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High Plasma 18β-Glycyrrhetinic Acid Levels Are Associated With More Intensive Antihypertensive Treatment, Resistant Hypertension and Apparent Mineralocorticoid Excess in Patients With Type 2 Diabetes

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A B S T R A C T

Background: Excessive consumption of glycyrrhizin (GL), a licorice-derived substance, can cause blood pressure (BP) elevation and apparent mineralocorticoid excess (AME). However, self-reported intake can be unreliable due to unrecognized sources of GL. Plasma levels of 18β-glycyrrhetinic acid (GA), a major metabolite of GL, may serve as a biochemical marker of exposure. Identifying individuals with high plasma levels of GA could be relevant in BP management in at-risk patients.
Objectives: To examine whether plasma levels of GA are associated with BP, antihypertensive treatment intensity, resistant hypertension, and biochemical markers of AME in patients with type 2 diabetes (T2D).
Methods: In this cross-sectional study, we measured GA in plasma from 1160 patients with T2D. Participants were divided into high GA (top quartile) and low GA (bottom 3 quartiles). Linear and logistic regression models assessed associations of GA levels with BP, antihypertensive treatment intensity (defined daily dose; DDD), resistant hypertension and markers of AME. Models were adjusted for confounders such as age, sex, sociodemographic, lifestyle, diabetes duration, estimated glomerular filtration rate, glycated hemoglobin, homoeostasis model assessment 2 for insulin sensitivity, and where appropriate systolic BP and treatment.
Results: High GA was not significantly associated with higher BP but with more intensive antihypertensive treatment (+0.28 DDD [0.03–0.52], $P = 0.03$) compared with low GA. High GA was also associated with higher risk of resistant hypertension (adjusted odds ratio: 1.91 [1.12–3.24], $P = 0.02$). Additionally, high GA was associated with markers of AME (lower aldosterone (−41.5 pmol/L [−63.1 to −20.0]; $P < 0.001$), lower potassium (−0.06 mmol/L [−0.10 to −0.01]; $P = 0.01$), lower cortisone (−6.08 nmol/L [−7.78 to −4.38]; $P < 0.001$), and higher cortisol/cortisone ratio (+1.26 [1.00–1.52]; $P < 0.001$)).
Conclusion: High GA levels, a possible marker of excessive licorice consumption, were associated with greater antihypertensive treatment intensity, resistant hypertension and biochemical markers consistent with AME in patients with T2D. These findings suggest that licorice-related exposure may be relevant to BP management in this population.

Keywords: licorice, 18β-glycyrrhetinic acid, hypertension, type 2 diabetes, apparent mineralocorticoid excess

Abbreviations: 11βHSD2, 11β-hydroxysteroid dehydrogenase type 2; AME, apparent mineralocorticoid excess; BP, blood pressure; CCB, calcium channel blocker; CV%, intermediate precision; DD2, Danish Centre for Strategic Research in Type 2 diabetes; DDD, defined daily dose; eGFR, estimated glomerular filtration rate; GA, 18β-glycyrrhetinic acid; GAD, 65-ab Glutamic Acid Decarboxylase Autoantibodies; GL, glycyrrhizin; HbA1c, glycated hemoglobin; HOMA2S, homeostasis model assessment 2 for insulin sensitivity; IDA, individually tailored treatment in type 2 diabetes; IQI, interquartile interval; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLOQ, lower limit of quantification; log_2GA, log_2-transformed GA; MR, mineralocorticoid receptor; MRA, mineralocorticoid receptor antagonist; OR, odds ratio; RAAS, renin-angiotensin-aldosterone system; SD, standard deviation; T2D, type 2 diabetes.

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Introduction

Excessive licorice consumption is a well-recognized cause of elevated blood pressure (BP) [1–3]. Therefore, identification of excessive licorice consumption could represent an important strategy for improving the management of hypertension. The active compound responsible for the BP elevation observed in line with excessive licorice consumption is glycyrrhizin (GL) [2]. GL is primarily found in licorice confectionery but is also widely consumed in medical products and as an aromatic additive in products not commonly associated with licorice, such as tea, chewing gum, and alcoholic beverages [4,5]. In addition, the sweetness of GL makes it a potential candidate as a natural sweetener [6,7]. Individuals with type 2 diabetes (T2D) represent a particularly relevant population in this context due to the high prevalence of hypertension and the increased cardiovascular disease risk associated with coexisting hypertension in this group [8]. T2D management often involves multiple lifestyle adjustments, including dietary modifications, which may increase the likelihood of exposure to GL through sugar-free products containing licorice-derived compounds. Furthermore, individuals with newly diagnosed T2D may be especially receptive to dietary advice and changes, making this a timely window to identify potentially modifiable contributors to poor BP control. Investigating GL intake in patients with T2D could therefore yield clinically meaningful insights relevant to both dietary guidance and BP in this high-risk group.

Despite the lack of an established safe dose, both the WHO and the European Union recommend limiting regular GL intake to no >100 mg/d [9,10]. Nevertheless, regular consumption at or below this provisional limit has been shown to cause clinically significant BP elevations [3,11]. Current guidelines for evaluating secondary hypertension recommend assessing the intake of substances affecting BP regulation, including licorice [8]. However, patients may underreport their licorice intake due to a lack of awareness about its potential health risks. Additionally, the variability in GL content across licorice products, and its presence in non-confectionery products, makes it difficult for patients and health care providers to estimate actual GL exposure accurately [3,12]. Therefore, a biochemical approach to detect excessive GL exposure may help address these challenges and could be relevant in the context of BP management.

GL is metabolized by intestinal bacteria into 18β-glycyrrhetinic acid (GA), which is readily absorbed into the bloodstream [13,14]. GA inhibits 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), an enzyme that inactivates glucocorticoids and protects the mineralocorticoid receptor (MR) from overactivation [2]. The enzyme is expressed in key tissues involved in BP regulation, including the distal nephron of the kidney, vascular wall, heart, and brain [2]. Inhibition of 11βHSD2 activity, among other physiological effects, causes MR overactivation in cells of the distal nephron of the kidney, leading to a cascade of effects, including BP elevation, renal potassium excretion, suppression of aldosterone, decreased cortisone levels, and increased cortisol/cortisone ratios [2,15]. This constellation of findings is referred to as apparent mineralocorticoid excess (AME)[15].

Given that concentrations of plasma GA correlate with in vivo 11βHSD2 inhibition [16,17], measuring plasma levels of GA could provide a reliable marker for excessive licorice consumption. Consequently, the primary aim of this study was to investigate the association of plasma GA levels with BP levels, intensity of antihypertensive treatment, resistant hypertension, and biochemical markers of AME in individuals with T2D. Secondary aims were to evaluate whether plasma GA levels could serve as a marker for high licorice intake by correlating GA levels with self-reported licorice consumption, and to assess whether licorice intake assessed using a questionnaire-based approach was associated with BP levels, intensity of antihypertensive treatment, resistant hypertension, and biochemical markers of AME.

Methods

Study design and population

This population-based cross-sectional study used blood samples and data from the "Individually tailored treatment in T2D" (IDA) study (Supplemental Figure 1)[18]. The IDA study recruited individuals with recently diagnosed T2D above 18 years of age from the "Danish Centre for Strategic Research in T2D" (DD2) cohort study [19]. Recruitment was facilitated by general practitioners participating in the IDA study. Eligible participants had a life expectancy above 2 y, were not diagnosed with type 1 diabetes (defined as age < 30 y at DD2 enrollment, fasting C-peptide < 300 pM and glutamic acid decarboxylase autoantibodies (GAD 65-ab) > 20 IU/mL) and were not enrolled in other clinical trials. A total of 1172 individuals participated in the IDA study. At baseline (2013–2018) participants completed a questionnaire, underwent clinical examinations, and provided plasma and serum samples. Afterward, samples were frozen immediately and kept at −80°C for future analysis. Plasma samples were not available from 12 subjects due to inadequate amount of tissue for all biochemical measurements. Therefore, a total of 1160 individuals were included in the study.

Participant characteristics and investigations

Participants' age and sex were derived from civil registration numbers, allowing linkage with Danish registries. Sociodemographic variables, including education level, work status, smoking habit, alcohol consumption, and licorice intake, were collected via a questionnaire. Licorice consumption was assessed by asking participants whether they consumed licorice and, if so, to quantify their weekly licorice intake. If they answered "yes," they were considered "self-reported consumers," whereas those who answered "no" were classified as "self-reported non-consumers." Self-reported weekly licorice intake was categorized into the following 5 categories: *1)* 0 g/wk ("non-consumers"); *2)* 1–10 g/wk; *3)* 11–50 g/wk; *4)* 51–100 g/wk; and *5)* >100 g/wk (for details see Supplemental Methods).

Clinical data, including diabetes duration, comorbidities, and prescribed medications, were collected via clinical interviews, patient records, and electronic medical records. Anthropometric measurements, including weight and height, were used to calculate BMI (kg/m^2). Ambulatory BP was measured using the Mobil-O-Graph system (IEM), with BP recorded every 3 min over a 30-min period. The mean of all readings was used as final systolic and diastolic BP [20].

Biochemical measurements

Plasma GA and aldosterone, as well as serum cortisol and cortisone levels were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a Waters Acquity Ultra-Performance Liquid Chromatographer coupled to a Xevo TQ-S tandem mass spectrometer. Samples were extracted on a Hamilton STARlet workstation using Waters Oasis MAX (10 mg) 96-well plates for aldosterone, Waters Oasis HLB (30 mg) 96-well plates for cortisol and cortisone, and protein precipitation with acetonitrile for GA. Deuterated internal standards were used for aldosterone, cortisone and GA, and C13 labeled internal standard for cortisol. The analyses were calibrated by in-house prepared calibrators and the intermediate precision for the internal controls were below 10%.

The LC-MS/MS method for measurement of plasma GA levels had a lower limit of quantification, defined as the lowest concentration at which intermediate precision (CV%) was $\leq \pm 20\%$ [21], was 2 μg/L (Supplemental Table 1). The preservation and freeze-thaw stability of plasma GA using a LC-MS/MS method have previously been deemed satisfactory by others [22].

For more details about measurements of blood levels of GA, aldosterone, cortisol, and cortisone, as well as other biochemical measurements see Supplemental Methods.

Antihypertensive medication standardization

Antihypertensive medication usage was quantified using the WHO Collaborating Center for Drug Statistics Methodology Defined Daily Dose (DDD) system [23], allowing standardized comparisons across drug classes. The DDD represents the assumed average maintenance dose per day for a drug when used for its main indication in adults [23].

Statistical analysis

Plasma GA concentrations are presented as medians with interquartile intervals (IQI) and minimal and maximal concentrations. Participants were categorized into high GA (top quartile) and low GA (bottom 3 quartiles), with the low GA group acting as controls. Participant characteristics are displayed as means with standard deviations (SD) for continuous variables and as numbers with percentages for categorical variables. Associations between GA category and outcomes were assessed using linear regression for continuous outcomes and logistic regression for binary outcomes, adjusted for relevant confounders. Complete-case analyses were conducted, excluding observations with missing data. Therefore, the number of individuals included varied across models and is reported in the tables. Primary outcomes were diastolic and systolic BP and antihypertensive DDD, whereas secondary outcomes were resistant hypertension (BP > 135/80 mmHg and treatment with $\geq$3 different types of BP-lowering drugs (which should include a diuretic, a renin-angiotensin-aldosterone system [RAAS] inhibitor, and a calcium channel blocker [CCB] [8]) and biochemical markers of markers of AME (aldosterone, potassium, cortisone, and cortisol levels, as well as cortisol/cortisone ratios). Age and sex adjusted models were adjusted for age and sex, whereas fully adjusted models additionally accounted for BMI, smoking, alcohol, education, diabetes duration, eGFR, HbA1c, and HOMA2S. In the fully adjusted models, additional confounders were included depending on the specific outcome: For analysis of BP, adjustment also included treatment (RAAS inhibitors, mineralocorticoid receptor antagonists (MRAs), non-MRA diuretics, and other antihypertensive drugs (including CCB, central adrenergic inhibitors and alpha and beta-blockers). For analysis of DDD, systolic BP was added to the model. For analyses of AME we additionally adjusted for both systolic BP and specific antihypertensive treatment; and for potassium analyses we further adjusted for potassium supplement. In supplementary analyses of the association between GA category and estimate untreated BP, 15 mmHg and 10 mmHg were added to systolic and diastolic BP levels in subjects treated with antihypertensive treatment, as recommended in modeling studies [24]. The interaction of GA category with age, sex, BMI, smoking, alcohol, education, diabetes duration, eGFR, HbA1c, and HOMA2S (and treatment for BP analysis, and systolic BP for DDD analysis) in the analyses of the association of GA and systolic and diastolic BP and DDD were also evaluated. A significance level of 0.05 was used for all statistical tests.

Association between GA and self-reported licorice intake was explored using continuous GA against categorical weekly licorice intake. This association was further assessed for interactions with above mentioned covariates. Analyses of primary and secondary outcomes were repeated using self-reported licorice intake as an independent variable (consumers vs. non-consumers, and a combined self-reported licorice intake and GA category variable, as independent variables).

Sensitivity analyses investigated the influence of antihypertensive treatment on associations between GA, BP levels, and AME markers, stratifying participants by antihypertensive usage. Odds ratios (ORs) were calculated for having DDD of $\geq$1, $\geq$2, $\geq$3 or $\geq$4, receiving $\geq$2 or $\geq$3 different types of antihypertensive drugs, and for specific antihypertensive medication groups. Dose-response relationships of plasma GA with outcomes were examined, using $\log_2$-transformed GA ($\log_2$GA) and quartiles.

Ethics

The IDA study has been approved by the Regional Committee on Medical Health Ethics (Region of Southern Denmark S-20120186). The study was conducted in concordance with the Helsinki declaration II.

Results

Plasma GA concentration

Median concentration was 5.7 μg/L (IQI: 0.5–37.1). The high GA group (n = 290) had median GA of 90.8 μg/L (IQI: 57.2–149.8]; min = 37.1 μg/L; max = 1631.0 μg/L), whereas the low GA group (n = 870) had median GA of 1.6 μg/L (IQI: 0.30–10.1]; min = 0.0 μg/L; max = 37.0 μg/L). Participant characteristic by GA category are presented in Table 1.

Plasma GA concentrations, BP, and antihypertensive medication

High GA concentrations were not associated with higher BP, but with an average increase of 0.28 antihypertensive DDD ([0.03–0.52]; P = 0.03; Table 2) in the fully adjusted model. For results from age and sex adjusted models, we refer to Supplemental Table 2. We performed additional analyses of the association between GA category and estimated untreated systolic and diastolic BP levels (15 mmHg and 10 mmHg added in subjects treated with antihypertensive medication). These showed significant association between high GA and both

TABLE 1
Participant characteristics by GA category.

Characteristic	Low GA	High GA	Total
n (%)	870 (75.0)	290 (25.0)	1160 (100.0)
Sex (Female), *n* (%)	354 (40.7)	119 (41.0)	473 (40.8)
Age, mean (SD)	61.6 (10.5)	59.2 (10.4)	61.0 (10.6)
BMI, mean (SD)	32.0 (6.2)	32.2 (6.5)	32.0 (6.3)
Smoke, n (%)			
Never smoker	358 (41.2)	113 (39.1)	471 (40.7)
Current smoker	173 (19.9)	52 (18.0)	225 (19.4)
Former smoker	338 (38.9)	124 (42.9)	462 (39.9)
Alcohol consumption, *n* (%)			
Below 7–14 standard drinks/wk	712 (81.8)	240 (82.8)	952 (82.1)
Above 7–14 standard drinks/wk	158 (18.2)	50 (17.2)	208 (17.9)
Education length, *n* (%)			
Primary school	292 (33.8)	81 (27.9)	373 (32.4)
Secondary education	91 (10.5)	28 (9.7)	119 (10.3)
Higher education	480 (55.6)	181 (62.4)	661 (57.3)
Diabetes duration (d), mean (SD)	1253 (932)	1263 (939)	1256 (933)
eGFR [mL/min/1.73m^2], mean (SD)	87 (16)	86 (17)	87 (17)
HbA1c, mean (SD)	51 (10)	52 (13)	51 (11)
HbA1c categorical, *n* (%)			
≤48	422 (48.6)	148 (51.0)	570 (49.2)
>48	447 (51.4)	142 (49.0)	589 (50.8)
HOMA2S, mean (SD)	3.60 (8.59)	3.36 (1.68)	3.54 (7.48)

Participants are categorized into 2 groups based on plasma levels of GA: low GA (bottom 3 quartiles) and high GA (top quartile).
Data are displayed as means with standard deviations (SD) for continuous variables and as numbers with percentages for categorical variables.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; GA, 18β-glycyrrhetinic acid; HbA1c, glycated hemoglobin; HOMA2S, homoeostasis model assessment 2 for insulin sensitivity.

systolic (2.12 mmHg [0.15–4.09]; $P = 0.04$) and diastolic BP (1.58 mmHg [0.22–2.95]; $P = 0.02$, Supplemental Table 3) in the fully adjusted models. A significant interaction between GA and smoking status on systolic BP was observed, with a more pronounced association in current smokers ($P = 0.01$, Supplemental Figure 2). A near-significant interaction was also seen with HbA1c ($P = 0.09$), suggesting a stronger effect in individuals with HbA1c ≥49 mmol/mol. No potential interactions on the association between GA and diastolic BP were observed (Supplemental Figure 3). Additionally, HbA1c modified the association between GA and DDD, with a greater effect in those with HbA1c ≥49 mmol/mol ($P = 0.01$; Supplemental Figure 4).

TABLE 2
Fully adjusted linear and logistic regression models for the association of GA category (high vs. low) with primary and secondary outcomes.

Primary outcomes	Estimated difference[1] (95% CI)	*P* value
Systolic BP (mmHg) (*n* = 1146)	1.46 (−0.33 to 3.26)	0.11
Diastolic BP (mmHg) (*n* = 1146)	1.26 (−0.01 to 2.52)	0.05
Antihypertensive DDD (*n* = 1143)	0.28 (0.03–0.52)	**0.03**
Secondary outcomes	**Estimated difference[1] (95% CI)**	***P* value**
Aldosterone (pmol/L) (*n* = 1145)	−41.5 (−63.1 to −20.0)	**<0.001**
Potassium (mmol/L) (*n* = 1110)	−0.06 (−0.10 to −0.01)	**0.01**
Cortisol (nmol/L) (*n* = 1135)	4.14 (−11.2 to 19.5)	0.60
Cortisone (nmol/L) (*n* = 1135)	−6.08 (−7.78 to −4.38)	**<0.001**
Cortisol/cortisone (*n* = 1135)	1.26 (1.00 to 1.52)	**<0.001**
	Odds ratio (95% CI)	***P* value**
Resistant hypertension[2](*n* = 1146)	1.91 (1.12–3.24)	**0.02**

Models are adjusted for age, sex, BMI, smoking, alcohol, education, diabetes duration, eGFR, HbA1c, and HOMA2S. BP analyses also adjusted for treatment (RAAS inhibitors, MRAs, non-MRA diuretics, and other antihypertensives [including calcium channel blockers, central adrenergic inhibitors, and alpha/beta-blockers]). DDD analyses additionally adjusted for systolic BP, whereas analyses of AME markers included both systolic BP and treatment (potassium supplements were added for potassium analysis).
The number of individuals included in each regression model is reported in the table, reflecting complete-case analysis where participants with missing data on confounders were excluded.
Statistically significant values ($P < 0.05$) are in bold type.
Abbreviations: AME, apparent mineralocorticoid excess; BP, blood pressure; DDD, defined daily dose [of antihypertensive medication]; GA, 18β-glycyrrhetinic acid; HOMA2S, homoeostasis model assessment 2 for insulin sensitivity; MRA; mineralocorticoid receptor antagonists; RAAS, renin-angiotensin-aldosterone system.
[1] Estimated difference between mean of each GA category (high vs. low).
[2] defined as BP > 135/80 mmHg and treatment with ≥3 different types of BP-lowering (including diuretic, RAAS inhibitor and calcium channel blocker).

High GA was also associated with increased likelihood of resistant hypertension (OR: 1.91 [1.12–3.24]; $P = 0.02$; Table 2) in the fully adjusted model.

Markers of apparent mineralocorticoid excess

In the fully adjusted model, high GA concentrations were associated with lower aldosterone (estimated difference: −41.5 pmol/L [−63.1 to −20.0]; $P < 0.001$), potassium (−0.06 mmol/L [−0.10 to −0.01; $P = 0.01$) and cortisone concentrations (−6.08 nmol/L [−7.78 to −4.38; $P < 0.001$), as well as higher cortisol/cortisone ratios (1.26 [1.00–1.52]; $P < 0.001$; Table 2). The cortisol concentrations were not significantly higher in the high GA group. The results did not change significantly using age and sex adjusted models (Supplemental Table 2).

Licorice intake and plasma GA concentrations

We observed a significant stepwise increase in plasma GA levels with higher self-reported weekly licorice intake (Supplemental Table 4). The association was significant both within each category of licorice consumption and in the overall stepwise relationship (overall $P < 0.001$). Potential interactions with smoking status (never, current, or former) and systolic BP (≥129 or <129 mmHg) were noted (P-interaction = 0.11 and 0.06 respectively, data not shown).

Self-reported licorice intake, BP, antihypertensive medication, and markers of AME

Self-reported licorice consumption was not associated with higher BP (Supplemental Table 5). Individuals, who reported consumption of licorice received insignificantly higher antihypertensive DDD than non-consumers in the fully adjusted model (0.16 [−0.05 to 0.38]; $P = 0.13$; Table 3). For age and sex adjusted models see Supplemental Table 6. No significant associations between self-reported licorice consumption and estimated untreated BP were observed (Supplemental Table 3). Self-reported licorice consumption was associated with lower levels of aldosterone (estimated difference: −19.6 pmol/L [−38.6 to −0.64]; $P = 0.04$) and cortisone (−2.66 nmol/L [−4.17 to −1.14]; $P = 0.001$), as well as higher cortisol/cortisone ratios (0.38 [0.14– 0.62]; $P = 0.002$; Supplemental Table 5) in the fully adjusted model. Self-reported licorice intake was not significantly associated with cortisol or potassium levels, nor likelihood of resistant hypertension.

Combined GA category and self-reported licorice intake

Individuals with high GA who also reported licorice consumption ($n = 211$) were treated with 0.38 more antihypertensive DDD ([0.07–0.68], $P = 0.01$; Table 3) compared with subjects with low GA who reported that they did not consume licorice ($n = 497$)(reference group). Among licorice consumers, high GA levels were linked to an increase of 0.29 antihypertensive DDD ([−0.02 to 0.60]; $P = 0.07$; data not shown) compared with low GA in the fully adjusted model. The results were not significantly different in the age and sex adjusted models (Supplemental Table 6).

Sensitivity analyses

When stratifying participants based on usage of BP-lowering medication, we found that the association of high GA with low aldosterone levels was only significant in those not receiving antihypertensive medication. In both treatment groups, high GA was significantly associated with lower cortisone levels and higher cortisol/cortisone ratios. High GA was not significantly associated with levels of BP, regardless of treatment status (Supplemental Table 7).

Participants with high GA exhibited numerically higher likelihood of being treated with ≥1, ≥2, ≥3 or ≥4 DDD (fully adjusted OR: 1.40 [1.02–1.91], $P = 0.04$; 1.32[0.98–1.77]; $P = 0.06$; 1.36[1.00–1.85]; $P = 0.05$; 1.42[0.99–2.01]; $P = 0.05$, Supplemental Table 8). They were also more likely to be prescribed ≥2 and ≥3 different types of antihypertensive drugs (fully adjusted OR: 1.80[1.34–2.44], $P < 0.001$; 1.48 [1.06–06]; P value = 0.02, Supplemental Table 8). Additionally, individuals with high GA more often received antihypertensive medication within all categories (RAAS inhibitors, non-MRA diuretics,

TABLE 3
Fully adjusted linear regression models of the associations of self-reported licorice intake (self-reported consumers vs. non-consumers), and combined self-reported licorice consumption and GA category, with antihypertensive DDD.

	Estimated difference of DDD[1] (95% CI)	P value
Self-reported licorice consumers[2] ($n = 1154$)	0.16 (-0.05 – 0.38)	0.13
Combined self-reported licorice intake and GA group (n = 1143)		
Self-reported non-consumers and low GA (42.8%)	reference	-
Self-reported consumers and low GA (6.8%)	0.09 (-0.16 – 0.34)	0.49
Self-reported non-consumers and high GA (32.2%)	0.16 (-0.28 – 0.59)	0.49
Self-reported consumers and high GA (18.2%)	0.38 (0.07 – 0.68)	**0.01**

Adjusted models accounted for age, sex, BMI, smoking, alcohol, education, diabetes duration, eGFR, HbA1c, HOMA2S, and systolic BP.
The number of individuals included in each regression model is reported in the table, reflecting complete-case analysis where participants with missing data on confounders were excluded.
Statistically significant values ($P < 0.05$) are in bold type.
Abbreviations: DDD, defined daily dose [of antihypertensive medication]; GA, 18β-glycyrrhetinic acid; HOMA2S, homoeostasis model assessment 2 for insulin sensitivity
[1] Estimated difference in mean antihypertensive DDD for each category compared with the reference (non-consumers or non-consumers and low GA).
[2] Self-reported consumers defined as participants answering "*yes*" to licorice consumption (50.3%) (compared with participants answering "no"; non-consumers [49.7%]).

MRAs, and other antihypertensive drugs, including CCB, central adrenergic inhibitors, and alpha and beta-blockers [fully adjusted OR: 1.43 [1.07–1.92], $P = 0.02$; 1.60 [1.19–2.17]; $P = 0.002$; 1.91 [0.94–3.90]; $P = 0.08$; 1.31 [0.98–1.75]; $P = 0.07$; Supplemental Table 8]).

Using linear regression analysis, $\log_2$GA was unrelated to DDD in the fully adjusted model (beta coefficient: 0.02 ([−0.01 to 0.05]; $P = 0.19$, data not shown). When categorizing participants into 4 groups based on percentiles of plasma GA, we found that for the primary outcomes none of the groups differed significantly from the reference group (Q1) in the fully adjusted model; however, Q4 showed a non-significant difference from Q1 for DDD and diastolic BP. For aldosterone and cortisol/cortisone only Q4 differed significantly from Q1 (Supplemental Table 9).

Discussion

In a study cohort of patients with recently diagnosed T2D, we found that plasma concentrations of GA above the 75th percentile were associated with more intensive antihypertensive treatment than in those with lower GA. Although high GA was not directly linked to higher BP, our findings suggest that individuals with high GA require more intensive antihypertensive treatment to achieve BP levels comparable to those with lower GA. Therefore, high GA may be indirectly associated with higher BP levels. Moreover, high GA was linked to increased likelihood of resistant hypertension and was associated with biochemical markers of AME, including lower aldosterone, potassium, and cortisone, alongside higher cortisol/cortisone ratios. The significant association between estimated weekly licorice intake and GA levels support the idea that GA could serve as a marker of consistent licorice consumption. Collectively, these findings support the hypothesis that GA may be a potential marker of excessive GL exposure. Notably, when licorice intake was assessed solely through a questionnaire, self-reported licorice consumption was associated with certain biochemical markers of AME, but showed no significant associations with BP levels, antihypertensive treatment intensity, or resistant hypertension.

The strengths of this study include the pathophysiological corroboration of our primary outcomes and the large sample size. Furthermore, plasma levels of GA were measured using a highly sensitive and selective LC–MS/MS method. Additionally, we adjusted for multiple demographic, clinical, and laboratory confounders to ensure robust and reliable findings. Our study has several limitations. First, individuals with recently diagnosed T2D are typically subjected to intensive treatment to manage hypertension [25]. Before enrollment, GPs may have optimized antihypertensive treatment in individuals' sensitive to GA-induced BP elevation, potentially masking the direct impact of high GA in this population. This could explain why we do not observe a direct association between high GA and higher BP. To address this confounding by indication, we included the intensity of BP-lowering treatment as a primary outcome. Furthermore, in supplementary analyses of the association between GA category and estimated untreated systolic and diastolic BP levels (15 mmHg and 10 mmHg added in subjects treated with antihypertensive medication), we found diastolic and systolic BP levels to be significantly higher in subjects with high GA (Supplemental Table 3). However, in our population 72% received BP lowering medication, some with multiple drugs, and these analyses probably still have a high degree of bias, even though modeling studies have suggested this method to have the lowest degree of bias [24]. Second, we assume that individuals with elevated GA are likely regular licorice consumers. Considering the relatively short half-life of GA (9–20 h [17,26–29]), some participants may have consumed large amounts of licorice shortly before blood sampling without being habitual licorice consumers. Follow-up measurements of GA would be ideal for confirming consistent licorice intake. Nevertheless, we observe a relationship between GA and estimated weekly licorice intake, indicating measurement of GA as a potential marker of high licorice consumption. Third, we did not find indications of dose-response relationship between GA concentration and DDD or markers of AME. Since not every individual with impaired activity of 11βHSD2 will develop signs of AME [17], substantial inter-individual variations in the clinical response to a certain GA concentration must exist, which may explain why a dose-response relationship was not observed. Fourth, blood sampling for aldosterone measurement was not conducted under standard conditions, and interfering medications were not paused or replaced beforehand, contributing to higher risk of preanalytical error in these measurements. To overcome confounding by interfering medication, we adjusted for relevant medication and performed stratified analyses based on antihypertensive treatment status. The stratified analyses revealed that high GA was indeed associated with lower aldosterone levels and higher cortisol/cortisone ratio in individuals not receiving BP-lowering medication. Fifth, participants were not required to fast before blood sampling, which could influence the variability of GA concentrations. This aspect was not controlled in our study design, and the fasting status was not recorded. Lastly, given the exploratory nature of this study and the number of statistical comparisons made, we cannot exclude the possibility that some of the observed associations may be due to chance, and the findings should therefore be interpreted with appropriate caution.

To our knowledge, this is the first study to investigate the associations of plasma GA levels with BP, antihypertensive treatment intensity, and resistant hypertension. Although the connection between regular licorice or GL consumption and BP elevation, as well as biochemical signs of AME is well established [1,3,16,17], our findings support the hypothesis that high plasma GA concentrations may serve as a surrogate marker of excessive GL exposure. This is underscored by the observed association with both clinical and biochemical indicators of 11βHSD2 inhibition. The underlying pathophysiological mechanism involves GA-mediated inhibition of intracellular 11β-HSD2, which reduces the conversion of active cortisol to inactive cortisone. This leads to inappropriate activation of the MR by cortisol, resulting in sodium retention, potassium excretion, and ultimately contributing to BP elevation and biochemical features consistent with AME [2,15]. Whether assessment of 11βHSD2 activity should be accompanied by measurement of 24-h urinary cortisol/cortisone ratios to confirm decreased 11βHSD2 activity is debatable, as GA also inhibits the widely distributed isoenzyme 11β-hydroxysteroid dehydrogenase type 1, which catalyzes the invert conversion of cortisone to cortisol [30]. As 24-h urinary samples were unavailable, we could not perform these measurements. Nevertheless, it has been reported that evaluation of cortisone and cortisol/cortisone in blood are at least as valuable as 24-h urinary measurements for identifying non-classical, such as licorice-induced cases [15,16].

The estimated GL dosages consumed in our study, based on self-reported weekly licorice intake, were markedly lower than those reported in earlier intervention trials that have investigated effect of regular licorice or GL consumption on BP and biochemical markers of AME. Earlier studies have suggested that clinically meaningful BP elevations can be induced after ingestion of ≥100 mg GL/day [1,3,31]. In our study the median self-reported licorice intake was 11–50 g/wk for individuals with high GA (Supplemental Table 10), which assuming an average GL content of 0.2% in licorice confectionery [12], means ~3–14 mg GL consumption per day. Although these findings should be interpreted with care, as self-reported consumption of licorice were based on free text reports and associated with uncertainty, our results challenge the assumption that a daily intake below 100 mg GL consumption per day is generally safe [9,10], a notion that has been proposed by others [3,11].

Enhanced GA sensitivity compared to healthy individuals included in prior intervention studies may explain the observed association between lower GL doses and clinically relevant BP changes. In our study, we investigated individuals with T2D and with a high prevalence of hypertension (72%). Essential hypertension has earlier been shown to be associated with heightened susceptibility licorice-induced BP changes [32]. Also, both insulin resistance and 11βHSD2 inhibition is linked to salt-sensitive hypertension [33–36], suggesting that licorice consumption in individuals with T2D could exacerbate their already heightened BP response to dietary salt. The observed interaction of HbA1c on the association between GA and antihypertensive treatment intensity supports the notion that individuals with poor glycemic control are particularly vulnerable to the harmful effects of licorice consumption.

Another discrepancy, which may explain how GL dosages below 100 mg/d can lead to clinically relevant BP changes, are potential differences in GL exposure duration. Although intervention studies typically last weeks [1,3], free-living individuals consuming licorice habitually, may have sustained this practice for years, which may result in accumulation of GA, due to enterohepatic cycling, leading to delayed plasma clearance [13,17,37]. This accumulation may explain how low-dose licorice intake can be sufficient to induce clinically relevant 11βHSD2 inhibition.

High plasma GA levels may result not only from consistent low-dosage licorice exposure, but also significant unintentional GL intake, e.g., through consumption of sugar-free alternatives using GL as a natural sweetener [7]. Notably, 27% of participants in the high GA reported no licorice consumption, suggesting considerable unintended GL exposure in this group (Supplemental Table 10). The GL content in such food products and its clinical implications remains to be further investigated.

Our results suggest that measuring plasma GA levels may be useful in identifying excessive licorice consumption. Future studies should focus on further exploring the association between GL exposure and plasma GA levels, and investigate whether dietary interventions aimed at reducing licorice consumption or plasma GA levels affect BP levels and the need for antihypertensive medication. Although, we only included participants with T2D, our findings may also be relevant for a broader population, e.g. individuals with metabolic syndrome. Therefore, the potential association between licorice intake, plasma GA levels, and hypertension risk in a more general population also warrants further investigation.

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Author contributions

The authors' responsibilities were as follows – JLW, TBO, JVS, AVS, AS, JSM, ERBP. were involved in the conception, design, and conduct of study and analysis and interpretation of results. AVS, AS, JLW, JSM: developed and validated the method for biochemical measurement of GA concentrations in plasma; JLW: wrote the first draft of the manuscript, and all authors edited, reviewed, and approved the final version of the manuscript; JVS, TBO, KH: were responsible for blood sampling and data for the IDA study; JVS did the statistical analyses; JLW, TBO, JVS: is the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis; and all authors: read and approved the final manuscript.

Conflicts of interest

The authors report no conflicts of interest.

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Data availability

The raw data supporting the results and conclusion of this manuscript will be made available without undue reservation upon request to the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2025.05.048.

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