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ORIGINAL ARTICLE

Subjects with essential hypertension are more sensitive to the inhibition of 11 β-HSD by liquorice

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In this intervention study, we have investigated if hypertensive patients are more sensitive to liquorice-induced inhibition of 11 β-hydroxysteroid dehydrogenase (11 β-HSD) type 2 than normotensive (NT) subjects and if the response depends on gender. Healthy volunteers and patients with essential hypertension (HT), consumed 100 g of liquorice daily, for 4 weeks, corresponding to a daily intake of 150 mg glycyrrhetinic acid. Office, 24-h ambulatory blood pressure (BP) and blood samples were measured before, during and after liquorice consumption. Effect on cortisol metabolism was evaluated by determining the urinary total cortisol metabolites and urinary free cortisol/free cortisone quotient (*Q*). The mean rise in systolic BP with office measurements after 4 weeks of liquorice consumption was 3.5 mmHg ($p<0.06$) in NT and 15.3 mmHg ($p=0.003$) in hypertensive subjects, the response being different ($p=0.004$). The mean rise in diastolic BP was 3.6 mmHg ($p=0.01$) in NT and 9.3 mmHg ($p<0.001$) in hypertensive subjects, the response also being different ($p=0.03$). Liquorice induced more pronounced clinical symptoms in women than in men ($p=0.0008$), although the difference in the effect on the BP was not significant. The increase in *Q* was prominent ($p<0.0001$) and correlated to the rise in BP ($p=0.02$). The rise in BP was not dependant on age, the change in plasma renin activity or weight. We conclude that patients with essential HT are more sensitive to the inhibition of 11 β-HSD by liquorice than NT subjects, and that this inhibition causes more clinical symptoms in women than in men.
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Introduction

The enzyme 11 β-hydroxysteroid dehydrogenase (11 β-HSD) may be involved in the pathogenesis of essential hypertension (HT).[1] The isoenzyme 11 β-HSD type 2, mainly found in the kidney, converts cortisol to cortisone, protecting the mineralocorticoid receptors (MR) from cortisol, which has the same affinity to MR as aldosterone.[2] Inhibition of the type 2 therefore leads to the syndrome of pseudohyperaldosteronism.[3] The type 1 isoenzyme, primarily found in the liver, has mainly the opposite effect. Both isoenzymes are to some extent also found in other organs and tissues.[4–9]

Partial mutation of 11 β-HSD has been reported to cause HT.[10] A genetic defect in the 11 β-HSD enzyme type 2 as in patients with apparent mineralocorticoid excess (AME), as well as inhibition of 11 β-HSD type 2 activity by glycyrrhetinic acid (GA) (the active constituent in liquorice) or carbenoxolone (a synthetic inhibitor), leads to elevated blood pressure (BP), decreased serum potassium concentration, and retention of fluid and sodium, a syndrome of pseudohyperaldosteronism.[3,11–13] GA is also known to inhibit the renin–angiotensin–aldosterone system (RAAS).[3,14,15]

The role of 11 β-HSD in HT is complex, since the enzyme has been found in the human heart as well as in blood vessels of both man and rat.[16–19] Cortisol-induced dermal vasoconstriction is increased in humans with inhibited or a defect 11 β-HSD activity,[20] and after topical application of cortisol, the dermal vasoconstriction has been shown to be increased in patients with HT compared to controls, indicating a decreased 11 β-HSD activity. This suggestion is supported by the finding of a prolonged half-life of cortisol in patients with HT.[21,22]

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The finding of a higher ratio of cortisol to cortisone metabolites in urine in HT patients, and elevated concentrations of 11 β-HSD inhibitory substances in the urine of patients with low-renin HT further stress the role of 11 β-HSD in the control of BP.[23,24] Earlier studies of endogenous 11 β-HSD inhibitory factors, GA like factors (GALF's), indicate that the 11 β-HSD type 2 is more important than the type 1 in the pathogenesis of HT.[24,25] Since liquorice is known to inhibit the type 2 of 11 β-HSD, it is suitable to use it as a tool for further studies of the interaction of 11 β-HSD in HT. Further, since previous studies from our group have shown a dose–response relationship between liquorice[26] and the rise in BP, a study on the effect of BP in HT subjects was indicated. Those studies have also implied that the liquorice effect depends on gender, as women seemed to experience more adverse effects after liquorice consumption than men.

Our hypothesis was that the decreased conversion of cortisol to cortisone in HT patients might be caused not only by a defect 11 β-HSD enzyme or increased concentrations of inhibitory factors, but also by an increased sensitivity to these substances. Another hypothesis was that the sensitivity of 11 β-HSD inhibitory factors may depend on gender.

Materials and methods

Healthy, normotensive (NT) individuals in the age range of 20–45 years without HT in accordance with the definitions given in the Framingham study,[26] and patients of the same age with treated essential HT with systolic BP (SBP) below 140 mmHg and diastolic BP (DBP) below 90 mmHg were recruited to the study. A prerequisite was that the BP had to be stable for the last 3 months in the HT group, defined as no need for change in medication.

Patients receiving other antihypertensive drugs than β-receptor inhibitors and/or Ca^{2+}-antagonists were excluded. Secondary HT, use of tobacco in any form or any hormone medication (even contraceptives) except thyroxin excluded participation. Women in early menopause as well as pregnant women were also excluded, and in order to confirm this, a pregnancy test was taken for every woman at the start of the study.

In total, 46 individuals were recruited to the study. Three HT and six NT individuals later changed their mind and thus did not enter the study protocol. One man reported that he quitted the study after 14 days of consumption because of heartburn without any further physical or biochemical examinations, and was therefore excluded. The final study comprised 25 healthy volunteers, 13 men and 12 women, with mean age 31.2 years (range 22–43), and 11 volunteers with HT, 8 men and 3 women, with mean age 40.7 years (range 33–44). Comparing the gender, all women (12 NT and 3 HT) constituted one group, the mean age being 35.0 years (range 22–44), and all men (13 NT and 8 HT) constituted another group the mean age being 33.5 years (range 24–44). Medication of the participants was as follows: one healthy woman was taking thyroxin because of hypothyroidism; 9 of the HT individuals were treated with β-receptor inhibitors and two with the combination of a β-receptor inhibitor and a Ca^{2+}-antagonist.

All participants in the study were asked for their alcohol habits, but no one reported excessive consumption during the study period, defined as more than one bottle of wine more than 2 days per week.

The study was approved by the ethical committee of the University of Gothenburg and a written informed consent was obtained.

Study protocol

The study period consisted of three parts, one run-in week with definition of baseline values, one 4-week period with a daily intake of 100 g of liquorice, that is, 150 mg GA, and finally a wash-out period of 4 weeks. Women started the consumption at day 1–4 in the menstrual cycle.

The office BP was measured after 5 min rest in the supine position with a standardized sphygmomanometer (STILLE Tourniquet 203-20197-8). Office BP and heart rate (HR) were measured on three different days during the run-in period, twice each time, and the mean of these six measurements was used as the baseline value. The 24-h ambulatory BP (24-h AM BP) (Space labs model 90207) and HR were measured two times during the control period and the mean of these measurements was used as the baseline value. The night was defined as the hours from 23.00 pm to 05.59 am and the day as the hours from 06.00 am to 22.59 pm. Office and 24-h AM BP as well as HR measurements were repeated after 14 ± 2 days of liquorice consumption, after 4 weeks consumption, and after the wash-out period. Office BP was also measured 2 weeks after finishing the consumption. On all of these occasions, the weight was measured and the body mass index (BMI) calculated. Blood samples and 24-h urine collections were taken at baseline, after 4 weeks of liquorice consumption and after the wash-out period (ie 4 weeks after the liquorice consumption ended). The same nurse throughout the 8 weeks study period measured the BP and took care of the 24-h BP measurements as well as the control of weight, height and sampling of blood and urine. All participants underwent an initial physical examination and were followed by the same doctor throughout the study period.

When comparing the effect of gender, all women (12 NT and 3 HT) constituted one group, the mean age being 35.0 years (range 22–44), and all men (13 NT and 8 HT) constituted another group, the mean age being 33.5 years (range 24–44).

Analytical methods

The serum sodium and potassium were measured by ion-selective electrode (Hitachi 917(Roche)) and the serum creatinine was measured by enzyme-photometric method (Hitachi 917(Roche)). The plasma renin activity (PRA) was measured by a radioimmunoassay (RIA) (ABBOTT, Diagnostic Division, Kista-Stockholm, Sweden). The serum cortisol was measured by an RIA (Orion Diagnostica, Orion Corporation Orion Diagnostica, Espoo, Finland). Urinary total cortisol metabolites (U-TCM) in 24-h urine collections were analysed by a simplified version of the method previously described.[27] Levels of urinary free cortisol and cortisone were determined by high-performance liquid chromatography and UV-detection (254 nm) following extraction and purification on a small octylsilane-bonded silica column. The imprecision of the method (including interassay variation) was evaluated by analysing 30 samples from the same urine pool at 15 different occasions. Expressed as the coefficient of variation, it was about 5% for cortisol (concentration 54 nmol/l) and 6% for cortisone (concentration 128 nmol/l). The accuracy was assessed by determining recoveries of cortisol and cortisone (70–2000 nmol/l) added to urine. These were essentially quantitative, that is, ranging from 97 to 105%. Details on the method will be published elsewhere.

Statistical methods

All values for office BP and HR are presented as the mean of two measurements, whereas the baseline value (obtained prior to treatment) is the mean of six measurements. The mean baseline value for the 24-h BP was calculated from the means of two 24-h measurements from each individual and the following mean 24-h values from the individual recordings.

A nonparametric test, Fisher's permutation test, was used for statistical analysis of dropouts and pairwise comparisons of all variables. In correlation calculations, the Pearsons's R was calculated for the whole group and the regression tested with a t-test. Correlation calculations accounted for the correlation of the difference between the baseline value and the value after 4 weeks of liquorice consumption for the respective parameter. Comparison between NT and HT groups with respect to BP was performed by elimination of the influence of gender and weight by use of Mantel's test, and in a similar way the effect of the variable NT/HT was eliminated in the comparison between the genders as well as the effect of age.[28] In order to evaluate the importance of the change in weight for the increase in BP, a regression coefficient was calculated. A professional statistician was consulted for the construction of the study as well as for all calculations.[29,30] A p-value less than 0.05 was considered significant. Results are described as mean $\pm$ SD throughout.

Results

Subjective complaints and clinical symptoms

The subjective effects of the liquorice consumption were lenient, starting after 2 weeks of consumption and disappearing when the liquorice consumption was ended. In all, 13 participants reported headache and nine reported oedema. Few reported diarrhoea, increased abdominal gas, slight dizziness or joint pain in fingers and wrists. The frequency of subjective complaints did not differ between the genders. However, the complaints seemed to be more pronounced in women, since quitting the liquorice consumption (marked BP elevation and/or oedema) was more frequent within the female group ($p = 0.0008$). Thus, six women (one with HT and five NT), but no man, had to quit the consumption earlier than 4 weeks: one woman with HT because of extreme rise in SBP and DBP (35.7 and 22.2 mmHg, respectively, after 14 days even though this did not give her any symptoms), two because of oedema and headache (after 9 and 14 days, respectively), one because of oedema, shortness of breath and tiredness (after 14 days), one because of headache (after 14 days), and one because of irregular menstruation (menstruation came too early and by continuing the liquorice consumption she would not have been comparable to the other women with a normal menstruation cycle). All women continued the study protocol considering physical and biochemical examinations throughout the study period ('*intention to treat—model*').

Office BP measurements

After 4 weeks of liquorice consumption, the mean rise in SBP was 3.5 mmHg ($p < 0.06$) in the NT group and 15.3 mmHg ($p = 0.003$) in the HT group, the response being different between the groups ($p = 0.004$) as during the wash-out period ($p < 0.03$). The DBP increment was also different between the groups ($p < 0.02$), lasting during 2 weeks of the wash-out period ($p < 0.006$). The significant increase in SBP and DBP did not differ between the genders.

In the NT group, the maximum increase in SBP was 23.7 mmHg, found in a woman who also increased by 13.3 mmHg in DBP, 3.3 kg in weight, and she experienced the most pronounced oedema. This woman consumed liquorice for 9 days. The maximum rise of DBP was 15.7 mmHg, noted in a man.

In the HT group, the maximum rise in SBP was 35.7 mmHg, found in a woman who increased by 22.2 mmHg in DBP and 2.4 kg in weight but without any side effects. She had consumed liquorice for only 14 days when she was asked to stop the consumption. The maximum rise of DBP was 26.3 mmHg, found in a man, who by that time complained of headache and oedema, and had consumed liquorice for 4 weeks. His weight had increased by 2 kg.

Table 1 24-h systolic BP

Group	n	*Baseline value*	*2 weeks with liquorice*[a]	p-*value*	*4 weeks with liquorice*[a]	p-*value*	*4 weeks after liquorice*	p-*value*
24-h ambulatory blood pressure (mmHg)								
SBP								
NT group	25	120.8 ± 9.4 (104.9–145.4)	+4.2 ± 6.8 (−12.1 to +19.5)	0.006	+5.6 ± 7.9 (−11.6 to +18.5)	0.003	−1.3 ± 6.2 (−15.1 to +12.9)	NS
HT group	11	129.2 ± 7.7 (118.1–141.5)	+9.8 ± 6.7 (−0.6 to +20.2)	0.002	+12.4 ± 5.1 (+3.2 to +18.2)	0.0006	+1.8 ± 4.9 (−7.6 to +10.0)	NS
Difference[b]		0.04		0.02		0.02		NS
DBP								
NT group	25	74.3 ± 4.7 (64.2–86.5)	+2.2 ± 4.6 (−12.5 to +12.5)	0.02	+4.2 ± 5.7 (−7.5 to +16.4)	0.002	−0.5 ± 4.4 (−13.7 to +9.2)	NS
HT group	11	84.2 ± 7.4 (71.1–95.8)	+6.5 ± 4.3 (+0.5 to +12.4)	0.002	+9.1 ± 4.5 (+4.0 to +18.3)	0.0005	+2.3 ± 4.0 (−4.7 to +9.1)	NS
Difference[b]		0.0006		0.014		0.04		NS

[a]Compared to baseline.
[b]Difference at baseline and difference in response between the NT and HT groups (Mantel's test).
All values are presented as the mean ± SD SBP, systolic blood pressure; DBP, diastolic blood pressure; NT, normotensive; HT, hypertensive. Range is shown in parenthesis below the value.
NS, not significant.

The 24-h, ambulatory blood pressure

After 4 weeks of liquorice consumption, the mean rise in 24-h SBP in the NT group and the HT group was different between the groups ($p = 0.01$) (Table 1). After the 4-week wash-out period, the mean SBP did not differ from the baseline value in any group (Table 1). Further, there was a difference in response between the groups considering DBP (Table 1).

Comparing the gender, the mean rise in SBP of 6.2 mmHg ($p = 0.003$) for women and 8.7 mmHg ($p = 0.0004$) for men after 4 weeks of liquorice consumption was not different between the groups. Neither was the mean rise in DBP. The HR did not change in any group.

By use of Mantel's test we could eliminate the effect of age, as the change in BP was not affected by age.

BMI and weight

BMI increased by a mean of 0.38 (SD = ± 0.42, range: −0.52 to +1.44) in the whole group (25.6 at baseline) with a maximum increase after 2 weeks of consumption, and was significant in the whole group (Table 2) as well as in all subgroups. No correlation was found between the change in BMI and the change in BP, and the change in BMI did not differ between the NT and HT groups or between the genders.

Weight increased significantly in all groups (Table 2). At baseline, men weighed more (87.2 ± 16.3 kg) than women (72.1 ± 11.0 kg, $p = 0.002$), and after 2 weeks of liquorice consumption, the increase in weight was greater in men (+1.4 ± 1.2 kg) than in women (+1.0 ± 1.5 kg, $p = 0.01$). No difference was found between the NT and HT groups in weight. There was a correlation between the change in SBP and the

Table 2 Variables are shown for the whole group (n=36)

Variable	*Baseline*	*Liquorice effect*	*Range*	P-*value*
Weight (kg)[a]	80.9 ± 16.0	1.2 ± 1.3	(−1.5–5.1)	<0.0001
Serum potassium (mmol/l)[b]	4.2 ± 0.2	−0.4 ± 0.3	(−0.9–0.2)	<0.0001
Serum creatinine (μmol/l)[b]	96.5 ± 10.9	−3.7 ± 7.9	(−21.0–10.0)	0.004
PRA (ng/ml/h)[b]	0.81 ± 0.73	−0.31 ± 0.77	(−1.87–1.83)	0.02
U-TCM[b]	7.04 ± 3.36	−0.87 ± 2.75	(−6.6–8.9)	<0.0001
Q[b]	0.33 ± 0.10	+0.35 ± 0.27	(0.002–1.30)	<0.001

[a]After 2 weeks of liquorice consumption compared to baseline.
[b]After 4 weeks of liquorice consumption compared to baseline.
Weight, serum potassium, serum creatinine, PRA, urinary cortisol metabolites (U-TCM) and the quotient *Q* (urinary free cortisol/free cortisone) are presented as the mean change from the baseline value ± SD U-TCM are measured by μmol/mmol creatinine.
NS, not significant.

change in weight ($p=0.04$) after the 4 weeks of liquorice consumption. At the same time, the mean increase in weight was 0.8 kg for the whole group and was not significant.

The effect of weight on the rise in BP was minimized, by calculating the regressions coefficients. For the whole group the regression lines were ($y=4.82+0.86\times X$), for the NT group it was ($y=2.03+1.72\times X$), and for the HT group it was ($y=10.67-0.70\times X$). Where y is the change in 24-h SBP between baseline and after the 4 weeks of liquorice consumption, that is, the rise in 24-h SBP, and X is the change in weight during the same period. None of the coefficients were statistically significant. Mantel's test also eliminated the effect of weight on the increase in SBP.

Serum sodium, potassium, creatinine and PRA

Serum sodium did not change with liquorice consumption, while serum potassium decreased significantly in the whole group (Table 2) as well as in all subgroups, and the change in serum potassium correlated inversely with the change in SBP ($r=-0.36$, $p=0.03$). Serum creatinine decreased with liquorice consumption and the change correlated inversely with the change in DBP ($r=-0.39$, $p=0.02$).

The HT group had significantly lower baseline values of plasma renin activity (PRA) (0.46 ± 0.44 ng/ml/h) than the NT group (0.96 ± 0.78 ng/ml/h, $p=0.03$) (Table 2 for the whole group). The PRA decreased with liquorice consumption and the change correlated inversely with the change in SBP ($r=-0.44$, $p<0.01$) and DBP ($r=-0.42$, $p=0.01$), considering the whole group.

No difference was found between the NT and the HT groups or between the genders concerning the change in serum potassium, creatinine, or PRA.

Cortisol metabolism

The serum cortisol did not change in any group. The decrement in U-TCM correlated inversely with the change in SBP daytime ($p=0.02$) and the DBP daytime ($p=0.01$). No difference was found between the NT and HT groups or between the genders concerning the change in the U-TCM. The quotient for the urinary free cortisol/free cortisone (Q) increased in all groups without a difference in response between the groups (Table 2 for the whole group). The Q quotient correlated with the change in serum creatinine ($r=-0.588$, $p=0.0002$), PRA ($r=-0.35$, $p<0.05$) and 24-h SBP ($r=0.40$, $p=0.02$). In the NT group, the increase in Q correlated with the increase in SBP ($r=0.50$, $p=0.007$), but this was not found in the HT group. Figure 1 shows the change in SBP and Q with liquorice consumption for the whole group. Figure 2 shows the correlation between the change in SBP and Q in the whole group, the NT and HT groups being distinguished.

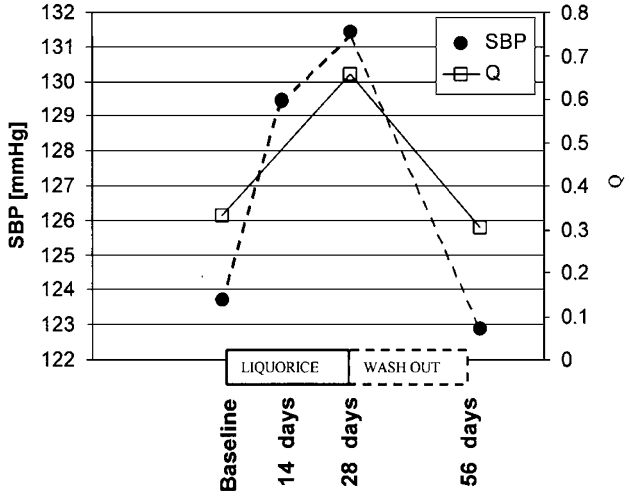

Figure 1 Liquorice-induced change in 24-h SBP for the whole group in relation to the change in Q (the quotient for urinary free cortisol/free cortisone), during the same period.

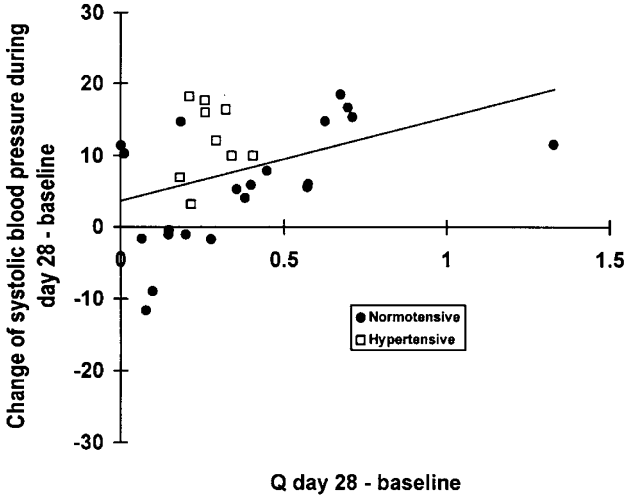

Figure 2 Regression plot showing the correlation of the change in Q (quotient for urinary free cortisol/free cortisone) and SBP for the whole group. The normotensive subjects (NT) are marked with filled dots and the hypertensive subjects (HT) with unfilled boxes. $y=3.69+11.68\times X$ ($r=0.40$) for the whole group.

Discussion

In this study, we found a more prominent increase in BP after inhibition of 11 β-HSD in patients with HT compared to NT individuals, which was independent of age or the change in PRA. The regression coefficient did not explain the rise in BP as a result of the increase in weight and neither did the Mantel's test, although this association has been established in the general population.[31] The increase in Q after liquorice consumption correlated to the

change in SBP emphasizing that the 11 β-HSD type 2 was affected. It is also interesting to notice that even though the given amount of GA in this study is considered to be moderate,[32] the rise in BP was quite prominent. Both SBP and DBP increased considerably in both groups.

In view of previous reports, we have designed this study with the goal of eliminating all factors known to influence 11 β-HSD as alcohol, tobacco, and furosemide, as well as factors influencing the RAAS as ACE-inhibitors, All-inhibitors, and diuretics.[3,14,33–38] The common use of drugs interfering with the RAAS, ACE, and AII complicated the inclusion of HT subjects, which resulted in smaller HT group with an uneven sex distribution. Nevertheless, the difference in response concerning BP was clear between the NT and HT groups. The change in PRA did not differ between the HT and NT groups even though it was lower at baseline in the HT group, indicating that the mechanism behind the more prominent rise in BP in the HT group is not explained by an increased salt sensitivity. Also, the liquorice used was sweet and not salted and therefore not escalating the sodium or fluid retention of GA.

An increased sensitivity to inhibition of 11 β-HSD in HT individuals is additionally supported by a report of a decrease in BP in spontaneously HT rats where intraperitoneal injections of dehydroepiandrosterone sulphate (DHEA-S) increased the activity of 11 β-HSD type 2 in the kidney while decreasing the enzyme activity in the liver.[39] DHEA-S-like cortisol is secreted from the adrenal cortex regulated by adrenocorticotrophic hormone (ACTH), but in contrast to cortisol, the secretion is reported to decrease with age.[40] DHEA-S was, in the study by Homma *et al*, found to have a peak value at the age of 30 with an annual decline of 2% during the following years. A decline of 11 β-HSD activity with age could thus be one reason why BP increases with age. In this context, it is important to state that even though our HT patients were older than the NT individuals ($p<0.002$), age did not explain the rise in BP.

The difference in BP increment between NT and HT individuals observed in our study may also have a genetic background, since we found that the adverse effects of liquorice consumption were more pronounced in women than in men. The lack of a significant difference in BP reaction after liquorice consumption between the genders could be explained by the fact that four out of the 15 women but none of the 21 men ended the consumption period earlier than the study plan, because of adverse effects (further two women were told to end the consumption period earlier than planned because of signs rather than symptoms, that is, high BP and irregular menstruation).

The decrease in serum creatinine, in spite of the fluid retention and change in electrolytes, might be influenced by the increased blood flow in the kidneys secondarily to the rise in BP, but might also be explained as a dilution effect caused by fluid retention.

This study has shown that individuals with essential HT are more sensitive to liquorice-induced rise in BP than NT individuals. Increased salt-sensitivity, age or the increase in weight did not explain the finding of a more prominent rise in BP in HT individuals. An increased sensitivity to the inhibition of 11 β-HSD in HT patients is postulated. This finding indicates that changes in the 11 β-HSD type 2 activity, owing to, for example, genetic diversity or exogenic factors, may be important in the pathogenesis of essential HT.

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Competing interests

None declared.

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