RESEARCH ARTICLE

Liquorice ingestion attenuates vasodilatation via exogenous nitric oxide donor but not via β$_2$-adrenoceptor stimulation

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Abstract

We examined the effect of liquorice ingestion on haemodynamic responses to exogenous nitric oxide donor (nitroglycerin) and β$_2$-adrenoceptor agonist (salbutamol), and 11β-hydroxysteroid dehydrogenase activity, in 21 volunteers and 21 reference subjects. Haemodynamic data was captured before and after sublingual nitroglycerin (0.25 mg) and inhaled salbutamol (400 µg) during orthostatic challenge utilising radial pulse wave analysis and whole-body impedance cardiography. The recordings were performed at baseline and following two weeks of liquorice intake (290–370 mg/d glycyrrhizin). Urinary cortisone and cortisol metabolites were examined. Liquorice intake elevated aortic systolic and diastolic blood pressure and systemic vascular resistance when compared with the reference group. Following research drug administration the liquorice-induced increase in systemic vascular resistance was observed in the presence of nitroglycerin (p<0.05) but no longer in the presence of salbutamol. Liquorice ingestion decreased cardiac chronotropic response to upright posture (p = 0.032) in unadjusted analysis, but when adjusted for age and sex the difference in the upright change in heart rate was no longer significant. The urinary cortisone to cortisol metabolite ratio decreased from 0.70 to 0.31 (p<0.001) after liquorice intake indicating significant inhibition of the 11β-hydroxysteroid dehydrogenase type 2. In the reference group the haemodynamic variables remained virtually unchanged. These results suggest that liquorice exposure impaired vasodilatation *in vivo* that was induced by exogenous nitric oxide donor but not that induced by β$_2$-adrenoceptor stimulation.

Trial registration: EU Clinical Trials Register 2006-002065-39 ClinicalTrials.gov NCT01742702.

Introduction

Hypertension and hypokalemia-induced secondary disorders are the main complications of liquorice ingestion reported in the literature [1]. Commercial liquorice extract is mainly obtained from the root of Glycyrrhiza glabra that is cultivated in temperate and semi-tropical parts of Europe and Asia [1,2]. The clinical effects of liquorice are mediated via inhibition of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) by the active metabolite glycyrrhetinic acid (GA), which leads to impaired conversion of active cortisol to inactive cortisone [1,3]. Increased cellular levels of cortisol bind both to the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) [1,3], while increased activation of both of these receptors can cause hypertension [4,5]. Normally aldosterone is the physiological agonist of the MR due to the protective effect of enzyme 11β-HSD2 [6,7], but following liquorice intake the action of cortisol is enhanced [1,3].

In the distal nephron, excessive MR activation by cortisol results in sodium and water retention, elevated blood pressure (BP), decreased plasma potassium concentration and suppression of the renin-angiotensin-aldosterone system [8]. The MR, GR and 11β-HSD2 are also expressed in the vascular wall, in both endothelial cells (EC) and the vascular smooth muscle cells (VSMC) [9–11]. Glucocorticoids may increase vascular tone via alterations within the endothelium including increased release of vasoconstrictors like angiotensin II and endothelin (ET)-1, and impaired endothelium-derived relaxation via nitric oxide (NO), or by direct actions within the VSMC including up-regulation of angiotensin II receptors [9]. MR antagonism has been found to reduce pulse wave velocity, a marker of arterial stiffness, in hypertensive subjects [12]. Both the MR and GR are found in cardiomyocytes, while the expression of 11β-HSD2 in the heart may be restricted to the coronary vasculature [13]. However, one report proposed the presence of 11β-HSD2 in human cardiac tissue [14]. Results from transgenic mouse models suggest that MR activation in cardiomyocytes contributes to progression of cardiac disease, while GR signalling is crucial for the maintenance of normal cardiac function [15,16].

Previously, we found that liquorice intake for two weeks increased BP via volume expansion, elevated peripheral arterial resistance and increased large arterial stiffness [17]. We also observed reduced chronotropic response and enhanced central wave reflection in the upright posture after liquorice exposure [17]. To examine the mechanisms underlying the elevation of systemic arterial resistance, here we investigated possible alterations in vasodilatory responses induced by exogenous NO donor and β2-adrenoceptor agonist after liquorice exposure in supine and upright positions. To evaluate the suppression of enzyme 11β-HSD2 activity, the urinary ratio of cortisone to cortisol metabolites was examined [8].

Methods

Ethical statement

All participants gave written informed consent, and the investigation conformed to the Declaration of Helsinki. The study was approved by the Ethics Committee of Tampere University Hospital on the 3rd of April 2007 (study code R07053M). The study is registered in the database of clinical trials (ClinicalTrials.gov, ID: NCT01742702), and is a part of an on-going investigation on haemodynamics (DYNAMIC-study code R06086M, EudraCT-number 2006-002065-39).

Study subjects and design

The study protocol (in Finnish) with an English summary have been published [18], and the CONSORT checklist is presented as supporting information (S1 CONSORT checklist). The

recruitment and characteristics of the subjects in the liquorice group and the study design were reported elsewhere [17,18]. The present results originate from the same liquorice intervention from which we published results concerning liquorice-induced changes in haemodynamics in the supine position [18] and during orthostatic challenge [17].

In the present study, the focus was on the mechanisms of vasodilatation induced by exogenous NO (nitroglycerin) and β_2-adrenoceptor stimulation (salbutamol) after 2 weeks of liquorice intake. The intervention group was the same as in earlier reports [17,18], while all findings concerning NO and β_2-adrenoceptor-induced vasodilatation are novel, and the reference group is new. The participants were recruited via announcements distributed at the Tampere University, Tampere University Hospital, and occupational health care units, and through notices in a local newspaper, and Varala Sports Institute. The study data were collected from May 2007 to June 2012 at the Departments of Internal Medicine and Clinical Physiology, Tampere University Hospital, Tampere, Finland. Medical examination was performed by a physician and the participants were interviewed for lifestyle habits and medical history [17,18]. Blood and urine samples were collected after ~12 hours of fasting and standard 12-lead electrocardiograms were recorded.

The final liquorice group included 22 healthy volunteers (14 women and 8 men) who consumed liquorice for two weeks [17]. Participant age ranged 23–58 years and the supine mean (standard deviation) (SD) brachial office BP was 119/70 (8/7) mmHg. Subjects with office BP > 140/90 mmHg or cardiovascular disease with drug therapy were excluded [17,18]. In addition, pregnancy or consumption of liquorice >300 grams per week excluded participation [17,18]. One male subject in the liquorice group did not receive sublingual nitroglycerin at the final visit due to nitroglycerin-induced headache after baseline measurements. Haemodynamic recordings were incomplete in one female subject after salbutamol inhalation at the final visit. Therefore, the statistical analyses of the head-up tilt table test included 22 subjects from visit 1 and 21 subjects from visit 2, while the analyses of laboratory values included all of the 22 subjects (Fig 1). The medications of the subjects in the liquorice group were reported elsewhere [17].

In order to distinguish haemodynamic changes induced by repeated measurements from the liquorice-induced changes, we investigated 21 volunteers maintaining their habitual diet. This group consisted of 3 women and 18 men, age 36–56 years with baseline supine mean (SD) brachial office BP 134/85 (9/7) mmHg. The reference subjects participated in our study on haemodynamics (DYNAMIC study, R06086M), and they were chosen so that all were without BP lowering medication, heart disease, diabetes, and cerebrovascular or peripheral arterial disease. The following clinical conditions with stable medications were present in the reference group: statin treatment for hypercholesterolemia (n = 3); proton pump inhibitor for gastro-oesophageal reflux (n = 2); selective serotonin reuptake inhibitor for depression (n = 2); thyroxine substitution (n = 1); intramuscular vitamin B_{12} replacement (n = 1); oral progestin contraceptive (n = 1); activated protein-C resistance without medication (n = 1); and cholelithiasis (n = 1). The liquorice group contained 4 present smokers and one previous smoker, while the reference group contained no present smokers and 9 previous smokers.

The study design was open-label [17,18]. During the 2-week liquorice intervention, the glycyrrhizin dose was 290–370 mg/d from commercial liquorice products (Halva liquorice™ or Kouvola liquorice™), corresponding to liquorice consumption from 120 to 300 g/d, respectively [17,18]. The use of two liquorice products was based on the recommendation of two research group members who piloted the intervention, as this protocol prevented aversion development towards liquorice [18]. During the exposure, the average intake of carbohydrates from the two liquorice products was ~150 g/d [18]. Before baseline measurements, all liquorice products were to be avoided for 3 weeks. In the reference group, the subjects were instructed
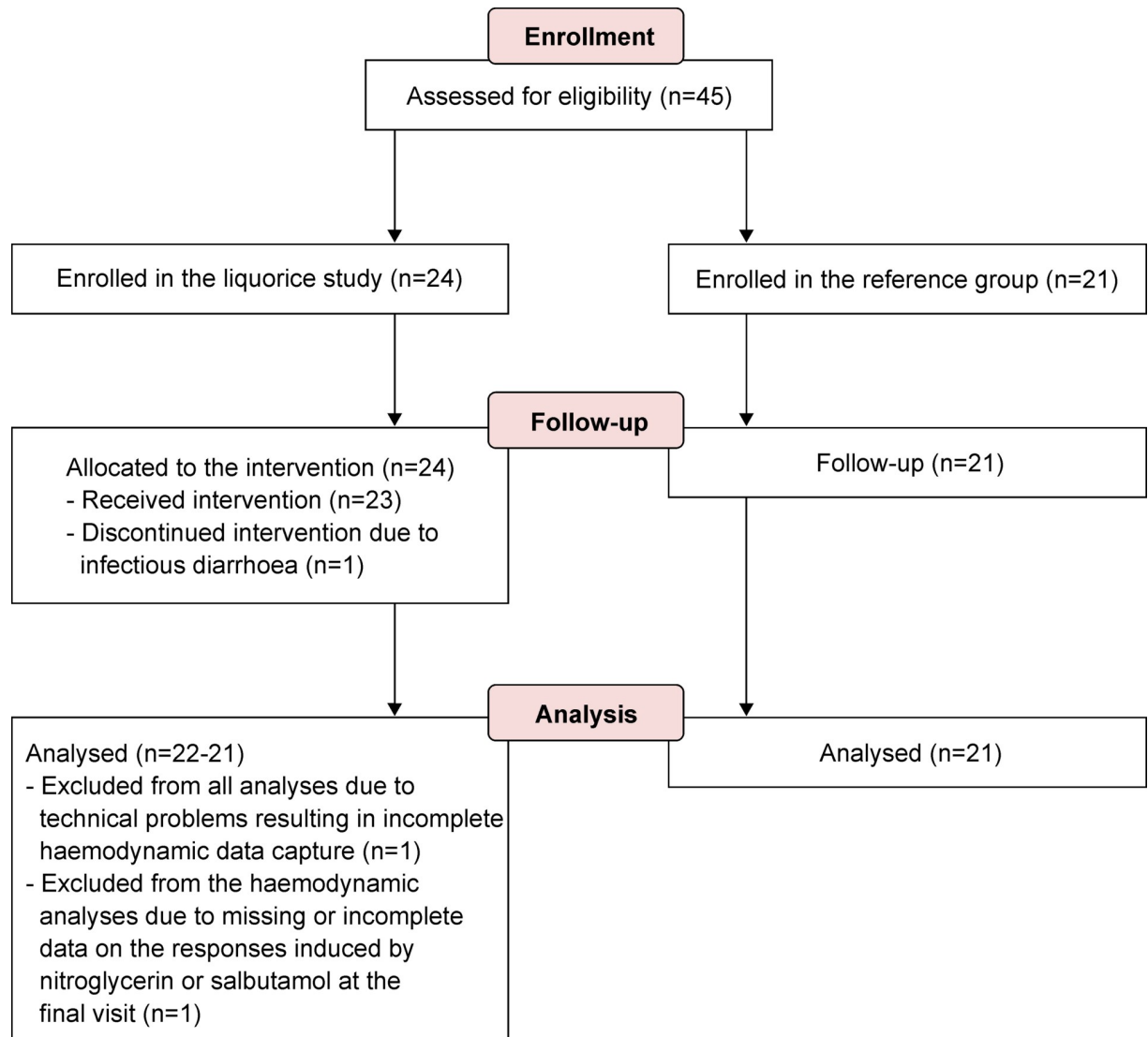
Fig 1. The CONSORT flow diagram of study participants.

to continue their conventional eating habits, and they were not aware of acting as a reference for the liquorice group [17,18]. The reported liquorice consumption frequency among the reference group was as follows: monthly or lower (n = 17), weekly (n = 4).

Haemodynamics were recorded before and after two weeks of liquorice exposure, and before and after 3 weeks (n = 16) or 10 months of follow-up (n = 5) in the reference group. For cortisone and cortisol metabolite analyses, 24-hour urine was collected at weeks 0 and 2 in the liquorice group.

Laboratory analyses

Blood count was determined using ADVIA 120 or 2120 (Bayer Health Care, Tarrytown, NY, USA), and plasma sodium, potassium, creatinine, glucose, triglyceride, and total, high-density, and low-density lipoprotein cholesterol concentrations were determined using Cobas Integra 700/800 (F. Hoffmann-LaRoche Ltd, Basel, Switzerland). Since plasma creatinine was within

the normal range, estimated glomerular filtration rate (GFR) was calculated with the RULE formula [19]. Plasma aldosterone was measured by radioimmunoassay (Aldosterone RIA Test DSL-8600, Diagnostics Systems Laboratories Inc, Webster, TX, USA) and renin activity utilising GammaCoat Plasma Renin Activity assay (Diasorin). Urinary tetrahydrocortisone (THE) and allo-tetrahydrocortisol plus tetrahydrocortisol (allo-THF+THF) concentrations were analysed using liquid chromatography-tandem mass spectrometry as described previously [20].

Pulse wave analysis

Radial BP and pulse wave form were continuously recorded by a tonometric sensor from the left radial pulsation (Colin BP-508T, Colin Medical Instruments Corp., San Antonio, Texas, USA). Contralateral brachial BP measurements were used to calibrate the radial BP recordings approximately every 2.5 min [21,22]. Continuous aortic BP was derived from the radial signal with the SphygmoCor PWMx pulse wave monitoring system (Atcor Medical, Australia) [23]. Augmentation index (AIx) (augmentation pressure/pulse pressure*100) was determined from the aortic pulse wave form.

Whole-body impedance cardiography

Beat-to-beat heart rate (HR), stroke volume, cardiac index (cardiac output/body surface area), pulse wave velocity (PWV) and extracellular water volume were measured with a whole-body impedance cardiography device (CircMon[R], JR Medical Ltd., Tallinn, Estonia) as previously described [24–26]. Systemic vascular resistance index (systemic vascular resistance/body surface area) (SVRI) was calculated from the tonometric BP and cardiac index recorded by CircMon[R]. The stroke volume and cardiac output values determined with the CircMon[R] show good correlation with values obtained using 3-dimensional echocardiography [27] and the thermodilution method [24,25]. In addition, the reproducibility and repeatability of the measurements are good [21,22].

Aortic-to-popliteal PWV was calculated by measuring the time difference between the onset of the decrease in impedance in the whole-body impedance signal and the popliteal artery signal with the CircMon[R] software [26]. The recorded PWV values show excellent correlation with values measured using either ultrasound or the tonometric SphygmoCor method [26,28].

Haemodynamic measurement protocol

The recordings were performed in a quiet, temperature-controlled laboratory by research nurses [21,22]. Prior to the measurements, caffeine products, smoking and heavy meal for ≥4 hours and alcohol for ≥24 hours were precluded. The electrodes for impedance cardiography were placed on the body surface, the tonometric sensor on the left radial artery pulsation, and a BP cuff to the right upper arm [21,22]. The left arm was abducted to 90 degrees and in an arm support at the level of the heart.

The beat-to-beat haemodynamic data was captured during six consecutive 5-min periods [22]. The 0.25 mg sublingual nitroglycerin resoriblet (Nitro resoriblet; Orion Pharma, Espoo, Finland) and 400 μg salbutamol inhalation (Ventoline; GlaxoSmithKline, Uxbridge, Middlesex, UK) were administered on the same day of measurements with at least one hour intermission between the drugs. Salbutamol was delivered by the use of a spacer device (Volumatic; Allen & Hanbury's, Uxbridge, Middlesex, UK) after guidance and supervision by the research nurse. The total recording time was 2 x 30 min: the subjects were first resting supine on the tilt table for 5 min, followed by 5 min of head-up tilt to >60 degrees, and then the tilt table was

restored to supine position. Then the research drug (nitroglycerin or salbutamol) was administered and the protocol (5 min supine—5 min upright—5 min supine) was repeated [22].

As the action of inhaled salbutamol begins from the first minute with stable plasma concentrations 5–20 minutes after administration [29], the measurement period of 15 min is sufficient to detect salbutamol-induced changes in haemodynamics. If the subject reported presyncopal symptoms and BP fell progressively during nitroglycerin-stimulated orthostatic challenge, the tilt table was restored to supine position before the 5 min was completed [30].

Statistical analyses

Means of the beat-to-beat values of each minute of the 30-min recordings were calculated and used in the statistics. If the head-up tilt was aborted after sublingual nitroglycerin before 5 min was completed, the missing upright values were replaced by the preceding values, providing that at least 2 min of upright recordings were available. Statistical analyses were performed using IBM SPSS Statistics Version 24 (IBM Corporation, Armonk, NY, USA). Variable values are reported as means with SDs, standard errors of the mean or 95% confidence intervals. The Shapiro-Wilk test and histograms were used to check the normal distributions of the variables.

To detect a 8 mmHg difference in the change in systolic BP from baseline (two-tailed alpha level 0.05, 80% power, SD of 9, power analysis method for two-sample t-test), the required minimum sample size was 20 controls and 20 experimental subjects. The area under the curve (AUC) was calculated utilising the IBM SPSS Statistics Version 24 [31] for every 5-min period in the supine and upright position during the recording (for aortic systolic and diastolic BP, HR, AIx, cardiac index, and SVRI), in both the absence and presence of the research drugs. Since there were significant differences in several variables between the liquorice and reference group at baseline (see below), the differences in the AUCs between visit 1 and visit 2 in the liquorice versus reference group were compared by the use of analysis of covariance. In order to avoid excessive loss of statistical power resulting from adjustment for multiple covariates [32,33], the outcomes were controlled for age [34] and sex [35], as these were considered the most important confounding factors. Wilcoxon signed-rank test was utilised to examine the within-group changes in urinary cortisone and cortisol metabolites, plasma aldosterone, potassium, and renin activity, and PWV. The mean supine values of PWV were calculated from the minutes 3–5 of the recordings when the signal was most stable. Non-continuous variables were analysed using Chi-square test. $P < 0.05$ was considered statistically significant.

Results

Characteristics of the study subjects at visit 1

The demographic and laboratory characteristics at baseline are shown in Table 1. The study groups differed in sex distribution and age: the majority of the participants in the reference group were men (18/3) and mean age was 12 years higher compared to the liquorice group. In addition, BMI, plasma fasting triglycerides, low density lipoprotein, glucose, haemoglobin and sodium were higher, and high density lipoprotein lower in the reference group than in the liquorice group.

At baseline, during the initial 15-min of the recording (in the absence of research drugs) aortic systolic ($p < 0.05$) and diastolic ($p < 0.01$) BP (Fig 2), and HR ($p < 0.05$, Fig 3) were higher in the reference group than in the liquorice group (univariate analysis of variance, adjusted for age and sex).

Table 1. Baseline characteristics of the study population.

	Reference (n = 20–21[a])		Liquorice (n = 22)		P value[b]
	Mean	**SD**	**Mean**	**SD**	
Number (percentage) of males	18 (86%)		8 (36%)		0.002[c]
Age (years)	47.3	6.2	34.9	9.2	<0.001
Body mass index (kg/m^2)	28.2	3.3	23.3	1.9	<0.001
Haemoglobin (g/l)	151	11	139	9	<0.001
Fasting plasma					
Cholesterol (mmol/l)	5.0	0.8	4.6	0.8	0.069
Triglycerides (mmol/l)	1.38	0.52	0.83	0.32	<0.001
High density lipoprotein (mmol/l)	1.31	0.28	1.83	0.36	<0.001
Low density lipoprotein (mmol/l)	3.2	0.7	2.4	0.6	0.001
Glucose (mmol/l)	5.5	0.5	5.2	0.4	0.008
Sodium (mmol/l)	141	1.4	140	1.3	0.001
Potassium (mmol/l)	3.8	0.3	3.9	0.3	0.264
eGFR[d] (ml/min per 1.73 m^2)	115	12	114	16	0.413

[a]Blood samples for fasting plasma values were not obtained from one subject.

[b]Mann-Whitney U-test

[c]Chi-square test

[d]eGFR, estimated glomerular filtration rate using the RULE formula [19].

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Influences of liquorice intake at visit 2

The purpose of the reference group was only to test the hypothesis whether the recorded hae-modynamic values change during repeated measurements. The reproducibility of the haemo-dynamic responses to upright posture during 12 weeks of follow-up has been previously shown in three parallel groups of subjects [36].

Renin and aldosterone levels and urinary glucocorticoid excretion. Two weeks of liquo-rice ingestion decreased plasma aldosterone concentration by -304 mol/l (95% CI -434 to -174, p<0.001), renin activity by -1.2 µg/l/h (-2.1 to -0.3, p<0.001), and potassium concentration by -0.2 mmol/l (-0.4 to -0.1, p = 0.012). At baseline, mean (SD) urinary THE excretion was 28.3 (18.9) nmol/l and allo-THF+THF 40.0 (24.1) nmol/l, and the ratio of THE to allo-THF+THF was 0.70 (0.26). After the liquorice intervention the mean change (95% CI) in THE was -16.9 nmol/l (-25.9 to -7.9, p = 0.001) and in allo-THF+THF -4.0 nmol/l (-14.1 to 6.2, p = 0.205). The ratio of THE to allo-THF+THF was decreased by -0.39 (from 0.70 to 0.31, p<0.001) fol-lowing liquorice indicating significant inhibition of 11β-HSD2 (Fig 4).

Pulse wave velocity. Mean values of PWV during the study are presented in Table 2. Two weeks of liquorice ingestion increased PWV (p = 0.046), while in the reference group PWV did not change (p = 0.689).

Liquorice and haemodynamics in the absence and presence of sublingual nitroglyc-erin. In the reference group, no significant changes in haemodynamics were detected during the follow-up (Figs 2 and 3 and 5–7 and S1). Two weeks of liquorice intake increased aortic systolic BP before nitroglycerin throughout the 15-min recording, and also after nitroglycerin during the orthostatic challenge and the 5-min supine period thereafter (Fig 2B). Aortic dia-stolic BP was increased before nitroglycerin during both 5-min supine positions, and after nitroglycerin during the final 5-min supine period (Fig 2D). The HR response to upright pos-ture was numerically but not significantly lower after liquorice consumption (unadjusted p = 0.067 in the absence and p = 0.064 in the presence of nitroglycerin, age and sex-adjusted
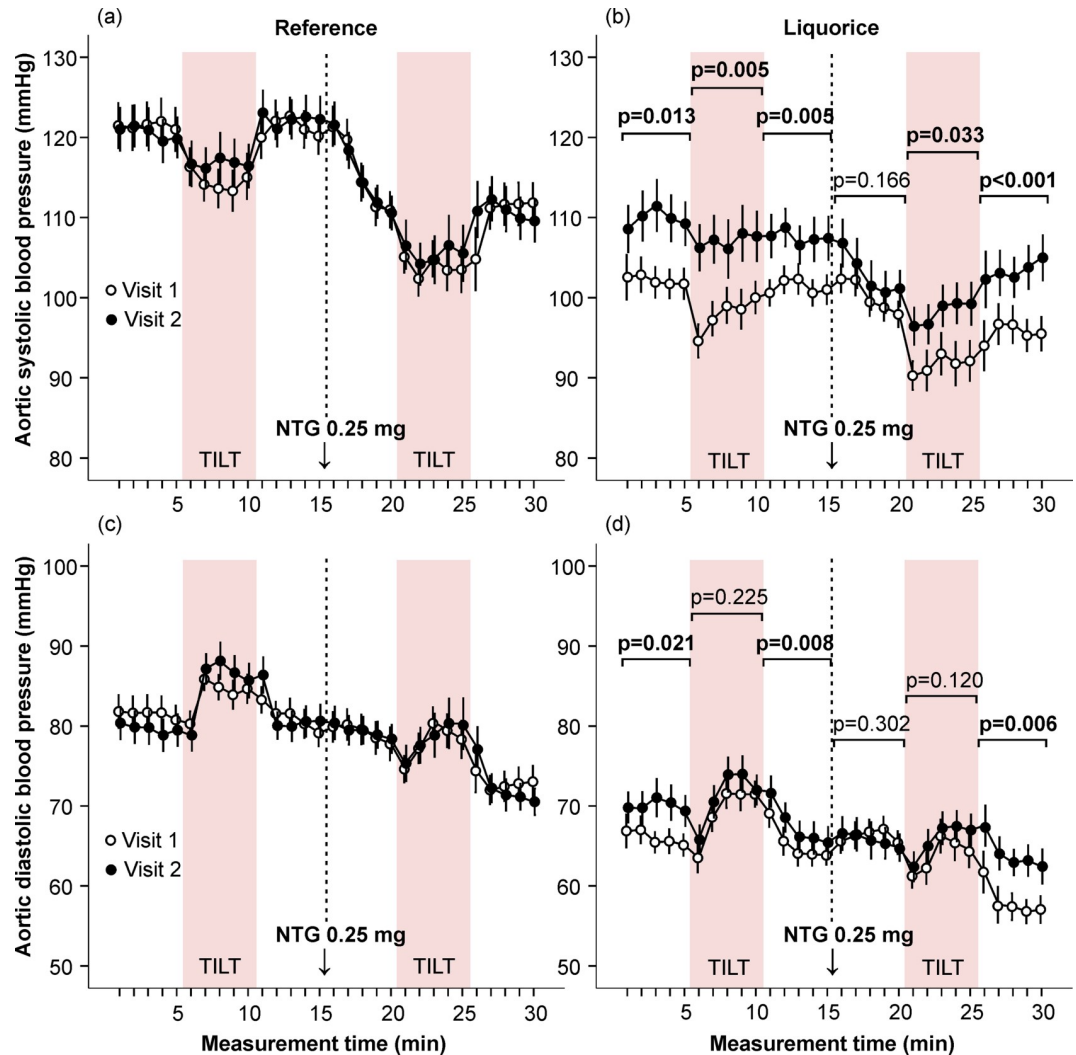
Fig 2. Aortic blood pressure at baseline and after the follow-up (reference group) or intervention (liquorice ingestion).
Systolic (a, b) and diastolic (c, d) blood pressure were measured in the absence (0–15 min) and presence (16–30 min) of
sublingual nitroglycerin during supine position and orthostatic challenge. The passive head-up tilt was carried out from 5 to
10 min, and 20 to 25 min. Mean and standard error of the mean, statistical analyses compared the difference of the area
under the curve between visit 1 and visit 2 in the liquorice versus the reference group, adjusted for age and sex.

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p = 0.291 and p = 0.225, respectively) (Fig 3C). An increase in AIx following liquorice diet was
detected before nitroglycerin during the first 5-min supine period and orthostatic challenge
(Fig 3D). Liquorice consumption had no significant effects on cardiac index (Fig 5B), but
higher SVRI was observed before nitroglycerin during the final 5-min supine period, and after
nitroglycerin during the orthostatic challenge and final 5-min in supine position (Fig 5D).

Liquorice and haemodynamics in the absence and presence of salbutamol inhalation.
The effects of liquorice ingestion on aortic systolic and diastolic BP before and after salbutamol
inhalation were corresponding to those detected before and after sublingual nitroglycerin (S1
Fig). In unadjusted analyses liquorice ingestion reduced HR response to upright posture before
(p = 0.032) but not after salbutamol (p = 0.060), and when age and sex were included as covari-
ates no significant differences were detected in cardiac chronotropic response to upright pos-
ture between the groups (Fig 6B). Liquorice intake slightly elevated AIx before and after
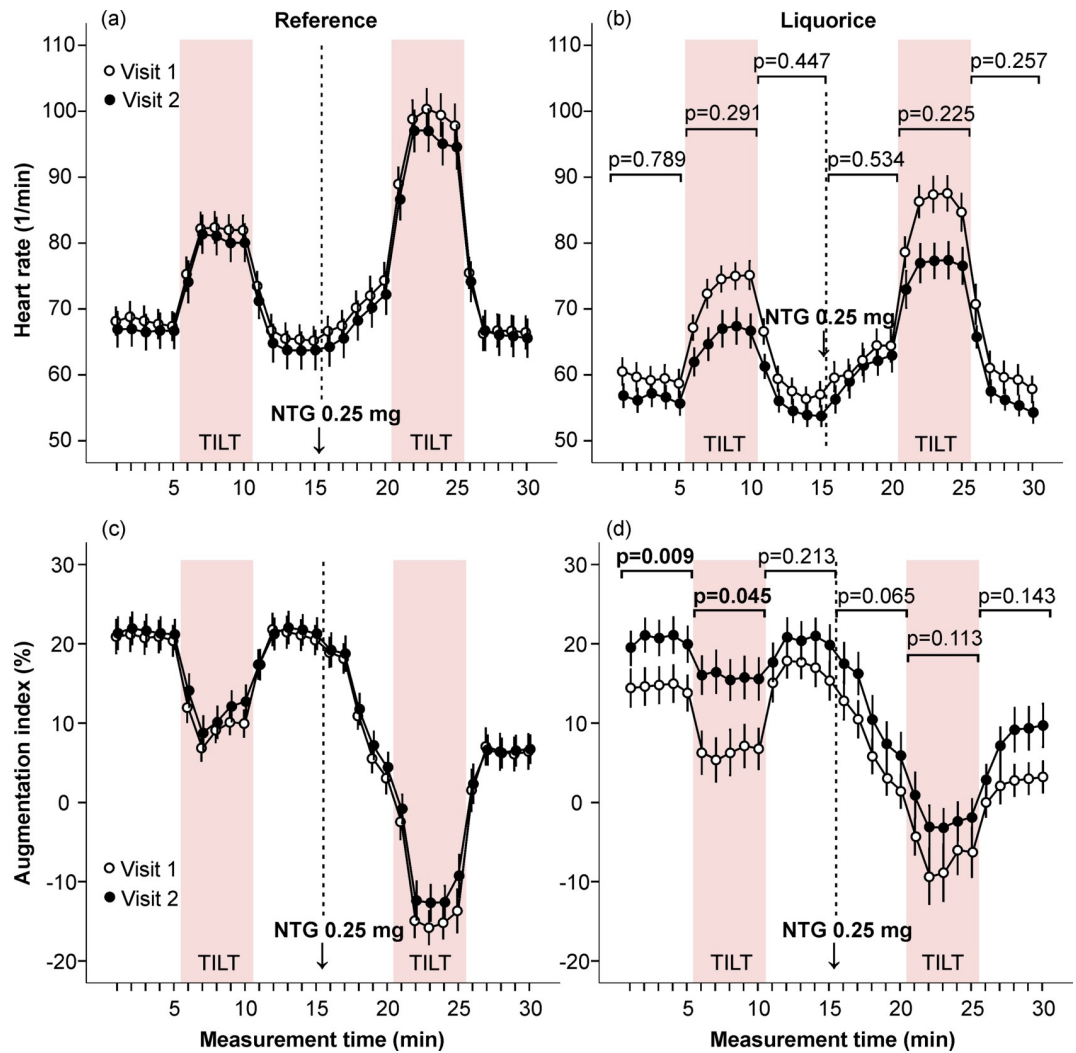
Fig 3. Heart rate and augmentation index in the absence and presence of sublingual nitroglycerin. Heart rate (a, b) and augmentation index (c, d) were measured at baseline and after the follow-up. Mean and standard error of the mean, the difference of the area under the curve between visit 1 and visit 2 was compared in the liquorice versus the reference group, adjusted for age and sex.

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salbutamol during two 5-min supine periods (Fig 6D). In the liquorice group, no significant changes were detected in cardiac index when compared to the reference group in the absence and presence of salbutamol (Fig 7B), while the liquorice-induced moderate increase in upright SVRI was abolished after salbutamol (Fig 7D).

Discussion

We investigated the effects of two-week-long liquorice ingestion on the haemodynamic changes elicited by exogenous NO donor (nitroglycerin) and β_2-adrenoceptor agonist (salbutamol) during orthostatic challenge. Liquorice exposure elevated aortic systolic and diastolic BP, and SVRI when compared with the reference group. In response to the research drug administration the liquorice-induced increase in SVRI was observed in the presence of nitroglycerin but no longer in the presence of salbutamol. Thus, liquorice intake attenuated the vasodilatory effect of exogenous NO donor, but not that of β_2-adrenoceptor agonist. Increased extracellular water volume
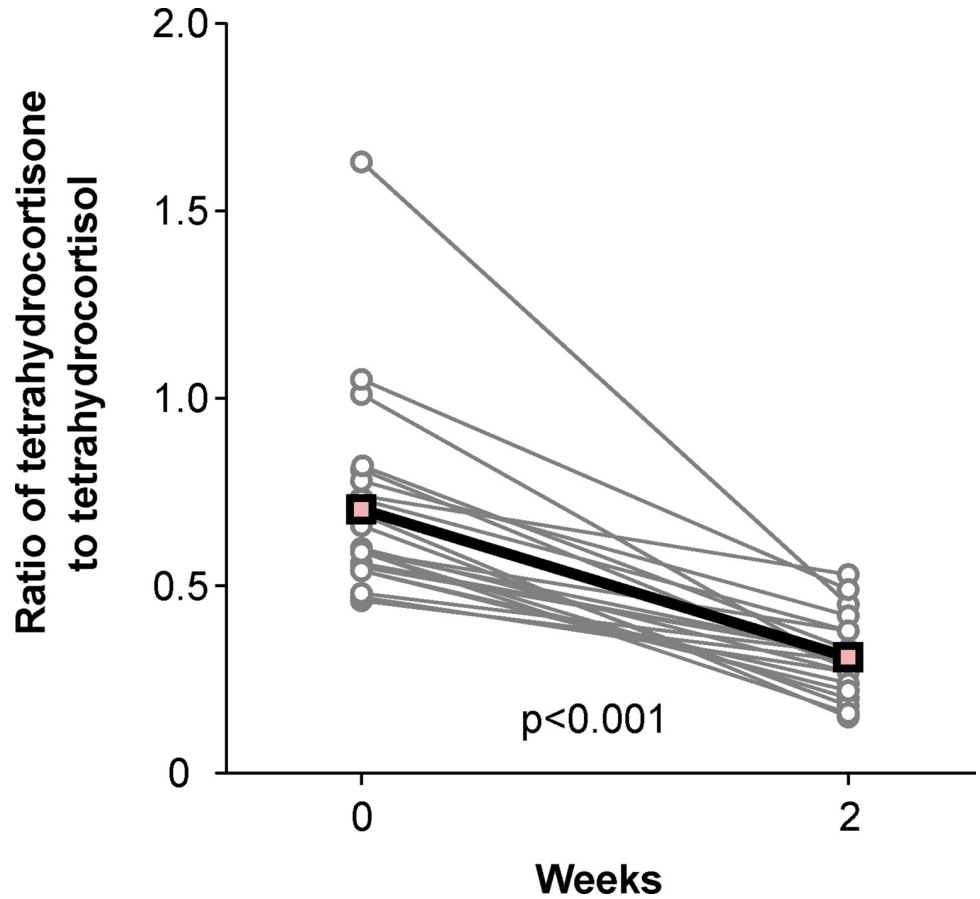
Fig 4. Liquorice intake and urinary glucocorticoid excretion. The urinary ratio of tetrahydrocortisone to tetrahydrocortisol at baseline and after two weeks of liquorice ingestion, grey lines depict each individual, thick black line depicts mean values, n = 22.

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does not seem like the probable explanation for these findings. The present results support the view that liquorice intake elevates BP via multiple mechanisms (Fig 8).

The inhibition of enzyme 11β-HSD2 was evaluated by examining the ratio of urinary cortisone to cortisol metabolites [8]. In this study, the liquorice-induced reduction in urinary THE excretion and ratio of THE to allo-THF+THF showed that the conversion of cortisol to cortisone was impaired [8,37]. Subsequently, plasma potassium and aldosterone concentrations were decreased, and plasma renin activity was reduced, in line with previous reports [8,38].

Table 2. Pulse wave velocity during the study. Mean values and standard deviations.

	Reference (n = 21)		Liquorice (n = 22)		P (ANOVA)[a]
	Mean	**SD**	**Mean**	**SD**	
PWV (m/s)					
Visit 1	8.7	1.1	7.1	0.7	0.066
Visit 2	8.7	1.2	7.4	0.8	0.625
Wilcoxon P value[b]	0.689		0.046		

[a]Adjusted for age and sex
[b]Comparison between visit 1 and visit 2.

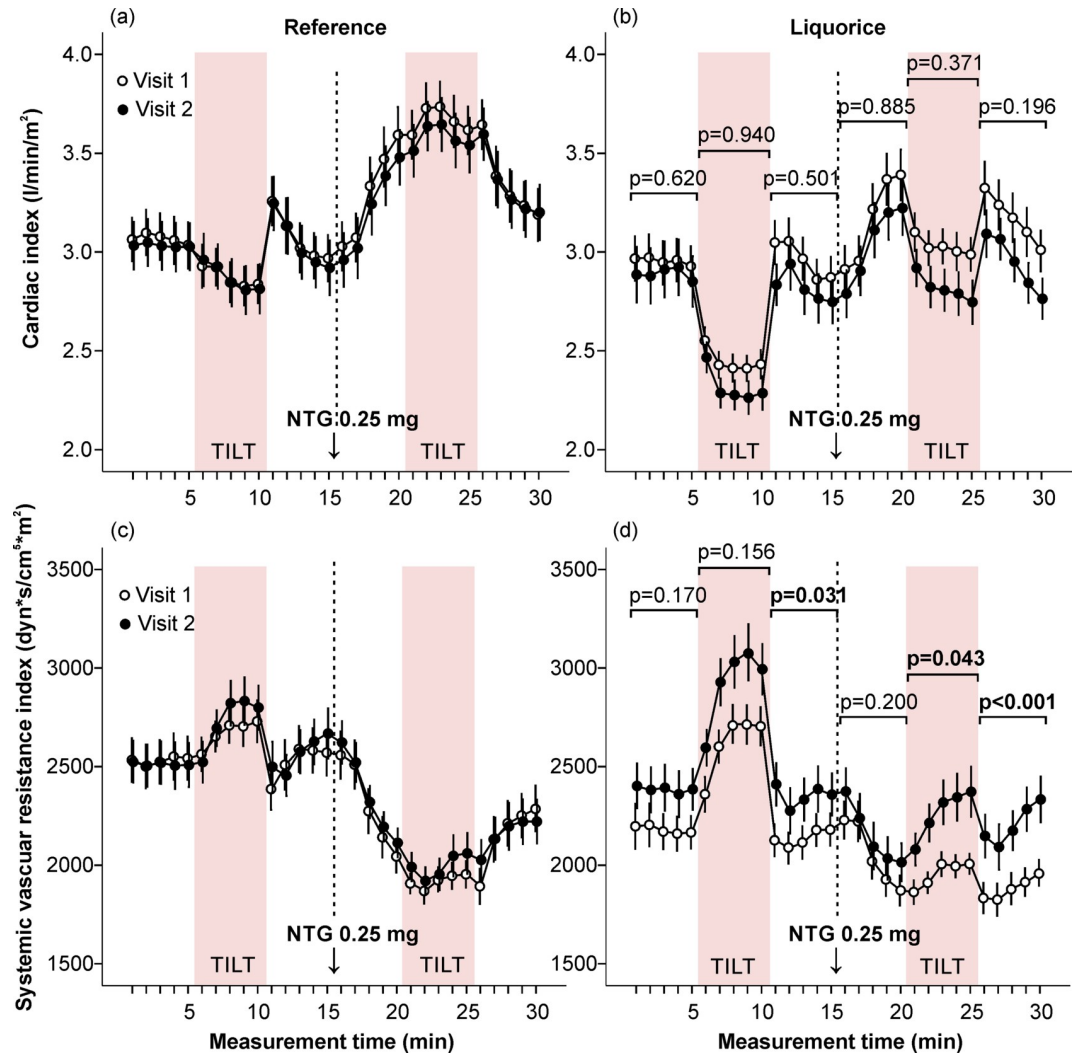https://doi.org/10.1371/journal.pone.0223654.t002

Fig 5. Cardiac index and systemic vascular resistance index in the absence and presence of sublingual nitroglycerin.
Cardiac index (a, b) and systemic vascular resistance index (c, d) were measured at baseline and after the follow-up. Mean
and standard error of the mean, the difference of the area under the curve between visit 1 and visit 2 was compared in the
liquorice versus the reference group, adjusted for age and sex.

These results suggest that MR activation was enhanced. Altered 11β-HSD activity in the vascu-
lar wall may also contribute to the elevation of BP [9]. In human VSMC, diminished 11β-
HSD2 activity enhanced cortisol-induced increase in angiotensin II binding and the upregula-
tion was inhibited by MR and GR antagonists [11]. In rats, inhibition of 11β-HSD2 induced by
GA supplementation caused endothelial dysfunction, activated the ET-1 system, and elevated
BP that was normalized by MR and ET$_A$-receptor antagonists [39,40]. Endothelial dysfunction
in 11β-HSD2 knockout mice may also result from additional indirect mechanisms related to
renal sodium retention and BP elevation, and not merely from increased activation of cortico-
steroid receptors in the endothelium [10]. The enzyme 11β-HSD2 is co-expressed with the MR
in the nucleus of the solitary tract and this might have a role in the central regulation of BP
[13]. Interestingly, deficient central 11β-HSD2 activity in mice increases their appetite for salt,
promotes salt-sensitivity, and enhances pressor responses to α_1-adrenoceptor stimulation [41].
In humans, liquorice ingestion induced a greater rise in BP in hypertensive than in
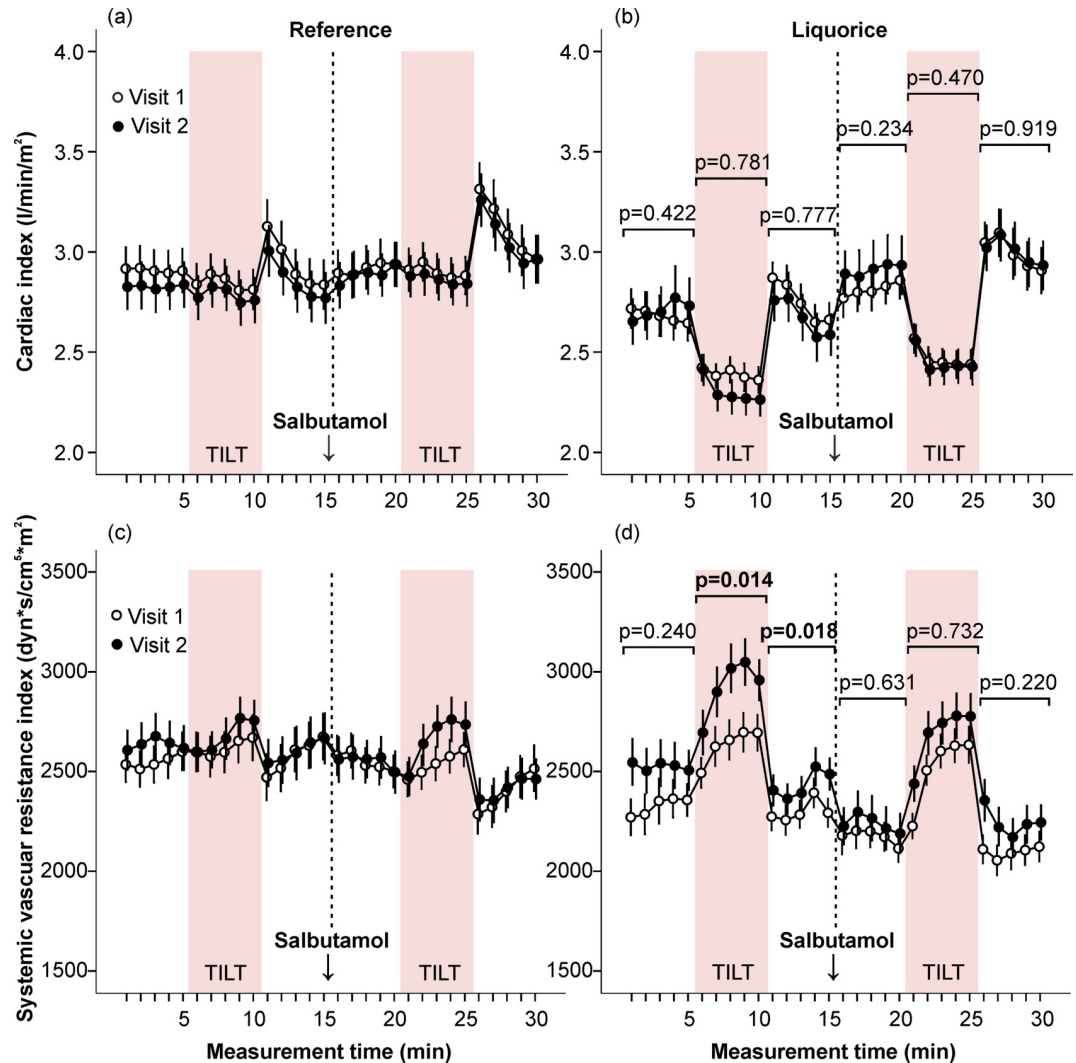
Fig 7. Cardiac index and systemic vascular resistance index in the absence and presence of salbutamol inhalation.
Cardiac index (a, b) and systemic vascular resistance index (c, d) were measured at baseline and after the follow-up. Mean
and standard error of the mean, the difference of the area under the curve between visit 1 and visit 2 was compared in the
liquorice versus the reference group, adjusted for age and sex.

normotensive subjects [42]. Other factors that increase susceptibility to liquorice-induced ele-
vation of BP are old age, female sex, hypokalaemia, prolonged gastrointestinal transit time,
decreased 11β-HSD2 activity, and anorexia nervosa [1]. Sigurjonsdottir et al. found that
enhanced BP response to liquorice in hypertensive subjects was not attributed to age, increased
salt sensitivity or weight, and the authors suggested that increased sensitivity to the inhibition
of the 11β-HSD2 was the underlying mechanism [42].

Nitroglycerin is a known exogenous NO donor and endothelium-independent vasodilator,
while the β2-sympatomimetic salbutamol induces vasodilatation that is mediated via a direct
relaxing action on VSMC and NO release from the endothelium [43,44]. Both sublingual
nitroglycerin and inhaled salbutamol can reduce systemic vascular resistance in humans [22].
In the present study, two weeks of liquorice ingestion moderately elevated SVRI in the pres-
ence of nitroglycerin but not in the presence of salbutamol. This suggests that liquorice intake
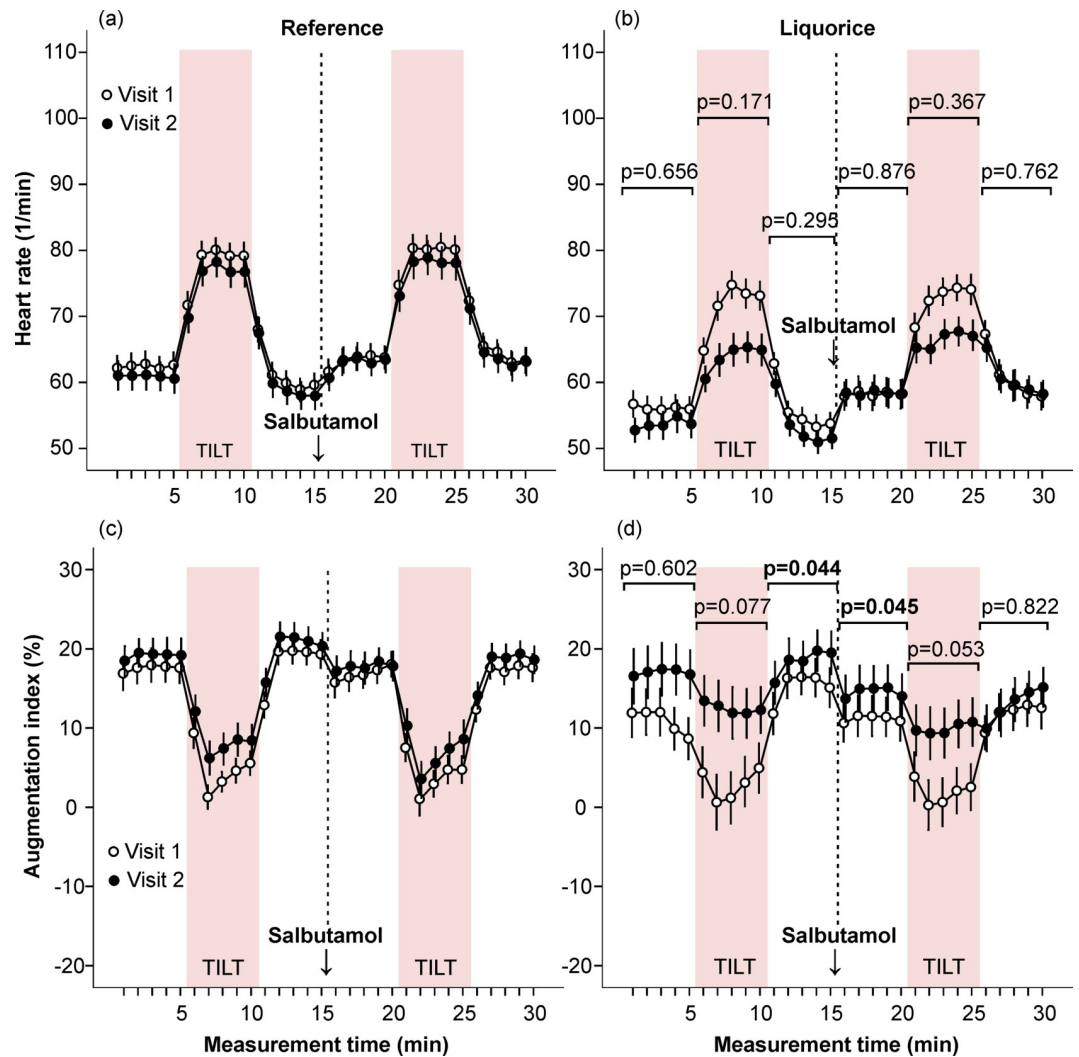impaired vasodilatation via exogenous NO in vascular smooth muscle, while the β2-

Fig 6. Heart rate and augmentation index in the absence and presence of salbutamol inhalation. Heart rate (a, b) and augmentation index (c, d) were measured at baseline and after the follow-up. Mean and standard error of the mean, the difference of the area under the curve between visit 1 and visit 2 was compared in the liquorice versus the reference group, adjusted for age and sex.

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adrenoceptor-mediated vascular relaxation was not affected. In accordance with our results, Sobieszczyk et al. demonstrated that GA attenuated endothelium-independent vasodilatation in the forearm blood flow in response to verapamil, while no significant influence was observed in the endothelium-dependent vasodilatation elicited by methacholine [45]. In addition, aortas from 11β-HSD2 knockout mice demonstrated impaired vasorelaxation to both endothelium-dependent and -independent agents [46]. In contrast, endothelium-dependent but not -independent relaxation was impaired in the aortas of GA-treated Wistar-Kyoto rats [39,40]. In the present study, PWV, a marker of arterial stiffness [47], was increased after liquorice ingestion. This was probably due to the elevation of BP, as the prevailing BP that is distending the blood vessels has a significant influence on the indices of arterial stiffness [47].

The level of AIx is influenced by arterial stiffness, but also height, gender, HR, systemic vascular resistance and stroke volume contribute to the magnitude of wave reflections [28,47,48]. The changes in AIx induced by salbutamol inhalation have been utilised with the idea that
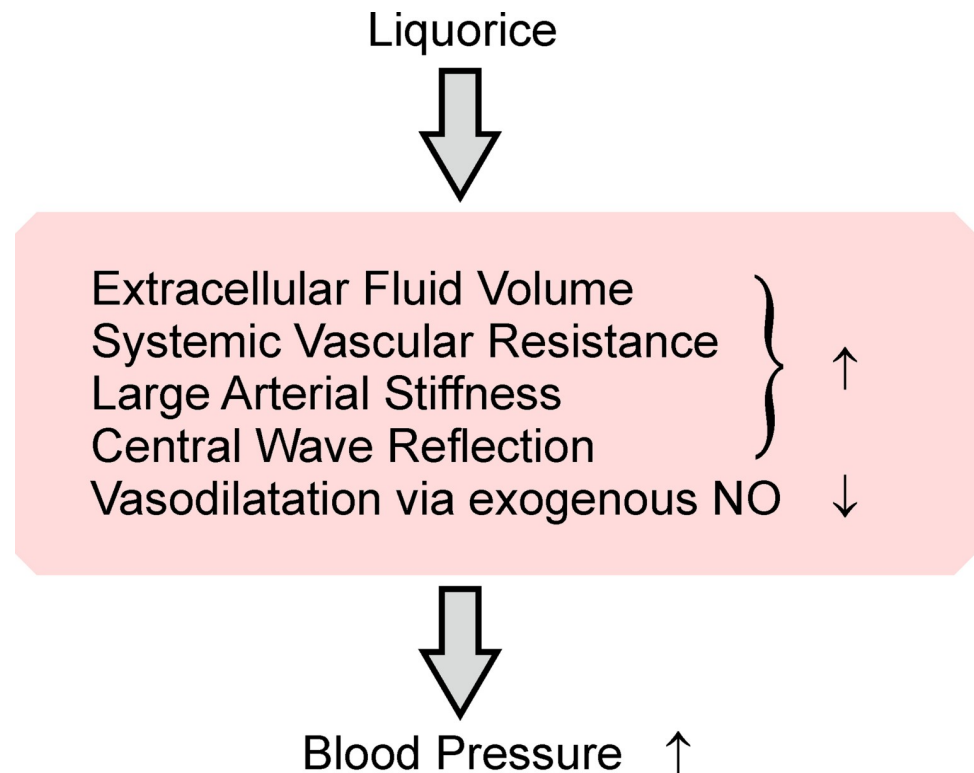
Liquorice

Extracellular Fluid Volume
Systemic Vascular Resistance
Large Arterial Stiffness   } ↑
Central Wave Reflection
Vasodilatation via exogenous NO   ↓

Blood Pressure ↑

Fig 8. Summary of the mechanisms of liquorice-induced elevation in blood pressure.

they reflect alterations in the endothelium-mediated vascular tone [29,49]. However, a recent study suggested that the reduction in AIx induced by salbutamol may be largely explained by the parallel increase in heart rate [50]. In our earlier report the liquorice-induced elevation of AIx during orthostatic challenge correlated with the simultaneous reduction in HR, increase in SVRI, and prolongation of ejection duration [17]. As a novel finding, AIx remained slightly elevated following salbutamol inhalation in the supine position in spite of the parallel reduction in SVRI. However, the liquorice-induced increase in AIx was abolished in response to nitroglycerin. This can probably be explained by the powerful vasodilatory effect of nitroglycerin, as AIx level is significantly influenced by systemic vascular resistance [28]. Corresponding to previous findings, the haemodynamic effects of salbutamol were more evident in the supine position, while those of sublingual nitroglycerin were accentuated in the upright position [22,51].

Sublingual nitroglycerin and inhaled salbutamol increase HR [22], and this was also observed in the present study. Our previous report found that cardiac chronotropic response to upright posture was reduced after liquorice diet [17]. Here, liquorice intake resulted in numerically but not significantly lower upright HR throughout the recording protocol in analyses adjusted for age and sex. The present study with a small number of subjects was probably underpowered to detect significant effects of liquorice intake on the upright regulation of HR in adjusted analyses [32].

Due to the sweet taste of glycyrrhizin, liquorice extract is used as a sweetener or flavouring agent [52]. Average daily glycyrrhizin consumption in the US population was estimated to be 0.027–3.6 mg/kg (or 1.6–215 mg) [2]. During a 12-month period, ~686,000 kg of liquorice root extract was imported to Finland (customs statistics in May 2013; www.tulli.fi) [18]. This

corresponds to a daily intake of ~21 mg glycyrrhizin if distributed per capita, but Finnish liquorice products are also exported [18]. A survey of 603 high school students in New Zealand indicated that 5.9% of the girls and 4.9% of the boys consumed at least 200 g of liquorice per week, while the highest consumption exceeded 1000 g per week [53]. Therefore, ingestion of glycyrrhizin products should always be considered as a putative cause for elevated BP in hypertensive patients to avoid unnecessary medical therapy.

Previously, we investigated the haemodynamic influences of liquorice in comparison with an age-matched control group [17,18]. However, repeated haemodynamic measurements with nitroglycerin and salbutamol administration were not performed in these control subjects. Therefore the results of the present study were analysed in parallel with another reference group. The reproducibility of the haemodynamic responses during head-up tilt was shown during 12 weeks of follow-up [36]. As a limitation, there were baseline differences between the present groups in demographic characteristics, laboratory values, and BP. The results were adjusted for age and sex, as these variables were considered the most relevant confounding factors influencing the haemodynamic profiles of the subjects. Aging is known to increase arterial stiffness, with subsequent increases in PWV, wave reflection, and central and peripheral systolic BP [34]. As a further sex-related difference, we previously found that the haemodynamic workload for the heart in the upright position was higher in men than in women [35]. The mean baseline brachial office BP of the reference group was high normal (134/85 mmHg), while the corresponding values in the liquorice group were optimal (119/70 mmHg) [54]. The purpose of the reference group was merely to examine whether the results of the haemodynamic recordings change during repeated measurements. Previously, liquorice ingestion has induced more pronounced clinical symptoms in women than in men, although the changes in BP were not different between the sexes [42]. However, another study suggested that men were more responsive to the liquorice-induced decrease in plasma aldosterone concentration than women [55]. Due to the small number of subjects, we did not analyse the influences of liquorice intake separately in male and female subjects. Future studies should compare the effects of gender on the influences of liquorice ingestion.

In conclusion, two weeks of liquorice ingestion resulted in the elevation of BP via multiple mechanisms. The present results suggest that liquorice exposure increased systemic vascular resistance via impaired response to NO in the vasculature, while the vasodilatory response to β_2-adrenoceptor stimulation was not significantly altered.

Supporting information

S1 Protocol. Trial protocol.
(PDF)

S2 Protocol. English summary of trial protocol.
(PDF)

S1 CONSORT checklist. CONSORT checklist.
(PDF)

S1 Fig. Aortic blood pressure in the absence and presence of salbutamol inhalation. Systolic (a, b) and diastolic (c, d) blood pressure were measured at baseline and after the follow-up (reference group) or intervention (liquorice ingestion) during supine position and orthostatic challenge. Mean and standard error of the mean, statistical analyses compare the difference of the area under the curve between visit 1 and visit 2 in the liquorice versus reference group, adjusted for age and sex.
(TIF)

S1 Dataset. Liquorice study demographic and clinical data.
(SAV)

S2 Dataset. Liquorice study haemodynamic data.
(SAV)

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