Original Article

Thiazide-induced hyponatraemia is associated with increased water intake and impaired urea-mediated water excretion at low plasma antidiuretic hormone and urine aquaporin-2

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Background: Hyponatraemia is a common, potentially life-threatening, complication of thiazide diuretics. The mechanism of thiazide-induced hyponatraemia is incompletely understood. Previous experiments have suggested a direct effect of thiazide diuretics on the plasma membrane expression of aquaporin (AQP)2.

Methods: We examined the effects of a single re-exposure to hydrochlorothiazide (HCTZ) 50 mg on water balance, renal sodium handling and osmoregulation in 15 elderly hypertensive patients with a history of thiazide-induced hyponatraemia and 15 matched hypertensive controls using thiazide diuretics without previous hyponatraemia.

Results: Patients with thiazide-induced hyponatraemia had significantly lower body weight and lower plasma sodium and osmolality at baseline. After HCTZ administration, plasma sodium and osmolality significantly decreased and remained lower in patients compared with controls ($P < 0.001$). Plasma antidiuretic hormone (ADH) and urine AQP2 were low or suppressed in patients, whereas solute and electrolyte-free water clearance was significantly increased compared with controls. Ad libitum water intake was significantly higher in patients (2543 ± 925 ml) than in controls (1828 ± 624 ml, $P < 0.05$), whereas urinary sodium excretion did not differ. In contrast, urea excretion remained significantly lower in patients (263 ± 69 mmol per 24 h) compared with controls (333 ± 97 mmol per 24 h, $P < 0.05$) and predicted the decrease in plasma sodium following HCTZ administration.

Conclusion: Thiazide diuretics are associated with markedly impaired free water excretion at low ADH and AQP2 in elderly patients. The higher water intake and lower urea excretion in patients points to an important role for polydipsia and urea-mediated water excretion in the pathogenesis of thiazide-induced hyponatraemia.

Keywords: antidiuretic hormone, aquaporin2, free water excretion, hyponatraemia, thiazide diuretics

Abbreviations: ADH, anti-diuretic hormone; AQP2, aquaporin 2; HCTZ, hydrochlorothiazide; PRA, plasma renin activity; TSH, thyroid-stimulating hormone

INTRODUCTION

Hyponatraemia is a potentially life-threatening complication of thiazide diuretics, especially amongst elderly patients [1,2]. In a recent observation study, the risk of hospitalization for hyponatraemia was 0.69 per 100 patient-years for chlortalidone and 0.49 per 100 patient-years for hydrochlorothiazide (HCTZ) [3]. In most cases, hyponatraemia occurs within several days after the institution of thiazide therapy. However, in one-third of patients, hyponatraemia develops after prolonged exposure to thiazide diuretics, making it difficult to identify patients at risk [4]. Various patient-related factors including female sex, advanced age and lower body weight have been associated with an increased risk of thiazide-induced hyponatraemia [4,5], but its pathogenesis is still incompletely understood. After a single re-challenge, patients with a history of thiazide-induced hyponatraemia have been shown to acutely develop hyponatraemia and impaired solute-free water excretion at low antidiuretic hormone (ADH) levels [6]. Solute-free water clearance depends on the transcellular osmotic gradient of collecting duct cells and the plasma membrane expression of the aquaporin-2 (AQP2) water channel. Following experimental evidence, it has been suggested that thiazide-induced hyponatraemia may be caused by direct effects on the plasma membrane expression of the water channel AQP2 [7–9]. A proportion of AQP2 is released in urine allowing assessment of the downstream effects of ADH [10–12]. Urine AQP2 doubles after an overnight fast and increases more than three-fold in

Journal of Hypertension 2015, 33:627–633

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Received 6 May 2014 **Revised** 15 September 2014 **Accepted** 16 September 2014

DOI:10.1097/HJH.0000000000000423

the pathological states of impaired water excretion characterized by nonsuppressible ADH and more than ten-fold after exogenous infusion of a synthetic vasopressin analogue [11,12]. We hypothesized that the impaired water excretion observed in patients with a history of thiazide-induced hyponatraemia is associated with increased sensitivity to ADH or expression of its downstream effector, AQP2.

Therefore, we assessed the effects of a single dose of HCTZ on water balance, renal sodium handling and osmoregulation in hypertensive patients with a history of thiazide-induced hyponatraemia and age-matched and sex-matched controls using thiazide diuretics for hypertension without hyponatraemia.

PATIENTS AND METHODS

Study participants

We compared 15 patients aged 60 years and older who were previously diagnosed with thiazide-induced hyponatraemia with 15 elderly individuals using thiazide therapy for hypertension without a history of hyponatraemia. Patients were recruited by searching the hospital database for presentations with hyponatraemia in the last 5 years according to the International Classification of Diseases 9 code (ICD-9), and by directly contacting physicians at our institute and regional hospitals.

Patients were eligible if they were previously diagnosed with thiazide-induced hyponatraemia (plasma sodium $\leq$128 mmol/l), had used thiazide diuretics for the treatment of hypertension and had normal plasma sodium levels ($\geq$135 mmol/l) after the cessation of therapy. Patients were excluded from participation if they had any other apparent cause for their hyponatraemia or if they used other drugs interfering with sodium and water homeostasis (see online supplementary methods, http://links.lww.com/HJH/A427). Controls used thiazide diuretics for the treatment of hypertension, but had no prior history of hyponatraemia. Controls were individually matched for age and sex with patients and recruited from the outpatient clinic of the study centre and affiliated hospitals.

Patients and controls were excluded if they had abnormal plasma sodium levels (<135 mmol/l) at the initial screening visit, an estimated creatinine clearance less than 50 ml/min according to the Cockcroft–Gault formula, a history of malignancy in the last 5 years, therapy-resistant or uncontrolled hypertension defined as blood pressure (BP) greater than 140/90 mmHg with three or more antihypertensive drugs, BP greater than 160/100 mmHg with two or more antihypertensive drugs or BP greater than 180/110 mmHg, known allergies for sulphonamides, or were unwilling or incapable to provide written informed consent. The study protocol was conducted in accordance with the Declaration of Helsinki and good clinical practice (GCP) guidelines. All participants gave written informed consent prior to the experiment. The study was approved by our local institutional medical ethics review board.

Study protocol

In controls, thiazide diuretics or thiazide combinations were stopped at least 6 weeks before the experiment. If possible, BP-lowering medication believed to interfere with the study outcome (e.g. potassium-saving diuretics, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers) were stopped, or replaced by peripheral alpha-blocking drugs (doxazosin 4 or 8 mg) or calcium-channel-blocking agents (nifedipine osmotic-release oral system (OROS) 30 mg) if BP was higher than 160/100 mmHg. Beta-blockers were continued if patients had an indication for beta-blocker therapy other than hypertension (i.e. rate control for atrial fibrillation or coronary artery disease).

Before the day of the experiment, patients were instructed to collect 24 h urine. On the day of the experiment, after an overnight fast, BP, heart rate and body weight were recorded. Baseline blood and urine samples were taken after 30 min rest. Thereafter, participants received a single dose of HCTZ 50 mg. Single dose re-exposure to HCTZ 50 mg in combination with amiloride 5 mg has previously been shown to cause an acute decrease in plasma sodium with impaired free water excretion in patients with thiazide-induced hyponatraemia without causing adverse effects [6]. As our aim was to study the effects of thiazide diuretics, we chose to conduct the experiment with HCTZ 50 mg only.

After 4, 8 and 24 h, measurement of BP, heart rate and body weight was repeated, and blood and urine samples were taken. BP was measured three times whilst seated after at least 5 min rest using a validated oscillometric device (Omron MX2, Omron Healthcare, Hoofddorp, the Netherlands). The last two BP readings were averaged and used for analysis. During the experiment, each participant registered food and fluid intake on diet lists with recording of the type and amount of solid foods and liquids. The amount of food and liquids was listed using a semiquantitative scale (i.e. slice of bread, glass of milk and cup of tea). Subsequently, the items were entered using the website of the Dutch food safety organization, which calculates the amount of calories, water, sodium, potassium, energy and protein for each item using the same semiquantitative scale. To familiarize participants with the diet list and limit the influence of diet registration on food and fluid intake, participants started recording 3 days prior to the experiment. Before and during the experiment, the correctness and completeness of the diet lists was assessed by the investigator. After 24 h, all patients resumed their original medication.

Laboratory analysis

Blood samples for the assessment of ADH and aldosterone were immediately put on ice before transportation to the laboratory. For the other measurements, blood samples were transported at room temperature and processed within 1 h. Sodium, potassium, glucose, creatinine, uric acid, urea and TSH were measured using a Modular P800 analyzer (Roche Diagnostics, Almere, the Netherlands). Plasma and urine osmolality were measured using Arkray Osmo Station OM-6050 (Menarini Diagnostics, Valkenswaard, the Netherlands). Cortisol was assayed with a chemiluminescent immunoassay on an Immulite system (Siemens Healthcare Diagnostics, Breda, the Netherlands). Urine samples for the measurement of AQP2 were stored at −80°C. Plasma renin activity (PRA), aldosterone and ADH were measured by radioimmunoassay. To quantify urine AQP2, urine creatinine equivalents were immunoblotted as

described [12,13]. Detailed description of the radioimmunoassays for PRA, aldosterone and ADH, and urinary AQP2 measurement are added as online data supplement, http://links.lww.com/HJH/A427.

The following formulas were used to calculate solute-free and electrolyte-free water clearance: solute-free water clearance $= V^*$ $(1 - U_{Osm}/P_{Osm})$, electrolyte-free water clearance $= V^*$ $[1 - (U_{Na} + U_K)/P_{Na}]$, in which V is urine production (ml/min), U_{Osm} and P_{Osm} are urine and plasma osmolality (mOsm/kg), respectively, U_{Na} and U_K are urinary sodium and potassium excretion, respectively (mmol/l), P_{Na} is plasma sodium (mmol/l) [14].

Sample size calculation and statistical analysis

On the basis of the results of the previous studies examining the effects of HCTZ on plasma sodium and free water excretion [6,15], we estimated that 15 patients and 15 controls would be sufficient to demonstrate significant differences in plasma sodium and allow detection of one SD difference in urine AQP2 with 80% power and alpha = 0.05. These differences are much smaller than previously demonstrated in the pathological states of impaired free water excretion and following exogenous administration of 1-desamino-8-D-arginine vasopressin (DDAVP) [11,12].

Baseline data were expressed as mean and SD for continuous variables because most parameters followed a parametric distribution. Log transformation was performed before analysis for variables with skewed distribution. Unpaired *t*-tests were used to compare data from patients and controls at baseline. Changes following HCTZ administration were assessed by repeated measures analysis. Multiple variable imputing was used in case data were missing. Univariate regression analysis was used to assess the baseline predictors of changes in plasma sodium at 4 and 8 h following HCTZ administration. The Statistical Package for Social Sciences (SPSS) 18.0 for Windows was used for data analysis. *P* less than 0.05 was considered significant.

RESULTS

Baseline clinical and laboratory data

The selection and clinical characteristics of included patients at the time of presentation with hyponatraemia are added as online data supplement, http://links.lww.com/HJH/A427. Of the 76 patients with possible thiazide-induced hyponatraemia, 24 patients had one or more exclusion criteria and 24 patients were unwilling or unable to participate, an additional seven patients had died and four patients had moved outside the region. Prior to the experiment, two patients left the study, one because of atrial fibrillation and one patient because the study burden was considered too great, leaving 15 patients for inclusion. Mean plasma sodium level of the included patients was 120 (range 102–128) mmol/l at initial presentation. Mean (range) time between presentation with hyponatraemia and start of the experiment was 13 (1–50) months. Baseline characteristics of the study participants are listed in Table 1. Patients and controls were well matched for age and sex. Body weight was significantly lower in patients compared with controls, whereas SBP and DBP did not differ between the groups. There were no significant differences in the frequency or type of BP-lowering medication. BP-lowering medication at baseline included calcium-antagonists (10 patients and five controls), alpha-blocking therapy (one patient and five controls) and beta-blocking agents (eight patients and three controls). Plasma sodium and osmolality were significantly lower in patients compared with controls at baseline, whereas ADH and was completely suppressed in 11 (73%) patients and in seven (47%) controls. Mean urine volume was 456 ml higher at baseline in patients compared with controls, whereas urine osmolality and urea excretion were significantly lower. Patients had lower urine AQP2, whereas solute-free and electrolyte-free water clearance tended to be higher. Mean plasma glucose, TSH and cortisol did not differ between the groups ($P > 0.20$ for all).

TABLE 1. Baseline characteristics

	Patients $n = 15$	Controls $n = 15$	*P*
Age (years)	75.3 ± 8.2	74.7 ± 6.1	0.83
Sex (male/female)	5/10	5/10	1.00
Body weight (kg)	62.6 ± 9.3	74.1 ± 14.3	<0.05
SBP (mmHg)	155 ± 20	153 ± 18	0.80
DBP (mmHg)	79 ± 10	82 ± 11	0.34
Antihypertensive therapy (%)	9 (60%)	13 (87%)	0.25
Plasma sodium (mmol/l)	139 ± 4.2	142 ± 3.1	<0.05
Plasma osmolality (mOsm/kg)	278 ± 5.8	285 ± 5.7	<0.005
Plasma potassium (mmol/l)	4.2 ± 0.3	4.1 ± 0.2	0.21
Plasma uric acid (mmol/l)	0.27 ± 0.06	0.29 ± 0.05	0.23
Plasma creatinine (umol/l)	75 ± 12	77 ± 16	0.74
Plasma urea (mmol/l)	5.3 ± 1.4	6.1 ± 1.7	0.18
Plasma ADH (pmol/l)	0.7 ± 0.8	1.3 ± 1.2	0.11
Plasma renin activity (μgA1/l/h)	0.9 ± 0.9	1.8 ± 2.5	0.16
Plasma aldosterone (nmol/l)	0.35 ± 0.35	0.36 ± 0.14	0.91
Creatinine clearance (ml/min)	72 ± 26	94 ± 37	0.07
Urine volume (ml per 24 h)	2369 ± 898	1913 ± 564	0.11
Urinary sodium excretion (mmol per 24 h)	124 ± 42	142 ± 62	0.35
Urinary potassium excretion (mmol per 24 h)	62 ± 28	86 ± 62	0.17
Urinary creatinine excretion (mmol per 24 h)	7.7 ± 3.0	10.3 ± 4.0	0.06
Urine osmolality (mOsm per 24 h)	630 ± 150	804 ± 261	<0.05
Urinary urea excretion (mmol per 24 h)	251 ± 61	346 ± 97	<0.005
Urinary AQP2 excretion	0.4 ± 0.4	1.0 ± 1.1	<0.05
Solute-free water clearance (ml/min)	−0.4 ± 1.0	−1.3 ± 1.3	<0.05
Electrolyte-free water clearance (ml/min)	0.23 ± 0.8	−0.16 ± 0.6	0.13

Data represented as mean ± SD unless indicated otherwise. Urine values were derived from the 24-h urine collection preceding the experiment. ADH, antidiuretic hormone.

Clinical and laboratory data following hydrochlorothiazide

The recordings of 24-h food and fluid intake after the start of the experiment are shown in Table 2. Diet during the experiment did not differ regarding the intake of sodium, potassium, protein (with or without correction for body weight), alcohol or energy content. Water intake was higher at all time intervals in patients compared with controls ($P < 0.001$ for trend), totalling 719 ml difference at 24 h. Table 3 and Fig. 1 show the effects of re-exposure to HCTZ

TABLE 2. Food and fluid intake during the experiment in patients previously diagnosed with thiazide-induced hyponatraemia and age-matched and sex-matched controls

Nutrient	Patients	Controls	*P*
Sodium (mmol/day)	114 ± 39	106 ± 32	0.59
Potassium (mmol/day)	63 ± 16	73 ± 18	0.15
Energy (kcal/day)	1434 ± 299	1592 ± 443	0.26
Water (ml/day)	2543 ± 925	1828 ± 624	<0.05
Protein (g/day)	63 ± 14	72 ± 24	0.18
Protein/body weight (g/kg per day)	1.0 ± 0.3	1.0 ± 0.3	0.79
Alcohol (g/day)	7.8 ± 9.2	5.9 ± 8.9	0.55

Data are expressed as mean ± SD.

50 mg at 4, 8 and 24 h from baseline. SBP and DBP remained similar throughout the experiment and did not significantly differ between patients and controls ($P = 0.86$ for SBP, $P = 0.30$ for DBP). Body weight tended to increase by 0.3 ± 0.8 and 0.1 ± 1.0 kg in patients at 4 and 8 h following HCTZ administration, whereas it tended to decrease in controls (0.1 ± 0.7 kg at 4 h and 0.4 ± 0.9 kg at 8 h). Plasma sodium maximally decreased from 139.2 ± 4.2 to 135.6 ± 4.0 mmol/l in patients and from 142.3 ± 3.1 to 140.3 ± 3.0 mmol/l in controls after 4 h (both $P < 0.01$), whereas plasma osmolality significantly decreased in patients (277.9 ± 5.8 to 274.3 ± 6.9 mOsm/kg at 4 h, $P < 0.01$) and tended to decrease in controls ($P = 0.06$). The difference in plasma sodium and osmolality between patients and controls was significant at all timepoints ($P < 0.005$). Plasma potassium and uric acid tended to decrease in both patients and controls at 4 and 8 h following HCTZ administration, but did not significantly differ (data not shown).

PRA did not significantly change during the experiment, whereas aldosterone decreased after 4 and 8 h in both patients and controls. Plasma ADH remained low or suppressed in patients, whereas in controls ADH increased from 1.3 ± 0.3 to 1.6 ± 0.4 pmol/l at 4 h. During the experiment, total urine volume was 775 ml higher in patients (2812 ± 1021 ml) than in controls (2037 ± 1850 ml, $P < 0.05$). Urinary sodium excretion during the experiment was 215 mmol per 24 h in patients and 211 mmol per 24 h in controls ($P = 0.90$), and did not differ at any of the timepoints. Total urea excretion remained lower in patients (263 ± 69 mmol per 24 h) compared with controls (333 ± 97 mmol per 24 h, $P < 0.05$). Urinary AQP2 excretion remained low in patients and significantly decreased in controls. Solute-free and electrolyte-free water excretion remained significantly higher in patients compared with controls during the experiment, indicating that water reabsorption in the patient group continued to be suppressed. Insensible loss, calculated as the difference between water excretion and intake, was 264 ml for patients and 209 ml for controls, and did not differ between groups ($P = 0.63$).

Predictors of hyponatraemia in patients and controls

Univariate predictors of the difference in plasma sodium at 4 h compared to baseline are depicted in Table 4. Lower body weight, PRA, urine osmolality and urinary urea excretion and higher plasma aldosterone values significantly predicted the decrease in plasma sodium at 4 h and also predicted the decrease in plasma sodium at 8 h following HCTZ administration (data not shown). Baseline plasma sodium, ADH, creatinine clearance and urinary excretion of sodium and AQP2 were not correlated with the changes in plasma sodium at 4 or 8 h following HCTZ administration.

DISCUSSION

Patients with a history of thiazide-induced hyponatraemia had significantly lower plasma sodium and osmolality at baseline, which further decreased after HCTZ administration. This occurred despite an increase in solute and electrolyte free water clearance at low or suppressed ADH and urine AQP2, indicating that in patients with previous thiazide-induced hyponatraemia, antidiuresis was appropriately suppressed. Therefore, our findings do not support increased sensitivity to ADH or expression of AQP2 as an explanation for the decrease in plasma sodium and osmolality following HCTZ administration.

TABLE 3. Plasma renin activity (PRA), plasma aldosterone, urine aquaporin (AQP)2, urine osmolality and electrolyte-free water clearance at 0, 4, 8 h and 24 h following administration of hydrochlorothiazide 50 mg in patients with a history of thiazide-induced hyponatraemia and controls

	0 h	4 h	8 h	24 h	*P* vs. control	*P* vs. baseline
Plasma renin activity (μgA1/l/h)						
Patient	0.9 ± 0.9	1.5 ± 1.6	1.5 ± 1.6	1.6 ± 1.6	0.13	0.28
Control	1.8 ± 2.5	1.9 ± 1.6	1.9 ± 1.7	3.1 ± 3.0		0.18
Plasma aldosterone (nmol/l)						
Patient	0.35 ± 0.35	0.25 ± 0.20	0.23 ± 0.14	0.51 ± 0.63	0.99	0.08
Control	0.36 ± 0.14	0.31 ± 0.16	0.24 ± 0.08	0.43 ± 0.16		<0.001
Urine AQP2 (ratio)						
Patient	0.4 ± 0.4	0.4 ± 0.2	0.4 ± 0.3	0.5 ± 0.7	0.23	0.88
Control	1.0 ± 1.1	0.7 ± 1.3	0.5 ± 0.5	0.3 ± 0.3		0.02
Urine osmolality (mOsm/kg)						
Patient	381 ± 143	349 ± 145	368 ± 165	303 ± 85	<0.005	0.09
Control	522 ± 112	462 ± 139	520 ± 129	444 ± 155		0.11
Electrolyte free water clearance (ml/min)						
Patient	0.23 ± 0.76	0.49 ± 1.17	0.70 ± 1.11	0.41 ± 0.36	<0.01	0.27
Control	−0.16 ± 0.61	−0.22 ± 0.46	−0.30 ± 0.44	0.12 ± 0.43		<0.05

Data are expressed as mean ± SD.

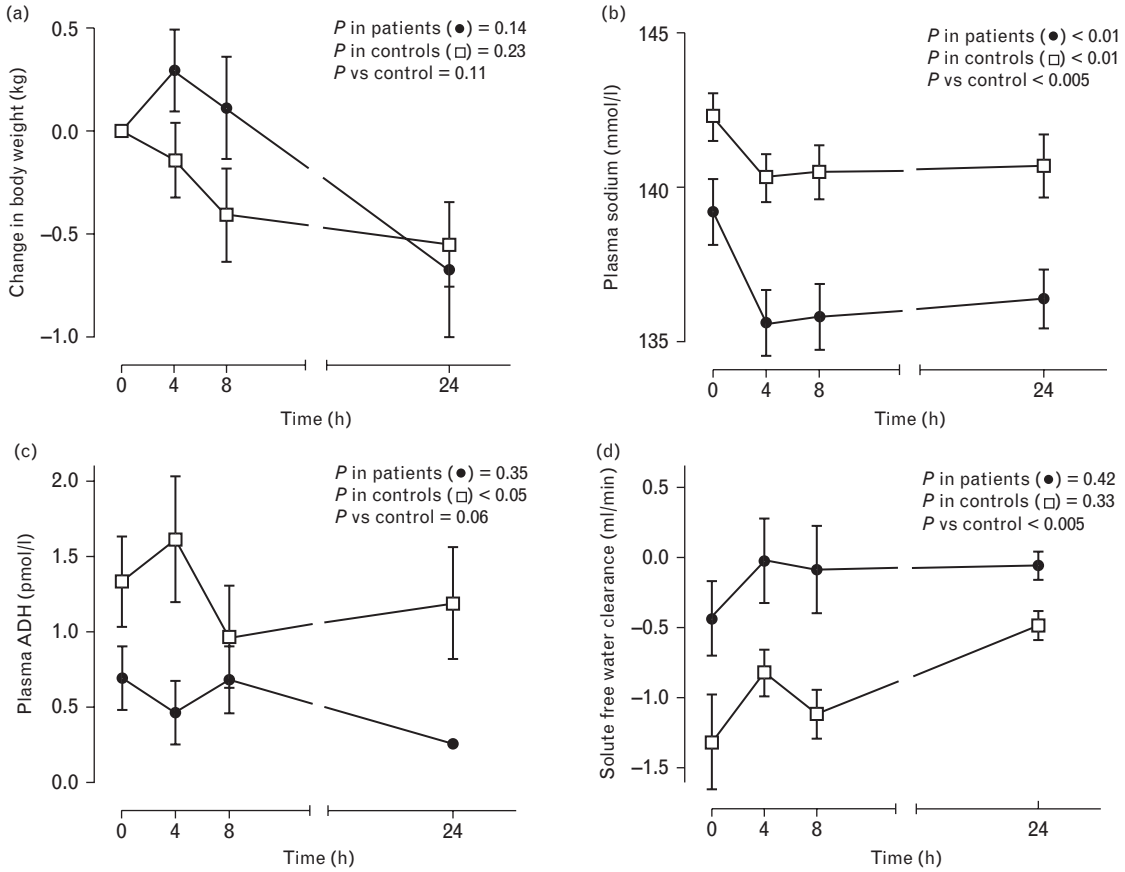

FIGURE 1 Body weight (a), plasma sodium (b), plasma ADH (c) and solute-free water clearance (d) at 0, 4, 8 and 24 h following administration of hydrochlorothiazide 50 mg in patients with a history of thiazide-induced hyponatraemia (●) and controls (□). Data are expressed as mean (SE).

The lower plasma sodium and osmolarity in patients with thiazide-induced hyponatraemia was associated with an increase in water intake and urine volume, both at baseline and during the experiment. Water intake usually has to exceed approximately 8 l per 24 h to cause significant hyponatraemia [16], considerably more than the 2.5–3 l per 24 h in the patients from our experiment. However, water intake only led to the development of hyponatraemia after exposure to HCTZ in these patients, suggesting that thiazide diuretics have an important effect on free water excretion.

TABLE 4. Univariate predictors of differences in plasma sodium at baseline and at 4 h following hydrochlorothiazide administration

Covariates at baseline	Beta	*P*
Age (years)	0.01	0.97
Sex (female)	0.32	0.08
Body weight (kg)	−0.40	<0.05
Plasma sodium (mmol/l)	0.25	0.19
PRA (μgA1/l/h)	−0.42	<0.05
Plasma aldosterone (nmol/l)	0.39	<0.05
ADH (pmol/l)	0.15	0.43
Creatinine clearance (ml/min)	−0.26	0.17
Urine sodium (mmol per 24 h)	0.03	0.89
Urine AQP2	0.13	0.50
Urinary urea excretion (mmol per 24 h)	−0.57	<0.01
Urine osmolality (mOsm/kg)	−0.41	<0.05

ADH, antidiuretic hormone.

This is in line with the results from a previous clinical experiment which showed that, especially in elderly patients, solute-free water excretion is greatly impaired after the administration of thiazide diuretics [6,15].

Solute-free water clearance is principally depending on the transcellular osmotic gradient of collecting duct cells and the plasma membrane expression of AQP2. By acting on the distal tubule, thiazide diuretics do not interfere with medullary tonicity and urinary concentrating capacity, resulting in the excretion of solutes in excess of water. The finding that solute-free water clearance remained negative in both patients and controls, despite low or suppressed ADH and urine AQP2, indicates that free water excretion at maximum physiological capacity was impaired in both groups after HCTZ and could not be compensated by enhanced water excretion. Whether the observed increase in water intake in patients with thiazide-induced hyponatraemia is a result of habit or may relate to differences in thirst sensation or osmosensing, such as recently demonstrated for a genetic variant in the transient receptor potential vanilloid 4 (TRPV4) channel [17], remains to be determined. The low or suppressed plasma ADH levels in patients, however, make an increase in ADH-stimulated water intake in patients less likely.

Next to the differences in water intake, urinary urea excretion was significantly lower in patients compared with controls, both at baseline and during the experiment, and predicted changes in plasma sodium in both groups. It is

well established that urea has the capacity to promote free water excretion [14]. In patients with the syndrome of inappropriate antidiuretic hormone secretion (SIADH), for example, oral urea is equally effective for the treatment of hyponatraemia compared to the vasopressin V(2)-receptor antagonists tolvaptan and satavaptan [18]. Urea production depends on the net protein intake and endogenous protein catabolism. The lower body weight in patients may reflect reduced muscle mass, which is an important contributor to endogenous protein turnover [19–21]. The lower urinary creatinine excretion, an estimate of muscle mass, in patients and the propensity for women and individuals with lower body weight to develop thiazide-induced hyponatraemia as observed by us and others is consistent with this finding [5]. In our experiment, the decrease in plasma sodium following HCTZ administration was not associated with changes in PRA or aldosterone. However, the increase in PRA with thiazide diuretics reaches its maximum only after 3 days [22]. A longer study period would be needed to appreciate the full effects of changes in PRA during thiazide treatment. For the present study, we excluded participants with an estimated glomerular filtration rate less than 50 ml/min, as a decrease in renal function could contribute to the development of hyponatraemia by an increase in water retention. However, creatinine clearance still tended to be lower in patients compared with controls. In univariate analysis, creatinine clearance was not a significant predictor of the decrease in plasma sodium, indicating that small decreases in renal function did not importantly affect the propensity to develop hyponatraemia following HCTZ administration.

Our study has several limitations. We studied the acute effects of HCTZ on water balance and renal sodium handling using a single dose of HCTZ 50 mg, whereas HCTZ 12.5 or 25 mg is sufficient to effectively lower BP. It should be noted, however, that all the patients in our study developed hyponatraemia during treatment with either HCTZ 12.5 mg or HCTZ 25 mg. Furthermore, the effects on sodium and water homeostasis may be different during chronic exposure to thiazide diuretics because of counter-regulatory mechanisms, most importantly the renin–angiotensin system [22]. As ADH and PRA levels were lower in patients than in controls, we consider it unlikely that angiotensin-II-mediated stimulation of ADH would affect patients more than controls during chronic administration of thiazide diuretics. Finally, plasma sodium and osmolality, albeit within the normal range, were already significantly lower at baseline in patients with thiazide-induced hyponatraemia. We did not correct for these baseline differences as it is conceivable that the determinants that contribute to thiazide-induced hyponatraemia are also responsible for the observed changes at baseline.

In conclusion, our results show that in elderly patients water excretion is markedly impaired after the administration of a thiazide diuretic, and suggests an important role for water intake and urea-mediated water excretion in the pathogenesis of thiazide-induced hyponatraemia. The present findings may also explain why the development of thiazide-induced hyponatraemia is often difficult to predict as water intake, insensible loss and protein catabolism may vary in time. Our data suggest that advice on water and protein intake may prevent thiazide-induced hyponatraemia in susceptible elderly patients.

ACKNOWLEDGEMENTS

This study was supported by the grant C06.218 from the Dutch Kidney Foundation to B.J.B. and a Marie Curie Intra European Fellowship grant (272375) to C.T. and P.M.T.D. P.M.T.D. is the recipient of the VICI grant 865.07.002 of the Netherlands Organization for Scientific research (NWO).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ashraf N, Locksley R, Arieff AI. Thiazide-induced hyponatremia associated with death or neurologic damage in outpatients. *Am J Med* 1981; 70:1163–1168.
2. Sonnenblick M, Friedlander Y, Rosin AJ. Diuretic-induced severe hyponatremia. Review and analysis of 129 reported patients. *Chest* 1993; 103:601–606.
3. Dhalla IA, Gomes T, Yao Z, Nagge J, Persaud N, Hellings C, *et al*. Chlorthalidone versus hydrochlorothiazide for the treatment of hypertension in older adults: a population-based cohort study. *Ann Intern Med* 2013; 158:447–455.
4. Sharabi Y, Illan R, Kamari Y, Cohen H, Nadler M, Messerli FH, *et al*. Diuretic induced hyponatraemia in elderly hypertensive women. *J Hum Hypertens* 2002; 16:631–635.
5. Chow KM, Szeto CC, Wong TY, Leung CB, Li PK. Risk factors for thiazide-induced hyponatraemia. *QJM* 2003; 96:911–917.
6. Friedman E, Shadel M, Halkin H, Farfel Z. Thiazide-induced hyponatremia. Reproducibility by single dose rechallenge and an analysis of pathogenesis. *Ann Intern Med* 1989; 110:24–30.
7. Kim GH, Lee JW, Oh YK, Chang HR, Joo KW, Na KY, *et al*. Antidiuretic effect of hydrochlorothiazide in lithium-induced nephrogenic diabetes insipidus is associated with upregulation of aquaporin-2, Na-Cl cotransporter, and epithelial sodium channel. *J Am Soc Nephrol* 2004; 15:2836–2843.
8. Loffing J. Paradoxical antidiuretic effect of thiazides in diabetes insipidus: another piece in the puzzle. *J Am Soc Nephrol* 2004; 15:2948–2950.
9. Cowtan T. Thiazide diuretics. *N Engl J Med* 2010; 362:659.
10. Deen PM, van Aubel RA, van Lieburg AF, van Os CH. Urinary content of aquaporin 1 and 2 in nephrogenic diabetes insipidus. *J Am Soc Nephrol* 1996; 7:836–841.
11. Saito T, Ishikawa SE, Ando F, Okada N, Nakamura T, Kusaka I, *et al*. Exaggerated urinary excretion of aquaporin-2 in the pathological state of impaired water excretion dependent upon arginine vasopressin. *J Clin Endocrinol Metab* 1998; 83:4034–4040.
12. Baumgarten R, van de Pol MH, Deen PM, van Os CH, Wetzels JF. Dissociation between urine osmolality and urinary excretion of aquaporin-2 in healthy volunteers. *Nephrol Dial Transplant* 2000; 15:1155–1161.
13. Trimpert C, van den Berg DT, Fenton RA, Klussmann E, Deen PM. Vasopressin increases S261 phosphorylation in AQP2-P262L, a mutant in recessive nephrogenic diabetes insipidus. *Nephrol Dial Transplant* 2012; 27:4389–4397.
14. Berl T. Impact of solute intake on urine flow and water excretion. *J Am Soc Nephrol* 2008; 19:1076–1078.
15. Clark BA, Shannon RP, Rosa RM, Epstein FH. Increased susceptibility to thiazide-induced hyponatremia in the elderly. *J Am Soc Nephrol* 1994; 5:1106–1111.
16. Hariprasad MK, Eisinger RP, Nadler IM, Padmanabhan CS, Nidus BD. Hyponatremia in psychogenic polydipsia. *Arch Intern Med* 1980; 140:1639–1642.
17. Tian W, Fu Y, Garcia-Elias A, Fernandez-Fernandez JM, Vicente R, Kramer PL, *et al*. A loss-of-function nonsynonymous polymorphism in the osmoregulatory TRPV4 gene is associated with human hyponatremia. *Proc Natl Acad Sci USA* 2009; 106:14034–14039.
18. Soupart A, Coffernils M, Couturier B, Gankam-Kengne F, Decaux G. Efficacy and tolerance of urea compared with vaptans for long-term treatment of patients with SIADH. *Clin J Am Soc Nephrol* 2012; 7: 742–747.

19. Young VR, Munro HN. Ntau-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. *Fed Proc* 1978; 37:2291–2300.
20. Morais JA, Gougeon R, Pencharz PB, Jones PJ, Ross R, Marliss EB. Whole-body protein turnover in the healthy elderly. *Am J Clin Nutr* 1997; 66:880–889.
21. Short KR, Vittone JL, Bigelow ML, Proctor DN, Nair KS. Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab* 2004; 286:E92–E101.
22. Griffing GT, Sindler BH, Aurecchia SA, Melby JC. The effects of hydrochlorothiazide on the renin–aldosterone system. *Metabolism* 1983; 32:197–201.