Journal of Human Hypertension (2004) 18, 437–443

www.nature.com/jhh

ORIGINAL ARTICLE

Is inappropriate left ventricular mass related to neurohormonal factors and/or arterial changes in hypertension? a LIFE substudy

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We investigated whether inappropriately high left ventricular (LV) mass, defined as observed LV mass exceeding the level of individual LV mass predicted from gender, height, and stroke work, may be associated with an imbalance between growth-promoting and growth-inhibitory factors and/or structural vascular changes. In 53 patients with hypertension and electrocardiographic LV hypertrophy, 24-h ambulatory blood pressure (BP); echocardiographic LV mass, stroke volume and stroke work; minimal forearm vascular resistance (MFVR); and intima–media cross-sectional area in common carotid arteries (IMA) were evaluated after 2 weeks of placebo treatment. Serum insulin, plasma epinephrine, norepinephrine, endothelin, angiotensin II, aldosterone, and brain natriuretic peptide (BNP) were also measured. High observed LV mass was related to high IMA ($r=0.46$, $P<0.001$), MFVR (in men: $r=0.36$, $P<0.05$), 24-h ambulatory systolic BP ($r=0.30$, $P=0.06$), and lower plasma angiotensin II ($r=-0.33$, $P<0.05$), but not to other circulating growth factors. Stroke work was similarly related to IMA ($r=0.42$, $P<0.01$), MFVR (in men: $r=0.41$, $P<0.05$), and plasma angiotensin II ($r=-0.32$, $P<0.05$). Inappropriate LV mass, identified by the ratio between observed LV mass and the value predicted for gender, height, and stroke work, was not significantly related to any of the arterial or neurohormonal variables. In this small series of older hypertensive patients, inappropriate LV mass was not significantly related to arterial changes or to measured circulating growth factors, although weak relations cannot be excluded. Alternatively, inappropriately high LV mass might be related to unmeasured factors such as local myocardial alterations in growth factors and/or genetic predisposition to develop excessive LV hypertrophy.
Journal of Human Hypertension (2004) **18**, 437–443. doi:10.1038/sj.jhh.1001720
Published online 11 March 2004

Keywords: left ventricular hypertrophy; vascular hypertrophy; growth factors; inappropriate left ventricular mass

Introduction

Left ventricular (LV) hypertrophy is often seen in patients with essential hypertension, and is, in part, thought to be a compensatory response to increased cardiac load. In population studies, it has been demonstrated that it is possible to predict LV mass from gender, height to the physiologic allometric power, and stroke work using an empiric formula.[1] In some subjects the observed LV mass is higher than that predicted, independent of the presence of arterial hypertension.[2] The inappropriately high LV mass in these subjects may theoretically be explained by at least three different mechanisms. One mechanism is the presence of a higher blood pressure (BP) load in the ascending aorta than indicated by the BP measured at the level of the brachial artery, due to changes in the peripheral pressure wave reflection in elderly hypertensive patients with vascular hypertrophy,[3] which may not be completely detectable by using resting stroke work as a single-beat index of cardiac workload. Another mechanism is an imbalance between growth-promoting and growth-inhibitory factors.[4–6] A third mechanism is genetic factors leading to a more vigorous growth response to a given pressure load.[7] Inappropriately high LV mass, de-

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Published online 11 March 2004

fined as observed LV mass higher than 128% of predicted LV mass,[1] may theoretically be especially serious because it might represent a kind of overcompensation, which might compromise the haemodynamics and exaggerate oxygen requirements of the heart. Therefore, we investigated whether inappropriate high LV mass was associated with higher 24-h BP load, increased arterial hypertrophy, and/or higher levels of growth-promoting circulating neurohormonal factors and BNP.

Methods

Subjects

After 2 weeks of placebo treatment, we studied 53 patients (14 women and 39 men), age 56–77 years, with essential hypertension and LV hypertrophy, as determined by screening electrocardiogram prior to study entry. All patients were participants in the Insulin Carotids US Scandinavia (ICARUS) substudy[8] of the Losartan Intervention For Endpoint reduction in hypertension (LIFE) trial, and met the study's inclusion and exclusion criteria.[9] There were 15 smokers, 21 exsmokers, and 17 nonsmokers. In all, 13 patients had recently diagnosed hypertension, of whom eight had never received antihypertensive treatment. Of the 45 previously treated patients, 33 patients had received vasodilators. The mean duration of known hypertension was 7 years, ranging from 0 to 35 years. Patient characteristics are listed in Table 1.

Protocol and methods

Echocardiography
Echocardiograms were performed following a previously employed protocol.[10] Standardized examinations included two-dimensional (2-D) guided M-mode echocardiograms and selected 2-D recordings. Studies were performed using a high-quality commercially available echocardiograph (VingMed CFM-800, General Electric) equipped with 3.0–3.5 MHz and 2.0–2.5 MHz probes and Super-VHS video recorder. Measurements were made blindly at the Echocardiography Reading Center at The New York Presbyterian Hospital–Weill Medical College of Cornell University in New York City by experienced physician readers using computerized review stations (Digisonics, Houston, TX, USA) equipped with digitizing tablet and monitor screen overlay for calibration and performance of each needed measurement. LV dimensions were measured according to recommendations of the American Society of Echocardiography.[11] When optimal orientation of the LV or atrial imaging views could not be obtained, correctly oriented 2-D linear dimension measurements were made by the leading-edge convention.[11] End-diastolic LV diameter and wall thickness were used to calculate relative wall thickness (RWT),[10] and LV mass by a formula that gives values closely related to autopsy LV weight ($r = 0.90$), and that showed excellent reproducibility from readings by this group of experienced physician readers.[12] Stroke volume was calculated as the difference between LV end-diastolic and end-systolic volume generated from the internal dimensions of LV using Teichholz' formula. Predicted LV mass was calculated using the formula described by de Simone *et al*.[1] ($-18.1 \times$ gender $+ 0.64 \times$ stroke work $+ 6.63 \times$ height $(m)^{2.7} + 55.37$), where gender = 1 if male and 2 if female, and stroke work is calculated as stroke volume $\times$ systolic BP $\times$ 0.0144. Inappropriately high LV mass was defined as LV $mass_{observed}$/LV $mass_{predicted} > 1.28$.[1]

Carotid artery ultrasound
Intima–media thickness (IMT) and the lumen diameter of the common carotid arteries were measured by ultrasound using the ACUSON 128XP/10*c* (Acuson Corporation, CA, USA) and a linear 7 MHz transducer. IMT of the right and left common carotid artery was measured in the 1 cm segment proximal to the dilation of the carotid bulb as described by Howard *et al*.[13] The longitudinal B-mode image was accepted as valid when a double line representing lumen–intima and media–adventitia interfaces was visualized in the longest possible segment of both near and far walls. At end-diastole, IMT of the far walls of the left as well as of the right common carotid artery were measured at 10 points 1 mm apart using the leading-edge convention, and the mean value was calculated. Based on end-diastolic IMT and lumen diameter, the intima–media cross-sectional area (IMA) was calculated.[14] The end-diastolic and end-systolic lumen diameters of the right and the left common carotid artery were measured using M-mode within the 1-cm segment proximal to the dilation of the carotid bulb avoiding measuring at plaques (defined as a focal thickness > 1.5 mm). For both arteries the relative change in lumen diameter was divided by the brachial pulse

Table 1 Patient characteristics

	Mean (95% CI)
Age (years)	65 (64–67)
Height (m)	1.70 (1.67–1.73)
Weight (kg)	83 (80–85)
Body mass index (m^2/kg)	28.6 (27.8–29.4)
Plasma glucose (mmol/l)	6.2 (5.7–6.8)
Serum cholesterol (mmol/l)	6.0 (5.7–6.4)
Systolic BP (mmHg)	176 (172–180)
Diastolic BP (mmHg)	96 (93–98)
Daytime ambulatory SBP (mmHg)	158 (152–163)
Daytime ambulatory DBP (mmHg)	90 (86–93)
Observed LV mass (g)	243 (227–259)
Observed LVMI (g/m^2)	125 (118–132)
Predicted LV mass (g)	188 (177–199)

CI = confidence interval; DBP = diastolic BP; LV = left ventricular; LVMI = left ventricular mass index; SBP = systolic BP.

pressure at the time of the investigation, and the mean value was used as a measure of carotid distensibility, the so-called pressure strain modulus. All readings were done on digitized pictures (Optimas 6.11, Optimas Corporation, USA) at The University of Michigan Medical Center in Ann Arbor. IMT and IMA/height were used to assess arterial hypertrophy, whereas the pressure strain modulus was used to assess arterial stiffness.

Forearm plethysmography
The maximal forearm blood flow was measured after 10 min of ischaemia[15] using strain-gauge plethysmography (Model EC5R, Hokanson Inc., Bellevue, WA, USA). A wrist-occluding cuff was used. Forearm blood flow was measured for 3 s every 6 s during the first minute of hyperaemia, and was expressed as millilitres flow per 100 m tissue per minute. At the same time BP was measured three times with a semiautomatic sphygmomanometer (Dinamap Model 1846 SX, Critikon, Johnson & Johnson Medical, Inc., USA). Among the 9–10 forearm blood flow measurements taken during the first minute of hyperaemia, the highest value was chosen to represent the maximal forearm blood flow. Minimal forearm vascular resistance (MFVR) was calculated as the corresponding mean arterial BP divided by the maximal forearm blood flow (MFVR = MABP/FBF). We analysed MFVR in women ($MFVR_{women}$) and men ($MFVR_{men}$) separately because MFVR values and standard deviations were higher in the more obese women (3.1 ± 1.42 *vs* 2.3 ± 0.48 mmHg min).[16] MFVR is thought to represent an integrated measure of arteriolar remodelling and capillary rarefaction in the forearm, and is used to assess anatomical peripheral vascular remodelling.

Myograph investigations
Under local anaesthesia, a subcutaneous biopsy measuring about 1 cm^3 was taken from the gluteal region. The biopsy was placed in a cold physiological saline solution. The small resistance arteries (100–500 μm) were isolated under microscope and mounted on a myograph (Automated Dual Wire Myograph System Model 500A, JP Trading I/S, Århus, Denmark). The media thickness and lumen were measured by microscope, and the vessels were exposed to increasing radial stretch calculating internal circumference at 100 mmHg distension pressure[17] for calculation of the normalized media:-lumen ratio (MLR). In most patients MLR represented the mean of two resistance arteries, and was used as a measure of resistance artery remodelling.

Ambulatory BP
After resting for 1 h, an automatic BP device that measured ambulatory BP using the cuff-oscillometric method (Takeda TM-2421, A&D Co. Ltd, Tokyo, Japan)[18] was applied to the left arm and worn for 24 h. Oscillometric BP were measured every half hour during daytime and every hour at night (2300 to 0600 hours). There were more than 20 readable measurements during 24 h in 33 men and seven women, considered sufficient[19] to calculate the median 24-h value.

Assays
After an overnight fast, all patients had a polyethylene cannula inserted into an antecubital vein for collection of blood samples. After lying supine in a quiet room with a constant temperature of 24–27°C for 1 h, the blood samples were drawn and collected in specially prepared containers. Plasma glucose concentrations were measured using a Beckmann glucose analyzer 2 (Beckman Instruments, Inc., Fullerton, CA, USA) and a glucose oxidase method. Serum insulin concentrations were determined by enzyme immunoassay, as described by Andersen *et al*.[20] Plasma concentrations of epinephrine and norepinephrine were determined by radioenzymatic labeling and high-pressure liquid chromatography.[21] Plasma endothelin concentrations were measured by radioimmunoassay using rabbit antiserum (RAS 6901, Peninsula Laboratories, Belmont, CA, USA), ^{125}I-endothelin (Amersham Life Science Ltd), and standards (Peptide Institute, Osaka, Japan). Blood was collected in EDTA aprotinin tubes and plasma was extracted on Sep-pak C18 prior to analysis. Active plasma renin concentrations were determined using the principle of antibody trapping[22] as modified by Millar *et al*.[23] Plasma angiotensin II concentrations were measured according to the method of Kappelgaard *et al*[24] with the modification that Sep-Pac C_{18} (Millipore Waters) was used for plasma extraction. Plasma aldosterone concentrations were measured using a commercial kit (DSL-8600, Diagnostic Systems Laboratories Inc., Webster, TE, USA). Plasma brain natriuretic peptide (BNP) was measured by Shionoria®BNP, an immunoradiometric assay kit using two different monoclonal antibodies.[25]

Statistical analysis

For data management and statistical analyses, Statistica 5.1 (StatSoft, Inc., Tulsa, OK, USA) was used. Parametric statistics were used calculating mean values and 95% confidence intervals. We performed simple linear regression analyses calculating the regression coefficient (r). When performing stepwise, backward multiple linear regression analyses calculating the standardized regression coefficient for each parameter (β) and the common adjusted coefficient of determination for the model (adj. R^2), all parameters shown to be significant in simple linear regression analyses entered the model. Two-tailed P-values < 0.05 were considered statistically significant.

Results

Observed LV mass was related to predicted LV mass ($r=0.61$, $P<0.001$), but it was significantly higher than predicted ($P<0.001$) (Table 1). As a result, 28 of the 53 patients had inappropriate LV mass. Patients with inappropriate LV mass had higher observed LV mass and relative wall thickness, as expected, but did not have significantly more pronounced vascular hypertrophy (Table 2) or higher levels of circulating growth factors (Table 3).

Observed as well as predicted LV mass were positively related to 24-h systolic BP, $MFVR_{men}$, IMT, IMA, and lumen of the common carotid arteries (Table 4), and negatively related to serum insulin and plasma angiotensin II levels (Table 5). Observed and predicted LV mass were unrelated to the other circulating growth factors (Table 5). The ratio between observed and predicted LV mass, a measure of inappropriate LV mass, was not related to structural vascular parameters, 24-h ambulatory BP, or circulating growth factors (Tables 4 and 5).

Stroke work, which is the main determinant of LV mass in adults, was related to observed LV mass ($r=0.55$, $P<0.001$). More interestingly, stroke work was also related positively to $MFVR_{men}$ ($r=0.41$, $P<0.05$) and IMA ($r=0.42$, $P<0.01$), and negatively to plasma angiotensin II ($r=-0.32$, $P<0.05$). In multiple regression analyses, high stroke work was independently associated with high IMA ($\beta=0.40$) and low plasma angiotensin II ($\beta=-0.30$) (adj. $R^2=0.22$, $P<0.01$), while it was unrelated to other circulating growth factors. Angiotensin II was negatively related to LV end-diastolic diameter ($r=-0.35$, $P<0.05$). We did not find any significant influence of previous antihypertensive treatment on plasma levels of the growth factors.

Discussion

More than half of the patients enrolled in this study had inappropriately high LV mass, despite the fact that most of the patients had previously received antihypertensive treatment for several years and were examined after only 2 weeks of placebo treatment during which BP rose to a higher level than the heart had recently been accustomed to. The

Table 2 Cardiovascular hypertrophy and BP related to inappropriateness of LV mass

	Observed/predicted LV mass Mean (95% CI)		
	< 1.28 (n = *25*)	*⩾ 1.28* (n = *28*)	P-*value*
24-h systolic BP (mmHg)	153 (143–162)	157 (151–164)	NS
24-h diastolic BP (mmHg)	86 (80–91)	88 (83–92)	NS
Predicted LV mass (g)	206 (188–223)	172 (159–184)	<0.01
Observed LV mass (g)	225 (205–244)	260 (235–284)	<0.05
Observed LVMI (g/m^2)	117 (107–126)	132 (122–143)	<0.05
RWT (ratio)	0.38 (0.36–0.40)	0.44 (0.42–0.47)	<0.001
IMT (mm)	0.97 (0.91–1.03)	0.95 (0.88–1.01)	NS
IMA/height (mm^2/m)	13.4 (12.1–14.6)	12.8 (11.9–13.7)	NS
Lumen (mm)	8.2 (7.7–8.7)	8.3 (7.9–8.6)	NS
MFVR (mmHg min 100)	2.5 (2.2–2.8)	2.9 (2.3–3.6)	NS
Media:lumen ratio (%)	11.4 (10.1–12.7)	11.0 (9.8–12.3)	NS

BSA = body surface area; CI = confidence interval; IMA = intima–media cross-sectional area of the common carotid arteries; IMT = intima–media thickness of the common carotid arteries; LV = left ventricular; LVMI = left ventricular mass index; MFVR = minimal forearm vascular resistance; NS = not significant; RWT = relative wall thickness.

Table 3 Circulating growth factors related to inappropriateness of left ventricular mass

	Observed/predicted LV mass mean (95% CI)		
	< 1.28 (n = *25*)	*⩾ 1.28* (n = *28*)	P-*value*
Serum insulin (pmol/l)	54 (37–71)	44 (34–54)	NS
Plasma epinephrine (mmol/l)	0.28 (0.24–0.32)	0.28 (0.23–0.33)	NS
Plasma norepinephrine (mmol/l)	1.29 (1.05–1.53)	1.25 (1.08–1.41)	NS
Plasma renin (mU/l)	9.4 (4.7–14.1)	10.5 (6.9–14.1)	NS
Plasma angiotensin II (pmol/l)	10.1 (7.7–12.5)	11.3 (8.5–14.1)	NS
Plasma aldosterone (pmol/l)	152 (116–188)	131 (99–162)	NS
Plasma endothelin (pmol/l)	1.26 (1.11–1.41)	1.20 (1.09–1.31)	NS
Plasma BNP (pmol/l)	37 (21–53)	37 (25–50)	NS

BNP = brain natriuretic peptide; CI = confidence interval; NS = not significant.

Table 4 Relationship between left ventricular mass and blood pressure and arterial hypertrophy

	Left ventricular mass					
	Observed		*Predicted*		*Observed/predicted*	
	r	P-*value*	r	P-*value*	r	P-*value*
24-h DBP	0.14	NS	0.15	NS	0.04	NS
24-h SBP	0.30	0.06	0.36	<0.05	0.21	NS
IMT	0.22	NS	0.44	0.001	−0.002	NS
IMA	0.46	<0.001	0.55	<0.001	0.10	NS
Lumen	0.53	<0.001	0.51	<0.001	0.06	NS
IMT/lumen	−0.12	NS	−0.07	NS	−0.04	NS
Distensibility	−0.04	NS	0.03	NS	−0.06	NS
$MFVR_{men}$	0.36	<0.05	0.31	0.08	0.02	NS
Media:lumen ratio	0.08	NS	0.22	NS	0.01	NS

DBP = diastolic BP; IMA = intima–media cross-sectional area of the common carotid arteries; IMT = intima–media thickness of the common carotid arteries; Lumen = internal diameter of the common carotid arteries; MFVR = minimal forearm vascular resistance; NS = not significant; SBP = systolic BP.

Table 5 Relationship between left ventricular mass and circulating growth factors

	Left ventricular mass					
	Observed		*Predicted*		*Observed/predicted*	
	r	P-*value*	r	P-*value*	r	P-*value*
Serum insulin	−0.29	0.07	−0.06	NS	−0.26	NS
Plasma epinephrine	0.04	NS	0.01	NS	0.04	NS
Plasma norepinephrine	0.01	NS	0.01	NS	−0.04	NS
Plasma renin	0.06	NS	0.15	NS	−0.02	NS
Plasma angiotensin II	−0.33	<0.05	−0.31	<0.05	−0.07	NS
Plasma aldosterone	−0.19	NS	−0.03	NS	−0.16	NS
Plasma endothelin	0.08	NS	0.19	NS	−0.01	NS
Plasma BNP	0.22	NS	0.20	NS	0.04	NS

BNP = brain natriuretic peptide; NS = not significant.

high prevalence of inappropriate LV mass may in part reflect the selection of patients having electrocardiographic LV hypertrophy.

As previously reported,[3] observed LV mass was directly related to vascular hypertrophy, whereas the deviation of LV mass from the predicted value was not. This suggests that vascular hypertrophy and the accompanying increased cardiac afterload due to changes in the peripheral pressure wave reflection may not explain the increase in LV mass beyond the values that would be compensatory for the individual body size and cardiac workload in middle-aged patients with hypertension and electrocardiographic LV hypertrophy. This observation is also consistent with the fact that the equation predicting LV mass, to some degree, takes into account vascular impact by calculating stroke work, which is influenced by peripheral resistance and arterial compliance.[26] Since we did not measure the peripheral pressure wave reflection, we cannot rule out the possibility that the use of brachial BP instead of aortic BP may contribute to the lower predicted LV mass in this group of older hypertensive patients. However, this did not seem to play a major role because we did not see any relation between inappropriate LV mass and the measured vascular changes. The lack of relationship between inappropriately high LV mass and vascular hypertrophy is probably correct and not due to difficulties in measuring vascular hypertrophy because the vascular changes previously have been shown to be related to both LV hypertrophy as well as high BP load.[3]

Moreover, the fact that inappropriate LV mass was not related to any of the circulating growth factors measured indicates that the excess of LV mass, at least in this selected group of patients, was not due to an imbalance between circulating levels of selected growth-promoting and growth-inhibitory factors. Although this lack of association could be due to some effect of previous therapy, a confounder that cannot be completely excluded by our analysis, we did not find any significant association of previous antihypertensive treatment with circulat-

ing growth factors. However, we cannot rule out an influence of previous antihypertensive treatment on local myocardial growth factor production. In fact, deviations of LV mass from the haemodynamically predicted LV mass may be related to local myocardial alteration in turnover of growth factors,[27,28] which is not reflected by circulating values. The negative relation between plasma angiotensin II and observed LV mass is consistent with previous studies,[29,30] and needs to be examined in view of the previous considerations and of the importance of volume load in the magnitude of hypertrophic response.[31]

Although no relation was found with previous treatment with ACE inhibitors, a possible influence cannot be excluded because of the opposite effects of ACE inhibitors to increase circulating angiotensin II concentration while reducing LV mass.

A third possibility is that the excess of LV mass in this group of patients is due to the selection of patients with LV hypertrophy on ECG leading to an overrepresentation of patients with a genetic predisposition for developing excessive LV hypertrophy in response to a given haemodynamic load.

Conclusion

Inappropriate high LV mass is common in middle-aged, hypertensive patients with electrocardiographic LV hypertrophy, and is independent of BP load, levels of circulating growth factors, and structural vascular changes, indicating that the deviation of LV mass from the haemodynamically predicted value cannot be explained by vascular changes or circulating growth factors. Therefore, we speculate that other factors, such as differences in genetics and local growth factors, may explain the high prevalence of inappropriate LV mass in this group of patients.

Acknowledgements

We acknowledge technical assistance by Lisa Krause in Ann Arbor, Michigan, USA, in measuring IMT, and Paulette A Lyle for assistance in preparation of the manuscript. This work was supported in part by the Grants from The Danish Medical Association Research Fund, Copenhagen, Denmark; The Becket-Foundation, Copenhagen, Denmark; Editor and Mrs. Anders Christian Kaarsens Foundation, Copenhagen, Denmark; and Merck & Co, Inc, West Point, PA, USA as part of the LIFE ICARUS substudy.

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