = + Carlen <i>et al</i> ¹⁰	Parsons-Smith criteria ⁵	8.5-12 = 0 7-8 = + 5-7 = ++ 3-5 = +++ <3 = ++++	<30 = 0 30-50 = + 51-80 = ++ 81-120 = +++ >120 = ++++ Conn <i>et al</i> ¹⁶	<60 = 0 60-80 = ± >80 = +

The peak frequency of the four consecutive CAEEG recordings on the same day varied only slightly (<0.2 Hz) in all but one patient, in whom changes up to 0.5 Hz were found.

TRAILMAKING TEST

All patients needed more than the normal time to complete the test when it was first administered. According to the criteria of Conn *et al*¹⁶ four patients scored grade 1, one grade 2, and three grade 3 (Table 4). Over the five week period a practice effect occurred. There were, however, no significant differences between the results during the vegetable and animal diets (Table 6).

NITROGEN METABOLISM

Fasting arterial ammonia levels on admission were slightly abnormal in four and mildly raised in the other four patients (Table 4). Subsequent non-fasting samples during the dietary protein manipulation rose at least once over the initial fasting value in all but the two patients (nos. 2 and 5) who had distal splenorenal shunts. These changes

did not relate to the dietary protein source. Furthermore, there was no relationship between the arterial ammonia concentrations and plasma amino acid levels.

The plasma amino acid patterns varied considerably in the four selected patients. For most amino acids, these changes did not relate to the diets. The basic amino acids tended to differ, however, for the vegetable and animal diets: arginine and ornithine concentrations tending to be higher, but lysine and citrulline levels lower during the vegetable than with the animal diet. Furthermore, the α -amino-N-butyric acid (AANB) concentration was higher during the animal ($1.92 \pm 0.23 \mu\text{mol}/100 \text{ ml}$) ($19.79 \times 10^{-2} \pm 2.37 \times 10^{-2} \text{ mg}/100 \text{ ml}$) than the vegetable diet ($0.39 \pm 0.09 \mu\text{mol}/100 \text{ ml}$, $p=0.02$) ($4.02 \times 10^{-2} \pm 0.92 \times 10^{-2} \text{ mg}/100 \text{ ml}$). The glycine concentration was lower during the animal ($0.26 \times 10^3 \pm 0.03 \times 10^3 \mu\text{mol}/100 \text{ ml}$) ($19.90 \pm 2.81 \text{ mg/ml}$) than the vegetable diet ($0.29 \times 10^3 \pm 0.04 \times 10^3 \mu\text{mol}/100 \text{ ml}$) ($22.22 \pm 3.18 \text{ mg/ml}$, $p<0.05$). Plasma methionine levels did not differ for the two diets.

All plasma branched chain over aromatic amino acid ratios were below 1.7, compared with a normal value of 3.0 to 3.5.⁵ The ratio varied slightly from week to week but none of these changes was statistically significant.

The plasma amino acid levels did not correlate with the amino acid intake.

The urinary 3-methyl-histidine excretion during the animal protein diet ($237 \pm 22.8 \mu\text{mol}/24 \text{ h}$) ($4.09 \pm 3.85 \text{ mg}/24 \text{ h}$) was significantly higher than during the vegetable diet ($153 \pm 19.4 \mu\text{mol}/24 \text{ h}$, $p=0.01$) ($2.59 \pm 3.35 \text{ mg}/24 \text{ h}$).

All stool samples for the nitrogen balances were complete, as were the urine collections, with three

Table 5 EEG results in patients and age- and sex-matched control subjects

	Control group	Patients	p-value paired t test
Conventional EEG			
Dominant frequency (Hz)	10.0±0.35	7.1±0.48	<0.001
PSE grade	0	1.4 (0-3.5)	<0.00001
CAEEG			
Peak frequency (Hz)	9.28±0.28	7.13±0.35	<0.0005

Table 6 Results during dietary manipulation

	Mixed	Vegetable	Animal	Veg vs ani p-value paired t test
Conventional EEG				
Dominant frequency (Hz)	7.1±0.48	6.8±0.52	6.8±0.40	0.92
CAEEG				
Peak frequency (Hz)	7.13±0.35	7.10±0.44	6.58±0.43	0.01
Trailmaking test				
Part A (s)	66±10.7	53±8.0	49±9.0	0.65
Part B (s)	173±48.9	141±32.7	143±40.3	0.32
Arterial ammonia (μmol/l)	109±14.4	115±15.2	110±12.6	0.42
BCAA				
AAA	1.13±0.11	1.06±0.12	1.11±0.11	0.45

exceptions, in which case an estimate was substituted based on the average urinary nitrogen output during the entire study.

Of the 40 nitrogen balances, 34 were positive (range -1.55 to +5.0 g/24 h) and, in only one patient (no. 1), three of five balances were negative.

Fig. 2 Apparent nitrogen balance and its components: urinary and faecal nitrogen excretion. Urinary nitrogen excretion was lower during vegetable than mixed ($p < 0.01$) and animal ($p < 0.07$) diets.

During the vegetable diet the apparent nitrogen balance tended to be more positive than during the mixed as well as the animal diets (Fig. 2). Faecal nitrogen excretion did not differ for the various diet periods. In contrast the urinary nitrogen excretion tended to be lower during the vegetable diet (6.1 ± 0.7 g/24 h) compared with the animal (7.4 ± 0.9 g/24 h, $p < 0.07$) and the mixed diet (7.6 ± 0.9 g/24 h, $p < 0.02$).

ALCOHOLIC VS POST-NECROTIC CIRRHOSIS

None of the tests differentiated the patients with alcoholic from those with post-necrotic cirrhosis.

Discussion

On admission only three patients had clinical evidence of portal-systemic encephalopathy, the other five having abnormal conventional EEG, CAEEG, Trailmaking Test and raised arterial ammonia – that is, subclinical portal-systemic encephalopathy.¹⁷⁻¹⁹

The mean peak frequency of the CAEEG during the pure animal protein diet was lower than during the vegetable and mixed diets. The conventional EEG failed to reflect this influence of the animal diet. This may be explained by the difference in discriminative power of the two forms of EEG analysis. Moreover, during the animal diet the mean peak frequency became lower than 7 Hz, a level that has previously been associated with the development of clinical portal-systemic encephalopathy.¹⁸ Our results therefore confirm the beneficial effect of vegetable over animal protein reported by Greenberger *et al.*¹ Pure vegetable and mixed protein diets, however, did not result in a different EEG response. The effect of the animal diet could not be explained by a change in liver function, as the biochemical liver function tests did not change during the study. Constipation was prevented in

these patients and also drugs were avoided that are known to precipitate portal-systemic encephalopathy, such as sedatives. Treatment with lactulose had also been discontinued. Finally, the CAEEG response could not be explained by differences in the caloric intake during the diets.

The arterial ammonia level, the only recognised biochemical test for portal-systemic encephalopathy,²⁰ was (mildly or moderately) raised in all patients, although the levels did not reflect the CAEEG changes seen with the dietary manipulation. Another proposed biochemical marker for portal-systemic encephalopathy, the plasma branched chain over aromatic amino acid ratio⁶ was abnormal in all patients. The amino acid ratio did not, however, correlate with the EEG abnormalities, nor with the results of the Trail-making Test. Nevertheless, diet-related changes in plasma amino acid levels were found in the basic amino acids, which remove ammonia *via* the Krebs-Henseleit cycle. Furthermore, the arginine intake was highest during the vegetable diet. Arginine may be beneficial in portal-systemic encephalopathy,²¹ although other workers have not confirmed this observation.²²⁻²³ During the animal diet the patients consumed more methionine, itself incriminated in the pathogenesis of portal-systemic encephalopathy²⁴ *via* its metabolite, mercaptans.²⁵

Despite a protein restricted diet these patients were generally in positive nitrogen balance. The vegetable diet tended to make the apparent nitrogen balance more positive than the mixed and animal diets. Although this effect was not statistically significant, it should not be disregarded, as it was fully accounted for by of a decrease in urinary nitrogen, without change in faecal nitrogen excretion. The more positive balance during the vegetable diet can in part be explained by the different nitrogen content of vegetable *vs* animal proteins. For the conversion from protein to nitrogen the average factor 6.25 was used.⁹ For vegetable protein, however, the true factor is closer to 6.4 and for animal protein closer to 6.15.⁹ The nitrogen intake during the vegetable diet was therefore probably approximately 0.4 g/24 h higher than during the animal diet. The remainder of the difference in apparent nitrogen balance, 0.6 g, could be explained by increased catabolism during animal protein ingestion as suggested by the higher level of AANB, a breakdown product of neutral amino acids,²⁶ the lower glycine levels, and increased urinary 3MH excretion.²⁷ The increased excretion during the animal diet, however, could be due solely to meat consumption.²⁸

The present findings indicate that a diet rich in animal protein may be deleterious in the manage-

ment of chronic mild portal-systemic encephalopathy. As a pure vegetable protein diet tended to bring these patients into more positive nitrogen balance, it would be reasonable to try the effect of a vegetarian diet in patients with the chronic resistant form of the disease. Otherwise, we suggest a well-balanced mixed protein diet, avoiding excessive animal protein intake, as the best long-term dietary regimen. This would also be most likely to ensure that the patients complied with the diet, as the majority of them needed great encouragement to finish their meals during the vegetable diet because of complaints of early satiety, abdominal distension with gas, and more frequent bowel actions. These side-effects, however, especially the latter, may be useful rather than harmful and, particularly with the more severe chronic patients, the diet may be worth persevering with.

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