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1 **ADRENAL ADAPTATION IN K DEPLETED MEN : ROLE OF PROGESTERONE?**

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21
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ABSTRACT

Background: In rodents the stimulation of adrenal progesterone is necessary for renal adaptation under potassium depletion. Here we sought to determine the role of progesterone in adrenal adaptation in potassium-depleted healthy human volunteers and compared our findings to data collected in patients with Gitelman syndrome, a salt-losing tubulopathy.

Methods: Twelve healthy young men were given a potassium-depleted diet for 7 days at a tertiary referral medical center (NCT02297048). We measured by LC-MS/MS plasma steroid concentrations at days 0 and 7 before and 30 min after treatment with tetracosactide. We compared these data to data collected in 10 Gitelman syndrome patients submitted to tetracosactide test.

Results: The potassium-depleted diet decreased plasma potassium in healthy subjects by 0.3 ± 0.1 mmol/l, decreased plasma aldosterone concentration by 50% ($p=0.0332$) and increased plasma 17-hydroxypregnenolone concentration by 45% ($p=0.0232$) without affecting other steroids. CYP17 activity, as assessed by 17-hydroxypregnenolone /pregnenolone ratio increased by 60% ($p=0.0389$). As compared to healthy subjects, Gitelman syndrome patients had 3-fold higher plasma concentrations of aldosterone, 11-deoxycortisol (+30%), and delta 4-androstenedione (+14%). Their post-tetracosactide progesterone concentration was 2-fold higher than that of healthy subjects and better correlated to plasma potassium than to plasma renin.

Conclusion: The increase in 17-hydroxypregnenolone concentration after mild potassium depletion in otherwise healthy human subjects suggests that 17 hydroxylation of pregnenolone prevents the increase in progesterone observed in potassium-depleted mice. The unexpected over-response of non-mineralocorticoid steroids to tetracosactide in Gitelman syndrome subjects suggests that the adrenal system not only adapts to sodium depletion but may also respond to hypokalemia.

246 WORDS

71 **INTRODUCTION**

72 Potassium is the most abundant cation in organisms, and the equilibrium between its low
73 extracellular and high intracellular concentrations influences cellular processes and
74 properties such as membrane resting potential and cell volume. The inability to maintain this
75 equilibrium, and particularly to restrain extracellular plasma potassium concentration in a
76 tight physiological range, is potentially life-threatening. The potassium balance is
77 coordinated by mechanisms for potassium storage into and release from internal stores
78 (internal balance) and mechanisms that regulate retention and excretion (external balance)
79 [1]. In this complex regulatory system, the kidney, mainly the distal part of the nephron,
80 plays an important role. In steady-state conditions, renal excretion of potassium is similar to
81 potassium intake. The distal nephron excretes potassium through ROMK and BK channels,
82 which require a favorable electrical gradient generated by the activity of the amiloride-
83 sensitive Na⁺ channel ENaC [2, 3]. Potassium reabsorption is mediated by the H,K-ATPases,
84 mainly the isoform HKA2 [4]. Excretion and reabsorption are functional under physiological
85 conditions [5]. In subjects on a low-potassium diet condition, the renal switch from a high
86 rate of potassium secretion to efficient potassium conservation is mediated by a number of
87 complementary mechanisms: 1) an increase in electroneutral (mediated by the Na-Cl
88 cotransporter NCC) versus electrogenic (mediated by ENaC) Na⁺ reabsorption [6]; 2) a
89 decrease in plasma aldosterone concentration, which induces a decreased activity of ENaC
90 [7]; 3) inhibition of ROMK through a pathway dependent on reactive-oxygen species [8]; 4)
91 the progesterone-dependent stimulation of the HKA2 [4, 9]; and 5) the proliferation of
92 tubular epithelial cells of the distal nephron, which occurs in chronic conditions [10].

93 |
94 In rodents in a potassium-depleted state, there is stimulation of adrenal secretion of
95 progesterone and progesterone-dependent stimulation of HKA2 expression suggesting that
96 progesterone is not just a sex hormone [9, 11]. This offers a new promising way in treating
97 or preventing renal hypokalemia by stimulating potassium reabsorption rather than
98 inhibiting its secretion. This potassium-sparing effect of adrenal progesterone has only been
99 described in rodent while the stimulation of adrenal Progesterone synthesis by K depletion
100 may not be effective in human. Indeed, the presence in human adrenal gland of the
101 cytochrome p450 family member CYP17A1, an enzyme that is not expressed in rodent [12],

102 metabolizes progesterone into 17-hydroxyprogesterone and may prevent progesterone
103 accumulation [13] (Figure 1).

104

105 To address the role of progesterone in renal adaptation to potassium depletion in humans,
106 we submitted healthy subjects to a potassium-depleted diet and studied their renal
107 adaptation to potassium depletion, the changes induced in plasma adrenal steroid
108 concentrations, and their responses to adrenocorticotrophic hormone (ACTH) stimulation
109 following tetracosactide (Synacthene®) administration. Finally, plasma adrenal steroids were
110 quantified in patients with chronic severe renal hypokalemia related to a congenital salt-
111 losing tubulopathy, the Gitelman syndrome.

112

113

114 **SUBJECTS AND METHODS**

115 **SUBJECTS**

116 The study was approved by the local Ethics Committee (P120906 - CPP Ile de France VI,
117 NCT02297048). All subjects gave informed consent for genetic analyses before participating
118 the study. The study was registered on clinical trial registry (NCT02297048).

119

120 The twelve healthy subjects included in the study were Caucasian males 18 to 35 years of
121 age. Inclusion criteria were BMI ranging from 18 to 30 kg/m², plasma ionogram and liver
122 panel within normal range, estimated glomerular filtration rate (MDRD) > 60 ml/min/1.73
123 m². Exclusion criteria were recent (within 7 days) diarrhea, gastric or duodenal ulcer, any
124 current drug other than acetaminophen, drug allergy history or any severe allergy, drug or
125 alcohol abuse, blood hemoglobin < 10 g/dl.

126

127 The ten patients with renal loss of potassium were Caucasian males 18 to 75 years old with
128 genetically proven Gitelman syndrome. Exclusion criteria were recent (within 7 days)
129 diarrhea, drug allergy history or any severe allergy, or blood hemoglobin < 10 g/dl.

130

131 **INVESTIGATIONS**

132 After a safety inclusion visit, healthy subjects were subjected twice to a 7-day period of
133 potassium depletion. Potassium depletion was induced by 30 g daily sodium polystyrene
134 sulfonates (Kayexalate®, Sanofi-adventis France) over two days, followed by 5 days under a
135 low-potassium diet (25 mmol/d). We attempted to maintain a constant level of calories and
136 of sodium intake during the diet period. A safety end-study visit was performed a 7-day
137 washout period after the second potassium-depletion interval.

138

139 The day before admission for each experimental visit, participants were asked to collect
140 urine for measurement of 24-h urinary output and determination of urinary concentration of
141 electrolytes (Na, K, Cl, and creatinine) and of steroid metabolites. At the beginning (day 1,
142 D1) or the end of potassium depletion (day 7, D7), blood samples were taken before drug
143 administration at about 9:00 a.m. after an overnight fast and after 1 h of rest in the sitting
144 position (time H0). Additional blood samples were taken 30 min after intravenous

145 administration of 250 µg tetracosactide (time H1; Synacthene[®], alfasigma). Samples at H0
146 allowed determination of plasma electrolytes including sodium, potassium, chloride,
147 bicarbonate, creatinine, and plasma renin concentration, plasma aldosterone, plasma ACTH,
148 and of steroids. At H1, plasma renin and plasma aldosterone were quantified. The patients
149 with Gitelman syndrome were given the tetracosactide test once; the same parameters that
150 were measured in the healthy volunteers were measured in the Gitelman syndrome
151 patients.

152

153 **METHODS**

154 Plasma concentrations of a panel of 15 steroids were measured using a liquid
155 chromatography coupled to tandem mass spectroscopy (LC-MS/MS) assay to avoid the risk
156 of interference between steroids as previously described [14]. The steroids evaluated were
157 pregnenolone, progesterone, corticosterone, deoxycorticosterone (DOC), aldosterone, 17-
158 hydroxypregnenolone, 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, cortisol,
159 cortisone, DHEA, delta 4-androstenedione, 11-β-hydroxyandrostenedione, testosterone.

160 Plasma renin concentration was assessed with commercial kit (Renin III from CisBio). Urinary
161 concentrations of cortisol and plasma concentrations of ACTH were determined using
162 chemiluminescent enzyme immunoassays (Immulite 2000 XPi, Siemens Healthineers).

163 Routine plasma and urinary aldosterone concentrations were also assessed with a
164 commercial kit (DPC Coat-a-Count assay from Diagnostic Products Corp).

165

166 **STATISTICAL ANALYSIS**

167 Our hypothesis was that potassium depletion would increase adrenal progesterone and/or
168 17-hydroxyprogesterone synthesis. The primary outcome was the change induced by
169 potassium depletion in the plasma progesterone concentration measured 30 min after
170 intravenous administration of 250 µg tetracosactide. This was a pilot study without
171 preliminary data in humans, therefore, there was no data that could be used to support the
172 calculation of necessary sample size for statistical significance. In male mice subjected to
173 potassium depletion, there was a stimulation of progesterone production by 300% [9].
174 Assuming only a 50% increase in progesterone after potassium depletion (mean difference
175 +0.03 [IC95% : +0.02; -0.08]; p < 0.2827), an estimated standard deviation (SD) of 0.05 pg/ml,

176 and a two-sided type 1 error rate of 0.05%, a sample size of 12 evaluable subjects would
177 yield 80% power.

178
179 Analysis of variance for normally distributed variables or the Kruskal-Wallis non-parametric
180 test for non-normally distributed variables were used to compare the characteristics
181 between genotypes. In most cases, data were log-transformed when they were not normally
182 distributed. To compare values from D0 to D7 or from H0 to H1, paired t tests were
183 performed, whereas independent sample t tests were used to compare values of Gitelman
184 patients with values of healthy subjects. All analyses were carried out using SAS Statistical
185 Software (version 9.4, SAS Institute), and $p < 0.05$ was considered to be significant. Results
186 are expressed as median [IQR: Q1;Q3].

187

188 **RESULTS**

189 **EFFECT OF POTASSIUM DEPLETION IN HEALTHY SUBJECTS**

190 As expected, when values were compared for healthy subjects on D0 versus D7, plasma
191 potassium concentration decreased (mean difference 0.3 ± 0.1 mmol/l, $p = 0.0180$, Table 1),
192 and subjects became slightly hypokalemic without change in plasma concentrations of
193 sodium, chloride, bicarbonate, creatinine, or estimated glomerular filtration rate (Table 1).
194 Potassium depletion induced a decrease (by a factor of 2) in daily urinary excretion of
195 potassium (Table 1) without affecting daily urinary excretion or fractional excretion of
196 sodium (Table 1).

197

198 As measured by immunoassay, plasma aldosterone concentration and urinary aldosterone
199 excretion decreased by -55% ([IC95: -72%;-28%]; $p = 0.0068$) and -69% ([IC95: -80%; -51%];
200 $p = 0.0001$), respectively, from D0 to D7 in healthy subjects (Table 1). This is likely due to the
201 inhibitory effect of potassium depletion on aldosterone secretion since the plasma renin
202 concentration was not significantly affected (Table 1). Plasma ACTH and urinary cortisol
203 excretion (nmol/mmol creatinine) (Table 1) and plasma cortisol (Table 2) were not affected
204 by potassium depletion.

205

206 A week of potassium depletion did not affect any of the plasma steroid concentrations as
207 assessed by LC-MS/MS (Table 2, healthy subjects, D0 vs. D7) with the exceptions of
208 aldosterone, which decreased by -50% ([IC95% -80%;-10%]; p=0.0332) and 17-
209 hydroxypregnenolone, which increased by 45% [IC95% 9%; +67%]; p=0.0232). Of note,
210 concentrations of plasma progesterone (mean difference 20% [IC95 % -24%; +49%],
211 p=0.283) and its metabolite 17-hydroxyprogesterone (mean difference +11% [IC95 % -9%;
212 +28%], p 0.237) were similar before and after a week of potassium, depletion (Table 2).

213
214 We then estimated enzymatic activity of adrenal steroidogenesis by calculating the ratio
215 between the product and substrate of enzymes (Figure 1, Supplemental Table 1). This
216 analysis identified two changes in important steps of the steroidogenesis in healthy subjects
217 after potassium depletion. First, the activity of CYP11B2, estimated as the ratio of
218 aldosterone to corticosterone was 3-4-fold decreased. Second, less expectedly, CYP17
219 activity, estimated as the ratio of 17-hydroxypregnenolone to pregnolone, was increased by
220 60%. The stimulation of CYP17 was associated with no change in estimated activity of 3- β -
221 hydroxysteroid dehydrogenase (3 β OHSD) in the glomerulosa and a decrease in estimated
222 3 β OHSD activity in the fasciculata, which prevented conversion of 17-hydroxypregnenolone
223 into 17-hydroxyprogesterone.

224
225 Intravenous administration of 250 μ g tetracosactide led to a 2-fold increase in plasma
226 cortisol, a 4-fold increase in plasma aldosterone, and a significant increase in all plasma
227 hormonal concentrations except renin, testosterone, and cortisone (Table 2). Post
228 tetracosactide concentrations of steroids measured under usual diet (D0) and after a week
229 under low potassium diet (D7) were similar.

230
231 **EFFECT OF CHRONIC POTASSIUM DEPLETION**

232 The effect of a severe and chronic depletion of potassium was addressed in patients
233 suffering of a salt and potassium losing nephropathy, the Gitelman syndrome (GS) (OMIM
234 #263800). As shown in Table 1, patients with GS were older than healthy subjects (p<
235 0.0001) but had similar BMI (p=0.1765), and their diastolic blood pressure was slightly higher
236 than that of healthy subjects (Table 1). Patients with the salt-losing tubulopathy had marked
237 activation of the renin angiotensin-aldosterone system and high sodium intake (as

238 demonstrated by higher levels of sodium excretion) as compared to controls (Tables 1 and
239 3). They had marked hypokalemia, abnormal levels of potassium excretion (i.e., K excretion >
240 40 mmol/24 h and fractional excretion of K higher than 5%). Their plasma ACTH was similar
241 to that of healthy subjects (Table 1).

242 Despite their chronic hypokalemia, GS patients did not display any differences in pre-
243 tetracosactide plasma steroid concentrations compared to healthy subjects with the
244 exceptions of lower levels of aldosterone (mean difference 69% [IC95 % +33%; +86%], p
245 0.0046) and of higher levels of 11-deoxycortisol and of delta 4-androstenedione (mean
246 differences +44% [IC95% +1%; +68%], p=0.0471 and 19% [IC95 % +0%; +34%], p=0.0485,
247 respectively) (Table 2).

248
249 GS patients had a stronger response to tetracosactide than healthy subjects (Table 3). As
250 expected, in GS patients post-tetracosactide plasma aldosterone concentration was
251 markedly higher than in healthy subjects (+67% [+44%; +80%], p=0.0003). In addition, the
252 post-tetracosactide concentrations of pregnenolone, progesterone, corticosterone, DOC,
253 and 11-deoxycortisol were about 40% higher in GS patients than in healthy subjects,
254 whereas cortisol and delta 4-androstenedione levels were 20-30% higher. This exacerbated
255 response to tetracosactide could be, depending of the steroid, related either to the
256 observed chronic stimulation of angiotensin II or to chronic potassium depletion. To
257 distinguish between these possibilities, we calculated the correlation between post-
258 tetracosactide plasma steroid levels and plasma renin (which are high when angiotensin II
259 activity is high) or plasma potassium values. We found that progesterone, corticosterone,
260 DOC, and aldosterone were correlated with both plasma renin and plasma potassium levels
261 (Supplemental Table 2, Figure 2 A-D), whereas pregnenolone and delta 4-androstenedione
262 only correlated with plasma potassium concentration (Figure 2 E-H). Interestingly,
263 aldosterone (Figure 2 A-B) and DOC (Supplemental Table 2) best fitted with plasma renin
264 concentration, whereas progesterone best fitted with plasma potassium level (Figure 2 C-D).

265

266 **DISCUSSION**

267 **MILD REDUCTION OF POTASSIUM INTAKE DOES NOT INFLUENCE PROGESTERONE AND 17-** 268 **HYDROXYPROGESTERONE CONCENTRATIONS IN MEN**

269 Although it is clear that steroid production by the adrenal gland changes in response to
270 sodium and potassium content of the diet [15, 16], almost nothing was known about
271 adaptation of humans to a low potassium diet except that plasma aldosterone concentration
272 falls markedly after 2-3 days of potassium depletion [17]. Elabida et al. demonstrated in mice
273 that potassium depletion but not sodium depletion, led to increase in adrenal progesterone
274 production [9]. Potassium depletion induced an increased expression of steroidogenic acute
275 regulatory protein (StAR), CYP11A1, and 3 β HSD1 and was associated with decreased in
276 CYP11B1 expression (a key enzyme in corticosterone synthesis) and in CYP11B2 expression
277 (a key enzyme for aldosterone synthesis). Using a mathematical model of steroidogenesis in
278 mice, Elabida et al. predicted that potassium depletion should result in an increase in plasma
279 progesterone, a prediction confirmed experimentally in male mice and male men [9]. In mice
280 progesterone regulates renal potassium handling both in males and females under
281 potassium restriction, independently of its role in reproduction, and this adrenal
282 progesterone was required for full adaptation to potassium depletion [9]. The mechanism
283 was mediated by stimulation of the H,K-ATPase type 2 and was prevented by the
284 progesterone receptor antagonist RU486. These results, however, were not immediately
285 transferable to man since human adrenal steroidogenesis strongly differs from that of
286 rodents as humans express CYP17, which metabolizes progesterone into 17-
287 hydroxyprogesterone, although Elabida et al. also established a weak correlation between
288 plasma progesterone in men and their potassium intake [9].

289 |
290 To directly address the contribution of progesterone on renal adaptation to potassium
291 depletion in human, we induced potassium depletion in healthy men. Although we did not
292 measure intracellular potassium levels, the marked inhibition of plasma and urinary
293 aldosterone concentrations were indicative of a state of potassium depletion. At the
294 beginning and the end of the period, we performed a tetracosactide test to stress the
295 adrenal response to potassium depletion and potentially reveal subtle modifications in
296 adrenal steroidogenesis. Neither progesterone nor 17-hydroxyprogesterone concentrations
297 measured before or after tetracosactide administration were affected by potassium
298 restriction. However the potassium depletion we induced may have been too weak
299 (decrease by 2-fold of potassium intake) or too short (7 days) or both as compared to what
300 has been done in mice (decrease by 20-fold of potassium intake for 1 week). In male mice,

301 the increase of progesterone appears to stimulate potassium reabsorption to counteract the
302 deleterious effect of prolonged potassium restriction. This step was not observed in healthy
303 subjects in our present study. In addition, the presence of a CYP17A activity in human
304 adrenal glands may have prevented progesterone accumulation. In support of this, we
305 observed a significant 50% increase in 17-hydroxypregnenolone and, unexpectedly, the
306 estimated CYP17 activity increased by 60%. Our results suggest that this increase in CYP17
307 activity leads to 17-hydroxypregnenolone accumulation but that conversions of
308 pregnenolone into progesterone and 17-hydroxypregnenolone into 17-hydroxyprogesterone
309 are prevented by low 3 β HSD activity (Figure 1). Our study was powered to demonstrate a
310 50% increase in plasma progesterone and 17-hydroxyprogesterone concentrations, whereas
311 150 subjects would be required to demonstrate that the observed 20% increase in pre-
312 tetracosactide progesterone concentration was significant.

313

314 **PATIENTS WITH SALT-LOSING TUBULOPATHIES HAD ALTERED ADRENAL RESPONSE TO** 315 **TETRACOSACTIDE BUT UNAFFECTED PROGESTERONE METABOLISM**

316 We also studied the plasma steroid profile in patients with a congenital renal loss of sodium
317 and potassium due to a Gitelman syndrome. These patients had a severe chronic
318 hypokalemia (median 2.7 mmol/l, Table 1), a renal loss of sodium, resulting in marked
319 stimulation in plasma renin and aldosterone concentrations, and high potassium intake. In
320 contrast to observations in rodents, despite profound hypokalemia, GS patients displayed a
321 hyperaldosteronism in response to their loss of salt and are under potassium
322 supplementation to limit their hypokalemia. The levels of plasma progesterone and 17-
323 hydroxyprogesterone were similar to those observed in healthy subjects. Interestingly, after
324 treatment with tetracosactide, levels of many steroids were more elevated in GS patients
325 than in healthy subjects. These results indicate that patients with GS have a higher ability
326 than healthy subjects to respond to ACTH stimulation in order to promote steroid
327 production. Since, these patients displayed stimulation of their renin-angiotensin-
328 aldosterone system (Tables 2 and 3), we suggest that the presence of high levels of
329 angiotensin II sensitizes the adrenal glands of GS patients to ACTH. This synergic effect of
330 angiotensin II and ACTH on steroid production was originally documented about 35 years
331 ago [18]. Indeed, angiotensin II not only increases the expression of CYP11B2, but also
332 indirectly stimulates the activities of 3 β HSD2 and CYP21 enzymes [19]. GS patients are,

333 however, in a paradoxical situation since they exhibit a profound hypokalemia that probably
334 counteracts, at least partially, the effect of angiotensin II. When ACTH is given to GS
335 patients, it disrupts this fragile equilibrium and reveals a “hidden” hyperactivity of the
336 adrenal glands, which produce steroids like progesterone with a good correlation to plasma
337 potassium level.

338 In conclusion, we provide evidence that potassium depletion induced in healthy men
339 enhances CYP17 adrenal activity, blunting progesterone accumulation. It is important to
340 note that our data do not exclude the possibility that an adrenal steroid-dependent pathway
341 is involved in adaptation to more severe potassium depletion. We unexpectedly observed
342 over-response to tetracosactide of non-mineralocorticoid steroids in GS patients suggesting
343 that adrenal glands not only adapt to sodium depletion but also to potassium depletion

344

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351

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398

399

400 **LEGENDS**

401 **FIGURE 1: Effect of potassium depletion on adrenal steroidogenesis.** Activities of the
402 main enzymes of adrenal steroidogenesis were estimated by calculation of the product to
403 substrate ratios, indicated in the circles. Significant changes after potassium depletion are
404 indicated by red circles when enzymatic activity was enhanced and by grey dashed circles
405 when activity was inhibited. As expected, potassium depletion dramatically depressed
406 CYP11B2 activity (and thus aldosterone production). It also increased CYP17 activity,
407 leading to an increase in 17-hydroxypregnenolone, and reduced 3 β HSD activity in the
408 fasciculate preventing transformation of 17-hydroxypregnenolone into 17-
409 hydroxyprogesterone. The decrease in 17-20 liase in the reticularis was not sufficient to
410 prevent DHEA accumulation (due to transformation of 17-hydroxy pregnenolone into
411 DHEA).

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413 **FIGURE 2: Correlation of post-tetracosactide concentrations of plasma hormones with**
414 **plasma renin or plasma potassium concentrations.** The individual concentrations of
415 plasma hormones were measured after administration of 250 μ g tetracosactide in healthy
416 subjects under normal diet or in Gitelman patients and correlated to the plasma renin
417 concentration or the plasma K level. Nonparametric Spearman coefficient r [CI95%]
418 and p values of the correlations were calculated using Prism 8 software.

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420 **Conflict of interests:** There are no conflicts of interests and the results presented in this
421 paper have not been published previously in whole or part.

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Table 1: Baseline characteristics of healthy subjects and GS patients and effects of potassium depletion on hemodynamic, plasma, and urinary electrolytes and hormones.^a

Parameters		Healthy subjects (n=12)		GS patients (n=10)
		D0	D7	
Age	yrs	27.4 [22.6;30.7]	-	46.2 [39.4;49.5] ^c
BMI	kg/m ²	23.9 [20.8;25.4]	-	25.2 [22.5;27.2]
SBP	mmHg (H0)	121 [115;127]	118 [117;127]	120 [115;122]
DBP	mmHg (H0)	65 [64;70]	70 [65;74]	81 [74;85] ^c
Plasma				
Sodium	mmol/L	139 [137;141]	140 [138;141]	138 [137;139]
K	mmol/L	3.6 [3.5;3.9]	3.4 [3.2;3.7] ^b	2.7 [2.0;3.1] ^c
Cl	mmol/L	105 [103;106]	104 [103;106]	97 [94;98] ^c
Bicarbonate	mmol/L	27 [26;28]	27 [27;29]	87 [72;96]
eGFR (MDRD)	ml/min/1.73m ²	103 [83;112]	100 [90;108]	
ACTH	pg/mL (H0)	18 [14;28]	19 [14;27]	18 [13;28]
Renin	mIU/L (H0)	14 [12;16]	12 [6;17]	159 [96;200] ^c
Aldosterone	pg/mL (H0)	67 [40;83]	23 [17;34] ^a	237[67;359] ^c
Urine				
Urine output	l/24h	1.7 [0.8;2.4]	1.5 [1.1;2.0]	2.2 [1.7;2.4]
Na	mmol/24h	136 [80;200]	155 [103;192]	231 [187;305] ^c
K	mmol/24h	57 [36;72]	25 [21;32] ^b	117 [98;138] ^c
Cl	mmol/24h	147 [89;185]	163 [105;198]	304 [256;383] ^c
Creatinine	mmol/24h	14 [12;18]	16 [15;18]	15 [13;18]
Na excretion	%	0.5 [0.4;0.6]	0.5 [0.5;0.6]	0.9 [0.7;1.1] ^c
K excretion	%	8.3 [5.8;10.6]	3.6 [2.9;3.9] ^b	23.2 [19.1;29.1] ^c
Aldosterone/creatinine	ng/mmol	0.52 [0.33;0.63]	0.19 [0.07;0.26] ^b	1.56 [0.96;3.07] ^c
Cortisol/creatinine	ng/mmol	14 [10;25]	16 [12;27]	43 [42;44]

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^a Potassium depletion was induced by 30 g daily polystyrene sulfonates for two days, followed by 5 days of a low-potassium diet (25 mmol/day). Blood and urine samples were taken at baseline (D0) and day 7 (D7). Results are median [IQR] and p value of geometric means. ^b p < 0.05 D7 vs. D0 ^c p < 0.05 GS vs. healthy subjects D0. Abbreviations: BMI, body mass index;SBP, systolic blood pressure;DBP, diastolic blood pressure;eGFR, estimated glomerular filtration rate;ACTH, adrenocorticotrophic hormone.



