Left ventricular geometry and endogenous ouabain in a Flemish population

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Background Endogenous ouabain, an endogenous digitalis-like steroid, is synthesized in the adrenal glands and possibly in the hypothalamus. *In vitro* and *in vivo*, endogenous ouabain triggers growth and proliferation of cardiomyocytes.

Objective We therefore investigated the association between left ventricular structure and function and plasma endogenous ouabain in a general population.

Methods We randomly recruited 536 individuals from a general population (50.7% women, mean age 53.1 years). Measurements included echocardiographic left ventricular structure and function, blood pressure, plasma endogenous ouabain, and the 24-h urinary excretion of sodium.

Results The geometric mean plasma endogenous ouabain was 95.5 pmol/l (interquartile range 79.4–120.2 pmol/l). We expressed effect sizes for a 1-SD increase in plasma endogenous ouabain (0.21 on the logarithmic scale), while accounting for important covariables. For a 1-SD increment in plasma endogenous ouabain, SBP, left ventricular posterior wall, the interventricular septum, and relative wall thickness increased by 1.59 mmHg ($P = 0.009$), 0.138 mm ($P = 0.003$), 0.152 mm ($P = 0.013$), and 0.71×10^{-2} ($P = 0.008$), respectively. In a sensitivity analysis, involving 431 individuals aged 29–71 years (10–90th percentile interval), a 1-SD increase in plasma endogenous ouabain was associated with opposite trends in ejection fraction (+0.90%, $P = 0.005$) and left ventricular systolic longitudinal strain (−0.48%, $P = 0.011$). Moreover, in individuals whose sodium excretion was above median (160 mmol/24 h), the aforementioned associations reached a higher level of statistical significance.

Conclusion Our population-based study suggested that endogenous ouabain might have a trophic effect on the myocardium, independent of blood pressure and other covariables. The clinical implication of these findings remains to be elucidated. *J Hypertens* 27:1884–1891 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

In 1982, Hamlyn *et al.* [1] demonstrated for the first time that a factor circulating in human plasma inhibited purified Na+,K+-ATPase and was correlated with higher blood pressure (BP). This observation initiated further research on endogenous digitalis-like steroids such as cardenolides and bufadienolides [2]. Endogenous ouabain is synthesized in the adrenal glands and possibly in the hypothalamus. Functionally, endogenous ouabain is a high-affinity, reversible, and specific inhibitor of the sodium pump, with inotropic and vasopressor activity [3]. In addition, endogenous ouabain activates signal transduction via the Src–epidermal growth factor receptor (EGFr)–extracellular signal-regulated kinase (ERK) pathway, and thereby triggers growth and proliferation of renal tubular cells and cardiomyocytes *in vitro* and *in vivo*, even at subnanomolar concentrations [4].

In never-treated [5] and untreated [6] hypertensive patients, left ventricular (LV) mass, stroke volume (SV), and relative wall thickness (RWT) increased and heart rate (HR) decreased with higher circulating endogenous ouabain level. In some studies, nearly 50% of patients with uncomplicated essential hypertension have elevated plasma endogenous ouabain [6]. Both LV remodelling and hypertension are important risk factors for congestive heart failure. To our knowledge, no population study has described the association between LV structure and function and plasma endogenous ouabain. We therefore investigated this

association in individuals randomly selected from a Belgian population.

Methods

Study participants

The Ethics Committee of the University of Leuven approved the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) [7]. As described elsewhere [7,8], from August 1985 until December 2005, we identified a random population sample stratified by sex and age from a geographically defined area in northern Belgium. The seven municipalities gave listings of all inhabitants sorted by address. Households, defined as those who lived at the same address, were the sampling unit. We numbered households consecutively, and generated a random-number list by use of the SAS random function (SAS Institute, Cary, North Carolina, USA). Households with a number matching the list were invited; household members older than 18 years were eligible.

From June 2005 until June 2008, we re-invited 845 former participants for a follow-up examination at our field centre, including echocardiography. After excluding 25 patients who were bedridden or institutionalized, we obtained informed written consent from 667 participants (participation rate 81.3%). We excluded 51 individuals because of LV remodelling due to myocardial infarction or coronary revascularization ($n = 16$); valvular heart disease ($n = 27$); atrial fibrillation ($n = 6$), or the presence of an artificial pacemaker ($n = 2$). We discarded a further 80 individuals from analysis because of missing endogenous ouabain measurements ($n = 75$) or because the echocardiogram was of insufficient quality ($n = 5$) Thus, the total number of participants statistically analyzed was 536.

Echocardiography

The participants refrained from smoking, heavy exercise, or drinking alcohol or caffeine-containing beverages for at least 3 h before echocardiography. The BP during echocardiography was the average of two readings, obtained with a validated OMRON 705IT device (Omron Corp., Tokyo, Japan) at the end of the examination.

Data acquisition

One experienced physician (T.K.) did the ultrasound examination according to the recommendations of the American Society of Echocardiography [9] using a Vivid7 Pro (GE Vingmed, Horten, Norway) interfaced with a 2.5–3.5-MHz phased-array probe. With the individuals in partial left decubitus and breathing normally, she obtained images, together with a simultaneous ECG signal, from the parasternal long and short axes and from the apical four and two-chamber long-axis views. All recordings included at least five cardiac cycles and were digitally stored for off-line analysis. M-mode echocardiograms of the left ventricle were recorded from the parasternal long-axis view under control of the two-dimensional image. The ultrasound beam was positioned just below the mitral valve at the level of the posterior chordae tendineae.

To record LV outflow tract velocities from the apical window, the observer positioned the Doppler sample volume at the LV outflow. To record mitral flow velocities from the apical window, the observer positioned the Doppler sample volume at the mitral valve tips. Using tissue Doppler imaging (TDI), she recorded low-velocity, high-intensity myocardial signals at a high frame rate (>190 FPS), while adjusting the imaging angle to ensure a parallel alignment of the ultrasound beam with the myocardial segment of interest. From the apical window, the sonographer placed a 5-mm Doppler sample at the septal, lateral, inferior, and posterior sites of the mitral annulus. The Nyquist limit was set as low as possible, avoiding aliasing.

Off-line analysis

The sonographer analyzed recorded images off-line, averaging three heart cycles for statistical analysis, using a workstation running the EchoPac software package, version 4.0.4 (GE Vingmed). The LV internal diameter and interventricular septal and posterior wall thickness were measured at end diastole from two-dimensional-guided M-mode tracings, as described in the American Society of Echocardiography guideline [9]. End-diastolic LV dimensions were used to calculate LV mass by an anatomically validated formula. RWT was calculated as the ratio of (interventricular septum + posterior wall)/LV internal diameter at end diastole. LV concentric remodelling was defined as RWT exceeding 0.43. LV end-systolic and end-diastolic volumes were calculated with the use of Teicholtz's method. Ejection fraction was calculated as the ratio of (end-diastolic volume − end-systolic volume)/LV end-diastolic volume. Intraobserver reproducibility was 2.2% for LV internal end-diastolic diameter, 4.6% for LV wall thickness, and 4.3% for LV mass. SV was calculated from the pulsed-wave Doppler velocity profile and the cross-sectional area at the LV outflow tract. Cardiac index (*CI*) was calculated as the ratio of (SV × HR)/body surface area [body weight$^{0.425}$ (in kg) × body height$^{0.725}$ (in cm) × 0.007184]. Total peripheral resistance index was assessed as the ratio of mean BP × 80/*CI*.

From the transmitral flow signal, the observer measured peak early diastolic velocity (*E*), peak late diastolic velocity (*A*), and the *E/A* ratio. From the TDI recordings, we determined peak early (E_a) and peak late (A_a) diastolic mitral annular velocities, and the E_a/A_a ratio at the four acquisition sites (septal, lateral, inferior, and posterior). As described elsewhere [10], we extracted longitudinal strain

curves off-line from colour myocardial Doppler images, using dedicated Software Package for Echocardiographic Quantification LEuven (SPEQLE) (version 4.6.2). We positioned the sampling volumes (10 mm) in the inferior and inferolateral walls at the level of the posterior chordae tendineae. To compute end-systolic longitudinal deformation, we averaged three consecutive cycles. Because there were no differences between the inferolateral and inferior walls in longitudinal strain, for statistical analysis we averaged these measurements and used their absolute values.

Biochemical measurements
On the day of the echocardiographic examination, after the individuals had rested in the supine position for at least 45 min, venous blood samples were collected into EDTA tubes. After centrifuging, the plasma was separated, aliquoted, and stored at $-70°C$.

Endogenous ouabain was extracted from plasma and measured using a specific radioimmunoassay (RIA) as described [11]. Briefly, plasma samples were mixed with one volume of 0.1% trifluoroacetic acid in water and centrifuged at 3000g for 30 min at 4°C. The supernatants were passed, under vacuum, over prewashed C18 columns. Following a wash cycle involving water and then 2.5% acetonitrile, endogenous ouabain was eluted with 25% acetonitrile. The eluate was dried under vacuum. The extracts were reconstituted in RIA buffer, incubated at room temperature for 15 h in the presence of 3H-ouabain and a rabbit polyclonal antiouabain antiserum. The incubation was interrupted by addition of stop solution and the rapid separation of bound from free-labelled ouabain by filtration over glass fiber filters. Trapped counts were determined by scintillation counting. The assay used a highly specific ouabain antiserum with low cross-reactivity for digoxin ($\sim$0.42%), spironolactone (<0.01%), canrenone (<0.01%), and canrenoate (0.07%) but has no measurable interaction with any of the antihypertensive medications used by the patients [12].

The N-terminal portion of pro-B-type natriuretic peptide (NT-proBNP) was measured in plasma samples by a competitive enzyme immunoassay (EIA) for research use (Biomedica Gruppe, Vienna, Austria). The standard range provided by the manufacturer of the EIA is from zero to 1000 pmol/l (median 208 pmol/l, 95th percentile 300 pmol/l).

The participants also collected a timed 24-h urine sample in a wide-neck plastic container for the measurement of sodium, potassium, creatinine, and aldosterone. Urinary aldosterone concentration was measured by commercial RIA (DSL-8600 Active; Diagnostic Systems Laboratories Inc., Webster, Texas, USA). Electrolytes in urine and plasma were determined by flame photometry.

Other measurements
At the examination centre, trained study nurses administered a questionnaire to collect detailed information on each patient's medical history, smoking and drinking habits, and intake of medications. Hypertension was defined as a BP of at least 140 mmHg SBP or 90 mmHg DBP (average of five consecutive readings at the examination centre) or as the use of antihypertensive drugs. Body mass index (BMI) was weight in kilograms divided by the square of height in meters.

Statistical methods
For database management and statistical analysis, we used SAS software, version 9.1 (SAS Institute). We normalized the distributions of plasma endogenous ouabain, NT-proBNP, and the urinary aldosterone excretion by a logarithmic transformation. We compared means and proportions using the large sample z-test and Fisher's exact test, respectively. Our statistical methods also included single and multiple linear regressions. We included in our models covariables with known physiological relevance for LV structure and function [10,13,14]. We applied a generalization of the standard linear model, as implemented in the PROC MIXED procedure of the SAS package, to investigate the associations between the echocardiographic measurements and explanatory variables, while accounting for relatedness amongst study participants and adjusting for covariables. We expressed multivariable-adjusted effect sizes for 1-SD increase in the explanatory variables.

Results
Characteristics of participants and endogenous ouabain distribution
The 536 participants included 272 (50.7%) women and 205 (38.2%) hypertensive patients, of whom 116 (21.6%) were on antihypertensive drug treatment. Twenty-seven women (9.9%) used oral contraceptives and seven (2.3%) took hormonal replacement therapy. The distribution of plasma endogenous ouabain departed from normality and was positively skewed ($P < 0.0001$) with a coefficient of skewness amounting to 5.02. The geometric mean plasma endogenous ouabain was 95.5 pmol/l [interquartile range (IQR) 79.4–120.2 pmol/l]. Table 1 shows the clinical characteristics of the study participants by quartiles of plasma endogenous ouabain. With higher plasma endogenous ouabain, there was an increase in SBP ($P = 0.014$, Table 1). The prevalence of hypertensive patients also increased with higher endogenous ouabain (Table 1). There were no significant differences amongst the endogenous ouabain quartiles in age, anthropometric characteristics, DBP, HR, and biochemical variables (Table 1).

As expected, in univariate regression analysis ($r = 0.13$, $P = 0.0028$, Fig. 1) and in an analysis adjusted for sex, age, age squared, BMI, antihypertensive treatment, and use of

Table 1 Clinical characteristics of participants by endogenous ouabain quartiles

Characteristic	Low ($n = 134$)	Medium−low ($n = 134$)	Medium−high ($n = 134$)	High ($n = 134$)	P
Limits (pmol/l)	<79.4	79.4−95.4	95.5−120.2	>120.2	−
Anthropometrics					
Age (years)	50.3 ± 14.0	50.3 ± 15.1	50.6 ± 14.9	53.6 ± 14.9	0.19
BMI (kg/m^2)	26.3 ± 3.6	26.3 ± 4.7	26.0 ± 3.7	26.1 ± 3.9	0.92
Sex, women (%)	69 (51.5)	72 (53.7)	67 (50.0)	64 (47.8)	0.44
SBP (mmHg)	126.7 ± 16.1	127.8 ± 16.2	127.8 ± 16.3	132.8 ± 18.5	0.014
DBP (mmHg)	78.9 ± 8.2	79.0 ± 8.2	79.8 ± 9.2	80.4 ± 10.8	0.45
HR (beats/min)	60.8 ± 9.4	61.4 ± 9.3	60.6 ± 8.9	60.2 ± 9.5	0.78
Questionnaire data					
Current smoking, n (%)	32 (23.9)	29 (21.6)	28 (20.9)	28 (20.9)	0.54
Drinking alcohol, n (%)	57 (42.5)	57 (42.5)	60 (44.8)	57 (42.5)	0.91
Hypertensive, n (%)	41 (30.6)	44 (32.8)	57 (42.5)	63 (47.0)	0.002
Treated for hypertension, n (%)	22 (16.4)	29 (21.6)	34 (25.4)	31 (23.1)	0.13
Biochemical data					
Total cholesterol (mmol/l)	5.28 ± 1.02	5.28 ± 0.99	5.32 ± 1.01	5.31 ± 1.03	0.98
Serum Na+ (mmol/l)	140.2 ± 1.94	140.1 ± 1.66	140.3 ± 1.91	140.1 ± 1.72	0.77
Serum K+ (mmol/l)	4.45 ± 0.35	4.39 ± 0.33	4.46 ± 0.41	4.38 ± 0.45	0.19
NT-proBNP (pmol/l)	209 (158 to 288)	219 (166 to 309)	219 (162 to 302)	219 (166 to 288)	0.89
Urinary volume (l/day)	1.78 ± 0.66	1.82 ± 0.83	1.76 ± 0.68	1.85 ± 0.80	0.77
Creatinine excretion (mmol/day)	149 ± 60	198 ± 73	149 ± 60	198 ± 73	
24-h urinary Na+ (mmol/day)	178 ± 76	159 ± 65	178 ± 77	173 ± 70	0.10
24-h urinary K+ (mmol/day)	72 ± 30	70 ± 26	76 ± 26	75 ± 26	0.30
Urinary Na+/K+ ratio	2.56 ± 0.93	2.40 ± 0.92	2.47 ± 0.98	2.36 ± 0.76	0.29
24-h aldosterone (mmol/day)	21.4 (15.1 to 31.6)	20.4 (14.8 to 30.9)	22.9 (14.8 to 31.6)	22.4 (14.1 to 33.1)	0.96

Values are mean (±SD), geometric mean (IQR), or number of individuals (%). P values are for differences across quartiles. HR, heart rate; IQR, interquartile range.

Fig. 1
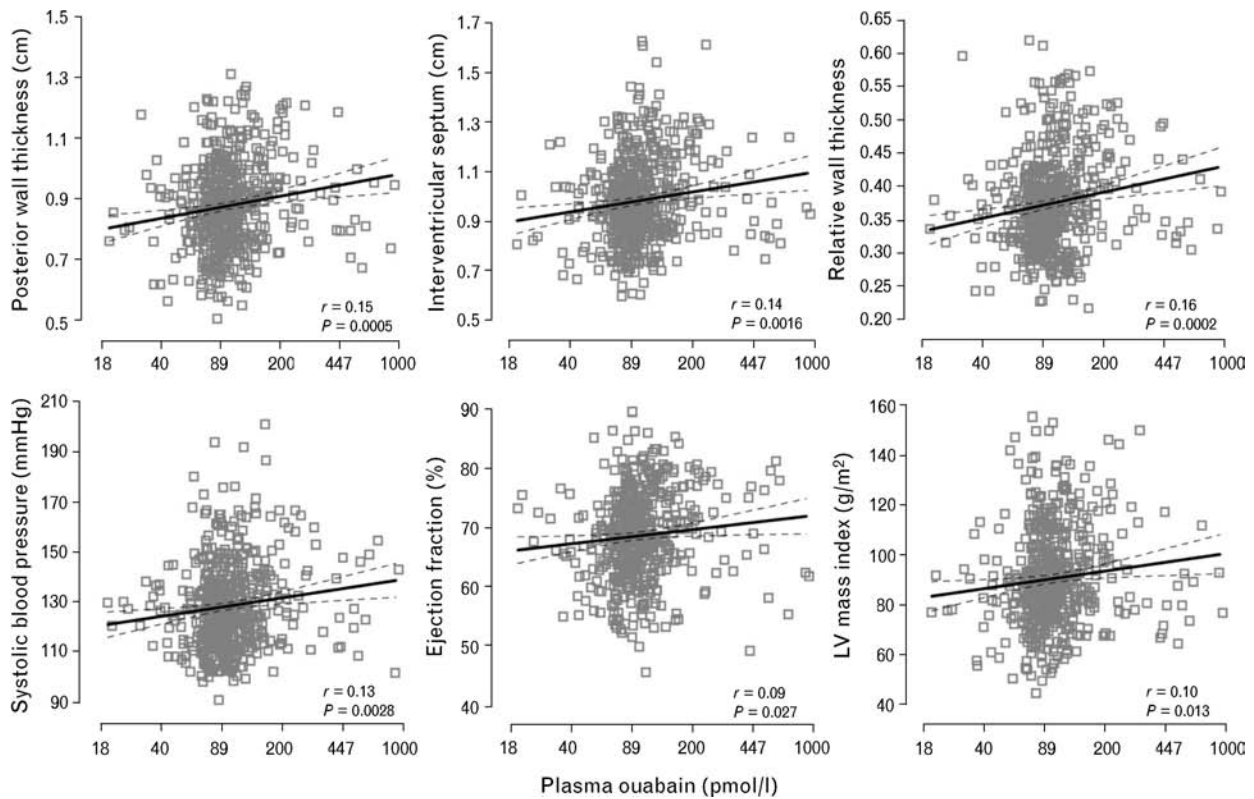
Scatter plots of the left ventricular wall thickness, relative wall thickness, left ventricular mass index, ejection fraction, and SBP versus plasma endogenous ouabain. The full and dotted lines represent the regression line and the 95% confidence interval, respectively.

Table 2 Echocardiographic characteristics of participants by endogenous ouabain quartiles

Characteristic	Low (n = 134)	Medium−low (n = 134)	Medium−high (n = 134)	High (n = 134)	P
Limits (pmol/l)	<79.4	79.4–95.4	95.5–120.2	>120.2	–
Conventional echocardiography					
Left atrial diameter (mm)	39.4 ± 5.2	39.2 ± 5.6	38.8 ± 5.1	39.6 ± 5.0	0.63
LV internal diastolic diameter (mm)	50.3 ± 4.8	50.4 ± 4.5	50.0 ± 4.5	49.8 ± 5.1	0.74
LV internal systolic diameter (mm)	31.4 ± 4.3	30.5 ± 4.0	30.5 ± 4.5	29.8 ± 5.4	0.044
Interventricular septum (mm)	9.6 ± 1.5	9.6 ± 1.6	10.0 ± 1.9	10.3 ± 1.8	0.002
Posterior wall (mm)	8.6 ± 1.4	8.5 ± 1.3	8.8 ± 1.4	9.2 ± 1.5	0.0001
RWT	0.36 ± 0.063	0.36 ± 0.063	0.38 ± 0.079	0.40 ± 0.077	0.0002
LV mass (g)	165 ± 46	165 ± 43	170 ± 43	177 ± 45	0.07
LV mass index (g/m²)	88.4 ± 20.5	88.3 ± 18.6	91.8 ± 19.3	94.8 ± 19.2	0.016
LV hypertrophy, n (%)	10 (7.5)	7 (5.2)	15 (11.2)	17 (12.7)	0.05
LV concentric remodelling, n (%)	14 (10.4)	13 (9.7)	30 (22.4)	44 (32.8)	<0.0001
Ejection fraction (%)	66.8 ± 7.0	69.3 ± 6.7	68.6 ± 7.4	70.1 ± 8.4	0.002
Doppler echocardiography					
SV (ml)	83.5 ± 15.7	83.9 ± 16.6	83.0 ± 15.9	86.2 ± 19.6	0.45
CI (l/min/m²)	2.72 ± 0.49	2.76 ± 0.53	2.72 ± 0.54	2.77 ± 0.50	0.75
Total peripheral resistance (dynes∗s∗cm⁻⁵∗m²)	2873 ± 527	2853 ± 597	2904 ± 597	2899 ± 554	0.87
Transmitral E/A ratio	1.27 ± 0.52	1.25 ± 0.52	1.26 ± 0.52	1.26 ± 0.52	0.99
TDI E_a/A_a ratio	1.22 ± 0.52	1.26 ± 0.52	1.24 ± 0.52	1.16 ± 0.52	0.53
E/E_a ratio	6.73 ± 0.52	6.86 ± 0.52	6.93 ± 0.52	7.22 ± 0.52	0.18
Longitudinal strain (%)	23.1 ± 3.47	23.3 ± 3.62	22.9 ± 4.06	22.4 ± 3.28	0.31

Values are mean (±SD), geometric mean (IQR range), or number of individuals (%). P values are for differences across quartiles. CI, cardiac index; IQR, interquartile range; LV, left ventricular; RWT, relative wall thickness; SV, stroke volume; TDI, tissue Doppler imaging.

oral contraceptives, there was a positive association between SBP and plasma endogenous ouabain. With adjustments applied, a 1-SD increase in plasma endogenous ouabain was associated with an increase in SBP by 1.59 mmHg ($P = 0.009$, Table 3).

Association between LV phenotypes and plasma endogenous ouabain

Table 2 shows the echocardiographic and hemodynamic data of the study population grouped according to plasma endogenous ouabain quartiles. In unadjusted analyses in which we treated the plasma endogenous ouabain as a continuous (Fig. 1) or categorical variable (Table 2), LV wall thickness, RWT, LV mass index, and ejection fraction increased with plasma endogenous ouabain level

($P \le 0.027$), whereas LV systolic diameter decreased with plasma endogenous ouabain ($P = 0.044$, Table 2). There were no significant differences amongst the endogenous ouabain quartiles in SV, CI, total peripheral resistance, or variables reflecting diastolic function and LV longitudinal strain (Table 2).

In multivariable-adjusted analyses accounting for sex, age, BMI, SBP, and use of antihypertensive drugs, only LV wall thickness and RWT significantly increased with plasma endogenous ouabain (Table 3). For a 1-SD increment in plasma endogenous ouabain, LV posterior wall, the interventricular septum, and RWT increased by 0.138 mm ($P = 0.003$), 0.152 mm ($P = 0.013$), and 0.71×10^{-2} ($P = 0.008$), respectively. LV concentric

Table 3 Adjusted LV traits and BP in relation to plasma endogenous ouabain in different age groups

Characteristic	All individuals (n = 536) Estimate (95% CI)	P	Age 20–80 years (n = 523) Estimate (95% CI)	P	Age 29–71 years (n = 431) Estimate (95% CI)	P	Age 42–62 years (n = 273) Estimate (95% CI)	P
Posterior wall (mm)	0.138 (0.046 to 0.229)	0.003	0.140 (0.046 to 0.233)	0.004	0.129 (0.028 to 0.230)	0.01	0.133 (0.006 to 0.260)	0.04
Interventricular septum (mm)	0.152 (0.032 to 0.273)	0.013	0.154 (0.032 to 0.275)	0.013	0.182 (0.051 to 0.313)	0.006	0.233 (0.073 to 0.393)	0.005
LV diastolic diameter (mm)	−0.227 (−0.567 to 0.110)	0.18	−0.256 (−0.601 to 0.090)	0.15	−0.338 (−0.703 to 0.028)	0.07	−0.609 (−1.05 to −0.175)	0.06
RWT (×10⁻²)	0.71 (0.19 to 1.23)	0.008	0.75 (0.22 to 1.28)	0.006	0.86 (0.29 to 1.43)	0.003	1.21 (0.53 to 1.90)	0.0006
LV mass (g)	2.07 (−0.59 to 4.73)	0.12	1.99 (−0.73 to 4.71)	0.15	1.97 (−0.98 to 4.93)	0.19	0.80 (−2.88 to 4.48)	0.67
LV mass index (g/m²)	1.01 (−0.40 to 2.43)	0.16	0.96 (−0.48 to 2.41)	0.19	0.89 (−0.67 to 2.46)	0.26	0.22 (−1.58 to 2.41)	0.68
Ejection fraction (%)	0.38 (−0.23 to 1.38)	0.22	0.54 (−0.07 to 1.16)	0.08	0.90 (0.28 to 1.52)	0.005	1.14 (0.34 to 1.94)	0.005
Longitudinal strain (%)	−0.31 (−0.65 to 0.04)	0.08	−0.36 (−0.72 to −0.02)	0.04	−0.48 (−0.85 to −0.11)	0.011	−0.57 (−1.05 to −0.08)	0.022
SBP (mmHg)	1.59 (0.39 to 2.80)	0.009	1.62 (0.39 to 2.85)	0.010	1.68 (0.33 to 3.03)	0.015	1.22 (−0.45 to 2.89)	0.15
DBP (mmHg)	0.46 (−0.23 to 1.16)	0.19	0.46 (−0.25 to 1.17)	0.20	0.43 (−0.34 to 1.21)	0.27	−0.02 (−1.00 to 0.96)	0.97

Effect sizes are expressed for a 1-SD increase in plasma endogenous ouabain (0.21 on the logarithmic scale). BP, blood pressure; CI, confidence interval; HR, heart rate; LV, left ventricular; RWT, relative wall thickness. LV traits were adjusted for sex, age, BMI (not applicable to LV mass index), SBP, and antihypertensive drug treatment. Ejection fraction was adjusted for sex, age, BMI, and HR. Longitudinal strain was adjusted for sex, age, and waist-to-hip ratio. BP was adjusted for sex, age, age squared, BMI, and use of antihypertensive drug treatment or oral contraceptives.

Table 4 Adjusted LV traits and BP in relation to plasma endogenous ouabain by sodium excretion

| Characteristic | Na < 161 mmol/24 h (n = 265) | | Na ≥ 161 mmol/24 h (n = 267) | |
	Estimate (95% CI)	P	Estimate (95% CI)	P
Posterior wall (mm)	0.103 (−0.016 to 0.223)	0.09	0.203 (0.058 to 0.350)	0.006
Interventricular septum (mm)	0.089 (−0.068 to 0.246)	0.26	0.227 (0.039 to 0.414)	0.018
LV diastolic diameter (mm)	−0.035 (−0.491 to 0.422)	0.88	−0.542 (−1.06 to −0.026)	0.040
RWT (x10^{-2})	0.32 (−0.36 to 1.01)	0.35	1.22 (0.43 to 2.02)	0.003
LV mass (g)	1.71 (−1.55 to 4.96)	0.30	2.10 (−2.21 to 6.41)	0.34
LV mass index (g/m^2)	0.86 (−0.95 to 2.67)	0.35	1.20 (−1.05 to 3.45)	0.29
Ejection fraction (%)	0.23 (−0.65 to 1.12)	0.60	0.41 (−0.41 to 1.24)	0.32
Longitudinal strain (%)	−0.10 (−0.58 to 0.38)	0.69	−0.50 (−1.00 to −0.005)	0.048
SBP (mmHg)	1.10 (−0.64 to 2.84)	0.21	2.25 (0.55 to 3.97)	0.010
DBP (mmHg)	0.002 (−0.95 to 0.96)	0.99	1.07 (0.036 to 2.10)	0.043

Effect sizes are expressed for a 1-SD increase in plasma endogenous ouabain (0.21 on the logarithmic scale). BP, blood pressure; CI, confidence interval; HR, heart rate; LV, left ventricular; RWT, relative wall thickness. LV traits were adjusted for sex, age, BMI (not applicable to LV mass index), SBP, and antihypertensive drug treatment. Ejection fraction was adjusted for sex, age, BMI, and HR. Longitudinal strain was adjusted for sex, age, and waist-to-hip ratio. BP was adjusted for sex, age, age squared, BMI, and use of antihypertensive drug treatment or oral contraceptives.

remodelling was more prevalent with higher plasma endogenous ouabain (P < 0.0001, Table 2), even after adjustment for covariables.

Sensitivity analyses
In sensitivity analyses, we narrowed the age range, first by excluding 13 participants younger than 20 or older than 80 years (1st–99th percentile interval), and next by only including in our analyses participants with an age range corresponding to the 10–90th percentile interval (29–71 years, n = 431) or to the IQR (42–62 years, n = 273). As shown in Table 3, the results of these sensitivity analyses, adjusted for covariables, were confirmatory for LV wall thickness, RWT, and SBP. Moreover, by narrowing the age range we noticed that a 1-SD increase in plasma endogenous ouabain was associated with opposite trends in ejection fraction (+0.90%, P = 0.005) and LV systolic longitudinal strain (−0.48%, P = 0.011). Similarly, we repeated our analyses after exclusion of individuals on antihypertensive drugs. In 420 untreated individuals, our findings remained consistent. SBP, LV wall thickness, and RWT increased with plasma endogenous ouabain (P ≤ 0.02).

In further sensitivity analyses, we treated the urinary sodium excretion as a continuous variable or a variable dichotomized by the population median (160 mmol/24 h). In individuals whose urinary sodium excretion was below median, the aforementioned associations of plasma endogenous ouabain with BP and the LV traits were in the same direction, but did not reach statistical significance (P ≥ 0.09, Table 4). In contrast, in participants with a sodium excretion above median, these associations reached a higher level of statistical significance (Table 4). We did not observe significant interaction between plasma endogenous ouabain and urinary sodium excretion in relation to any trait under study (P ≥ 0.09).

Discussion
The key finding was that, with adjustments applied, SBP and LV wall thickness increased with plasma endogenous ouabain. The significant positive association of RWT with plasma endogenous ouabain rested on increased LV wall thickness and a slightly decreased LV internal end-diastolic diameter. Our findings are in line with previous studies in selected groups of hypertensive patients. In 92 never-treated hypertensive patients, Pierdomenico *et al.* [5] noticed that plasma endogenous ouabain was significantly higher in patients with concentric remodelling than in those with normal geometry or concentric hypertrophy. In 128 untreated hypertensive patients, Manunta *et al.* [6] found that LV mass, SV, and RWT increased and HR decreased with higher circulating endogenous ouabain level. In this study, nearly 50% of patients with uncomplicated essential hypertension had elevated plasma endogenous ouabain compared with normotensive individuals.

In the current study, sensitivity analyses in untreated individuals or in a limited age range group provided confirmatory results. Moreover, by controlling the age range, and thereby decreasing the variability in LV systolic function, we observed that higher plasma levels of endogenous ouabain were associated with an increased ejection fraction and a decreased LV systolic longitudinal deformation. The effect of endogenous ouabain on LV systolic function might be explained by the observed association between RWT and plasma endogenous ouabain. Indeed, in our previous report [10], longitudinal end-systolic strain significantly and independently decreased with age and RWT, whereas ejection fraction increased with age and RWT.

In the current study, the associations of plasma endogenous ouabain with LV traits and BP were tighter at higher sodium excretion irrespective of whether sodium excretion was treated as a continuous variable or dichotomized by median. At variance with a previous report [15], we did not find significant interaction between sodium excretion and circulating endogenous ouabain in relation to BP. In the previous study [15], plasma endogenous ouabain apparently behaved as a BP-modulating factor.

In individuals with plasma endogenous ouabain values below the median (140 pmol/l), SBP and DBP increased by 2.2 and 1.4 mmHg, respectively, for each 50 mmol/24 h increment in urinary sodium excretion ($P < 0.01$). No association between BP and urinary sodium excretion was found when plasma endogenous ouabain exceeded the median. However, in the previous study [15], conditions of measurement were different. Blood samples were obtained in the sitting position, and BP was measured at individuals' homes. In never-treated hypertensive patients [16] and in healthy individuals [17], acute interventions that promoted loss of body sodium (administration of diuretic) raised circulating endogenous ouabain. To our knowledge, no previous studies investigated the interaction between plasma endogenous ouabain and sodium excretion in relation to the structural and functional properties of the left ventricle.

Experimental studies suggested that endogenous ouabain originates from the adrenocortical gland and possibly from the hypothalamus. Endogenous ouabain has been isolated from bovine adrenals and hypothalamus [18,19]. Cultured human and bovine adrenocortical cells secrete endogenous ouabain into the culture fluid [20]. In conscious catheterized dogs, the endogenous ouabain content was approximately 5–6-fold higher in adrenal venous blood than in arterial blood [21]. With prolonged adrenalectomy and cortisone replacement in rats, the plasma concentration of endogenous ouabain was not measurable [22].

The mechanisms underlying the present association of LV geometry with plasma endogenous ouabain remain to be elucidated. Endogenous ouabain circulates in plasma in subnanomolar concentrations, which might be too low to inhibit Na+,K+-ATPase [23]. In-vitro studies demonstrated that ouabain at very low concentrations (10^{-10} mol/l) promoted cell growth via activation of the ERK1/2 pathway in rat cardiomyoctyes [24] and in proximal tubular cells [25], as well as in human umbilical vein smooth muscle cells [26]. Furthermore, Ferrandi et al. [4] induced cardiac growth in rats by infusing ouabain at a rate of 15 μg/kg/day for 18 weeks, which doubled the circulating ouabain level from 0.3 to 0.7 nmol/l and increased BP by 20 mmHg and the weight of the left ventricle by 11%. These in-vivo effects are associated with significant enrichment of α1, β1, γa Na+,K+-ATPase subunits together with Src and EGFr in isolated renal caveolae membranes, and with activation of ERK1/2 [4]. On the contrary, Rossoni et al. [27] showed that treatment of rats with a higher concentration of ouabain (8 μg/day) for a shorter period (5 weeks) induced hypertension without LV hypertrophy or impaired function of the sarcoplasmic reticulum. These authors also showed positive inotropic and lusitropic effects on the hearts of ouabain-treated rats, which were associated with an increment in the activity of myosin ATPase and in the expression of the catalytic subunit of the Na+,K+-ATPase. More recently Jiang et al. [28] described ouabain-induced cardiac hypertrophy in rats before the BP started to increase.

Since the original report published in 1991 [29], many laboratories, albeit not all, were able to detect endogenous ouabain in tissues and isolate it from tissues and plasma, using a variety of immunoassays (RIA, enzyme-linked immunosorbent assay, EIA, and dissociation-enhanced lanthanide fluorescent immunoassay). Comparable with hormones circulating in plasma at subnanomolar concentrations, such as NT-proBNP, the normal range of endogenous ouabain varied between laboratories [30,31]. Variability in reported plasma endogenous ouabain levels might be explained by differences in the immunoassay radioactive label, the sensitivity of the ouabain antibodies, and cross-reactivity with other glycosides or steroid hormones with similar polarity [30,31]. In our population study, we used a RIA, which was carefully standardized as previously reported [11]. In the current study, the geometric mean concentration of plasma endogenous ouabain was 95.5 pmol/l, which is lower than reported previously in our Flemish population (140 pmol/l) using the same RIA [15]. Body position and prolonged rest in the supine position might explain the lower level. Indeed, in the present study, we collected plasma after the participants had rested lying for at least 45 min. In humans and dogs, physical exercise raises the circulating endogenous ouabain level [32].

The present study must be interpreted within the context of its limitation. Variables reflecting LV structure and function are quantitative traits prone to measurement error. However, only one experienced observer performed all ultrasound examinations. We excluded patients with overt heart disease that change cardiac structure. Extensive studies have been performed to validate the RIA for plasma endogenous ouabain used in the current study [33]. Amongst 21 patients, a positive correlation ($P < 0.001$) has been observed between plasma endogenous ouabain measured by both HPLC–mass spectrometry and RIA (Ferrandi M, Hamlyn J, Manunta P; unpublished observation).

Conclusion

Our population-based study suggested that endogenous ouabain might have a trophic effect on the myocardium, independent of BP and other covariables. Elevated circulating ouabain might be a factor that contributes to an increased risk of LV remodelling and hypertrophy amongst patients with essential hypertension. These findings need replication in other populations and patient cohorts as well as in longitudinal studies. Approximately one-third of essential hypertensive patients show an increased level of circulating ouabain, which is associated with renal and cardiac remodelling [31]. A new compound, rostafuroxin, which antagonizes the pathological molecular effects of endogenous ouabain, might open

new possibilities for the therapy of hypertension and the related target organ damage [34].

Acknowledgements

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