Glycerol clearance in alcoholic liver disease

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SUMMARY Glycerol clearance was studied by a primed dose-constant infusion technique in 14 patients with alcoholic liver disease and six normal control subjects. Fasting blood glycerol concentrations were raised in the alcoholic subjects (0.09 ± 0.01 vs 0.06 ± 0.01 $\mu\text{mol/l}$, $P < 0.05$) and glycerol clearance was impaired (24.5 ± 1.9 vs 37.5 ± 3.2 ml/kg/min , $P < 0.005$). Endogenous production rate of glycerol and distribution space at steady state were similar in alcoholic and control subjects. The metabolic clearance rate of glycerol correlated negatively with basal glycerol concentrations. Thus tissue uptake of glycerol is impaired in liver disease. As glycerol is metabolised primarily in the liver by conversion to glucose, these data suggest a defect of gluconeogenesis in alcoholic liver disease.

The liver is the principal organ of glucose production in the fasting state^{1 2} with small contributions from the kidney and small intestine. Hypoglycaemia may complicate acute severe liver disease or end-stage chronic liver damage,³ but in most patients with chronic liver disease fasting blood glucose concentrations are either normal or mildly raised.^{4 5} Nonetheless, defects in glycogen storage and glycogen degradation are apparent from catheterisation experiments⁶ or analysis of the glycaemic response to glucagon in some^{7 8}—although not all^{9 10}—studies. Little is known about gluconeogenesis in chronic liver disease but catheterisation studies suggest a compensatory increase in cirrhotics.¹¹ This may also be inferred from radioactive tracer experiments showing normal rates of total glucose production after an overnight fast,¹² although other investigators have shown a small decrease in glucose production.¹³

Glycerol is highly gluconeogenic in man and animals and is preferentially metabolised to glucose in favour of other gluconeogenic precursors.¹⁴⁻¹⁶ In fasting, 40–100% of circulating glycerol is converted to glucose,^{15 17} conversion increasing as blood concentrations rise.¹⁸ The liver is responsible for 70–90% of glycerol uptake^{19 20} with the remainder metabolised by the kidneys,^{21 22} small intestine, and other tissues.²³ Renal uptake of glycerol is not increased in cirrhotic man.⁶

Clearance of an intravenous glycerol load may thus provide an index of liver dysfunction and an estimate of gluconeogenic capacity in cirrhotic man. We have investigated glycerol clearance in patients with alcoholic liver disease by a primed dose-constant infusion technique to steady state, with analysis of decay of blood glycerol levels on cessation of the infusion.

Methods

PATIENTS

Fourteen patients with alcoholic liver disease (11 male, three female, age 36–70 years) were compared with six normal controls (five males, one female, age 22–64 years). Eight patients had hepatic cirrhosis and six alcoholic hepatitis or fatty liver on biopsy (Table 1). The duration of heavy intake of alcohol admitted by the patients ranged from five to 30 years. Patient number 2 had mild chronic hepatic encephalopathy but no other patient was clinically in liver failure at the time of study. Patients were on a variety of medications (Table 1) but no drugs were taken on the day of study. Four patients had spontaneous portal-systemic shunting as shown by oesophageal varices on barium swallow or endoscopy, and four patients had clinically evident ascites. No patient had evidence of renal impairment. Control subjects were normal laboratory personnel or their acquaintances and none was on any drug therapy, or suffering from any known disorder. Informed consent was obtained from all subjects for the study, which was approved by the Southampton Hospitals Ethical Committee.

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Table 1 Clinical features of alcoholic patients and controls

No.	Liver biopsy diagnosis	Age (yr)	Sex	%IBW	Ascites	Varices	Aspartate amino-transferase (IU/l)	Lactate dehydrogenase (IU/l)	Bilirubin (μ mol/l)	Albumin (g/l)	Therapy (daily dose)
1	Cirrhosis	38	M	99	—	—	72	280	28	29	—
2	Cirrhosis	64	M	101	—	+	75	369	32	27	Neomycin (4 g)
3	Cirrhosis	49	F	93	—	—	41	189	21	46	—
4	Cirrhosis	52	M	104	—	—	29	178	15	43	Digoxin (0.25 mg)
5	Cirrhosis	70	M	127	—	—	86	286	112	42	Digoxin (0.0625 mg)
6	Cirrhosis + alcoholic hepatitis	48	M	119	—	+	110	334	56	35	Spironolactone (100 mg)
7	Cirrhosis	53	M	88	—	—	67	202	8	34	—
8	Cirrhosis	64	M	125	+	+	112	310	28	27	Spironolactone (200 mg)
9	Alcoholic hepatitis	51	M	103	—	—	35	402	4	40	—
10	Alcoholic hepatitis	45	F	85	—	—	31	232	4	43	—
11	Alcoholic fatty liver	36	M	111	—	—	102	270	10	45	—
12	Alcoholic hepatitis	60	F	127	+	—	175	458	115	38	Spironolactone (100 mg)
13	Alcoholic hepatitis	61	M	119	+	—	153	269	16	39	—
14	Alcoholic fatty liver + hepatitis	58	M	127	—	—	82	316	21	32	—
Controls (n = 6)		22–64	5M 1F	98–115	—	—	(5–42)	(100–225)	(0–17)	(32–50)	—
		Mean 45		Mean 105							

%IBW: percentage ideal body weight, by Metropolitan Life Assurance figures.
Reference ranges shown in parentheses.

Studies were performed on recumbent subjects after an overnight (10–12 hours) fast. Subjects had abstained from alcohol for at least 48 hours and had a daily carbohydrate intake in excess of 250 g for this period. Teflon intravenous cannulae (Venflon, Viggo, Helsingborg) were sited in an antecubital vein on each side and patency was maintained by flushing with 0.154 mol/l saline after every sample. Thirty and 40 minutes after cannulation basal samples were taken. After an initial loading dose (100 $\mu\text{mol/kg}$ body weight diluted with water to isotonicity and administered over two minutes), a continuous intravenous infusion of glycerol, 19 $\mu\text{mol/kg/min}$ (Boots, Nottingham, 1.1 mol/l), was begun through the contralateral cannula and continued for 40 minutes with a Harvard continuous infusion pump. Blood samples were taken two minutes after the loading dose and at 10, 7.5, 5, and 2.5 minutes before the continuous infusion ended. Additional blood samples were taken 0, 2.5, 5, 7.5, 10, 12.5, 15, 20, 30, 45, and 60 minutes after the infusion ended. All urine produced over the experimental period was collected after the last blood sample, its volume measured and a 20 ml aliquot taken for glycerol analysis.

Venous blood (1.5 ml) was mixed immediately with 5 ml of 5% (V/V) ice-cold perchloric acid. Blood glycerol, glucose, lactate, pyruvate, and alanine were measured by an automated fluorimetric enzymatic method (Lloyd *et al.*, 1978²⁴). Urine glycerol was estimated by a spectrophotometric enzymatic method.²⁵ Plasma aspartate aminotransferase, lactate dehydrogenase, bilirubin, and albumin were measured by standard Autoanalyser techniques (Technicon).

Results are expressed as mean $\pm$ SEM. Statistical analysis was performed using Student's paired and unpaired *t* tests where appropriate. Correlations were sought by Spearman's ranking method. The distribution of certain data was skewed (plasma aspartate aminotransferase and bilirubin) and statistical analysis was performed on log-transformed data.

MATHEMATICAL ANALYSIS

A steady state for blood glycerol was accepted if a change of <5% in blood glycerol concentrations occurred over the last 10 minutes of the intravenous infusion. The metabolic clearance rate (MCR, l/kg/min) was calculated as the infusion rate (mmol/kg/min) divided by the increase in blood glycerol at steady state above the basal glycerol concentration (Gss, mmol/l).

$$\text{MCR (l/kg/min)} = \frac{\text{Infusion rate (mmol/kg/min)}}{\text{Gss (mmol/l)}}$$

The decay of blood glycerol when the infusion ended was closely approximated by a double ex-

ponential function of the form, $Ae^{-k_1t} + Be^{-k_2t}$ where A, B, k_1 , k_2 are constants. Curve fitting was performed by computer to the points of the blood glycerol decay curve, after the basal glycerol concentration had been subtracted, by minimising the sum of squares of deviations. The area under the curve (area, mmol/l $\times$ min) was calculated using the polyexponential equation. The excess amount of glycerol (Qg, mmol/kg) in the body at steady state was calculated as the product of the metabolic clearance rate and the area under the decay curve.

$$\text{Qg (mmol/kg)} = \text{MCR (l/kg/min)} \times \text{area (mmol/l} \times \text{min)}$$

The apparent volume of distribution of glycerol at steady state (V, l/kg) was calculated as the amount of glycerol present divided by the increase in blood glycerol above basal at steady state (modified from Riegelman *et al.*²⁶).

$$\text{V (l/kg)} = \frac{\text{Qg (mmol/kg)}}{\text{Gss (mmol/l)}} = \frac{\text{MCR (l/kg/min)} \times \text{area (mmol/l} \times \text{min)}}{\text{Gss (mmol/l)}}$$

The endogenous production rate of glycerol (PR, $\mu\text{mol/kg/min}$) was calculated as the product of the basal glycerol concentration (Gb, mmol/l) and the metabolic clearance rate.

$$\text{PR (\mu mol/kg/min)} = \text{Gb (mmol/l)} \times \text{MCR (l/kg/min)} \times 1000$$

It is assumed in these calculations that the endogenous production rate of glycerol remains constant throughout the experimental period.

Results

Basal blood metabolite concentrations are shown in Table 2. Fasting blood glucose concentrations were similar in patients and controls. Blood lactate concentrations were increased by 27% ($P < 0.02$) in the patient group, but the blood lactate:pyruvate ratio was similar in patients and controls. Blood alanine levels did not differ in the two groups. Fasting blood glycerol concentration was raised in the alcoholic patients (0.09 ± 0.01 vs 0.06 ± 0.01 mmol/l, $P < 0.05$).

Data obtained during glycerol infusion are shown in Table 3. At 2.5 minutes after administration of the loading dose, blood glycerol concentrations ranged from 0.35 to 1.22 mmol/l. In the patient group, glycerol infusion produced steady state glycerol concentrations of 0.91 ± 0.05 mmol/l. In the controls, steady state blood glycerol levels were 0.58 ± 0.05 mmol/l, $P < 0.002$. The metabolic clearance rate of glycerol was decreased in the alcoholics (24.5 ± 1.9 vs 37.5 ± 3.2 ml/kg/min, $P < 0.005$). The apparent volume

Table 2 Blood glucose and gluconeogenic precursor concentrations in basal state and at steady state glycerol levels in patients and controls

	Basal blood metabolite concentrations (mmol/l)			Blood metabolite concentrations (mmol/l) at glycerol steady state			
	Patients	Controls	P1	Patients	Controls	P2	P3
Glucose	5.43±0.21	5.35±0.31	NS	5.68±0.23	5.65±0.30	<0.02	<0.005
Lactate	0.89±0.04	0.70±0.04	<0.02	1.02±0.05	0.85±0.06	<0.001	<0.02
Pyruvate	0.09±0.01	0.08±0.04	NS	0.09±0.01	0.08±0.01	NS	NS
Lactate	10.3±0.4	9.1±0.5	NS	11.7±0.5	10.5±0.3	<0.001	<0.01
Pyruvate							
Alanine	0.27±0.01	0.30±0.02	NS	0.27±0.01	0.32±0.02	NS	NS

P1 is the significant level for patient group different from control (unpaired test).

P2 is the significance level for patient group concentration at steady state different from basal concentration (paired test).

P3 is the significance level for control group concentration at steady state different from basal concentration (paired test).

NS: not significant.

For blood glucose, values—mg/100 ml are 18×mmol/l.

Table 3 Glycerol infusion data in alcoholic patients and controls

	Steady state glycerol concentration (mmol/l)	Metabolic clearance rate (ml/kg/min)	Distribution space (l/kg)	Endogenous production rate (μmol/kg/min)
<i>Patient no.</i>				
1	0.79	24.5	0.564	2.08
2	0.96	20.8	0.837	2.66
3	1.07	23.0	0.866	2.25
4	0.69	29.0	0.633	1.31
5	0.98	20.1	0.845	1.63
6	0.86	23.7	0.379	1.52
7	1.14	18.6	0.638	2.38
8	1.09	18.6	0.726	1.73
9	0.71	30.4	0.749	2.61
10	0.50	45.6	0.821	2.87
11	0.79	27.4	0.531	1.51
12	1.21	16.8	0.315	1.44
13	1.03	21.5	0.433	2.06
14	0.86	22.4	0.653	1.32
Patient mean±SEM	0.91±0.05	24.5±1.9	0.642±0.048	1.96±0.14
<i>Control no.</i>				
1	0.70	27.5	0.537	1.51
2	0.71	32.4	0.746	2.20
3	0.54	44.0	0.576	3.87
4	0.65	33.3	0.536	1.20
5	0.50	39.5	0.886	2.69
6	0.39	48.3	0.781	1.64
Control mean±SEM	0.58±0.05	37.5±3.2	0.677±0.060	2.19±0.40
Significance	P<0.002	P<0.005	NS	NS

Decay of blood glycerol with time at end of the infusion was well represented by a double exponential function:

$y = 0.30e^{-0.23t} + 0.53e^{-0.03t}$ for patients;

$y = 0.27e^{-0.30t} + 0.24e^{-0.03t}$ for controls.

Calculations were performed as outlined in the Methods section.

of distribution of glycerol at steady state was similar in alcoholics and controls (0.642±0.048 vs 0.677±0.060 l/kg respectively).

Analysis of the decay of blood glycerol when the infusion ended showed it to be well represented by a double-exponential function (Figure). Values for the exponential constants k_1 and k_2 are shown in Table 3 with the corresponding half-life for each exponent of the decay. The half-life of the first exponent ranged from 1.4 to 9.5 minutes in the patient group and 1.8 to 3.1 minutes in the controls. The half-life for the second exponent was similar in both groups

(27.0±2.4 minutes, patients; 28.3±3.0 minutes, controls).

Within the patient group, correlations were sought between the metabolic clearance rate and indices of liver function and metabolic status. Metabolic clearance rate correlated significantly and negatively with the plasma aspartate amino-transferase concentration ($r_s = -0.64$, $P < 0.02$) and plasma bilirubin ($r_s = -0.62$, $P < 0.05$) but not with the other standard tests of liver function performed. A significant negative correlation was also obtained between the metabolic clearance rate and the basal blood glycerol

Figure Decay of blood glycerol at the end of continuous intravenous infusion. Blood glycerol expressed as change from basal values. Values shown are mean $\pm$ SEM. $\circ$ — $\circ$ Alcoholic liver disease. $\bullet$ — $\bullet$ Control. Glycerol infusion stopped at time 0.

concentration ($r_s = -0.57$, $P < 0.05$) but no relationship was obtained with the other fasting metabolites that were estimated.

Glycerol infusion produced a rise in blood lactate concentrations (15% in the patients, 21% in the controls at steady state) but blood pyruvate levels were unaltered (Table 2). Blood lactate:pyruvate ratios rose in both patients and controls. Blood glucose concentrations were increased by glycerol infusion to a similar degree in patients and controls.

Urinary glycerol loss ranged from 0.1 to 1.2 mmol/experiment and was never more than 2% of the total glycerol infused.

Discussion

Circulating concentrations of the gluconeogenic precursors, lactate, pyruvate, and glycerol, are raised in a variety of liver diseases after an overnight fast,^{3,4} and in the present study in patients with alcoholic liver disease, fasting blood lactate and glycerol concentrations were raised. These findings have usually been interpreted as secondary to diminished hepatic uptake. Impaired lactate clearance in cirrhosis has been known for many years²⁷ and recently reaffirmed²⁸ but information on clearance of other gluconeogenic precursors is lacking.

The metabolic clearance rate for blood glycerol (0.038 ± 0.003 l/kg/min) obtained in the present study compares with plasma clearances in the dog of 0.038 to 0.055 l/kg/min over a range of circulating glycerol levels from 0.09 to 0.61 mmol/l (calculated from the tracer studies of Winkler and colleagues.²⁹) Similar plasma glycerol clearances have been obtained in normal man from radioactive turnover data (0.032 to

0.049 l/kg/min, calculated from data of Havel,³⁰ assuming body weight of 70 kg), although somewhat lower values have also been obtained (0.024 to 0.032 l/kg/min, calculated from the data of Bortz *et al.*¹⁵ for lean subjects after a six to 14 hour fast). Estimates of clearance rates from plasma by the continuous infusion technique may be expected to produce lower values than from whole blood as the exogenous glycerol is presumably infused directly into plasma water. Equilibration of glycerol between plasma and red cells is, however, so rapid that a difference of only 10% between plasma and whole blood clearance rates may be anticipated.²¹ In the present study, whole blood glycerol clearance was estimated, as this may give a more accurate measure of organ uptake.³¹⁻³³

The rate of glycerol uptake for metabolism in the dog is proportional to the plasma glycerol level over a plasma range of 0.06 to 1.40 mmol/l, suggesting that the clearance rate is constant within this range.²⁹ At higher glycerol levels, the linear relationship is not preserved as uptake becomes saturated and significant renal glycerol excretion appears. The infusion rate of glycerol in the present study was chosen to give blood glycerol concentrations ranging from 0.5 to 1.3 mmol/l. The small amounts of glycerol in the urine in the present study, which were ignored in the calculations, suggest that glycerol disposal was through metabolism rather than urinary excretion. It was also considered desirable to avoid very high circulating glycerol levels even transiently, in view of the reports of renal damage after administration of massive amounts of glycerol.^{34,35} Much higher doses than those used in the present study may be administered therapeutically to man without adverse

effects³⁶ and no subject showed any evidence of renal impairment in this report.

The metabolic clearance rate is the best index of glycerol disappearance in this kind of experiment, as its measurement (the ratio of infusion rate to incremental glycerol concentration at steady state) is independent of any descriptive compartmental model and its estimation is valid even if glycerol elimination occurs outside the sampling compartment.³³ Glycerol clearance was decreased by 35% in patients with alcoholic liver disease and clearance correlated negatively with the other indices of liver dysfunction, plasma aspartate aminotransferase, and bilirubin concentrations. Diminished clearance was found in patients with alcoholic hepatitis and fatty liver as well as in cirrhotics.

Hepatic extraction of glycerol is high. At serum levels below 1 mmol/l there is almost complete extraction in a single transhepatic passage.³⁷ The clearance of substances with such high hepatic extraction is profoundly influenced by changes in hepatic blood flow⁵³ such that, if metabolism were purely hepatic and extraction 100%, then the metabolic clearance rate would provide an estimate of hepatic blood flow.¹⁹ With these assumptions, the clearance rate obtained in the normal subjects (0.03 l/kg/min) corresponds to a hepatic blood flow of 2.0–2.5 l/min, as opposed to estimates by other means for normal man of 1.5–2.0 l/min.³⁸ Glycerol administered into a peripheral, as opposed to portal, vein, however, will also be available for metabolism by other tissues, particularly the kidneys,²² so that its clearance rate from the body will overestimate hepatic blood flow. Although no formal assessment was obtained in the alcoholic patients in this report, hepatic blood flow will undoubtedly have been diminished in some patients.³⁹ The decrease in metabolic clearance of glycerol in alcoholic liver disease may therefore result from direct hepatocellular damage, diminished hepatic blood flow, or both. It is possible that a decrease in peripheral glycerol uptake may contribute, although this must be quantitatively small and there is no evidence to support this hypothesis.

It is assumed in our calculations that endogenous production rate of glycerol was unaltered during bulk glycerol infusion. This assumption may not be entirely valid as insulin secretion may have been stimulated by glycerol itself or the consequent rise in blood glucose.⁴⁰ The resultant error in calculation of clearance rates for glycerol during bulk infusion would, however, be small. Assuming that endogenous production rate for glycerol declined to zero, an error of less than 5% in clearance estimation would be anticipated at circulating glycerol levels of 0.5 mmol/l.²⁹ Possible variations in endogenous glycerol production were therefore ignored in clearance estimations.

The calculated endogenous production rate of glycerol in normal subjects (2.19 ± 0.40 $\mu\text{mol/kg/min}$) was similar to values from plasma data obtained in man using radioactively labelled glycerol (1.50 ± 0.23 $\mu\text{mol/kg/min}$ for lean subjects; 1.63 ± 0.22 $\mu\text{mol/kg/min}$ for obese subjects; calculated from the data of Bortz *et al.*¹⁵) Production rates of glycerol were similar in patients with alcoholic liver disease and controls, contrasting with the increase in lipolysis inferred from studies showing increased plasma non-esterified fatty acid (NEFA) concentrations in alcoholic liver disease.⁴¹ Plasma NEFA turnover studies after adequate dietary preparation are necessary to resolve this problem. In the present report, the significant negative correlation between glycerol clearance and fasting glycerol concentrations further suggests that the raised basal glycerol levels are related to diminished uptake.

The decay of blood glycerol concentration when the infusion ended was closely approximated by a double exponential function and this was used to calculate the area under the curve. The calculated apparent volumes of distribution for glycerol by the area method⁴² (0.642 ± 0.048 l/kg in alcoholics; 0.677 ± 0.060 l/kg in controls) agree well with estimates in other reports suggesting distribution of glycerol within the total body water.³⁰ If the first phase of decay of glycerol is ignored, much higher apparent volumes of distribution would be obtained in the present study, similar to values obtained for apparent glycerol space (up to 2.1 l/kg) by this method in the rat.⁴³ Such calculations of distribution volume are, however, inherently subject to error.⁴⁴ The volumes of distribution of glycerol in the present report were similar in alcoholics and controls, suggesting that the differences in glycerol elimination were not secondary to an altered distribution space for glycerol in alcoholic subjects.

Despite a similar rise in blood glucose in patients and controls, diminished glycerol uptake for gluconeogenesis by the diseased liver is the likely cause for the decrease in glycerol clearance in the alcoholics. Although glycerol is quantitatively a minor gluconeogenic substrate in fasting,⁴⁵ conversion to glucose is the major metabolic fate.^{15 17} A decreased gluconeogenic capacity in liver disease is not apparent under normal conditions, where blood glucose is either normal or mildly raised.^{4 5} Catheterisation studies suggest that, although splanchnic glucose production is decreased in liver disease, this decline results primarily from a decrease in glycogenolysis.^{11 16} Diminished splanchnic lactate uptake and glucose output in response to pharmacological doses of glucagon have, however, been reported in cirrhosis,⁴⁶ although there was considerable individual variation. Gluconeogenesis is also impaired in animal models of

liver disease such as diet-induced cirrhosis,⁴⁷ galactosamine hepatitis,⁴⁸ and bile duct ligation.⁴⁹

Glycerol infusion caused a minor rise in blood lactate concentrations and the ratio of lactate to pyruvate in peripheral blood in both alcoholics and controls. Metabolism of glycerol increases the lactate to pyruvate ratio in the liver⁵⁰ reflecting a more reduced hepatic cystolic redox state, as dihydroxyacetone phosphate is formed from glycerol-3-phosphate and enters the glycolytic-gluconeogenic pathway.²³ The change in lactate:pyruvate ratio in peripheral blood may reflect these changes in the liver.

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