Natriuretic and aquaretic effects of intravenously infused calcium in preascitic human cirrhosis: physiopathological and clinical implications

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Background: Preascitic cirrhosis is characterised by subtle renal sodium retention. Calcium inhibits $\text{Na}^+/\text{K}^+-2\text{Cl}^-$ cotransport in the Henle's loop and could potentially correct sodium-handling abnormalities at that site.

Aim: To investigate the effects of calcium infusion on sodium handling in 10 patients with preascitic cirrhosis and nine healthy controls after 1 week of sodium loading of 200 mmol sodium/day.

Methods: All patients underwent a 3 h supine determination of inulin, para-aminohippurate, lithium and free-water clearances, absolute and fractional excretions of sodium, potassium and calcium and plasma concentrations of renin, aldosterone, norepinephrine and vasopressin. The same were repeated over a further 3 h supine period including 60 min intravenous infusion of 33 mg/min calcium gluconate.

Results: After sodium loading, the 24 h urinary sodium excretion in patients with cirrhosis was lower than that in controls ($p<0.03$). Calcium infusion significantly decreased plasma norepinephrine levels ($p<0.03$), and induced greater increases in fractional delivery of sodium to the Henle's loop ($p<0.5$) in those with cirrhosis than in controls. This was associated with a decreased fractional reabsorption of sodium beyond the proximal tubule ($p<0.03$), resulting in greater urinary volume, sodium excretion and free-water clearance in those with cirrhosis than in controls (all with $p<0.05$). Because the aldosterone-driven potassium secretion, as assessed by the computation of tubular-capillary gradient of $[\text{K}^+]$ in the collecting duct, was similar in the two groups and unaffected by calcium, sodium retention must have occurred in the Henle's loop in those with cirrhosis.

Conclusion: Calcium is natriuretic in patients with preascitic cirrhosis; it also decreases norepinephrine release, which could be responsible for decreased reabsorption of sodium in the Henle's loop.

Sodium-handling abnormalities are present in patients with preascitic cirrhosis. When sodium loaded with an oral intake of 200 mmol/day, unlike in healthy people, patients with preascitic cirrhosis maintain positive sodium balance for at least 3 weeks.¹ These patients retain sodium mainly in the upright posture, despite normal serum aldosterone levels.^{2–3} The supine posture seems to be a compensatory mechanism that induces a natriuresis, but is inadequate when compared with controls.² Therefore, there is an increase in the total circulatory volume⁴ and central blood volumes^{5–6} in the supine posture, leading to decreased activity of the renin–angiotensin–aldosterone system⁷ in the same posture, but without suppressing the plasma norepinephrine.⁸

This subtle sodium retention occurs despite normal or increased glomerular filtration rate (GFR),^{9–10} indicating inappropriate sodium retention in some tubular portion of the nephron.^{11–12} Lithium clearance (an established index of distal delivery of tubular fluid) has been shown to be slightly reduced or normal both in supine patients with preascitic cirrhosis^{6–13–14} and in animal models of cirrhosis.¹⁵ The mechanisms involved in tubular sodium-handling abnormalities include excess intrarenal production of angiotensin II¹³ in the erect posture, leading to an increase in the proximal renal tubular reabsorption of sodium occurring in that posture.^{13–16} In supine patients with preascitic cirrhosis, there can be sodium retention beyond the proximal tubule despite suppressed serum aldosterone levels.⁶ A bile duct ligation model of preascitic cirrhosis in rats has shown that the Henle's loop—that is, a nephron segment beyond the proximal tubule—could be involved in excess sodium retention. These preascitic cirrhotic rats had hypertrophy of the thick ascending limb (TAL) of Henle's loop and

increased sensitivity to the effects of furosemide.^{17–18} Furthermore, in rats with carbon tetrachloride (CCl_4)-induced cirrhosis, a large increase in the expression of $\text{Na}^+/\text{K}^+-2\text{Cl}^-$ cotransporter was found in the TAL of Henle's loop,¹⁹ suggesting a role of this tubular segment in sodium homeostasis in cirrhosis.

Studies assessing sodium handling at Henle's loop in human cirrhosis are not available to date. Most data on tubular sodium handling in human cirrhosis have been obtained through lithium clearance and fractional excretion technique. As lithium clearance approximates the delivery of fluid from the proximal convoluted tubule to the descending limb of Henle's loop, this method provides only indirect measurements of sodium metabolism in the distal nephron, which includes, according to this technique, Henle's loop, distal convoluted tubule and collecting duct.^{20–21} As no clearance technique is able to assess the contribution of these three tubular segments in the reabsorption of sodium in the distal nephron, we propose to use calcium infusion as a tool to evaluate sodium handling in the TAL of Henle's loop. When the plasma concentration of calcium is raised, this ion binds to calcium-sensing receptors located on the basolateral membrane of the cells lining the TAL of Henle's loop²² (fig 1). This leads to local generation of arachidonic acid metabolites^{22–23} that inhibit the potassium channel in the luminal membrane.²⁴ Inhibition of potassium

Abbreviations: AVP, arginine vasopressin; CIN, inulin steady-state plasma clearance; CK, potassium clearance; CNa, sodium clearance; DFRNa, distal fractional reabsorption of sodium; FELi, lithium fractional excretion; GFR, glomerular filtration rate; IN, inulin; PAH, para-aminohippurate; RPF, renal plasma flow; TAL, thick ascending limb; TTKR, trans-tubular concentration ratio of potassium in the cortical collecting duct; UNaV, urinary sodium excretion rate

Figure 1 Mechanism of calcium-dependent inhibition of $\text{Na}^+\text{--K}^+\text{--}2\text{Cl}^-$ cotransport in the thick ascending limb of Henle's loop. At the loop of Henle, binding of extracellular calcium to specific calcium-sensing receptors (CaSR) localised in the basal domain of the cell membrane causes (inside the cell) a prompt but short-lived cytochrome p450-dependent generation of 20-hydroxyeicosatetraenoic acid (20-HETE) and a prolonged cyclo-oxygenase (COX) 2-dependent generation of PGE_2 . In turn, 20-HETE inhibits both the apical sodium-potassium-chloride cotransporter and the secretory potassium channel (whose activity is needed to speed up tubular reabsorption of sodium). Prostaglandin E_2 (PGE_2) directly inhibits the reabsorption of electrolytes through the same apical sodium-potassium-chloride channel. The final result is increased electronegativity of the lumen leading to a reduction in the reabsorption of calcium, and this compensates for systemic hypercalcaemia. This increased electronegativity also reduces the reabsorption of sodium. AC, adenylyl cyclase; P450, cytochrome P450; TAL, thick ascending limb; $\oplus$, positive charge; $\ominus$, negative charge; X, blockage of action.

recycling through the potassium channel reduces the reabsorption of sodium chloride through $\text{Na}^+\text{--K}^+\text{--}2\text{Cl}^-$ cotransporter^{25–27} (fig 1). Thus hypercalcaemia is natriuretic.

We hypothesise that in preascitic cirrhosis, the Henle's loop is at least partly responsible for the subtle sodium retention. Therefore, intravenous calcium should improve sodium excretion in these patients by decreasing reabsorption of sodium at the loop of Henle. We have chosen to study patients in the supine posture with a suppressed renin-angiotensin-aldosterone system to eliminate any potential confounding effects due to proximal or aldosterone-dependent tubular retention of sodium, as may occur in the upright posture, while maintaining some degree of sodium retention in that posture.

MATERIALS AND METHODS

Ethics approval for the study was granted by the Ethics Committee of the Toronto General Hospital, University Health Network, Toronto, Ontario, Canada. All study subjects gave informed consent for the study.

Patients

Ten patients (nine men and one woman) with biopsy-proven liver cirrhosis were recruited from the Liver Clinics of the Toronto General Hospital. None of the patients had a history of ascites or diuretic use. Absence of ascites was confirmed by ultrasound examination before enrolment. Oedema was also absent in all patients. Therefore, these patients were referred to as patients with preascitic cirrhosis. The mean (SD) age of the study patients was 52.1 (9.9) years. The causes of cirrhosis were alcohol misuse (four patients), viral hepatitis C infection (two patients), viral hepatitis B infection (one patient), Wilson's disease (one patient), autoimmune hepatitis (one patient) and primary biliary cirrhosis (one patient). All were stable

ambulatory patients without a history of gastrointestinal bleed before entry into the study. All patients with alcoholic cirrhosis had abstained from alcohol for at least 6 months before enrolment. Patients with intrinsic renal or cardiovascular disease on history or on examination were excluded, as were patients with abnormal urine analysis, renal ultrasonography, chest radiograph or ECG. No steroids, prostaglandin synthesis inhibitors, amines or drugs for hypertension were administered for at least 1 month before the study. Nine age-matched volunteers (seven men and two women, mean age 47.3 (13.1) years) with no history of liver, renal or cardiac diseases served as controls. Table 1 shows the demographics of the two study groups.

Study design

This study was conducted in the Physiology Laboratory at the Toronto General Hospital. All study subjects underwent a run-in period of 1 week during which they were maintained on 200 mmol sodium, 1.5 l fluid restriction/day diet. A 200 mmol sodium/day diet was chosen because this would allow some degree of sodium retention while in the supine posture. On the last day of high-sodium diet (day 7), a 24 h urine collection to measure urinary sodium excretion rate (24 h UNaV) was obtained. To ensure dietary compliance, patients and controls were instructed to consume only food items permitted on their dietary instruction sheets and to complete a daily dietary diary. Caffeine-containing beverages and food items were withheld during the study period and all study subjects were asked to refrain from smoking.

On the actual day of the study (day 8; fig 2), at 6:00 h, all participants received 300 mg of lithium carbonate orally. Lithium clearance was used as a measure of tubular delivery of fluid from the proximal tubule to the Henle's loop.^{20–21} Patients and controls were admitted fasting at 7:00 h. Participants, after voiding, were asked to remain supine in a quiet place for the whole 6 h study period, lasting from 8:00 h to 14:00 h. Two intravenous catheters, one for infusion and another for blood withdrawal, were then inserted. After drawing blood for prothrombin time, international normalised ratio, serum lithium, calcium, sodium, potassium, and creatinine, complete blood count, and liver function tests, intravenous priming doses of 5 mg/kg body weight for para-aminohippurate (PAH) and 20 mg/kg body weight for inulin (IN) were administered.

Table 1 Demographics of the two study groups

	Controls (n = 9)	Patients with cirrhosis (n = 10)
Mean (SD) age (years)	47 (13)	52 (9)
Mean (SD) body weight (kg)	65 (10)	73 (10)
Aetiology		
Alcoholic		4
Hepatitis C virus		2
Hepatitis B virus		1
Autoimmune hepatitis		1
Wilson's disease		1
Primary biliary cirrhosis		1
Serum bilirubin (<1.5 mg/dl)	0.8 (0.2)	1.8 (0.6)*
INR (0.9–1.1)	1.02 (0.03)	1.39 (0.06)†
Serum albumin (>3.8 g/dl)	4.1 (0.8)	3.9 (0.9)
Alkaline phosphatase (<110 U/l)	67 (56)	75 (72)
ALT (<40 U/l)	35 (16)	129 (43)†
Platelet count ($150\text{--}400 \times 10^9/\text{l}$)	266 (112)	74.2 (43.6)†
Pugh score	–	5.8 (0.3)

ALT, alanine aminotransferase; INR, international normalised ratio.

* $p < 0.05$ vs controls;

† $p < 0.03$ vs controls.

Data are given as mean (normal values), unless stated otherwise.

Thereafter, a constant infusion of IN and PAH diluted in 500 ml 5% glucose solution was started (respective infusion rates: 30 and 8 mg/min) to measure GFR and renal plasma flow (RPF) by means of their respective steady-state plasma clearances (CIN and CPAH).^{28, 29} There were two 3 h urine collections, from 8:00 h to 11:00 h and from 11:00 to 14:00 h. At 8:00 h and 11:00 h blood withdrawals were repeated for serum electrolytes, serum lithium, plasma norepinephrine, vasopressin (AVP), aldosterone, active renin, osmolality and steady-state IN and PAH levels. Thereafter, a 60 min infusion of 33 mg/min calcium gluconate diluted in 100 ml of 5% glucose solution was given. Mean arterial blood pressure (measured through arm sphygmomanometry) and heart rate were taken just before and at the end of the 60 min infusion of calcium gluconate solution. At noon, the infusions of both calcium gluconate and IN/PAH were stopped and the following blood withdrawals were repeated: steady-state plasma concentrations of IN and PAH, serum electrolytes including calcium and plasma hormones and osmolality. As IN was determined colorimetrically by anthrone method,^{28, 30} which measures fructose after IN hydrolysis, blood samples for IN were also analysed for basal fructose concentrations and subtracted from the final IN reading. As stated above, all study subjects remained supine until 14:00 h when a final blood sample was drawn for serum lithium. The study was designed so that the volumes of "free-water"—that is, 5% glucose solution, administered over the two 3 h supine urine collection periods were very close: 180 ml between 8:00 and 11:00 h and 160 ml between 11:00 h and 14:00 h. The urine collections for these two periods were used to determine urinary osmolality and excretions of sodium, potassium, calcium and lithium.

Laboratory determinations

Serum and urine concentrations of sodium, potassium, lithium and calcium were measured by flame photometry. Serum creatinine was determined colorimetrically. Human active renin, aldosterone, AVP and norepinephrine levels were collected in EDTA tubes: active renin was measured using the Renin III Generation Pasteur immunoradiometric procedure, aldosterone was evaluated by RIA (Coat-A-Count Aldosterone kit, Diagnostic Products Corporation, Los Angeles, California, USA) and norepinephrine was determined using high-performance liquid chromatography as described by Eriksson and Persson³¹ and by Weicker and colleagues,³² with some modifications. AVP plasma concentrations were measured by RIA (Vasopressin Direct RIA, Buhlmann Laboratories AG, Postfach, Switzerland). IN, PAH and basal fructose concentrations in plasma were determined colorimetrically.^{28–30}

CALCULATIONS

Sodium and potassium clearances (respectively, CNa and CK) were calculated by the conventional formula

$$C_{-x} = (U_{-x} \times V) / P_{-x}$$

where U_{-x} is the urinary and P_{-x} is the plasma concentration of x and V the urinary output (ml/min). Inulin and PAH clearances were calculated from the formula for their steady-state plasma clearances:

$$C_{-x} = \text{infusion rate}_{-x} / \text{ssP}_{-x}$$

where ssP_{-x} is the steady-state plasma concentration of x . CIN and para-animohippurate steady-state plasma clearance were taken, respectively, as measures of GFR and RPF.^{28, 29}

The filtration fraction (FF) was calculated by:

$$\text{FF} = \text{GFR} / \text{RPF} \times 100$$

Fractional sodium excretion was calculated by the ratio of CNa and of CK to CIN $\times 100$.

Free-water clearance (F-WCl) was calculated, following Rose and Post,³³ using the formula:

$$\text{F-WCl} = V - C_{\text{osm}}$$

where V is the urinary output (ml/min); C_{osm} is the osmolar clearance, which was computed using the formula:

$$C_{\text{osm}} = (U_{\text{osm}} \times V) / P_{\text{osm}}$$

where U_{osm} and P_{osm} are urine and plasma osmolalities, respectively.

Serum lithium concentrations were calculated by the formula^{7, 20, 21}:

$$\frac{S\text{-Li (1)} - S\text{-Li (2)} / 2.3 \times \log S\text{-Li (1)}}{S\text{-Li (2)}}$$

where $S\text{-Li (1)}$ and $S\text{-Li (2)}$ were the lithium serum concentrations at the beginning and at the end of each lithium clearance period. Assuming that lithium is reabsorbed in the proximal tubule in parallel with sodium and water and that it is neither secreted nor reabsorbed beyond the proximal tubule,^{20, 21} from urinary sodium excretion (UNaV), lithium clearance, CNa and CIN the following parameters were derived^{20, 21}:

Absolute distal fluid delivery (DD) = Lithium clearance (CLi) (ml/min)

Lithium fractional excretion (FELi) = $\text{CLi} / \text{CIN} \times 100\%$

Absolute distal sodium delivery (DDNa) = $\text{CLi} \times \text{serum sodium (mmol/min)}$

Distal absolute reabsorption of sodium (DRNa) = $\text{DDNa} - \text{UNaV (mmol/min)}$

Distal fractional reabsorption of sodium (DFRNa) = $\text{DRNa} / \text{DDNa} \times 100\%$

Figure 2 Actual design of the study day (see Materials and methods section). iv, intravenous; PAH, para-aminohippurate.

Finally, to evaluate aldosterone function on the distal tubular nephron, the transtubular concentration ratio of potassium in the cortical collecting duct (TTKR) was calculated in both clearance periods according to the following formula^{34, 35}:

$$TTKR = \frac{[K^+]_{urine} / (U/P)_{osm}}{[K^+]_{serum}}$$

This parameter provides a semiquantitative reflection of the ratio of $[K^+]$ in the tubule fluid to that in the plasma of the adjacent vessels at the cortical collecting duct, an aldosterone-dependent segment of the tubular nephron.^{34–36}

Mean arterial pressure was calculated from the formula:

$1/3$ (systolic blood pressure—diastolic blood pressure)+diastolic blood pressure.

Statistical analysis

The difference between controls and patients with preascitic cirrhosis was determined by Student's *t* test. Differences between baseline values and values after the infusion of calcium for each of the parameters were determined in each group by analysis of variance, followed by *t* test with Bonferroni correction.

RESULTS

Pre-study week

On consuming a 200 mmol sodium/day diet for 1 week, patients with preascitic cirrhosis gained 1.2 (0.7) kg in weight. This contrasts with the controls, who maintained their mean body weight. The 24 h UNaV in patients with preascitic cirrhosis at the end of 1 week of 200 mmol sodium/day diet was significantly lower than in healthy controls (154 (21) vs 177 (16) mmol/24 h/1.73 m² body surface area; *p*<0.03). The 24 h urine volume was similar in patients and controls (*p*>0.05; table 2).

Baseline supine period

GFR and RPF were similar in patients and controls (respectively, 126 (28) vs 116 (16) ml/min; *p*>0.05 and 662 (27) vs 738 (101) ml/min; *p*>0.05). Filtration fraction, however, was higher in patients (19.1% (2.0%) vs 16.0% (2.5%); *p*<0.01) compared with controls (table 3).

Serum sodium, potassium and calcium concentrations were similar in patients and controls (all with *p*>0.05). In the supine position, the baseline 3 h urine volume was not significantly different (377 (148) vs 498 (328) ml; *p*>0.05) between the patients with cirrhosis and controls. The same was also true for the 3-hour sodium and potassium excretion rates (table 3). However, the pattern of tubular sodium metabolism was very different in patients than in controls. FELi (ie, the fraction of filtered sodium load escaping proximal tubular reabsorption and reaching the Henle's loop) was not significantly different in patients and controls (respectively, 24% (4%) vs 22% (8%);

p>0.05) (table 4). The fraction of the sodium load delivered to the Henle's loop that is reabsorbed distally (ie, DFRNa) was significantly higher in the patients' group (97.5% (1.9%) vs 94.7% (1.5%); *p*<0.05; table 4, Fig 3). The trans-tubular potassium concentration ratio (TTKR) was very similar between patients and controls, ruling out significant aldosterone-dependent hyper-stimulations of potassium secretion and reabsorption of sodium in the distal convoluted tubule. Plasma osmolality and free-water clearance were also very similar in patients and controls (all with *p*>0.05).

The supine plasma renin and aldosterone levels in the patients with preascitic cirrhosis were lower than those in the controls, but the difference did not reach significance, probably owing to the small number of patients. The supine AVP levels were not significantly different between patients and controls, but the supine plasma norepinephrine levels were significantly higher in patients (15.2 (2.2) vs 9.1 (2.2) nmol/l; *p*<0.05) than controls (table 5).

Supine period following calcium intravenous infusion

GFR, RPF and filtration fraction remained unaffected by the intravenous infusion of calcium gluconate both in patients and in controls (tables 3–5).

Intravenous infusion of calcium significantly increased the serum calcium concentrations in both patients with cirrhosis and in controls (respectively, from 2.0 (0.1) to 2.4 (0.2) mmol/l; *p*<0.001, and from 2.1 (0.4) to 2.5 (0.1) mmol/l; *p*<0.001) and 3 h urinary calcium excretion rate (3 h UCav; table 3). Patients with cirrhosis experienced significant increases in 3 h urine volume (from 377 (148) to 514 (155) ml; *p*<0.01), urinary sodium excretion (from 11.5 (6.2) to 18.2 (8.4) mmol/h; *p*<0.01) and fractional sodium excretion (from 1.1% (0.5%) to 1.8% (0.7%); *p*<0.001). Although the controls also had increases in urinary excretion rate and urinary sodium excretion in response to the calcium infusion, the magnitude of the increases in urinary excretion rate (36% (5%) vs 18% (7%); *p*<0.03) and urinary sodium excretion (57% (4%) vs 34% (7%); *p*<0.03) were significantly higher in those with cirrhosis than in controls (fig 4). With respect to the tubular effects of calcium, those with cirrhosis experienced an increase in FELi, associated with a significant decrease in the fractional reabsorption of sodium by the distal nephron (DFRNa), from 97.5% (1.9%) to 93.0% (0.8%; *p*<0.03; fig 3). In contrast, only minimal changes in these parameters were reported in the control group. The values of TTKR, which represent the function of the aldosterone-dependent segments of the distal tubule (distal convoluted tubule and collecting ducts), were unaffected by the calcium load.

Plasma osmolality, serum sodium concentrations and free-water clearance were significantly increased by calcium gluconate, but mainly in those with cirrhosis.

Intravenous calcium led to a significant decrease in norepinephrine release, with plasma concentrations falling from 15.2 (2.2) to 5.9 (1.5) nmol/l (*p*<0.01) in the cirrhotic group. In contrast, the decrease in the control group was not significant. All the other hormone concentrations remained unaffected by the calcium load.

Calcium infusion resulted in no significant changes in mean arterial blood pressure both in controls (from 99 (18) to 95 (22) mm Hg, *p*>0.05) and in patients with cirrhosis (from 95 (23) to 97 (12) mm Hg, *p*>0.05). In addition, the heart rates of both the patients and the controls were also unaffected by calcium infusion.

DISCUSSION

This study confirms the previous observation of the occurrence of sodium retention in patients with preascitic cirrhosis, as

Table 2 Renal function and electrolyte handling in the pre-study period

	Controls (n = 9)	Patients with cirrhosis (n = 10)
Serum creatinine (mg/dl)	0.6 (0.2)	0.6 (0.3)
Serum sodium (mmol/l)	141 (2)	136 (8)
Serum potassium (mmol/l)	4.2 (0.5)	4.2 (0.6)
24 h urine volume (ml)	1511 (560)	1430 (401)
24 h UNaV (ml/24 h/1.73 m ²)	177 (16)	154 (11)*

UNaV, urinary sodium excretion rate.

Data are given as mean (SD). **p*<0.03 vs controls.

Table 3 Serum electrolytes and renal function at baseline and after calcium loading (supine subjects)

	Baseline	Calcium loading
GFR (ml/min)		
Controls	116 (16)	111 (37)
Cirrhosis	126 (28)	115 (36)
RPF (ml/min)		
Controls	738 (101)	787 (104)
Cirrhosis	662 (127)	653 (111)
Filtration fraction (%)		
Controls	16.0 (2.5)	14.2 (2.4)
Cirrhosis	19.1 (2.0)*	17.9 (2.2)
Serum sodium (mmol/l)		
Controls	141 (2)	142 (2)
Cirrhosis	136 (5)	142 (3)†
Serum potassium (mmol/l)		
Controls	4.2 (0.5)	4.0 (0.3)
Cirrhosis	4.2 (0.6)	3.8 (0.2)
Serum calcium (mmol/l)		
Controls	2.1 (0.4)	2.5 (0.1)‡
Cirrhosis	2.0 (0.1)	2.4 (0.2)‡
3 h urine volume (ml)		
Controls	498 (328)	591 (326)†
Cirrhosis	377 (148)	514 (155)§
3 h UNaV (mmol/h)		
Controls	10.4 (4.3)	14.1 (6.3)*¶
Cirrhosis	11.5 (6.2)	18.2 (8.4)‡
3 h UKV (mmol/h)		
Controls	3.7 (1.5)	2.3 (1.1)
Cirrhosis	3.7 (2.4)	2.7 (1.4)
3 h UCaV (mmol/h)		
Controls	0.15 (0.09)	0.40 (0.26)‡
Cirrhosis	0.23 (0.16)	0.36 (0.15)‡
Plasma osmolality (mOsm/kg)		
Controls	284 (8)	287 (10)
Cirrhosis	280 (12)	289 (11)†
Free-water clearance (ml/min)		
Controls	0.049 (0.07)	0.61 (0.17)†
Cirrhosis	0.024 (0.08)	0.13 (0.08)†

GFR, glomerular filtration rate; RPF, renal plasma flow; UCaV, urinary calcium excretion rate; UKV, urinary potassium excretion rate. Data are given as mean (SD).

* $p < 0.01$ vs controls, † $p < 0.03$ vs baseline, ‡ $p < 0.001$ vs baseline, § $p < 0.01$ vs baseline, ¶ $p < 0.05$ vs baseline.

shown by the lower urinary sodium excretion than in controls and by their weight gain while assuming a 200 mmol sodium/day diet for 1 week.

The evaluation of renal tubular function performed during the baseline supine period confirmed previous data,^{6,7} locating excess reabsorption of sodium in some segments of the nephron distal to the proximal convoluted tubule. This is demonstrated by the significantly higher distal fractional reabsorption of sodium (DFRNa; table 4, fig 3), which represents, according to the principles of lithium-clearance technique,^{20,21} the fraction of distal sodium delivery that is reabsorbed in the whole distal nephron (ie, Henle's loop, distal convoluted tubule and collecting duct).

Intravenous calcium loading, as performed in this and other studies,²⁶ was used to help in assessing the contribution of the ascending limb of Henle's loop to distal sodium retention. Calcium may affect sodium handling via multiple mechanisms. Calcium decreases plasma norepinephrine by directly decreasing neuronal release of catecholamines (table 5),³⁷ and indirectly by improving the effective arterial blood volume through a positive inotropic action.³⁸ This leads to a reduction in the reabsorption of sodium in the proximal convoluted tubule, causing an increase in the fractional delivery of sodium to the distal nephron (table 4). Calcium also selectively inhibits sodium-potassium-chloride cotransport at the loop of Henle²²⁻²⁷ (fig 1). Calcium can also affect water metabolism by antagonising the AVP-dependent reabsorption of water in the collecting duct,

Table 4 Renal tubular function at baseline and after calcium loading (supine subjects)

	Baseline	Calcium loading
3 h FENa (%)		
Controls	1.1 (0.6)	1.5 (0.8)*
Cirrhosis	1.1 (0.5)	1.8 (0.7)†
FELi (%)		
Controls	22 (8)	23 (3)
Cirrhosis	24 (4)	30 (5)*
DFRNa (%)		
Controls	94.7 (1.5)	94.7 (1.3)
Cirrhosis	97.5 (1.9)‡	93.0 (0.8)§
TTKR		
Controls	5.7 (2.3)	4.0 (1.0)
Cirrhosis	5.6 (2.4)	4.8 (2.1)

FENa, fractional excretion of sodium; FELi, fractional excretion of lithium; DFRNa, distal fractional reabsorption of sodium; TTKR, trans-tubular concentration ratio of potassium in the cortical collecting duct. Data are mean (SD).

* $p < 0.05$ vs baseline, † $p < 0.001$ vs baseline, ‡ $p < 0.05$ vs controls, § $p < 0.03$ vs baseline.

mediated both by reduction of medullary interstitial tonicity³⁹ and by reduced expression of water channels in the collecting duct.⁴⁰ Using the intravenous calcium has allowed us to confirm that in supine patients with preascitic cirrhosis, there is excess reabsorption of sodium by the ascending limb of Henle's loop, as (1) the intravenous infusion of calcium salts resulted in a significant decrease in DFRNa (ie, the fractional reabsorption of sodium beyond the proximal tubular nephron) only in the patient group (table 4, fig 3), and (2) the trans-tubular concentration ratio of potassium was normal in the aldosterone-sensitive cortical collecting duct (table 4), ruling out any aldosterone-dependent hyperstimulation of reabsorption of sodium in exchange with potassium in these segments of the distal tubule.

Sodium retention at the loop of Henle in cirrhosis has not been previously recognised in human cirrhosis. However, Jonassen and coworkers^{17,18} recently showed, in rats with preascitic cirrhosis, selective hypertrophy of the TAL of Henle's loop and increased sensitivity to the effects of furosemide, suggesting that increased reabsorption of sodium indeed occurs in this tubular segment. Our data strongly support this view. It is possible that structural changes at the loop of Henle such as hypertrophy of TAL cells may provide more functional mass of sodium-retaining unit at the Henle's loop, and increased expression of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter was found in the TAL of Henle's loop at least in rats with CCl_4 -induced liver cirrhosis.¹⁹

Table 5 Hormonal status at baseline and after calcium loading (supine subjects)

	Baseline	Calcium loading
Plasma renin (ng/l)		
Controls	23.5 (12.9)	16.9 (11.0)
Cirrhosis	11.2 (6.5)	10.1 (6.8)
Plasma aldosterone (pmol/l)		
Controls	120 (61)	111 (51)
Cirrhosis	98 (72)	90 (88)
Plasma vasopressin (pmol/l)		
Controls	7.4 (4.0)	10.3 (4.0)
Cirrhosis	7.9 (6.1)	6.9 (3.9)
Plasma norepinephrine (nmol/l)		
Controls	9.1 (2.2)	6.1 (1.2)
Cirrhosis	15.2 (2.2)*	5.9 (1.5)†

Data are given as mean (SD).

* $p < 0.05$ vs controls, † $p < 0.01$ vs baseline.

Figure 3 Distal fractional reabsorption of sodium (DFRNa) in controls and patients with cirrhosis, before and after the intravenous infusion of calcium. * $p < 0.05$; † $p < 0.03$.

The significantly increased levels of circulating norepinephrine found in our patients while on a high-sodium diet (table 5), an observation also reported by others,⁴¹ could also contribute to the increased reabsorption of sodium in the loop of Henle, as adrenergic stimulation has been shown to cause avid reabsorption of sodium in this tubular segment.⁴²

From our results, it seems that the mineralocorticoid-sensitive tubular segments of the nephron are not yet involved in sodium retention at this early stage of cirrhosis, as the TTKR values were similar between the controls and those with preascitic cirrhosis. This is despite the previous finding that the activity of 11 α -hydroxysteroid dehydrogenase, an enzyme that normally protects the mineralocorticoid receptor from stimulation by glucocorticoids, is significantly decreased in cortical collecting tubule in rats with experimental cholestatic liver cirrhosis. This would lead to glucocorticoid-dependent stimulation of mineralocorticoid receptors.^{43–44} This, together with the normal TTKR in the presence of low to normal aldosterone levels in those with preascitic cirrhosis would suggest that these patients might have an increased sensitivity to aldosterone, as suggested by Bernardi *et al.*⁴ As stated above, our findings also rule out this mechanism of sodium retention occurring at this stage of cirrhosis.

The extent of the natriuretic and diuretic actions of exogenous calcium in the patients with cirrhosis, although significant, seemed quantitatively lower than in healthy controls.²⁶ This could be due to the relatively small amount of calcium gluconate we administered (2 g over 60 min during the second 3 h clearance period), as compared to what is reported in the literature—that is, 9 g of calcium gluconate over 180 min of infusion.²⁶

In conclusion, we have now identified that sodium retention occurs at the Henle's loop in patients with preascitic liver cirrhosis, at least in the supine position. As calcium is acutely effective in increasing sodium and water excretion in preascitic cirrhosis, as already shown in patients with salt-sensitive arterial hypertension,^{27–45} further studies dealing with its potential benefits in clinical practice are warranted.

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Figure 4 Calcium-dependent changes in urine volume, sodium and potassium excretion in controls and patients with cirrhosis. * $p < 0.03$ vs healthy controls.

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EDITOR'S QUIZ: GI SNAPSHOT

A rare infection of the gastric mucosa

Robin Spiller, editor

Clinical presentation

A 63-year-old woman was referred for an open access gastroscopy with a three decade history of gastro-oesophageal reflux-like symptoms. She had a history of "Q fever" 5 years previously following her holiday in Gran Canary islands in Spain. She was on long term treatment with a proton pump inhibitor for her reflux symptoms. She had also used antacids for many decades on a symptomatic basis.

Her gastroscopy showed antral gastritis and hiatus hernia with grade B oesophagitis. Figure 1 shows the histological assessment of the gastric mucosa.

Question

What is the infection evident on histology? What is the possible underlying predisposing factor to this infection?

See page 1163 for answer

This case is submitted by:

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Figure 1 Histological assessment of the gastric mucosa (haematoxylin-eosin $\times 200$).

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